Mathematical models of microbial growth and metabolism: a whole-organism perspective

OLGA A. NEV AND HUGO A. VAN DEN BERG

Mathematics Institute, University of Warwick, Coventry CV4 7AL, UK

ABSTRACT

We review the principles underpinning the development of mathematical models of the metabolic activities of micro-organisms. Such models are important to understand and chart the substantial contributions made by micro-organisms to geochemical cycles, and also to optimise the performance of bio-reactors that exploit the biochemical capabilities of these organisms. We advocate an approach based on the principle of dynamic allocation. We survey the biological background that motivates this approach, including nutrient assimilation, the regulation of gene expression, and the principles of microbial growth. In addition, we discuss the classic models of microbial growth as well as contemporary approaches. The dynamic allocation theory generalises these classic models in a natural manner and is readily amenable to the additional information provided by transcriptomics and proteomics approaches. Finally, we touch upon these organising principles in the context of the transition from the free-living unicellular mode of life to multicellularity.

Keywords: *microbial growth, mathematical models, regulation, chemostat*

Olga Nev holds two BSc degrees —in Applied Mathematics and in Business Studies— from Nizhny Novgorod State University, as well as an MSc in Mathematical Economics from the St. Petersburg School of Economics. She recently obtained a PhD in Interdisciplinary Sciences at the University of Warwick. Her research focusses on the application of stochastic dynamical systems to mathematical biology. *email:* O.Nev@warwick.ac.uk

Hugo van den Berg teaches mathematical biology at the University of Warwick. In addition to several textbooks, on evolutionary dynamics and on the general theory & practice of mathematical modelling, he has written fourscore papers on energetics & homeostasis, T-cell immunity, evolutionary biology, bioinformatics, and the physiology of uterine contractility. *email:* hugo@maths.warwick.ac.uk

Olga Nev was funded through EU Research Framework programme 7 *Marie Curie Actions*, grant 316630 Centre for Analytical Science – Innovative Doctoral Programme (CAS-IDP)

1 Introduction

Life on Earth can be divided into several kingdoms[1], with the prokaryote domains of the Archaea and the Bacteria standing out by dint of their vast metabolic diversity and versatility which is belied by the paltry morphological variation encountered in these domains[2] (Fig. 1). By contrast, a stunning variation in morphology is exhibited by the eukaryote kingdoms Plantae and Animalia, but there it is coupled to a range of metabolic variability which seems quite modest when compared to that of the prokaryotes: fungi, plants, and animals make do with a fairly standard core metabolism (and even so, owe much of their metabolic versatility to endosymbiontic prokaryotes[3]), while bacteria are not only able to derive their molecular building blocks from a huge range of compounds, organic as well as inorganic, but also to catalyse reduction-oxidation reactions with a wide range of substrates, deriving energy from inorganic salts, light, or organic compounds[2]. Equally impressive is the ability of microbial life to adapt to extremes in temperature and salinity[2].

The biochemical versatility of microbes is not only apparent as we survey different species, but also often occurs (albeit in a more restricted form) within the individual cell, which is typically able to adapt its metabolism to the prevailing conditions, often to a far greater extent than are cells in multicellular organisms[4]. We shall return to this contrast in Section 6 and meanwhile fix our attention on the dynamic metabolic adaptability of the individual microbial cell.

Our ultimate goal is to be able to describe quantitatively how the cell responds to changing environmental conditions, not only in terms of the biochemical compounds it produces (which comprise the material substance of their own cells as well as substances secreted into the ambient medium), but also in terms of the compounds that are removed from the environment in the process. To appreciate the importance of this goal, one need only consider the role played by micro-organisms in tempering the impact of global climate change[5], for instance their involvement in sequestration of carbon dioxide[6]. Moreover, achieving the goal of accurate quantitative description can aid the optimisation of bioreactors and thus improve the efficiency of biotechnological-industrial applications.

Figure 1: **Shapes of unicellular bacteria.** The basic shapes are bacilli (rods, i.e. cylinders capped by hemispheric domes), cocci (actually short rods), vibrios (curved rods), and spirilla ("corkscrews"). If cells remain attached they can form stacked or grape-like clusters, or elongated filaments (known as trichomes, called streptococci when the individual cells are cocci). Cellular inclusions can distort the bacillus into a club-shape (far right).

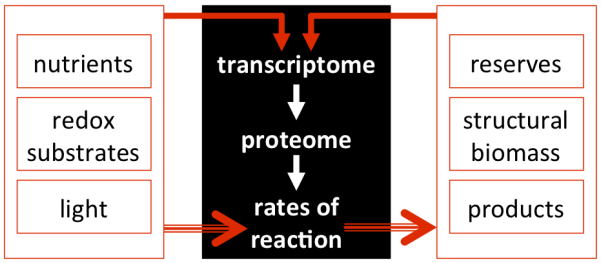

Figure 2:
Flow of information in a microbial cell.

2 The *dynamic allocation* approach

One way of understanding both the challenges the microbe faces and the ways in which it opposes these challenges, is to conceive of the cell as an agent that is constantly solving an *allocation problem*: that of distributing the molecular building blocks which it has at its disposal among the various kinds of catalytic machinery that are encoded in its genome[7]. At the molecular-mechanistic level, this distribution is dependent on the rates at which the various genes are being transcribed. Thus, to respond in an adaptive manner to changes in the environment, such as increases or decreases in the availability of nutrients, effectively means adjusting these rates of expression (and hence the building block allocation among different types of molecular machinery) to the prevailing conditions. Moreover, we should also expect these gene expression rates to be adjusted in response to the internal status of the cell, in particular the levels of intracellular stores of building blocks.

Combining these ideas, we arrive at the information flow diagram shown in Fig. 2. Ambient (external) conditions, listed on the left, serve as inputs to gene expression rates, which govern the transcriptome, which determine the proteome, which determines the rates at which the cell's biochemical reactions proceed. Indicated here are the *availability* conditions, which themselves are affected by additional environmental factors such as temperature, pH, water activity, salinity, and so on[2]. In addition to expression-level regulation, there is also a more locally acting mode of regulation in which the activity of the machinery is regulated, often by covalent addition of moieties such as phosphoryl groups[8].

The realised catalytic activities of the cell result in bioproduction of three major kinds of compounds: reserves, "structural biomass," and products that are secreted into the ambient medium. The densities of the reserves (i.e., reserves normalised by structural biomass) also serve as information inputs to this central pathway, closing a regulatory loop.

The meaning of "structural biomass" may not be immediately clear. Intuitively, it comprises everything inside the cell except the reserves—but this makes for a circularity when we define reserves, in turn, as components that do not belong to the structural biomass. The latter step is not necessary in those cases where reserves can be clearly distinguished on the basis of their biochemical identity, and often also on the basis of the fact that they occur in discrete sub-cellular structures called *inclusions*[8–10], examples of which are shown in Fig. 3. However, there are also awkward cases that are less clear-cut and this prompts us to define reserves formally as "non-structural." To

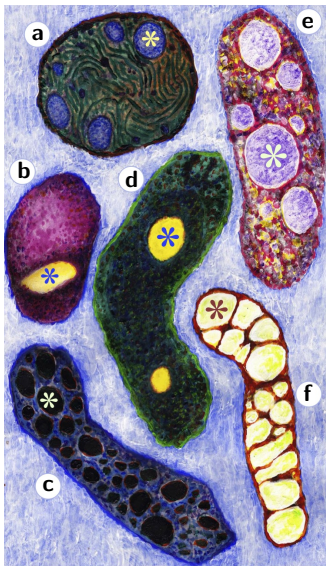

Figure 3: **Reserve inclusions.** In each cell depicted, an asterisk indicates one of the storage inclusions. (a): cyanophycin, *Anabaena variabilis*[13]; (b): sulfur, *Thermoanaerobacter sulfurigignens*[14]; (c): glycogen, *Methylacidiphilum fumariolicum*[15]; (d): polyphosphate, *Campylobacter jejuni*[16]; (e): polyhydroxybutyrate, *Rhodovibrio sodomensis*[17]; (f): triacylglycerol, *Rhodococcus opacus*[18].

avoid logical circularity, we then define the structural biomass as the bare minimum compatible with life, corresponding to a state that can be induced experimentally by starving the cell of all essential nutrients. Droop[11,12] introduced the idea of *subsistence* biomass (or cell composition, etc.) to express the same idea; thus, subsistence biomass and functional-structural biomass are two names for the same concept.

The central regulatory pathway in Fig. 2 has been depicted in a "black box" to call to mind that these steps have traditionally not been readily observable. By contrast, the quantities to the left and the right were more easily measured, and hence classic models of microbial growth and metabolism have always focussed on interrelating these "white box" quantities. With the advent of "high through-put" molecular-biological techniques, this black box has become more transparent, although there remain serious limitations as regards the achievable resolution, e.g., spatial, temporal, and in terms of molecular speciation.

Our general goal is to formulate mathematical models that accurately describe the relationships between the quantities enumerated in Fig. 2. Our discussion thus far suggests that we sharpen this goal with the following objectives: first, we wish to have a theory that is sufficiently flexible to accommodate (and take advantage of) any data that may be available regarding what has been depicted as a "black box" in Fig. 2; second, we do not want to lose contact with the classic models that deal with "white box" quantities only; and finally, we wish to have a theory that satisfies fundamental physicochemical laws and constraints, such as conservation principles and biochemical stoichiometry.

We believe that approaching the subject as an allocation problem is a natural and elegant way of fulfilling all of these desiderata[7,19–21]. At the heart lies a simple principle, expressed by the following equation:

$$\alpha_i = r_i / r_\Sigma \ , \tag{1}$$

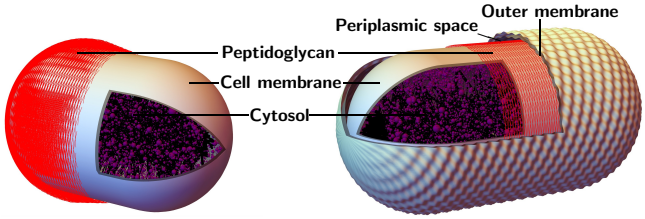

Figure 4:
General structure of Gram-positive (left) and Gram-negative (right) bacteria.

where α_i is the fraction of molecular building blocks devoted to the synthesis of machinery of type i, r_i is a *regulatory law* (or r-*function*, for short), and $r_\Sigma = \sum_i r_i$ is a normalising constant which assures that $\alpha_i \in [0, 1]$ and $\sum_i \alpha_i = 1$. This approach based on the perspective of "allocation" or "investment" has been gaining widespread acceptance in recent years, no doubt prompted by advances in high-throughput transcriptomics and proteomics which make this point of view seem almost self-evident [22–25].

The indexing in eqn (1) can be fine-grained, with i corresponding to one particular protein, or more coarse-grained, with i corresponding to a co-regulated class of proteins, all involved in a particular metabolic or physiological function. The quantity r_i may be a function of one or more quantities describing the cell's internal state, or its ambient medium. These functions may be chosen so as to let the resulting overall relationships accord with the classic models, or alternatively, r_i may be linked to transcriptomic data. The latter connection is made explicit if we imagine r_i as the amount of mRNA (per cell) encoding machinery if type i; eqn (1) then describes the fraction of "ribosome time" being dedicated to machinery if type i. This correspondence is, incidentally, rather imperfect, since different mRNA species have different turn-over times, different affinities for the ribosome, and so on. These effects tend to skew the effective allocation fraction and we should really think of r_i as the mRNA concentration after we have adjusted for such distortions.

Since the quantities on which the r_i are assumed to depend are in general allowed to be time-varying, we refer to this approach as the *dynamic allocation theory*. We enunciated three desiderata: the possibility of connecting with transcriptomics/proteomics, the possibility of connecting with classic models, and accordance with conservation principles and suchlike. The first two have been accounted for; as regards the latter, it turns out that the recipe "physico-chemical principles plus r-functions" suffices in many cases to specify a model completely [19,20]. In the next section we will review various aspects of microbial physiology that justify and motivate the dynamic allocation theory.

3 Selected aspects of microbial physiology

Microbial cells exhibit an astonishing degree of adaptability in the face of an often harsh environment. Responsiveness to changing conditions and an exquisitely optimised investment in internal stores are among the key traits that underpin this adaptability. We presently review these traits in more detail.

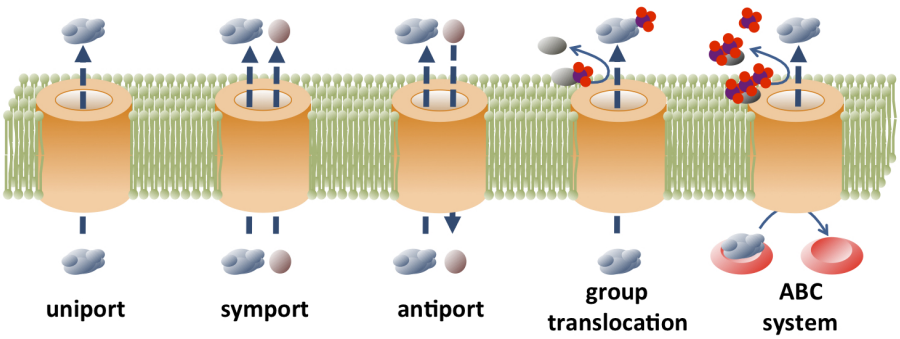

Figure 5: **General modes of action in nutrient uptake machinery.**

3.1 Nutrient uptake

In order to be processed by the cell's metabolic pathways and become available as fuel or molecular building blocks, nutrients must first traverse the cellular envelope. There are two basic ways in which this envelope is organised in prokaryotes (Fig. 4), traditionally named for the way in which they respond to the Gram stain[26], which remains an important tool in microbial microscopy to the present day. In Gram-positive cells, a rigid polysaccharide layer called peptidoglycan forms the bulk of the cell wall, whereas in Gram-negative bacteria the peptidoglycan layer is diaphanous and there is a second bilipid membrane called the *outer membrane*[8]. The area between outer and cytoplasmic membranes is occupied by a special substance with a high protein concentration, which is called the *periplasm*[27].

Although some hydrophobic molecules can pass through the membrane by diffusion, most nutrients require a dedicated pathway to be translocated across the membrane[28]. The latter is mediated by means of membrane-spanning transport proteins that function individually or in complex assemblies, depending on the mode of transport they effectuate (Fig. 5). The substrates may be transferred by themselves (*uniport*), or along with another substance, which may undergo net translocation in the same direction as the target molecule (*symport*) or in the opposite direction (*antiport*)[17]. Transport may be driven by the ΔG derived from the gradient of the substrate itself (i.e. facilitated diffusion in uniport) or that of the co-transported species (in symport and antiport), whereas in more complex systems the required ΔG is derived from group translocation or hydrolysis of adenosine-5′-triphosphate (ATP); group translocation involves the chemical modification of the transported substances during their uptake across the membrane[29].

Gram-negative bacteria have to overcome a special challenge, since the nutrient molecule has to negotiate both an outer and an inner (cytoplasmic) membrane. This challenge is met by the so-called *ATP Binding Cassette (ABC) system*[30,31]. Transport across the outer membrane is mediated by passive uniport through *porins*; once inside the periplasm the nutrient is swiftly bound by high-affinity binding proteins which deliver their cargo to an ATP-hydrolysing transporter.

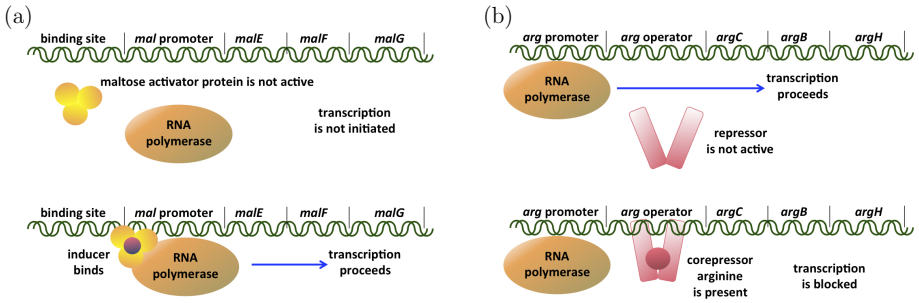

Figure 6: **Examples of regulatory mechanisms in bacteria. (a)** Transcription of the maltose transport system gene is initiated when the inducer maltose binds the activator protein, which engages the RNA polymerase and allows it to start transcription. **(b)** Transcription of the arginine biosynthetic pathway gene is blocked when the corepressor arginine binds to the repressor protein, leading to cessation of transcription.

3.2 Regulation of gene expression

Microbial cells regulate the expression levels of their uptake machinery so as to make (near-)optimal use of their limited supply of molecular building blocks [32,33]. Regulation at the level of transcription is typically mediated by *regulatory proteins* that bind the DNA. Binding of such a protein may stimulate the expression of the gene, or inhibit it; examples of both modes of regulation are shown in Fig. 6. In Fig. 6a, it can be seen that the presence of the nutrient substrate maltose leads to expression of the enzymes that constitute a biochemical pathway specifically dedicated to feeding this molecule into the pathways of core metabolism. In Fig. 6b, on the other hand, it is the presence of the product of a biosynthetic pathway, in this case arginine, that represses the production of enzymes involved in its synthesis. A design feature that these and many other examples have in common is that DNA-binding proteins serve as an adaptor between the substrate (maltose) or the product (arginine) to transform the signal (i.e. the concentrations of those compounds) into the propensity with which RNA polymerase initiates transcription.

In both these examples, a single control points governs the expression of a suite of enzymes, which makes functional sense since the enzymes catalyse subsequent steps in a particular biochemical pathway, and are hence needed (or not needed) in unison. A collection of protein-encoding domains of DNA (cistrons) that is under common regulatory control is called an *operon*. This modular organisation of gene expression underpins the notion that the index i in eqn (1) denotes functional units rather than individual proteins. There will also be enzymes that will be shared by several pathways, in which case their regulation may be more complex (or indeed more simple, when expression is *constitutive*, i.e. always "on").

The mechanisms of induction and repression shown in Fig. 6 can be generalised to the notions of positive feedforward and negative feedback, as illustrated in Fig. 7a. This figure also shows negative feedforward, in which two alternate substrates inhibit

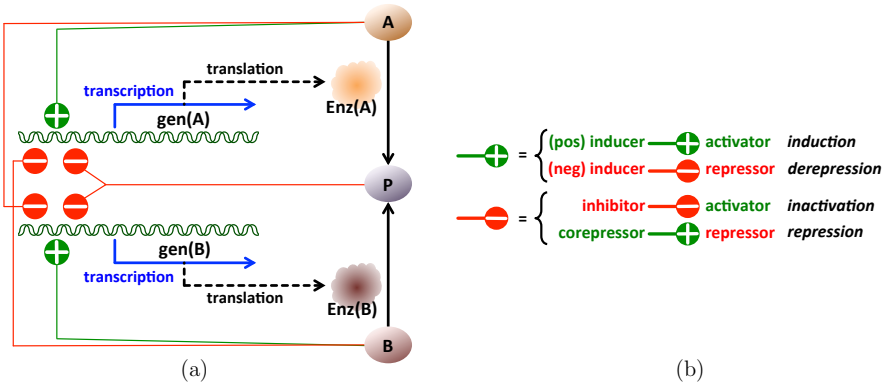

Figure 7: **Regulatory mechanisms in bacteria. (a)** Diagram illustrating modes of control in prokaryotic gene regulation. Two alternative substrates (**A** and **B**) are both converted to a common product (**P**), via reactions catalysed by specific enzymes (**enz(A)** and **enz(B)**) which are encoded by genes (**gen(A)** and **gen(B)**). Both positive and negative control are possible, as indicated. **(b)** Modes of regulation of gene expression. Stimulation of the expression of a given gene can be mediated by activation, in which an activator protein interacts with the upstream regulating region of the gene; this involves interaction with an inducer. Stimulation may also be effected via derepression, in which a repressor (an inhibitory transcription factor) is rendered less effective by an inducer. Inhibition of gene expression can likewise be achieved in two ways, either via inactivation of the activator protein via interaction with an inhibitor, or via activation of the repressor by binding to a corepressor. Inducers, inhibitors, and corepressors are often metabolites (substrates or products) but can also be components of an intracellular signalling cascade.

one another's processing pathways (a phenomenon known as *catabolite repression*[34]). Thus ample availability of one of them, say A, favours the expression of the enzymes that are dedicated to the utilization of A. These various modes can operate together, mediated via several regions (interaction sites) upstream from the promoter. Thus, several activating and repressing proteins may simultaneously interact with the DNA and modify the rate of assembly of the RNA polymerase at the promotor.

Each of these positive or negative interactions can be realised in two different ways, according to the familiar algebraic rules $+ \times + = +$, $- \times - = +$, $- \times + = -$, and $+ \times - = -$. The somewhat byzantine biological terminology (glossed in Fig. 7b) tends to obscure these elegant basic principles.

There are additional modes of regulation that come into play under conditions of extreme stress. Nutrient shortage in the environment induces an increase in the number of uncharged tRNAs in the cell, and as the ratio of uncharged to charged tRNA increases, the ribosome wastes a greater portion of its time interacting with uncharged tRNA, which leads to a stall of the ribosome, along with a production of small nucleotides called *alarmones*. These initiate the so-called *stringent response* characterised by a

cessation of rRNA and tRNA synthesis and ribosome production, as well as by a decrease in protein and DNA synthesis and amino acid production[8,35]. Another stress response involves *heat shock proteins* that help the cell recover from physico-chemical challenges such as high temperatures, radiation damage, or exposure to corrosive chemicals[36].

3.3 Cell division and growth

Bacteria multiply by division, and exhibit exponential growth curves which microbiologists call logarithmic. Prior to cell division, the cell elongates to approximately twice its original length while its genetic material is replicated. Once this is complete, filamentous temperature sensitive (Fts) proteins, chief among which is FtsZ, assemble to form the cell division apparatus (divisome) by polymerizing and thus forming the so-called Z-ring, which localises to the middle of the cell[37–39]. New cell envelope material is synthesised and the two chromosomes are pulled apart. The Z-ring subsequently depolymerises and a partition called the *septum* is formed, which divides the cell in two approximately equal parts.

The time required to produce a single generation may be referred to as the generation time T_g; it depends on environmental conditions as well as genetic traits. During a time span over which T_g is constant, growth is said to be *exponential*: after a period of time equal to $t = nT_g$, there will have been n doublings and the number of cells will be $N = N_0 2^n$, where N_0 and N denote, respectively, the initial and final number of cells. If W denotes the biomass of the population, then $\dot{W} \equiv dW/dt$ is its growth rate and $\dot{W}/W$ is the *specific growth rate*. Exponential growth is characterised by the condition $\dot{W}/W = \text{constant} = \mu$, which yields $W(t) = W(0)e^{\mu t}$. If we assume that $N/N_0 = W/W_0$, we find $2^n = e^{\mu t} = e^{\mu n T_g}$ and so $T_g = (\ln 2)/\mu$.

4 Classic experimental methods and models

As intimated in Section 2, the classic mathematical models for microbial growth and metabolism are closely tied to what can be readily observed in the standard laboratory systems for microbial growth. Accordingly, we briefly review the latter before discussing these models in more detail.

4.1 Culture systems

There are various ways of cultivating micro-organisms in bioreactors. The main methods of cultivation are *batch cultivation* and *chemostat cultivation*.

4.1.1 Batch culture

A batch culture is an enclosed vessel in which micro-organisms are able to grow as they consume substrate[40]. The biomass-specific substrate consumption rate q_s per unit of biomass of the population in the batch culture can be described as a function of the

nutrient concentration [N] by means of the Michaelis-Menten equation:

$$q_s = q_{\text{s,max}} \left(1 + K_m/[\text{N}]\right)^{-1} \ , \tag{2}$$

where $q_{\text{s,max}}$ represents the maximum substrate consumption rate and K_m denotes the concentration of the substrate at the half-maximal consumption rate[41]. The Pirt equation[42] describes the relation between q_s and the specific growth rate μ:

$$q_s = \mu/Y_{\text{s,max}} + m_s \ , \tag{3}$$

where $Y_{\text{s,max}}$ is the maximum yield of biomass, and m_s is a maintenance coefficient. With $\mu = \dot{W}/W$ (cf. Section 3.3), it follows that:

$$\dot{W} = Y_{\text{s,max}}(q_s - m_s)W \ , \tag{4}$$

and together with eqn (2) this yields:

$$\dot{W} = Y_{\text{s,max}} \left(q_{\text{s,max}} \left(1 + K_m/[\text{N}]\right)^{-1} - m_s\right) W \ . \tag{5}$$

The following conservation law applies if intracellular reserves do not vary:

$$[\text{N}]_0 = [\text{N}] + W/Y_{\text{s,max}} \ ,$$

where $[\text{N}]_0$ expresses the initial nutrient concentration. This allows us to obtain an autonomous differential equation for W:

$$\dot{W} = Y_{\text{s,max}} \left(q_{\text{s,max}} \frac{[\text{N}]_0 - W/Y_{\text{s,max}}}{K_m + [\text{N}]_0 - W/Y_{\text{s,max}}} - m_s\right) W \ . \tag{6}$$

Numerical solutions of this equation have a sigmoid appearance that accords well with the experimental data. However, as pointed out by Kooijman[43], the resulting sigmoid growth curve is indistinguishable from the curve that results if reserve dynamics are taken into account; only the time course of the nutrient $[\text{N}](t)$ will allow us to distinguish between these two scenarios.

4.1.2 Continuous culture

In sundry applications it is advantageous if cultures can be maintained in constant environmental conditions for long periods of time, which is not possible with closed batch cultures which are continually undergoing dynamical change as the culture matures and eventually runs out of fresh substrate. However, such steady-state conditions can be achieved in a continuous-flow culture, or chemostat (Fig. 8). In such a device there is a continuous supply of fresh medium combined with the withdrawal of an equal flux of cultivation broth, allowing the cultivation volume to remain constant[44]. Although much can be gleaned from transient dynamic behaviour, the tradition has been to wait until this transient dies out and the system attains an equilibrium, which is then investigated in detail.

In the chemostat, both the growth rate and the population density can be controlled independently and simultaneously. To this end, the experimenter manipulates two key

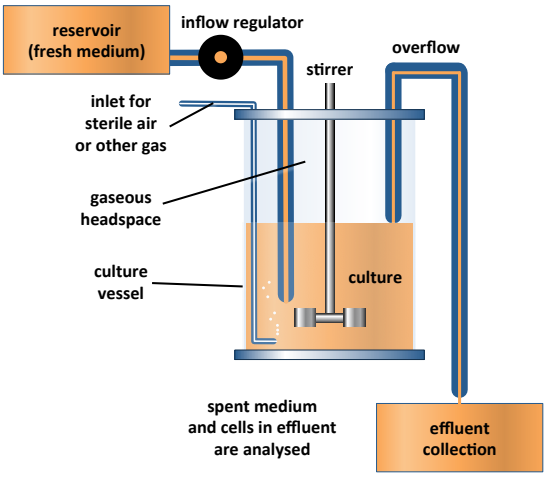

Figure 8:
Continuous culture system, "chemostat." The main vessel contains a well-stirred culture medium, which is replenished from the reservoir. The culture vessel maintains a constant volume of culture, since the inflow of a fresh medium from the reservoir equals the outflow of spent medium (overflow) from the vessel.

parameters: (i) the dilution rate $D = F/V$, where V is the volume of the main vessel, and F is the volumetric rate at which fresh medium is supplied; and (ii) the concentration of (limiting) nutrient $[\mathrm{N}]_R$ in the reservoir. Varying D, different growth rates can be achieved, with an eventual standing stock dictated by $[\mathrm{N}]_R$. We can see this by pursuing the following analysis[41]. We begin by considering biomass balance:

$$\frac{dW}{dt} = \mu W - \frac{F}{V} W \tag{7}$$

as well as a similar balance equation for the nutrient concentration [N]:

$$\frac{d[\mathrm{N}]}{dt} = \frac{F}{V}\left([\mathrm{N}]_R - [\mathrm{N}]\right) - \widetilde{\sigma}_W \mu W \ , \tag{8}$$

where $\widetilde{\sigma}_W$ is a stoichiometric coefficient. Equilibrium is defined by the conditions $dW/dt = 0$ and $d[\mathrm{N}]/dt = 0$. Hence we obtain the following steady-state chemostat equations:

$$\mu = D \ ; \qquad [\mathrm{N}] = [\mathrm{N}]_R - \widetilde{\sigma}_W W \ . \tag{9}$$

The first equation shows that by setting the dilution rate D of the chemostat to a certain value, we are able to cultivate micro-organisms at a specific growth rate equal to D. This is why the chemostat is such an important laboratory tool, affording a view of the physiology of microbes under well-defined conditions (constant growth rate, constant environmental conditions) or to examine how the growth rate affects, for instance, the rate of product formation. Specifically, if we assume the Monod equation[45], $\mu([\mathrm{N}]) = \widehat{\mu}\,(1 + K_S/[\mathrm{N}])^{-1}$, we find:

$$W = \left([\mathrm{N}]_R - \frac{DK_S}{\widehat{\mu} - D}\right)\widetilde{\sigma}_W^{-1} \ ; Y = D\left([\mathrm{N}]_R - \frac{DK_S}{\widehat{\mu} - D}\right)\widetilde{\sigma}_W^{-1} \ ; [\mathrm{N}] = \frac{DK_S}{\widehat{\mu} - D} \ . \tag{10}$$

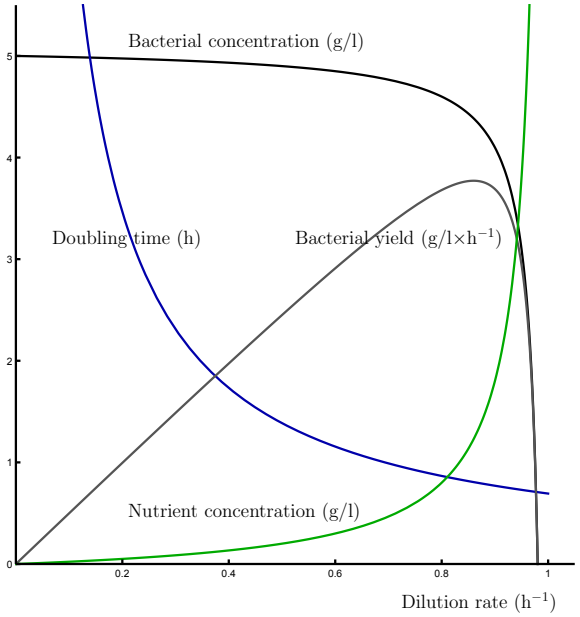

Figure 9:
Equilibrium relationships in the chemostat. Relationships between the dilution rate and, respectively, bacterial concentration, bacterial yield, doubling time, and nutrient concentration, as described by eqns (10) with parameter values $\widehat{\mu} = 1$ h^{-1}, $K_S = 0.2$ g/l, $\widetilde{\sigma}_W = 1$, $[N]_R = 10$ g/l.

These relationships have been plotted in Fig. 9; we can see that, as D approaches $\widehat{\mu}$, a "wash-out catastrophe" occurs.

Chemostat systems allow us to maintain exponential growth for a long period of time, as well as to repeat experiments under the same conditions, which facilitates the study of competitiveness of different strains and species under the same environmental conditions. Competition between different strains of a single species can be exploited to eliminate all but one, and thus the chemostat can be used as a tool to isolate specific types of bacteria, which can subsequently be studied in more detail[17]. Moreover, under certain specific conditions, such as nutrient shortage, long-term chemostat cultivation leads to adaptation of the cells to these conditions, which suggests that the chemostat might also be used in "evolutionary" engineering.

4.2 Mathematical models of microbial growth

In the previous section we encountered the Monod equation. More generally, models of bacterial growth can be formally represented as follows[41]:

$$\dot{W} = \mu(\mathbf{x}, \mathbf{u})W \ , \tag{11}$$

where $W \in \mathbb{R}^+$ is a suitable measure of biomass, $\mathbf{x} \in \mathbb{R}^p$ represents the internal state, $\mathbf{u} \in \mathbb{R}^q$ represents external conditions that affect growth and metabolism, μ is a function $\mathbb{R}^{p+q} \mapsto \mathbb{R}^+$, called the *model*, and the dot indicates differentiation with respect to time[35]. Here, W, $\mathbf{x}$, and $\mathbf{u}$ are all allowed to be functions of time t. Classic models tend to be characterised by $p = 0$ or $p = 1$ (i. e. they have little or no structuring

in terms of the internal state), by contrast, in fine-grained *systems biology* or *in silico* models, p may be on the order of many thousands[25].

4.2.1 Classic macroscopic models

Models are specified by the choice of the function $\mu : \mathbb{R}^{p+q} \mapsto \mathbb{R}^+$. We have already seen the Monod model[45]:

$$\mu([\mathrm{N}]) = \widehat{\mu}\,(1 + K_S/[\mathrm{N}])^{-1} \ , \tag{12}$$

where [N] is the ambient concentration of the limiting nutrient and $\widehat{\mu}$ and K_S are positive parameters[45]. As Monod pointed out[45], the parameter K_S should not be confused with the parameter K_m in eqn (2). In terms of our general description, eqn (11), we here have $p = 0$ and $q = 1$, as there are no state variables other than W and there is a single environmental variable, [N], on which the specific growth rate μ depends. If we allow [N] to vary in time, we have $W(t) = W_0 \exp\left\{\int_0^t \mu\left([\mathrm{N}](\tau)\right) d\tau\right\}$. One way to extend this model to $q > 1$, but still with $p = 0$, is to posit a multiplicative form $\mu(u_1, u_2, \dots) = \widehat{\mu} f_1(u_1) f_2(u_2) \cdots$ [46,47], where the $u_1, u_2, \dots$ are relevant environmental factors (such as levels of light, nutrients, and redox substrates) and the $f_1, f_2, \dots$ are appropriate functions $\mathbb{R}^+ \mapsto [0, 1]$ that express how these factors affect growth.

Pirt's model, eqn (3), incorporates maintenance costs. The concept of *endogenous metabolism* as the biological correlate of maintenance requirements was introduced by Herbert[48]. He proposed the following equation:

$$\dot{W} = (\widetilde{\mu} - a)W \ , \tag{13}$$

where the term aW represents a drain on growth due to the cost of maintenance; the correspondence between Pirt and Herbert can be expressed as $a = \widetilde{\mu} - \mu = Y_{\mathrm{s,max}} m_s$. Marr et al.[49] proposed that a could be estimated on the basis of the following empirical formula for the biomass at chemostat equilibrium:

$$W = W_{\max}\,(1 + a/D)^{-1} \tag{14}$$

where D is the dilution rate, as before, and $W_{\max}$ is the value that would be observed if $a = 0$.

In the opposite situation, when growth is assumed to depend on the internal state of the micro-organism only, so that $p = 1$ and $q = 0$, we are dealing with a class of models whose most famous representative is the Droop equation[11]:

$$\mu(Q) = \widehat{\mu}(1 - Q_0/Q) \ . \tag{15}$$

Here, Q characterises the internal state of the cell, namely, the internal nutrient 'pool' called the cell quota, which comprises the particle species of interest in any of its biomolecular speciations (e.g. free molecules, part of polymers, machinery etc.). The parameter Q_0 is the so-called *subsistence quota*, which can be interpreted as the minimum cell quota required by the cell to maintain its structural integrity. Growth at a non-zero rate requires $Q > Q_0$. Caperon[50] rendered the Droop equation as follows:

$$\mu(q) = \widehat{\mu}(1 + Q_0/q)^{-1} \ , \tag{16}$$

where $q = Q - Q_0$. The popularity of the rectangular hyperbola among classic modellers is evident, and may partially be due to the "transformation to linearity" trick this hyperbola permits (incidentally, a statistically highly unstable technique that should have fallen into disuse a long time ago).

4.2.2 Contemporary approaches: microscopic models

In sharp contrast to the classic whole-organism descriptions, which have few dynamic degrees of freedom, stand the models that explicitly represent individual molecular species; biochemistry being what it is, the natural consequence of this approach is $p \gg 1$; for instance, with $p \sim 10^8$ molecules inside the cell, a naive "molecular dynamics" type of approach would result in computationally highly demanding and analytically barely tractable models. Thus, the construction and analysis of models capable of describing the cell as a whole system is challenging. One good example is the model of bacterial metabolism, where the state variables are metabolite concentrations, gene expression levels, transcription factor activities, metabolic fluxes, and biomass concentration[25]. However, in many cases the aim is not to describe the whole organism but instead to focus on specific subsystems of the cell, such as the assembly of the Z-ring[37], or the electron transport chains of mitochondria[51] and purple non-sulfur bacteria[52].

Depending on the specific properties of the given biological network under consideration, different formalisms can be employed to simulate its dynamic behaviour. Signalling and regulatory networks concern signal flows, whereas metabolic networks deal with mass and energy flows. Metabolic networks have been analysed using flux balance analysis[53], metabolic flux analysis, pathway analysis by elementary modes, or extreme pathways[54,55]. Signalling networks can likewise be addressed via a variety of techniques, ranging from ODEs[56,57] to Boolean networks in global cellular models[58,59].

5 The dynamic allocation theory as a bridge between classic and contemporary approaches

In Section 2 we advocated the dynamic allocation approach as a generalisation of classic approaches that would be more amenable to modern "big data" settings. The approach rests on the principle expressed by eqn (1), but it stands to reason that this equation does not suffice by itself to meet these desiderata. We need to augment the allocation principle with dynamic compositional reckoning in terms of stoichiometric components.

5.1 Stoichiometric components

A stoichiometric component is a fixed linear combination of chemical species (which may be molecules, ions, functional groups), up to a multiplicative constant. By definition, such a component has a fixed empirical formula. To fix the multiplicative constant, we can normalise by the dominant chemical element (e.g., it is customary to think of

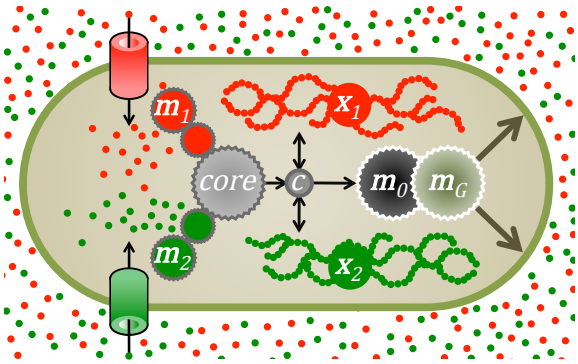

Figure 10:
Model diagram. Transporters and dedicated processing machinery (m_1 and m_2) feed the material into *core* metabolism, which leads to a central hub (c) that communicates with reserve polymers (x_1 and x_2) and feeds biosynthesis which is carried out by synthetic machinery m_0; growth is driven by growth machinery m_G.

carbohydrates as multiples of $C(H_2O)_m$, where $m \approx 1$; the number of multiples then counts as a number of *C-moles*). Alternatively, when the component corresponds to a single chemical species, it is more natural to think in terms of the number of moles of the discrete particles (molecules) of this species. Taking such stoichiometric components as a starting point has the great advantage that the dynamical behaviour of the model is almost self-evident in terms of the stoichiometric components; all that is really required is careful book-keeping, which is linear by its very nature.

Models with $p = 0$, such as Monod and Pirt-Herbert-Marr, represent an extreme case in which the biomass is accounted for as a single component. To make this connection, we have to impute to these models the assumption of strict compositional homeostasis, so that biomass, as such "all-included," has a fixed empirical formula. In the next step up, for the Droop-Caperon model, we regard the various reserves as separate components, along with a "subsistence biomass" and each of these components is assumed to have a fixed empirical formula. The *conical hull* (in the linear algebra sense) of these empirical formulas is the set of allowed chemical compositions at the level of the whole organism. In "microscopic" models with $p \gg 1$, the components correspond to single molecules or aggregates of molecules that co-vary in terms of their copy numbers per cell, as will be the case, for example, with enzymes encoded by a single operon (Section 3.2).

The choice of the number of components is thus tied up with, on the one hand, the assumptions regarding compositional homeostasis one is willing to entertain, and, on the other hand, the data available, in particular as regards the observed variation of the studied organism as regards its chemical composition. If a component i takes the form $\sum_k \kappa_{ik} \mathrm{P}_k$ where the coefficient κ_{ik} represents the preponderance of protein P_k in component i, then the bridge to proteomics is immediate[22], and the bridge to transcriptomics takes the general form $r_i = \sum_k \kappa_{ik} \sum_\ell \zeta_{k\ell} \mathrm{R}_\ell$, where R_ℓ is the level of the ℓth species of mRNA and the coefficient $\zeta_{k\ell}$ is a conversion factor.

A sensible default position halfway between the extremes can be negotiated by combining Grover's[60] *Variable-Internal-Stores* approach with a fairly coarse-grained partitioning of catalytic machinery as suggested by Scott and co-wrokers[61,62]; the re-

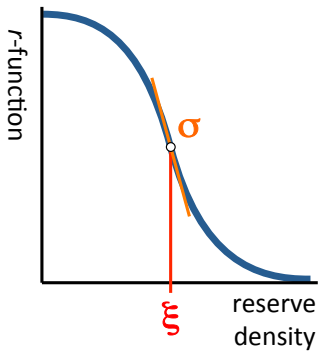

Figure 11:
Generic r-function. The simplest type of r-function depends on the density of one selected reserve, and governs the expression of the machinery required for the uptake and processing of the nutrient stored in that reserve. The sigmoid curve has two key parameters, location of the midpoint (ξ) and slope at the midpoint (σ); the values of these parameters determine if the model behaves more "Monod-like" or "Droop-like."

sulting model is represented schematically in Fig. 10 for the case of two essential nutrients, here represented as red and green dots.

5.2 Regulatory laws

To tie the stoichiometric dynamics to the allocation principle, eqn (1), we need to postulate regulatory laws which we call r-functions. A generic example is shown in Fig. 11: a decreasing sigmoid function which transforms the density of a nutrient x_i into the term r_i corresponding to allocation α_i of building blocks toward the machinery needed to acquire the nutrient corresponding to reserve i.

The precise mathematical form of this sigmoid is relatively unimportant: the salient point is that such a sigmoid can be parametrized by a midpoint parameter ξ and a slope parameter σ. It can be shown that the model will behave like the Monod/Pirt models, or like the Droop model, depending on the values that are selected for these parameters [19].

The configuration in which reserve densities govern the regulatory law corresponds to what we identified as negative feedback control in Section 3.2, with the internal store (x_i in Fig. 10) corresponding to the product P in Fig. 7.

The formalism proposed here extends effortlessly to any number of reserves, and to the inclusion of maintenance costs and how the dynamics change when these costs can no longer be covered by the available reserves or external supplies [19,20]. However, the reserves have to correspond to essential (non-substitutable) nutrients, and there are many known cases where alternative (substitutable) nutrients converge on a common reserve polymer. The regulation of assimilatory machinery for such alternative substrates is more involved. The general pattern is that the organism's gene regulation is set up to utilise a "preferred" compound in the first instance, and only express transporters and/or enzymes dedicated to "less preferred" compounds when the preferred one is no longer available, giving rise to characteristic multi-phasic growth curves $W(t)$ (in batch cultures) in which the bacteria are inoculated together with two or more alternative substrates, but regulate their machinery in such a way as to deplete these nutrients one after the other [34]; in the case of two nutrients this growth curve is called *diauxic*. Of course, these terms do not express actual states of mind of a bacteria, but rather for which nutrient the overall yield (efficiency of converting nutrient into biomass) is greatest [8].

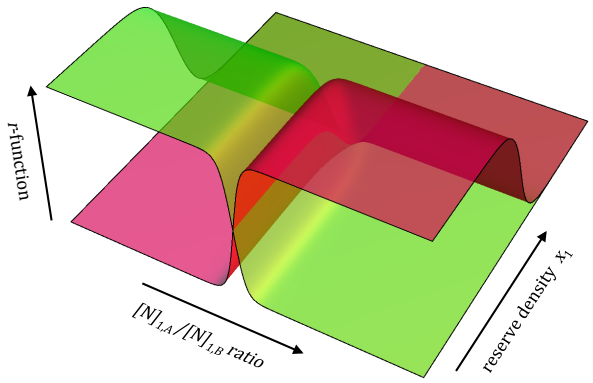

Figure 12:
Cross-regulation. Two substitutional nutrients with ambient concentrations $[\mathrm{N}]_{1,A}$ and $[\mathrm{N}]_{1,B}$ are converted into reserve type 1. The corresponding r-functions $r_{1,A}$ and $r_{1,B}$ are functions of $[\mathrm{N}]_{1,A}/[\mathrm{N}]_{1,B}$ and of x_1. The red surface is a graph of $r_{1,A}$ and the green surface is a graph of $r_{1,B}$.

The genetic circuit underlying this behaviour was shown in Fig. 7, where alternative substrates (A and B in Fig. 7) exert crossed negative feedforward control over their respective operons. To represent this behaviour, the environmental concentrations of the alternate nutrients have to be sensed somehow, either directly as the intracellular concentration reflects the ambient one, as with the maltose operon depicted in Fig. 7, or else via an intracellular signalling cascade; the so-called two-components systems, for instance, which constitute a large group of signalling pathways in prokaryotes[63]. In either case the functional behaviour can be captured by an r-function of the form shown in Fig. 12: besides depending on the reserve density in the original sigmoid fashion, there is also a change-over dependence on the ratio of environmental concentrations. Thus, the regulatory law in this case acquires characteristics of both feedback and feedforward, and can be interpreted as a smooth (or "fuzzy") rendition of a Boolean operation[64].

6 Outlook: living together as one

As the cells absorb nutrients and redox substrates from the ambient medium and secrete products back into this medium, they change the conditions they are faced with and to which they are adapting. It is precisely this causal loop which necessitates the careful

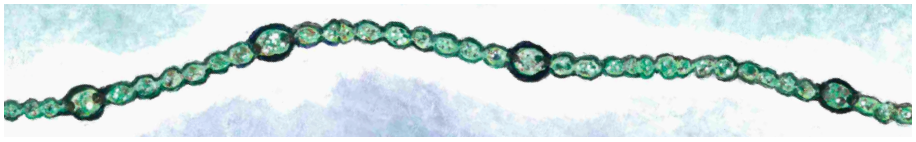

Figure 13: **Diazotrophic filament of *Anabaena* OCC7120.** The difference between the photosynthetic vegetative cells (small) and nitrogen-fixing heterocysts (large) is conspicuous[65].

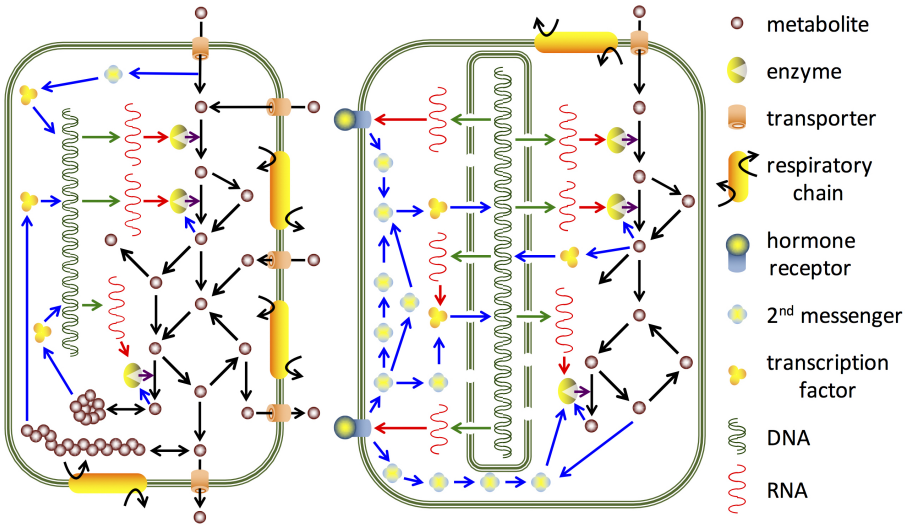

Figure 14: **Uni- versus multicellular lifestyles.** Left: a free-living unicellular organism; it has a high degree of metabolic versatility and adaptability, and the signalling pathways that control gene expression are predominantly geared to availability of nutrients and substrates in the ambient environment; on the right, a cell that is part of a multicellular conglomerate: such a cell is shielded from some of the environment's fluctuations due to the organism's homeostasis of the *milieu intérieur* and accordingly is metabolically less versatile; signalling pathways controlling gene expression are dominated by extracellular "first messenger" signals that arise elsewhere in the organism and coordinate the cell's specialised activities within the multicelled whole.

analysis of culture conditions (both batch and chemostat) which we briefly reviewed in Section 4.1.

In its crudest form, this environmental feedback loop has a detrimental, competitive character (*Verlendung*). However, when species with different metabolic capabilities live together, one species' product can be another species' substrate: symbiotic cycles arise that allow the agglomeration of species to thrive. Microbial mats, for instance, are consortia of several species with distinct biochemical functionalities, undertaking intricate cooperative interactions to harvest energy and matter from the environment[66].

This differentiation into complementary, metabolic capabilities can also occur within the colony, that is, within the group of descendants of a common progenitor cell. Cyanobacterial heterocysts (Fig. 13), for instance, arise in the absence of combined nitrogen (e.g. nitrate, ammonia) in the environment, and express *nitrogenase*, which allows them to convert dinitrogen into ammonia and supply the vegetative cells with fixed nitrogen in the form of glutamate and β-aspartyl-arginine; the latter fix carbon dioxide and supply the heterocysts with carbohydrates such as sucrose[67]. This division of labour allows both nitrogen and carbon fixation to occur at the same time and nearly the same place; cyanobacteria employ oxygenic photosynthesis to fix carbon,

and nitrogenase is inactivated by oxygen[67]. The cells exchange organic molecules via a shared fluid phase, comprising the unstirred water layer surrounding the filament, the glycocalyx, and possibly a shared periplasmic space[65]. Moreover, the heterocysts secrete the pentapeptide PatS that inhibits heterocyst differentiation, thus ensuring a regular spacing of heterocysts rather than random clustering[65].

These are the hallmarks of true multicellularity: (i) cells are exposed to a milder version of the ambient medium, as a result of metabolites they excrete into this medium, as part of (ii) their differentiation toward specialised metabolic or physiological tasks, among which are (iii) growth and reproduction. The metabolites in the shared *interstitial* medium have beside their 'raw' biochemical function, also a "token" function in mediating cell fate (e.g., PatS); these tokens are often modified intermediaries of energy metabolism, such as ppGpp (an alarmone) or cAMP.

The phylogenetic development of multicellularity is merely an elaboration of these traits: differentiation into more subtypes, a delicate conditioning of the interstitial medium, and gradual shift towards the "token" function as hormones and cell-adhesion molecules coordinate the organisation into tissues (which drives the morphological richness we evoked in Section 1).

Given the success of the "physico-chemical principles plus r-functions" approach in free-living unicellulars, we may well wonder if these principles transfer to the whole-organism level in multicellular organisms. There are several key differences (Fig. 14). As a result of homeostasis of the interstitium, individual cells are no longer adapting to a harsh medium; their gene expression is governed, to a far greater degree, by stimuli from first messengers; and the accumulation of internal stores has become one of the specialised functions. We should like to retain the idea of starting from physico-chemical principles, and augment it with appropriate regulatory laws, which must now be concerned with the composition of the interstitium, the status of the storage tissues, and the control of somatic growth and development versus germline reproduction.

References

[1] Ruggiero, M. A., D. P. Gordon, T. M. Orrell, N. Bailly, T. Bourgoin, R. C. Brusca, T. Cavalier-Smith, M. D. Guiry, and P. M. Kirk (2015). A higher level classification of all living organisms. *PLoS ONE 10 10*, e0119248.

[2] Schlegel, H. G. and C. Zaborosch (1993). *General Microbiology* (Seventh ed.). Cambridge: Cambridge University Press.

[3] Margulis, L. (1993). *Symbiosis in Cell Evolution: Microbial Communities in the Archean and Proterozoic Eons* (Second ed.). New York: W. H. Freeman.

[4] van den Berg, H. A. (1998). Modelling the metabolic versatility of a microbial trichome. *Bull. Math. Biol. 60*, 131–150.

[5] Hallegraeff, G. M. (2010). Ocean climate change, phytoplankton community responses, and harmful algal blooms: A formidable predictive challenge. *J. Phycol. 46*, 220–235.

[6] Holligan, P. M., E. Fernández, J. Aiken, W. M. Balch, P. Boyd, P. H. Burkill, M. Finch, S. B. Groom, G. Malin, K. Muller, D. A. Purdie, C. Robinson, C. C. Trees, S. M. Turner, and P. van der Wal (1993). A biochemical study of the coccolithophore, *Emiliania huxleyi*, in the North Atlantic. *Global Biochem. Cycles 7*, 879–900.

[7] van den Berg, H. A. (2001). How microbes can achieve balanced growth in a fluctuating environment. *Acta Biotheor. 49*, 1–21.

[8] Neidhardt, F. C., J. L. Ingraham, and M. Schaechter (1990). *Physiology of the Bacterial Cell: A Molecular Approach*. Sunderland, Massachusetts: Sinauer Associates.

[9] Beveridge, T. J. (1989). The structure of bacteria. In J. S. Poindexter and E. R. Leadbetter (Eds.), *Bacteria in Nature III: Structure, Physiology, and Genetic Adaptability*, pp. 1–65. Plenum.

[10] Preiss, J. (1989). Biochemistry and metabolism of intracellular reserves. In J. S. Poindexter and E. R. Leadbetter (Eds.), *Bacteria in Nature III: Structure, Physiology, and Genetic Adaptability*, pp. 189–258. Plenum.

[11] Droop, M. R. (1983). 25 years of algal growth kinetics – a personal view. *Botanica Marina 24*, 99–112.

[12] Droop, M. R. (1968). Vitamin B12 and marine ecology. IV. The kinetics of uptake, growth and inhibition in *Monochrysis lutheri*. *J. Mar. Biol. Assoc. 48*, 689–733.

[13] Ziegler, K., D. P. Stephan, E. K. Pistorius, H. G. Ruppel, and W. Lockau (2001). A mutant of the cyanobacterium *Anabaena variabilis* ATCC 29413 lacking cyanophycin synthetase: Growth properties and ultrastructural aspects. *FEMS Microbiol. Lett. 196*, 13–18.

[14] Lee, Y.-J., A. Prange, H. Lichtenberg, M. Rohde, M. Dashti, and J. Wiegel (2007). In situ analysis of sulfur species in sulfur globules produced from thiosulfate by *Thermoanaerobacter sulfurigignens* and *Thermoanaerobacterium thermosulfurigenes*. *J. Bacteriol. 189*, 7525–7529.

[15] Khadem, A. F., M. C. F. van Teeseling, L. van Niftrik, M. S. M. Jetten, H. J. M. O. den Camp, and A. Pol (2012). Genomic and physiological analysis of carbon storage in the verrucomicrobial methanotroph "*Ca.* Methylacidiphilum fumariolicum" SolV. *Front Microbiol. 3*, 345.

[16] Gangaiah, D., I. I. Kassem, Z. Liu, and G. Rajashekara (2009). Importance of Polyphosphate Kinase 1 for *Campylobacter jejuni* viable-but-nonculturable cell formation, natural transformation, and antimicrobial resistance. *Appl. Environ. Microbiol. 75*, 7838–7849.

[17] Madigan, M. T., J. M. Martinko, P. V. Dunlap, and D. P. Clark (2009). *Brock Biology of Microorganisms* (Twelfth ed.). San Francisco, CA: Pearson Benjamin Cummings.

[18] Alvarez, H. M. (2016). Triacylglycerol and wax ester-accumulating machinery in prokaryotes. *Biochimie 120*, 28–39.

[19] Nev, O. A. and H. A. van den Berg (2017a). Variable-Internal-Stores models of microbial growth and metabolism with dynamic allocation of cellular resources. *J. Math. Biol. 74*, 409–445.

[20] Nev, O. A. and H. A. van den Berg (2017b). Microbial metabolism and growth under conditions of starvation modelled as the sliding mode of a differential inclusion. *Dyn. Systems*, DOI: 10.1080/14689367.2017.1298726.

[21] van den Berg, H. A., Y. N. Kiselev, and Orlov (2002). Optimal allocation of building blocks between nutrient uptake systems in a microbe. *J. Math. Biol. 44*, 276–296.

[22] Kramer, G., R. R. Sprenger, M. A. Nessen, W. Roseboom, D. Speijer, L. de Jong, M. J. T. de Mattos, J. Back, and C. G. de Koster (2010). Proteome-wide alterations in *Escherichia coli* translation rates upon anaerobiosis. *Mol. Cell. Proteomics 9.11*, 2508–2516.

[23] Liebermeister, W., E. Noor, A. Flamholz, D. Davidi, J. Bernhardt, and R. Milo (2014). Visual account of protein investment in cellular functions. *Proc. Natl. Acad. Sci. U.S.A. 111*, 8488–8493.

[24] O'Brien, E. J., J. A. Lerman, R. L. Chang, D. R. Hyduke, and B. O. Palsson (2013). Genome-scale models of metabolism and gene expression extend and refine growth phenotype prediction. *Mol. Syst. Biol. 9*, a693.

[25] Ederer, M., S. Steinsiek, S. Stagge, M. D. Rolfe, A. T. Beek, D. Knies, M. J. T. de Mattos, T. Sauter, J. Green, R. K. Poole, K. Bettenbrock, and O. Sawodny (2014). A mathematical model of metabolism and regulation provides a systems-level view of how *Escherichia coli* responds to oxygen. *Front. Microbiol. 5*, 124–135.

[26] Gram, H. C. (1884). Über die isolierte Färbung der Schizomyceten in Schnitt- und Trockenpräparaten. *Fortschr. Med. 2*, 185–189.

[27] Goodsell, D. S. (2009). *The Machinery of Life* (Second ed.). New York: Springer.

[28] Kaback, H. R. (1986). Active transport in *Escherichia coli*: Passage to permease. *Annu. Rev. Biophys. Biophys. Chem. 15*, 279–319.

[29] Boos, W. (1974). Bacterial transport. *Annu. Rev. Biochem. 43*, 123–146.

[30] Higgins, C. F. (1992). ABC transporters: From microorganisms to man. *Annu. Rev. Cell Bioi. 8*, 67–113.

[31] Davidson, A. L. and J. Chen (2004). ATP-binding cassette transporters in bacteria. *Annu. Rev. Biochem. 73*, 241–268.

[32] Decker, K. B. and D. M. Hinton (2013). Transcription regulation at the core: Similarities among bacterial, archaeal, and eukaryotic RNA polymerases. *Annu. Rev. Microbiol. 67*, 113–139.

[33] Lee, D. J., S. D. Minchin, and S. J. W. Busby (2012). Activating transcription in bacteria. *Annu. Rev. Microbiol. 66*, 125–152.

[34] Fisher, S. H. and A. L. Sonenshein (1991). Control of carbon and nitrogen metabolism in *Bacillus subtilis*. *Annu. Rev. Microbiol. 45*, 107–135.

[35] Dawes, E. A. (1989). Growth and survival of bacteria. In J. S. Poindexter and E. R. Leadbetter (Eds.), *Bacteria in Nature III: Structure, Physiology, and Genetic Adaptability*, pp. 67–187. Plenum.

[36] Yura, T., H. Nagai, and H. Mori (1993). Regulation of the heat-shock response in bacteria. *Annu. Rev. Microbiol. 47*, 321–350.

[37] Dow, C. E., A. Rodger, D. I. Roper, and H. A. van den Berg (2013). A model of membrane contraction predicting initiation and completion of bacterial cell division. *Integr. Biol. 5*, 778.

[38] Dow, C. E., H. A. van den Berg, A. Rodger, and D. I. Roper (2015). Biological insights from a simulation model of the critical FtsZ accumulation required for prokaryotic cell division. *Biochemistry 54*, 3803–3813.

[39] Broughton, C. E., D. I. Roper, H. A. van den Berg, and A. Rodger (2015). Bacterial cell division: Experimental and theoretical approaches to the divisome. *Science Progress 98*, 131–345.

[40] Meyer, H.-P., O. Käppeli, and A. Fiechter (1985). Growth control in microbial cultures. *Ann. Rev. Microbiol. 39*, 299–319.

[41] van den Berg, H. A. (2011). *Mathematical Models of Biological Systems*. New York: Oxford University Press.

[42] Pirt, S. J. (1965). The maintenance energy of bacteria in growing cultures. *Proc. Roy. Soc. Lond. 133*, 300–302.

[43] Kooijman, S. A. L. M. (2000). *Dynamic Energy and Mass Budgets in Biological Systems* (Second ed.). Cambridge: Cambridge University Press.

[44] Dykhuizen, D. E. (1990). Experimental studies of natural selection in bacteria. *Annu. Rev. Ecol. Syst. 21*, 373–398.

[45] Monod, J. (1949). The growth of bacterial cultures. *Annu. Rev. Microbiol. 3*, 371–394.

[46] Gottschal, J. C. (1992). Continuous culture. In J. Lederberg (Ed.), *Encyclopaedia of Microbiology*, Volume 1, pp. 559–572. San Diego, CA: Academic Press.

[47] de Wit, R., F. P. van den Ende, and H. van Gemerden (1995). Mathematical simulation of the interactions among cyanobacteria, purple sulfur bacteria and chemotrophic sulfur bacteria in microbial mat communities. *FEMS Microbiol. Ecol. 17*, 117–136.

[48] Herbert, D. (1958). Some principles of continuous culture. In G. Tunevall (Ed.), *Recent Progress in Microbiology*, Stockholm, pp. 381–396. Almquist & Wiksell.

[49] Marr, A. G., E. H. Nilson, and D. J. Clark (1962). The maintenance requirement of *Escherichia coli*. *Ann. N. Y. Acad. Sci 102*, 536–548.

[50] Caperon, J. (1968). Population growth response of *Isochrysis galbana* to nitrate variation at limiting concentration. *Ecology 49*, 866–872.

[51] Beard, D. A. (2005). A biophysical model of the mitochondrial respiratory system and oxidative phosphorylation. *PLoS Comput. Biol. 1*, e36.

[52] Klamt, S., H. Grammel, R. Straube, R. Ghosh, and E. D. Gilles (2008). Modeling the electron transport chain of purple non-sulfur bacteria. *Mol. Syst. Biol. 4*, 156–174.

[53] Varma, A., B. W. Boesch, and B. O. Palsson (1993). Stoichiometric interpretation of *Escherichia coli* glucose catabolism under various oxygenation rates. *Appl. Environ. Microbiol. 59*, 2465–2473.

[54] Schilling, C. H., D. Letscher, and B. Ø. Palsson (2000). Theory for the systemic definition of metabolic pathways and their use in interpreting metabolic function from a pathway-oriented perspective. *J. Theor. Biol. 203*, 229–248.

[55] Price, N., J. Reed, J. Papin, S. Wiback, and B. O. Palsson (2003). Network-based analysis of metabolic regulation in the human red blood cell. *J. Theor. Biol. 225*, 185–194.

[56] Kholodenko, B., M. B. Yaffe, and W. Kolch (2012). Computational approaches for analyzing information flow in biological networks. *Sci. Signaling 5*, re1.

[57] Hlavacek, W. S., J. R. Faeder, M. L. Blinov, R. G. Posner, M. Hucka, and W. Fontana (2006). Rules for modeling signal-transduction systems. *Sci. STKE 2006*, re6.

[58] Morris, M. K., J. Saez-Rodriguez, P. K. Sorger, and D. A. Lauffenburger (2010). Logic-based models for the analysis of cell signaling networks. *Biochemistry 49*, 3216–3224.

[59] Karlebach, G. and R. Shamir (2008). Modelling and analysis of gene regulatory networks. *Nat. Rev. Mol. Cell Biol. 9*, 770–780.

[60] Grover, J. P. (1991). Resource competition in a variable environment: Phytoplankton growing according to the Variable-Internal-Stores model. *Amer. Nat. 138*, 811–835.

[61] Scott, M., C. W. Gunderson, E. M. Mateescu, Z. Zhang, and T. Hwa (2010). Interdependence of cell growth and gene expression: Origins and consequences. *Science 330*, 1099–1102.

[62] Scott, M., S. Klumpp, E. M. Mateescu, and T. Hwa (2014). Emergence of robust growth laws from optimal regulation of ribosome synthesis. *Mol. Syst. Biol. 10*, 747.

[63] Whitworth, D. E. and P. J. A. Cock (2008). Two-component signal transduction systems of the Myxobacteria. In D. E. Whitworth (Ed.), *Myxobacteria: Multicellularity and Differentiation*. ASM Press.

[64] Kompala, D. S., D. Ramkrishna, and G. T. Tsao (1984). Cybernetic modelling of microbial growth on multiple substrates. *Biotechnol. Bioeng. 26*, 1272–1281.

[65] Kumar, K., R. A. Mella-Herrera, and J. W. Golden (2009). Cyanobacterial heterocysts. *Cold Spring Harb. Perspect. Biol. 2*, a000315.

[66] van Gemerden, H. (1993). Microbial mats: A joint venture. *Mar. Geol. 113*, 3–25.

[67] Burnat, M., A. Herrero, and E. Flores (2014). Compartmentalized cyanophycin metabolism in the diazotrophic filaments of a heterocyst-forming cyanobacterium. *Proc. Natl. Acad. Sci. U.S.A. 111*, 3823–3828.