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Nonequilibrium thermodynamic considerations of the efficiency, control, and regulation of microbial growth

Hans V. Westerhoff

E.C. Slater Institute, University of Amsterdam and Division of Molecular Biology, Netherlands Cancer Institute, Plesmanlaan 121, NL-1066 CX Amsterdam, The Netherlands

Abstract

The, submaximal, thermodynamic efficiency of aerobic microbial growth depends on whether no consumption / production of O_2, or no carbon product other than biomass, is admitted into the assimilation equation. When the growth substrate has a low degree of reduction, the former efficiency approaches 22 % whilst the latter approaches 54 %. Expectations for maximal growth rate at optimum efficiency and for maximum economic growth rate at optimum efficiency are 24 and 54 %, respectively. A new control analysis method is derived that may be used to deduce properties of intracellular processes from the control properties of the cell as a whole.

THERMODYNAMIC EFFICIENCIES

For rates to be high, Gibbs (free-) energy must be dissipated, which leads to a reduction in the thermodynamic efficiency. Using mosaic non equilibrium thermodynamics (*MNET*) one may calculate which states are optimal with respect to (combinations of) various criteria, such as efficiency, high growth rate, Gibbs-energy rich products, and this in the context of the mechanisms used by the organisms to approach such optimum states [1]. For aerobic growth on substrates that are "rich in Gibbs energy", experimental thermodynamic efficiencies were low [1, cf., 2, 3], but in correspondence with optimization for maximum growth rate disregarding thermodynamic efficiencies. In the case of substrates that carry little oxidative Gibbs energy per carbon atom, the thermodynamic efficiency appeared to correspond to optimization in view of both efficiency and growth rate [1, see however below].

The thermodynamic efficiency discussed here serves to compare microbial growth to other (bio)machines that transduce Gibbs energy from one form to another. Consequently, it addresses systems in which there is a well-defined input reaction and a well-defined output reaction. Ideally, these are chemically independent of one another. For growth of microbes, one could think of cases where the Gibbs-energy source is photons, and the carbon substrate is unable to yield ATP through substrate level phosphorylation. Then the input reaction is the dissipation of photon Gibbs energy, where the photons have a chemical potential μ_γ, at a rate, J_γ. The output reaction is growth with the Gibbs energy difference of the assimilation equation, called ΔG_a, and a rate J_a (in microbiological literature often called μ). The thermodynamic efficiency for growth is then defined as:

$$\eta = \frac{J_a \cdot \Delta G_a}{J_\gamma \cdot \mu_\gamma} \qquad (1)$$

To growth on two carbon substrates of which one is exclusively used for catabolism and one is exclusively used for anabolism, the analogous definition applies. Such cases of growth are rare however. Most often, catabolism and anabolism are intertwined and an unequivocal, mechanistic

discrimination between catabolic and anabolic processes is impossible. A case in point is growth on a single carbon substrate, part of which is used to provide carbon for biomass synthesis, and part of which is used to be oxidised to CO_2, in an exergonic reaction coupled to the synthesis of ATP.

Earlier, we have used "brute force" in order to distinguish catabolism from anabolism: we defined the anabolic reaction as the reaction that has the growth substrate as the substrate *and biomass as the ONLY carbon containing product* [1]. In this philosophy, any CO_2 appearing in the anabolic reaction would betray amalgamation with catabolism. Here the thermodynamic efficiency obtained in this way will be called the *ancarbogenic* efficiency.

Let us consider growth on the substrate $C(H_2O)_nH_m$, where m may be negative. For simplicity, biomass will be taken to have the chemical formula CH_2O. The degrees of reduction of the substrate (γ_S) and of biomass are then 4+m and 4, respectively [1]. Measuring flows in terms of C-moles, substrate consumption rate J_S, growth rate, J_a, and CO_2 production rate, J_C, obey the conservation relationship:

$$J_S = J_a + J_C \tag{2}$$

Input and output flow of the microbe envisioned as Gibbs-energy transducer have been defined [1] by the requirement that they correspond to the carbon flowing to biomass and the carbon not flowing to biomass (i.e., to CO_2 in this example), respectively. The corresponding chemical reaction equations are:

$$C(H_2O)_nH_m + \left(\frac{m}{4}\right) O_2 \quad \text{-------> } CH_2O + \left(n\text{-}1+\frac{m}{2}\right) H_2O \tag{3}$$

for anabolism and:

$$C(H_2O)_nH_m + \left(1+\frac{m}{4}\right) O_2 \quad \text{-------> } CO_2 + \left(n+\frac{m}{2}\right) H_2O \tag{4}$$

for catabolism. The Gibbs-energy differences of these two reactions may be denoted by ΔG_a and ΔG_C, respectively. The thermodynamic efficiency is defined as the output power divided by the input power, hence as [1]:

$$\eta = \frac{J_a \cdot \Delta G_a}{J_C \cdot \Delta G_C} \tag{5}$$

This *ancarbogenic* thermodynamic efficiency has been calculated for a number of cases of aerobic growth on a single carbon substrate and found to vary from 22 % (oxidized substrates) to -90 % (reduced substrates) [1]. For theoretical Gibbs-energy transducers, 24 % corresponds to optimisation for maximum growth rate at optimum efficiency, the negative efficiencies correspond to optimisation for maximum growth rate only [1].

When the substrate is more oxidised than biomass ($m < 0$ in Eq. 3), the chemical equation for the output process (anabolism) involves *production* of molecular oxygen [4,3,5]. Since the organisms under consideration are unable to produce molecular oxygen, this is mechanistically unrealistic. As more often however, the thermodynamics of a process can be considered, independently of mechanism. Indeed, the *overall* reaction of input plus output as described by the non equilibrium thermodynamic model, is consistent with reality and it is not obvious why the conceptual splitting of the overall process into catabolism and anabolism should be equally consistent. When the "biomachine" catalysing microbial growth is to be compared energetically to other such machines, only the overall process and its efficiency may matter, and how the machines actually catalyse the overall process may be irrelevant.

The problem that oxygen is produced in the anabolic reaction equation whenever the substrate is more oxidised than biomass, is directly related to the requirement that no other carbon product than biomass be product in this process. Let us consider what would happen if one would relax this definition of anabolism. Part of the excess oxygen in the substrate (when compared to biomass) may be used to oxidize some more substrate and this process may be comprised in the anabolic reaction, which then also produces carbon dioxide. In other words, the new (to be indicated by a prime, ') anabolic reaction is the original one plus a fraction (α) of the catabolic reaction:

$$(1+\alpha)\cdot C(H_2O)_nH_m+\{(\tfrac{m}{4})\cdot(1+\alpha)+\alpha\}\cdot O_2\text{----->}CH_2O + \alpha\cdot CO_2 + \{\alpha\cdot(n+\tfrac{m}{2})-1\}\cdot H_2O \quad (6)$$

The rate and Gibbs-energy difference of the new anabolic reaction are given by:

$$J_a' = J_a \quad (7)$$

$$\Delta G_a' = \Delta G_a + \alpha \cdot \Delta G_c \quad (8)$$

The reaction equation of catabolism remains Eq. 4. Hence:

$$\Delta G_c' = \Delta G_c \quad (9)$$

However, the flux through this reaction is reduced because some of it now flows in the anabolic reaction:

$$J_c' = J_c - \alpha \cdot J_a \quad (10)$$

It is important that the total rates of substrate consumption, CO_2 production, oxygen consumption and biomass production are independent of α. Also the rate of Gibbs-energy dissipation is independent of α:

$$\Phi = J_a' \cdot -\Delta G_a' + J_c' \cdot \Delta G_c' = J_a \cdot -\Delta G_a + J_c \cdot \Delta G_c \quad (11)$$

The thermodynamic efficiency however, does vary with α, i.e., with how much of catabolism is mixed into anabolism:

$$\eta' = \frac{J_a' \cdot \Delta G_a'}{J_c' \cdot \Delta G_c'} = \frac{\eta + \alpha \cdot J_a/J_c}{1 - \alpha \cdot J_a/J_c} \quad (12)$$

Allowing for reduction of an arbitrary amount of excess anabolic oxygen by substrate and (unlike in the above, ancarbogenic consideration of the efficiency) comprising that process in the anabolic "output process"), makes the thermodynamic efficiency of microbial growth undefined. i.e., a function of α [3]. Moreover the anabolic ("output") reaction then produces CO_2 and obtains catabolic characteristics.

It is possible to replace the definition that the output processes only produce carbon in the form of biomass with the requirement that anabolism does not consume or produce molecular oxygen. This then sets the value of α to (from Eq. 6):

$$\alpha = -\frac{m}{4+m} = \frac{4}{\gamma_S} - 1 \quad (13)$$

Because for the anoxygenic case $\alpha = 0$, combination of Eqs. 12 and 13 yields:

$$\eta_{anoxygenic} = \{\eta_{ancarbogenic} \cdot (1\text{-}Y) - Y + 4 \cdot \frac{Y}{\gamma_S}\} / \{1 - 4 \cdot \frac{Y}{\gamma_S}\} \quad (14)$$

where Y is the growth yield (in C-mole biomass per C-mole substrate):

$$Y = \frac{J_a}{J_a + J_c} \quad (15)$$

Using data for microbial growth summarized in ref. [1], I have calculated this anoxygenic efficiency as a function of the degree of reduction of the substrate. The open squares in Fig. 1 show how this efficiency varies with the degree of reduction of the substrate, the open circles give the "ancarbogenic" thermodynamic efficiencies $\eta_{ancarbogenic}$. I conclude that the anoxygenic efficiency approaches 54 % rather than the 22 % attained by the ancarbogenic efficiency as the substrate becomes more and more oxidized. When the growth substrate is rich in Gibbs energy, growth efficiency is negative; also with this definition growth is downhill and efficiency is no point of consideration for the organism.

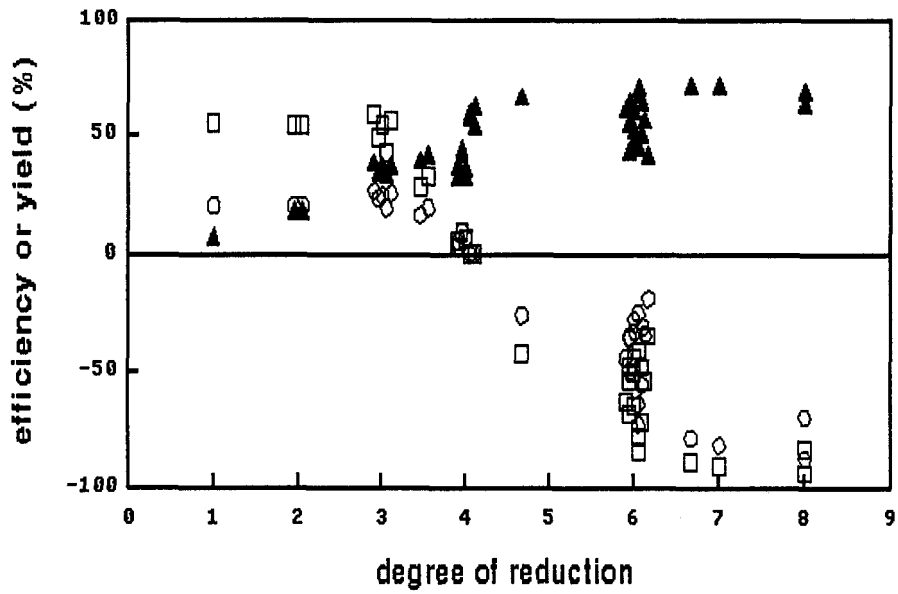

Fig. 1. Comparison of the anoxygenic to the ancarbogenic efficiencies of aerobic microbial growth. Growth yield (▲), degree of reduction (slightly spread out), and ancarbogenic efficiency (O) were taken from ref 1, the anoxygenic efficiency (□) was calculated using Eq. 14.

For a theoretical, proportional and symmetrical, Gibbs-energy transducer, Stucki [6] has considered varying (in a specific order [1]) of both the degree of coupling and the ratio of output to input Gibbs energy difference. Independent of any assumption on the degree of coupling, he calculated that the state leading to maximum growth rate at optimum efficiency has an efficiency of 24%. Optimizing towards the state of maximum 'economic' output flow at optimum efficiency lead to an efficiency of 54% [6, 1]. It remains to be seen whether the correspondences between these theoretical numbers and the ancarbogenic and anoxygenic efficiencies calculated above, should be taken to indicate that in terms of the anoxygenic

efficiency microbial growth is optimized for maximum 'economic' growth rate. What *should* remain from these calculations is that the efficiency of microbial growth should not be expected to be close to 100 %, but considerably less, given that microbes should not be expected just to be optimized for efficiency.

Recently, we observed a phenomenon that is reminiscent of this conclusion: the activity of the H^+-ATPase in wild type *E. coli* appears to be optimal for microbial growth rate, whilst growth yield is not [7,8] nor is thermodynamic efficiency [1].

Already when its black box is only slightly opened, microbial growth is a composite of a number of processes, e.g. catabolism (coupled to the synthesis of ATP from ADP and inorganic phosphate), anabolism (i.e., biomass synthesis coupled to the hydrolysis of ATP or the dissipation of proton gradient across the plasma membrane) and ATP leak [1]. Accordingly, the overall thermodynamic efficiency can be written as the product of the thermodynamic efficiency of catabolism (η_c), the thermodynamic efficiency of anabolism (η_a) and the rate of ATP hydrolysis in anabolism divided by the rate of ATP synthesis in catabolism [9]. The latter ratio is equal to the actual "yield on ATP" divided by the "theoretically maximum yield on ATP" ($\frac{Y_{ATP}}{Y^{max}_{ATP.theor}}$). Along these lines and translated to this case of microbial growth, Gnaiger [10, 11] has pointed out that if anabolism were to be optimal hence require an efficiency η_c of x % and anabolism were to be optimal hence require an efficiency η_a of x, the overall process would at most have an efficiency of x^2 %%which is much smaller than x%, hence incompatible with the overall process being optimal. Indeed, for oxidative phosphorylation in rat-liver mitochondria this is a relevant contention: if , as deduced from experimental determinations [12], mitochondrial oxidative phosphorylation in starved rat liver cells were optimized for maximal economic rate of ATP synthesis at optimal efficiency, hence would exhibit an efficiency of 54%, then the metabolism of the liver cell could not operate at that efficiency and could itself not be optimized for maximum output rate at optimal efficiency. It would then be unclear why the mitochondria and not the entire organism or liver cell would have been optimized so as to attain the efficiency of 54%.

Clearly, addressing the question of optimization implies that one defines what is the important function to consider and at what level of organisation (i.e., organism, tissue, cell or organelle) one should consider it [11]. For (prokaryotic) microorganisms this is comparatively simple however, since organisms, tissue, cell and organelle tend to coincide. Moreover, the function of these organisms is readily defined: what is important for, e.g., *E. coli* is the fitness of the entire cell, not of any of its component processes. Consequently, what one needs to consider is the optimization of the growth rate, growth yield and growth efficiency of the entire cell; the efficiency of oxidative phosphorylation alone in *E. coli* is not likely to be subject to an independent optimization argument. This is what we have done above and in the past [1, 9] and that is why we consider the above argumentation pertinent to microbial growth.

CONTROL ANALYSIS

A different category of properties for which biological systems may have been optimized, is distribution of control and regulability. It may be useful for a living system to have regulation of a metabolic pathway by one of its products and this may have implications for other properties such as flux and thermodynamic efficiency. For instance, the control coefficient of the *E. coli* H^+-ATPase for growth rate is close to zero [7, 8]. Because the sum of the flux control coefficients is limited to 1, this may reflect a shift of control to where it should be: at the substrate input. It may have been achieved

by having a stimulative effect on respiration of a reduced cellular ATP level in the presence of an increased membrane potential [7]. Interestingly, this extra regulation requires the coupling between respiration and phosphorylation to be loose, hence yield and thermodynamic efficiency to be smaller than maximal.

The analysis of the control and regulation of microbial physiology is greatly facilitated by the application of the well defined concepts of metabolic and hierarchical control theory. One of these is the extent to which a physiological process such as growth is controlled by a biochemical / biophysical reaction, such as substrate uptake. The corresponding flux control coefficient, C_1^J, is the percentage by which growth rate increases when the activity of the uptake system is increased by 1% [for more complete descriptions of the application of control theory to microbial physiology see refs 13-15]:

$$C_1^J = \left\{ \frac{dJ/J}{de_1/e_1} \right\}_{sys} = \left\{ \frac{d\ln|J|}{d\ln e_1} \right\}_{sys} \qquad (16)$$

The second part of the definition refers to the log-log derivative, which equals the slope in a double logarithmic plot of growth rate (J) versus the activity (e_1) of the uptake system. Analogous concentration control coefficients quantify the extent to the uptake process controls steady-state concentrations. Because in general growth rate is not only determined by the activity of the uptake systems but also by other cellular processes, there are additional control coefficients relating growth rate to those processes. Except for a number of well-defined cases [16, 17], the sum of the control coefficients referring to a single flux over all processes, amounts to 1. Therefore, the question of how growth rate is controlled is answered only if sufficient control coefficients have been determined so as to testify to a total control of 1.

The extent to which the uptake process determines steady growth depends on the kinetic properties of all processes involved in growth. The important properties are the so-called elasticities of the cellular processes with respect to the concentrations of the intracellular metabolites. The elasticity coefficients are defined as the percentage increase in the rate of one of the processes (e.g., process i) if one of its effectors (e.g., chemical X_j) increases 1% in concentration. In precise terms:

$$\varepsilon_{X_j}^i = \left\{ \frac{(\partial v_i/v_i)}{(\partial X_j/X_j)} \right\}_{enz} = \left\{ \frac{(\partial \ln|v_i|)}{(\partial \ln X_j)} \right\}_{enz} \qquad (17)$$

For systems of rather general complexity the control properties and the elasticities are just two sides of the same medal: the control coefficients are the inverse of the elasticity coefficients, be it that the simultaneous inverse must be taken and that the elasticity coefficients must be written into a matrix as defined by which reactions are connected with which other reactions. This matrix has been called the generalized *r x m* elasticity matrix, **E** (*r* and *m* are the number of processes and the number of independent metabolite concentrations, respectively). The *r x r* matrix of control coefficients, **C**, which then contains all the (*m x r*) concentration control coefficients as well as a number (*r-m* x *r*) of flux control coefficients is then equal to the matrix inverse of the elasticity matrix:

$$\mathbf{C} = \mathbf{E}^{-1} \qquad (18)$$

Eq. 18 demonstrates that the control properties of microbial growth can be understood on the basis of the properties (notably the elasticity coefficients of) all the processes that are involved in it. There are two corollaries: On the one hand one may predict the control properties once one knows (approximately) the elasticities of the processes (this may involve such considerations as that a certain reaction is virtually substrate saturated, hence has an elasticity close to zero with respect to its substrate). On the other hand, once one knows the control properties of an existing case of microbial growth, one may use the inverse of Eq. 18:

$$\mathbf{E} = \mathbf{C}^{-1} \qquad (19)$$

to calculate kinetic properties of the processes within the microbe (i.e., the elasticity coefficients). Indeed, the biotechnologist may use Eq. 19 to devise a microbe with desired growth control properties.

To obtain matrices **C** and **E**, one may follow a systematic procedure: First the number of independent steady-state fluxes [the rank, $n = r\text{-}m$, of stoichiometry matrix **N**; cf., 18] is determined and n independent flux properties of interest are selected. The control coefficients of each of these fluxes with respect to all r enzymes is written as a row matrix and these n row matrices are combined to yield an r x n flux control matrix, $\boldsymbol{\phi}'$. This matrix differs from the r x r matrix of elemental flux control coefficients, $\boldsymbol{\phi}$, each row of which contains the control coefficients of all enzymes (elemental processes) with respect to the steady-state flux through any such process. Then the $n \times r$ transformation matrix **S** is determined that transforms $\boldsymbol{\phi}$ to $\boldsymbol{\phi}'$:

$$\boldsymbol{\phi}' = \boldsymbol{\phi} \cdot \mathbf{S} \tag{20}$$

Subsequently, one determines a set of percentage increases for each reaction rate in the system such that steady state is maintained immediately. One of these may be that all rates are increased by 1%. One should find n such sets and write these as n column matrices next to one another so as to constitute the matrix $\boldsymbol{\kappa}$ [$\boldsymbol{\kappa}$ contains a complete basis for the Kernel of the normalized stoichiometry matrix **n**; Westerhoff, in preparation, cf., 18]. Defining $\boldsymbol{\kappa}'$ by:

$$\boldsymbol{\kappa}' = \boldsymbol{\kappa} \cdot (\mathbf{S} \cdot \boldsymbol{\kappa})^{-1} \tag{21}$$

$$\mathbf{C} = \{ \boldsymbol{\phi}' \mid \boldsymbol{\gamma} \}^{T} \tag{22}$$

$$\mathbf{E} = \{ \boldsymbol{\kappa}' \mid -\boldsymbol{\varepsilon} \} \tag{23}$$

$\boldsymbol{\varepsilon}$ is the $r \times m$ matrix of elasticity coefficients, and $\boldsymbol{\gamma}$ is the $m \times r$ matrix of concentration control coefficients. The superscript T refers to the transpose of the matrix. The matrices **C** and **E** of Eqs. 22 and 23 fulfil Eq. 18, showing that indeed the control coefficients can be expressed into the elasticity coefficients and are the inverse of the latter. Also Reder [18] has expressed the (in that case nonnormalized) control coefficients into (in that case nonnormalized) elasticity coefficients, but this was not in the sense of a single matrix inversion.

I conclude that efficiency has more than a single definition, that it is just one of the properties that should be optimal for a living system and that the requirement for simultaneous optimization of a great number of properties may have led to the compromise found in the natural systems, with lower than maximal efficiencies. Further control analyses along the lines developed here or by others, may help us further understand the deviations from maximal efficiency [19].

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