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Thermodynamic efficiency of microbial growth is low but optimal for maximal growth rate

(nonequilibrium thermodynamics/growth yield/*r*- and *K*-selection/uncoupling/energy wastage)

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ABSTRACT **Thermodynamic efficiency of microbial growth on substrates that are more oxidized than biomass approaches 24%. This is the theoretical value for a linear energy converter optimized for maximal output flow at optimal efficiency. For growth on substrates more reduced than biomass, thermodynamic efficiencies correspond to those predicted for optimization to maximal growth rate (or yield) only.**

Microbial growth yields (Y, expressed in grams of cells produced per mole of substrate consumed) are some 50% less than they theoretically could be (1–4). Calculation of this theoretical growth yield ("Y_{theor}") was based on stoichiometries of production and utilization of ATP in catabolism and anabolism, respectively, and the chemical composition of microbial cells (4–8). The experimental Y falling short of Y_{theor} has been taken to indicate that anabolism is incompletely coupled to catabolism due to "maintenance" (9–13), "overflow metabolism" (14, 15), or "futile metabolic cycles" (16–18). Alternatively, less than the assumed amount of ATP may be generated in catabolism, or more ATP may be needed in anabolism than has been assumed, so that Y_{theor} has been overestimated (e.g., see ref. 17). Moreover, Y/Y_{theor} is not necessarily lower than 1: a specific organism might have adjusted its ATP reaction stoichiometries (19–21) so as to increase its growth yield.

The second law of thermodynamics dictates that the free enthalpy (Gibbs free energy) yield or thermodynamic efficiency (η; Eq. 1) cannot exceed 1 (100%) (22, 23). However, like the growth yield, thermodynamic growth efficiency falls short of its maximum value (4, 24–27).

Kedem and Caplan (28) have shown that, paradoxically, efficiencies <100% may be useful: at 100% efficiency, a system could only "operate" at zero rate. Even uncoupling may be advantageous (29, 30). Interpretations of the deficient efficiency of microbial growth along these lines have been hampered (26) by using a differently defined efficiency (25), by confusing thermodynamic efficiency with growth yield (31) or enthalpy yield (32), or by equating phenomenological stoichiometries to mechanistic ones (33). We report here that the low thermodynamic efficiencies of microbial growth can be understood to be optimal, either for maximal growth rate when the substrate is more reduced than the biomass is (efficiency is extremely low) or for maximal growth rate at optimal efficiency when the substrate is more oxidized than the biomass is (efficiency $\simeq$ 23%).

Thermodynamic efficiencies of microbial growth: 26% and less

In most determinations of microbial growth yields, a single substrate provided both the free enthalpy and the carbon atoms needed for growth. Such a culture (Fig. 1*A*) differs from the energy converters considered by Kedem and Caplan (28) and Stucki (29, 30) (Fig. 1*C*). However (but cf. ref. 26), if all flows are expressed in terms of moles of carbon (C-moles), the rate of substrate consumption J_s equals the sum of the rate of biomass formation J_b ($\equiv$ the anabolic flow $-J_a$) and the rate of catabolic product ($e.g., CO_2$) formation J_{pr} ($\equiv$ the catabolic flow J_c) and can be subdivided into J_{s1} ($\equiv J_c$) and J_{s2} ($\equiv -J_a$) (Fig. 1*B*). Because the free enthalpy difference of catabolism per C-mole (ΔG_c) equals $\mu_s - \mu_{pr}$ and the free enthalpy difference of anabolism per C-mole (ΔG_a) equals $\mu_b - \mu_s$, the scheme of single-substrate microbial growth (Fig. 1*A*) is transformed here into the scheme of a free enthalpy transducer (Fig. 1*C*) to which nonequilibrium thermodynamic principles (22, 23, 25–30, 34–37) should apply. Note that here μ_x denotes the chemical potential of substrate x; it should not be confused with the growth rate.

The thermodynamic efficiency η of such an energy converter is defined (26, 28) as

$$\eta = \frac{-J_a \cdot \Delta G_a}{J_c \cdot \Delta G_c}. \qquad [1]$$

The results of yield studies are usually expressed in amounts of biomass produced (preferably in C-moles) per amount of substrate consumed (in C-moles). For growth on single carbon-containing substrates (Fig. 1*A*), the yield $Y'' = -J_a/J_s$, so that

$$\eta = \frac{Y''}{1 - Y''} \cdot \frac{\Delta G_a}{\Delta G_c}. \qquad [2]$$

Linton and Stephenson (ref. 38; cf. ref. 39) stressed the positive correlation between the heat of combustion of a substrate and the growth yield. Using the number of electrons per carbon atom produced upon complete oxidation of the substrate [the degree of reduction (25, 40)] as a measure of the heat of combustion (25, 26), we have summarized the table of Linton and Stephenson (38) in the first four columns of Table 1. Using standard free enthalpies of substrates, of biomass (cf. refs. 25 and 41), and of catabolic products (bicarbonate and water), as listed by Roels (25), and taking into account that the dependence of these free enthalpy values on concentration is negligible (25, 26, 42, 43), we calculated $\Delta G_a/\Delta G_c$ for each substrate in Table 1 (fifth column) and the growth efficiency (sixth column).

Because the degree of reduction (as defined by ref. 25; see Table 1) of biomass is about 4, thermodynamic efficiency is about 23% when the substrate is significantly more oxidized than biomass but becomes negative when the substrate is significantly more reduced than biomass.

"Negative thermodynamic efficiency" may need some clarification. Usually, the input process of energy conversion pro-

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Abbreviation: C-mole(s), mole(s) of carbon.

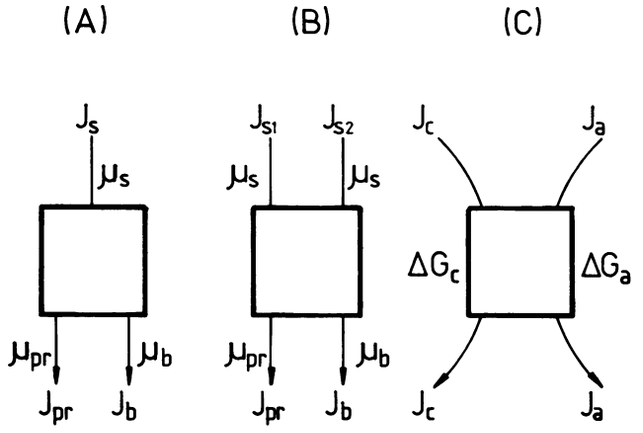

FIG. 1. Three equivalent schemes for microbial growth on one substrate. (*A*) The microbe is regarded as a free enthalpy converter into which a substrate of chemical potential μ_s flows at rate J_s, part of which substrate is combusted to products at chemical potential μ_{pr} (production rate J_{pr}). The remainder of the substrate is transformed to biomass at chemical potential μ_b (rate of biomass production J_b). (*B*) Here it is made explicit that J_s can be thought of as consisting of two parts, one (J_{s1}) leading to combustion and the other (J_{s2}) leading to biomass synthesis. (*C*) is fully equivalent to *B*, with the following translation: $J_c = J_{s1} = J_{pr}$ (the catabolic flow), $-J_a = J_{s2} = J_b$ (the anabolic flow), $\Delta G_c = \mu_s - \mu_{pr}$ (the free enthalpy of catabolism), and $\Delta G_a = \mu_b - \mu_s$ (the free enthalpy of anabolism).

vides the free enthalpy that is necessary to drive the output process against its own free enthalpy. An example is the combustion of petrol to move a car up a hill. Part of the free enthalpy consumed in petrol combustion is recovered in the form of increased gravitational potential energy of the car: this is a process with a positive ratio of output and input free enthalpies and has, therefore, a positive thermodynamic efficiency. The car also can operate at a negative efficiency: when going downhill, pressing the gas pedal will increase the free enthalpy of combustion of petrol and, thus, by increasing the velocity of the car, will increase the rate of free enthalpy loss of the car in the gravitational field. Yet this can be useful: the car reaches its destination more quickly.

Fig. 2 shows that microbial growth on oxalate is an "uphill" process (ΔG_a = "μ_b" − "$\mu_{oxalate}$" = positive). The free enthalpy needed for the process comes from the consumption of oxalate under formation of the products bicarbonate and water: ΔG_c = "$\mu_{oxalate}$" − "$\mu_{products}$" > 0. Consequently, the efficiency of microbial growth on oxalate is positive. (Here the μs are placed between quotation marks because they are composite μs, as indicated in the legend to Fig. 2.) The free enthalpy of methane exceeds the free enthalpy (per C-mole) of biomass. Therefore, conversion of methane to biomass is a "downhill" process (ΔG_a = "μ_b" − "$\mu_{methane}$" < 0), and its thermodynamic efficiency is bound to be negative whenever growth occurs. The free enthalpy generated in the consumption of 37% of the methane [i.e., (1–0.63)·100%; see Table 1] may serve to accelerate growth, but it does not serve the purpose of providing the free enthalpy needed for an uphill output reaction.

Microbial growth described by phenomenological nonequilibrium thermodynamics

In microbial growth (see Fig. 1*C*), the anabolic processes take place at a rate $-J_a$ against the free enthalpy difference (ΔG_a) between biomass and the substrate for anabolism. The free enthalpy needed is provided by that released in catabolism (ΔG_c) taking place at a rate J_c. Thus, microbial growth is analogous to the free enthalpy converters of nonequilibrium thermodynamics with relationships between "flows" (rates of anabolism and catabolism) and "forces" (ΔG_a and ΔG_c) as described (28–30, 34):

$$J_c/(L_{cc}\cdot\Delta G_c) = 1 + q\cdot x, \quad [3]$$

$$J_a/(Z\cdot L_{cc}\cdot\Delta G_c) = q + x. \quad [4]$$

The normalized force ratio x is defined in ref. 30 as

$$x \overset{\text{def}}{=} Z\cdot\Delta G_a/\Delta G_c. \quad [5]$$

The phenomenological coefficient L_{cc}, the degree of coupling

Table 1. The thermodynamic efficiency of microbial growth on substrates with different energy content

Organism	Substrate	Degree of reduction of substrate	Y"*	$\Delta G_a/\Delta G_c$	Thermodynamic efficiency, %
Pseudomonas oxalaticus OX1	Oxalate	1	0.07	2.57	20
	Formate	2	0.18	0.89	20
Paracoccus denitrificans	Fumarate	3	0.37	0.43	25
	Citrate	3	0.38	0.43	26
	Malate	3	0.37	0.41	24
	Succinate	3.5	0.39	0.27	16
Pseudomonas sp.	Acetate	4	0.44	0.12	9
Escherichia coli	Glucose	4	0.62	0.00	0
Pseudomonas sp.	Benzoate	4.3	0.48	0.00	0
Aerobacter aerogenes	Glycerol	4.7	0.66	−0.13	−26
Candida utilis	Ethanol	6.0	0.55	−0.28	−36
Pseudomonas C.	Methanol	6.0	0.67	−0.32	−64
"Job 5"†	Propane	6.7	0.71	−0.32	−79
	Ethane	7	0.71	−0.35	−82
Methylococcus capsulatus	Methane	8	0.63	−0.42	−70

The thermodynamic efficiency was calculated as described in the text for growth yield data (aerobic; NH_3 as the source of nitrogen) compiled by Linton and Stephenson (38). The degree of reduction of a substrate with formula $(CH_hO_o)_c$ was defined as $4 + h - 2o$ (25). In the calculations, standard free enthalpies and biomass composition ($CH_{1.8}O_{0.5}N_{0.2}$) were used as given by Roels (25).

* Expressed as ratio of biomass C-moles to substrate C-moles.

† From ref. 38.

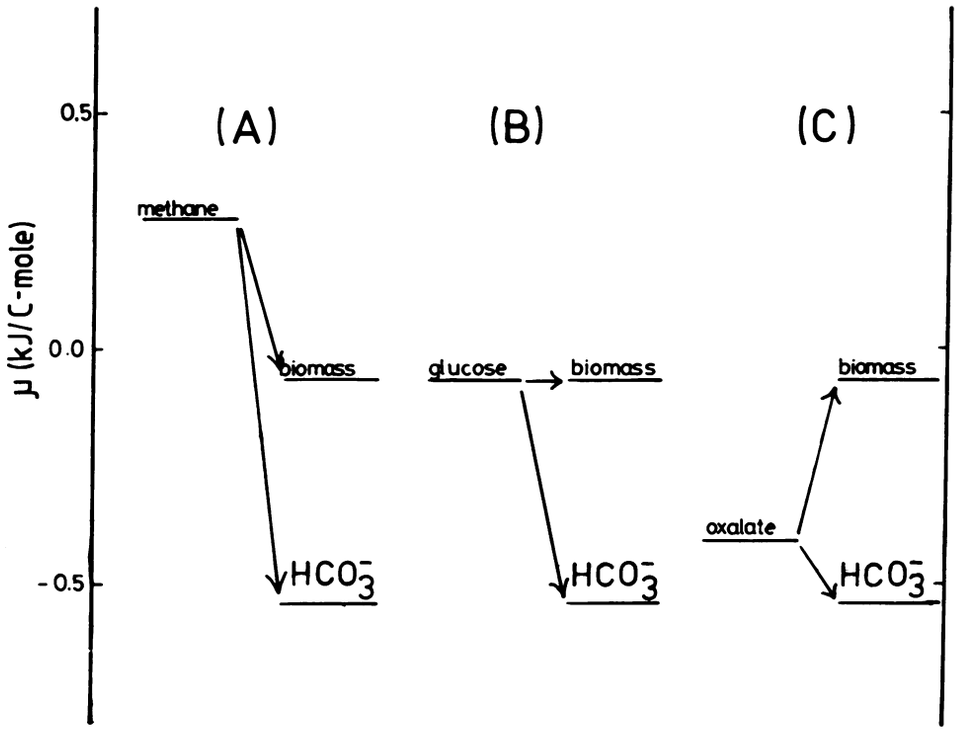

FIG. 2. Positive and negative thermodynamic efficiencies in microbial growth. "Formal" chemical potentials of methane (*A*), glucose (*B*), and oxalate (*C*) are compared to the formal chemical potentials of biomass and CO_2. Thus, growth on oxalate (*C*) is "uphill" and growth on methane is "downhill". Therefore, the corresponding efficiencies are positive and negative, respectively. Formal chemical potential of glucose was taken as the free enthalpy of ⅙ mol of glucose/0.2 mol of NH_4^+/0.2 mol of H^+/−0.4 mol of H_2O/−0.05 mol of O_2; of methane was taken as that of 1 mol of methane/0.2 mol of NH_4^+/−0.2 mol of H^+/−1.4 mol of H_2O/+0.9 mol of O_2; of oxalate was taken as that of 0.5 mol of oxalate anion (2−)/0.2 mol of NH_4^+/0.8 mol of H^+/0.1 mol of H_2O/−0.8 mol of O_2; and of CO_2 was taken as that of 1 mol of HCO_3^-/0.2 mol of NH_4^+/0.8 mol of H^+/−0.4 mol of H_2O/−1.05 mol of O_2. The formula for biomass was $CH_{1.8}O_{0.5}N_{0.20}$. All free enthalpy values were taken from ref. 25.

q, and the phenomenological stoichiometry Z are constants (i.e., independent of ΔG_a and ΔG_c) that are characteristic for the free enthalpy converter. At any given force ratio x, q is a measure of the extent to which catabolism entails the synthesis of biomass: when q equals zero, no synthesis results from catabolism; when q equals -1, anabolism and catabolism are strictly and proportionally coupled to one another. In the present description, q can be > -1, which means that processes in the microbe that do not lead to synthesis of progeny (e.g., "maintenance" in refs. 9–13 and "futile cycling" in refs. 16–18) are taken also into account; microbial growth is described as a partly coupled free enthalpy converter.

The performance of such a free enthalpy converter can be characterized by a number of output functions (29, 30), one of which is the thermodynamic efficiency η (28):

$$\eta = \frac{-J_a \cdot \Delta G_a}{J_c \cdot \Delta G_c} = -\frac{q + x}{q + 1/x}. \qquad [6]$$

Other relevant normalized state functions also can be expressed as functions of the normalized force ratio (x) and the degree of coupling (q) only (30): the (normalized) rate of biomass synthesis $[-J_a/(Z \cdot L_{cc} \cdot \Delta G_c)]$, the (normalized) flow ratio $[-J_a/(J_c \cdot Z)$; comparable to the growth yield on the basis of the catabolic substrate], and the (normalized) rate at which free enthalpy in the form of biomass is generated ($-J_a \cdot \Delta G_a / L_{cc} \cdot \Delta G_c^2$; the power output).

Optimal thermodynamic efficiencies

With the simple description of microbial growth as a partly coupled free enthalpy converter, it should be possible to calculate the optimal conditions with respect to a specific output function. At full coupling ($q = -1$), the efficiency would be less than 100%, unless, it would seem, the free enthalpy of biomass synthesis ΔG_a would exactly match the free enthalpy of catabolism ΔG_c [see Eqs. 5 and 6, with $Z = 1$ when $q = -1$ (28)]. In that case, however, the growth rate $-J_a$ would be zero (see Eq. 4), again decreasing the efficiency, now to 0%. For growth to occur, the normalized force ratio must become <1 (see Eq. 4), with a concomitant decrease in thermodynamic efficiency to $<100\%$. Moreover, in general, at all degrees of coupling, some thermodynamic efficiency may be sacrificed to make the process run faster.

By expressing the normalized output functions as functions of q and x, for every value of q, the value of x can be calculated for which that output function is maximal. We did this calculation for a fully coupled free enthalpy converter ($q = -1$) for each of the six output functions in the heading of Table 2. We inserted the resulting values of x into Eq. 6 (at $q = -1$) and obtained the efficiencies that are optimal at full coupling with respect to each of these six output functions (Table 2, first line).

It turned out that the efficiency of energy conversion must be as negative as possible for a fully coupled system to have a maximal output flow (the rate of biomass synthesis). This corresponds to $\Delta G_a/\Delta G_c$ being as small (or as highly negative) as possible. If x (and thus the free enthalpy of biomass synthesis) were to have a value so that the power production (i.e., the free enthalpy production rate $-J_a \cdot \Delta G_a$) were maximal, the thermodynamic efficiency should be as high as 50% (Table 2, second column, first line). Two other output functions are accompanied by thermodynamic efficiencies of 50% and 67%, respectively, when they are maximal: the economic biomass synthesis de-

Table 2. Theoretical thermodynamic efficiencies (in percentage) for 12 cases of optimal microbial growth

Condition	Rate of biomass synthesis, C-moles/hr $[-J_a]_{max}$	Power production, W $[-J_a \cdot \Delta G_a]_{max}$	Economic biomass synthesis, C-moles/hr $[-J_a \cdot \eta]_{max}$	Economic power production, W $[-J_a \cdot \Delta G_a \cdot \eta]_{max}$	Force ratio $[\Delta G_a/\Delta G_c]_{max}$	Growth yield $[1/(1 - J_c/J_a]_{max}$
$q = -1$	$\ll 0$	50	50	67	(100)	$\ll 0$
Optimal efficiency	24	41	54	62	100	100

The first line gives the theoretical thermodynamic efficiency at the degree of coupling of −1 and a force ratio chosen so that the function given in brackets in the heading is maximal. The second line gives the theoretical thermodynamic efficiency for the states reached when the function in the heading is maximized (again by varying the force ratio) while the growth efficiency is kept optimal by adjusting the degree of coupling to the force ratio. In the optimization calculations, the functions were normalized by $ZL_{cc}\Delta G_c$, $L_{cc}(\Delta G_c)^2$, $ZL_{cc}\Delta G_c$, $L_{cc}(\Delta G_c)^2$, Z and Z, respectively (30, 26) (see text). The efficiency of 100% corresponding to maximal force ratio for a fully coupled system is put between parentheses because it corresponds to the unrealistic growth rate of (precisely) zero.

fined as $-J_a \cdot \eta$ and the economic power production defined as $-J_a \cdot \Delta G_a \cdot \eta$ (30). If the system were to produce the highest possible output force (in this case biomass with the highest possible free enthalpy content), thermodynamic efficiency would be zero because the output flow (the rate of biomass synthesis) would be zero (see Eq. 4). (Note that this is a singular point.) Growth yield and flow ratio $Z \cdot (-J_a/J_c)$ show similar optimization behavior. For the growth yield to be maximal at any given degree of coupling, the force ratio would have to be infinitely negative, entailing a negative infinite thermodynamic efficiency. As in the case of maximal rate of synthesis of biomass (see above), this would in practice mean a finite but negative efficiency.

To facilitate comparison of the experimental efficiencies (Table 1) to the theoretical efficiencies (Table 2) we have drawn Fig. 3, in which the former are represented by dots and the latter by dashed lines: the efficiency values of the first line of Table 2 are represented by the lines at 67% and 50%. The two cases of negative (but not exactly predictable) efficiencies are represented by the shaded area in Fig. 3. Only the latter two theoretical values correspond to experimental ones: growth on highly reduced substrates (negative efficiencies) corresponds to optimization for the maximal rate of biomass synthesis or optimization for the maximal growth yield. The thermodynamic efficiency of about 23% for growth on highly oxidized substrates does not correspond to any of the theoretical efficiencies in the first line of Table 2.

In the calculation of optimal conditions above, the force ratio x has been allowed to vary, whereas the degree of coupling q was kept equal to −1. Stucki (29) proposed that, in addition to x, also q might be varied to obtain a state that would be optimal

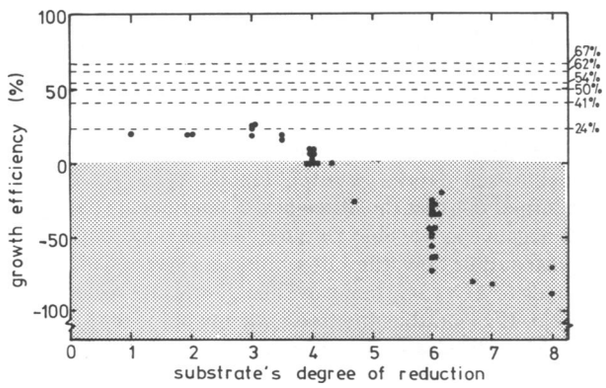

FIG. 3. The experimental and theoretical thermodynamic efficiencies of microbial growth. ●, Experimental values shown in Table 1 and values for additional cases of microbial growth calculated in the same way (26); ----, theoretical values from Table 2. The shaded area indicates the efficiencies predicted as $\ll 0\%$.

for the system. He calculated how x must depend on q to ensure maximal efficiency at every degree of coupling. Inserting this relationship into the dependence of each (normalized) output function on q and x, he wrote those functions as functions of q only. For the first four output functions given in the heading of Table 2, he then calculated (30) the value of q for which that function would be maximal. We have extended these calculations to include the last two output functions. The second line of Table 2 gives the thermodynamic efficiency that belongs to each of the six states. Comparison with the experimental values in Table 1 (see also Fig. 3) suggests that the experimental growth efficiency of about 23% on highly oxidized substrates corresponds to the maximal rate of biomass synthesis at optimal efficiency.

The results placed in an evolutionary context

We wish to stress that the observed thermodynamic efficiencies of microbial growth are well below 100%. However, these low efficiencies can be understood as optimal with respect to growth rate. For highly reduced substrates, the growth efficiencies are negative, which reflects the negative efficiency of a free enthalpy converter optimized for maximal output flow or for maximal yield (output flow divided by input flow). For substrates that are more oxidized than the biomass produced, the efficiencies lie around 24%, the value characteristic for a free enthalpy converter that is optimal with respect to maximal output flow at optimal efficiency.

It seems understandable that evolutionary pressure on microorganisms may have resulted in selection of the fastest growing mutants, termed "*r*-selection" (44). Highly reduced substrates contain more free enthalpy than is necessary for the formation of biomass (see Fig. 2 and refs. 14, 16, 25, 31, 32, and 38): even with a yield of 1 mol of biomass carbon per substrate carbon for microorganisms growing on highly reduced substrates, thermodynamic efficiency would be irrelevant (or even a hindrance) for survival. In the case of growth on more oxidized substrates, with free enthalpies lower than the free enthalpy of the biomass, some of the substrate must be combusted to yield the free enthalpy necessary for the conversion of substrate into biomass. In that case, the efficiency of free enthalpy conversion becomes important, so that a selection for mutants growing as fast as possible at optimal efficiency of free enthalpy conversion would not seem unexpected [cf. "*K*-selection" (44)].

However, an important objection could be raised here. In an ecosystem containing a population of similar microorganisms, the fastest growing microorganism will outgrow its competitors that grow more slowly, even when the substrates are highly oxidized, and irrespective of the efficiency of free enthalpy of conversion. Hence, it could be argued that even with highly oxidized substrates, the efficiency of growth is irrelevant. The answer may be that evolution has not been a smooth pro-

cess. Periods of excess substrate have probably been interrupted by periods of famine. Under conditions of starvation, microbial cultures survive by degrading part of the biomass to provide the maintenance free enthalpy that is necessary for survival. The culture that has grown most efficiently during the period of excess substrate will contain most free enthalpy (in the form of biomass) and will, therefore, survive longest during starvation. Consequently, the highest possible efficiency may have been the most stringent criterion imposed by evolution: of populations of closely related microorganisms all having the same degree of coupling, the surviving population will be the one with the highest efficiency. However, although it is the most important criterion, efficiency of growth may not have been the only important one.

Let us suppose that, at a certain moment in time, a mutant with a different degree of coupling becomes isolated from the wild-type microorganism and forms its own colonies. Because of the importance of high growth efficiency, the free enthalpy of the mutant colonies will adapt to the condition of maximal efficiency at the new degree of coupling. Both the wild-type colonies and the mutant colonies operate at efficiencies that are maximal with respect to their specific degree of coupling; however, not only may these efficiencies differ, but also any other output function will differ, such as the rate of biomass synthesis. The colonies with the highest rate of biomass synthesis will become most widespread. Thus, this scenario of the evolution of microbes, speculative as it may seem, presents us with a rationalization for the optimization for maximal growth rate at optimal efficiency.

The contention that, at high degrees of reduction of the substrate, there is more free enthalpy than necessary to convert all substrate carbon to biomass suggests that thermodynamic growth efficiency for carbon dioxide-fixing organisms must be positive rather than negative (as with non-CO_2-fixing organisms), even with highly reduced substrates. It turns out that this is true for *P. denitrificans* growing on methanol (45, 46) because this bacterium is known to fix carbon dioxide under this condition. The thermodynamic efficiency indeed proved to be positive: about 33% (26). A number of refinements can be made to our treatment (26, 42, 43) (nonsymmetric flow–force relationships, concentration-dependent chemical potentials, dependence on uncoupling mechanism, and variation of the sequence of optimization). However, we consider the correspondence between theoretical and experimental efficiencies to be too close to be merely fortuitous. More refined calculations and suitable experiments are required to substantiate its relevance.

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