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Review

Does microbial life always feed on negative entropy? Thermodynamic analysis of microbial growth

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Abstract

Schrödinger stated in his landmark book, *What is Life?*, that life feeds on negative entropy. In this contribution, the validity of this statement is discussed through a careful thermodynamic analysis of microbial growth processes. In principle, both feeding on negative entropy, i.e. yielding products of higher entropy than the substrates, and generating heat can be used by microorganisms to rid themselves of internal entropy production resulting from maintenance and growth processes. Literature data are reviewed in order to compare these two mechanisms. It is shown that entropy-neutral, entropy-driven, and entropy-retarded growth exist. The analysis of some particularly interesting microorganisms shows that enthalpy-retarded microbial growth may also exist, which would signify a net uptake of heat during growth. However, the existence of endothermic life has never been demonstrated in a calorimeter. The internal entropy production in live cells also reflects itself in the Gibbs energy dissipation accompanying growth, which is related quantitatively to the biomass yield. An empirical correlation of the Gibbs energy dissipation in terms of the physico-chemical nature of the growth substrate has been proposed in the literature and can be used to predict the biomass yield approximately. The ratio of enthalpy change and Gibbs energy change can also be predicted since it is shown to be approximately equal to the same ratio of the relevant catabolic process alone.

Keywords: Thermodynamics; Entropy; Gibbs energy dissipation; Heat; Calorimetry; Biomass yield

Contents

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1. Introduction

The second law of thermodynamics may be formulated in many different ways, but it predicts a dull state of equilibrium, the inevitable 'heat death' of the universe. Indeed Clausius's statement "the entropy of the universe tends to a maximum" may be regarded as one form of the second law of thermodynamics. However, as we see, the world displays an exciting pattern of life that is constantly generating impressively ordered structures, ranging from microorganisms to flowers to even human beings. So how is it that a living organism can come into existence, survive and grow rather than decay? How can it swim against an entropic stream, which tends to carry everything to an inert state of equilibrium and eventually to heat death?

These intriguing questions were already addressed by Schrödinger in 1944 with the publication of his famous book, *What is Life?* [1]. He stated, "How does the living organism avoid decay? The obvious answer is: by eating, drinking, breathing and (in the case of plants) assimilating. The technical term is called metabolism." However, Schrödinger [1] pointed out that metabolism per se does not explain anything: "Any atom of nitrogen, oxygen, sulfur, etc. is as good as any other of its kind, what could be gained by exchanging them?" The same appears to be true for the energy content of the exchanged metabolites: "Since, surely, any calorie is worth as much as any other calorie, one cannot see how a mere exchange could help". Thus Schrödinger arrived at his famous remark, "What an organism feeds upon is negative entropy. Or, to put it less paradoxically, the essential thing in metabolism is that the organism succeeds in freeing itself from all the entropy it cannot help produce while alive."

There is no doubt that with his book *What is Life?* Schrödinger made a seminal contribution to modern biology. But his concept of 'negative entropy' or 'negentropy' gave rise to everlasting discussion and criticisms concerning its meaning and significance [2–8]. Even 50 years after the publication of *What is Life?*, symposia were still organized, and collections of papers published, that centered around Schrödinger's question [9,10].

Schrödinger's negative entropy may be interpreted

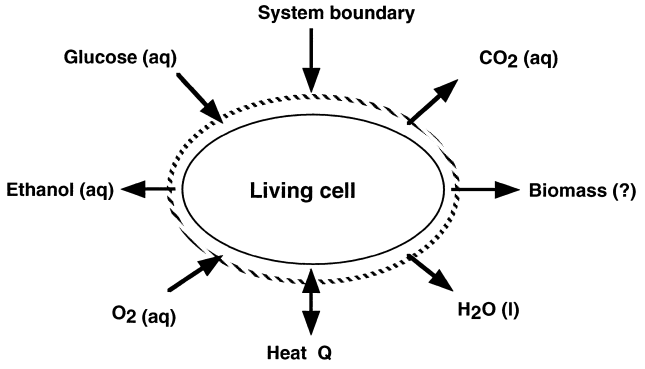

Fig. 1. Living cells as open systems.

based on an entropy balance around the living cell (Fig. 1).

$$\frac{dS}{dt} = \frac{d_{int}S}{dt} + \frac{d_eS}{dt} = \dot{S}_{Prod} + \frac{\dot{Q}}{T} + \sum_i \bar{s}_{e,i}\cdot\dot{n}_{e,i} \quad (1)$$

In this balance, which considers living organisms as open systems, $\dot{n}_{e,i}$ represents the rate of exchange of the i-th chemical species (nutrients and products) through the cell wall, with a partial molar entropy of $\bar{s}_{e,i}$ at the system boundary.

According to the second law of thermodynamics and given the fact that life and growth are clearly irreversible processes, entropy is always generated internally, i.e. $\dot{S}_{prod}$ is always positive. As pointed out by Schrödinger, living organisms must maintain a state of high organization, and avoid an increase in entropy, i.e. maintain $(dS/dt) \leq 0$. Therefore, internal entropy production ($\dot{S}_{prod}$) must be transferred to the environment, either by means of entropy exchange associated with heat ($\dot{Q}/T < 0$) or/and by yielding products of higher entropy than the nutrients. In the latter, the entropy carried away by the products would outweigh the import of entropy with nutrients, and thus, a net transfer of entropy to the environment would occur. It is generally agreed that this second possibility was meant by Schrödinger when coining his 'negative entropy (negentropy)'.

It is obvious that Schrödinger's statement does not sufficiently allow for the first mechanism of entropy export, i.e. simply giving off heat. Heat production may be observed directly with calorimetric experiments, or calculated based on energy balances. This paper aims to review calorimetric data available in the literature, and consequently, to assess to what extent microbial growth feeds on *negentropy*, and to compare this to heat exchange as a means for microorganisms to rid themselves from the internal entropy production.

As explained later in this paper, the two mechanisms for entropy export can also be unified and analyzed in terms of the Gibbs energy dissipation that accompanies microbial growth. Therefore, another aim of this paper is to review some of our knowledge about the Gibbs energy dissipated by growing microbial cultures and its relationship to heat generation, entropy production and growth yield.

2. Theory

As already pointed out, the export of entropy by heat and by changes of chemical entropy may be unified in terms of Gibbs energy changes. To this effect, it is convenient first to simplify the entropy balance Eq. 1 by assuming that the stoichiometry for microbial growth is known. In the simplest case, microorganisms grow according to a constant stoichiometry in which the whole process can be described by a 'macrochemical equation' (see Eqs. 9 and 10). In other more complex cases, the stoichiometric coefficients may vary, but it is then often possible to consider the microbial growth process as the net result of several global metabolic reactions of constant stoichiometry, such as catabolism, anabolism and maintenance reactions. In either case, molar balances can be written for each chemical species and combined with Eq. 1 (see Appendix A) to yield:

$$C_P\frac{dT}{dt} - T\left(\frac{\partial V}{\partial T}\right)_P\cdot\frac{dP}{dt} + T\cdot\sum_i \bar{s}_i\sum_j \nu_{ij}\cdot\dot{\xi}_j = \dot{Q} + T\cdot\sum_i(\bar{s}_{e,i} - \bar{s}_i)\cdot\dot{n}_{e,i} + T\dot{S}_{Prod} \quad (2)$$

In this equation, $\bar{s}_{e,i}$ represents the partial molar entropy of the exchanged species i, i.e. under the conditions of the system boundary, which is usually taken at the cell surface (Fig. 1), whereas $\bar{s}_i$ represents the partial molar entropy of i within the cell. Although cells are highly compartmentalized such

that any chemical species could exist in many different states within cells, the biomass has been assumed to be 'pseudo-homogeneous' [11], such that any given variable may be assigned a single, average value. Also, it has been assumed that the temperatures within and outside the cell are equal. ν_{ij} represents the stoichiometric coefficient of chemical species i in the j-th global metabolic reaction which proceeds at a rate $\dot{\xi}_j$.

The rate of heat exchange $\dot{Q}$ may be predicted from an enthalpy balance, which adopts a form similar to Eq. 2 (for a detailed derivation, see Appendix A):

$$C_P\frac{dT}{dt}-T\left(\frac{\partial V}{\partial T}\right)_P\cdot\frac{dP}{dt}+\sum_i\bar{h}_i\sum_j\nu_{ij}\cdot\dot{\xi}_j = \dot{Q}+\dot{W}+\sum_i(\bar{h}_{e,i}-\bar{h}_i)\cdot\dot{n}_{e,i} \quad (3)$$

Combination of Eqs. 2 and 3 permits the elimination of $\dot{Q}$, and yields the following balance:

$$\sum_j\sum_i\nu_{ij}\mu_i\dot{\xi}_j = \dot{W}+\sum_i(\mu_{e,i}-\mu_i)\cdot\dot{n}_{e,i}-T\dot{S}_{prod} \quad (4)$$

where μ_i is the chemical potential of chemical species i and defined as:

$$\mu_i = \bar{h}_i-T\bar{s}_i \quad (5)$$

Since in all but very special circumstances microbial cultures do not do any useful work, $\dot{W}$ can usually be disregarded. At this point, the equation may be simplified further by introducing the important concept that the entropy of a living cell should not increase. This may be done by assuming that the cellular composition remains constant and thus by considering the live cell as a catalytic unit operating at a steady state. The new cells (biomass) formed by growth would then be regarded as yet another metabolic product. In this case, (dn_i/dt) in the molar balance (Eq. A7) may be set to zero. The result is that $\dot{n}_{e,i}$ must be equal to $(-\Sigma_j\ \nu_{ij}\dot{\xi}_j)$, the net consumption or production of chemical species i by all reactions occurring in the cell. As a consequence, the second term of the right side of Eq. 4 can be incorporated into the left side of Eq. 4 to give:

$$\sum_j\Delta_{rj}\ G\cdot\dot{\xi}_j = -T\dot{S}_{prod} \quad (6)$$

with

$$\Delta_{rj}G\equiv\sum_i\nu_{ij}\cdot\mu_{e,i} = \sum_i\nu_{ij}\bar{h}_{e,i}-T\sum_i\nu_{ij}\bar{s}_{e,i} = \Delta_{rj}H-T\Delta_{rj}S \quad (7)$$

Eqs. 6 and 7 are important results of this analysis and may be interpreted as follows. The left side of Eq. 6 indicates the rate of Gibbs energy change due to the occurrence of irreversible reactions within the cell. While such irreversible phenomena constantly generate entropy at a rate of $\dot{S}_{prod}$, Eq. 6 shows that they lead to a constant disappearance, or dissipation, of Gibbs energy at a rate of $T\dot{S}_{prod}$. According to Eq. 6 the entropy production, or the Gibbs energy dissipation of any given process will be the greater the more its $\Delta_{rj}G$ is negative, i.e. the further it is removed from the equilibrium state. Since $\Delta_{rj}G$ reflects the irreversibility of a given reaction, it is usually considered to represent the driving force of this reaction. Some individual processes, such as those involved in biosynthesis, may have a positive $\Delta_{rj}G$, but they must be coupled to other exergonic reactions such that Gibbs energy change $(\Sigma_j\Delta_{rj}G\cdot\dot{\xi}_j)$ of the overall growth process remains negative.

As may be seen from Eqs. 6 and 7, $\Delta_{rj}G$ has to be formulated with the chemical potentials of the chemical substances i at the cell surface, $\mu_{e,i}$, rather than with μ_i inside the cell. The driving forces, $\Delta_{rj}G$, must therefore exist in the growth medium, and the growth process will modify the Gibbs energy at a rate of $(\Sigma_j\Delta_{rj}G\cdot\dot{\xi}_j)$ in the growth medium, and not within the cell. The Gibbs energy dissipation, and by the same token the entropy production, is seen to be exported from the cell into the medium. This is a consequence of the steady state assumption for the cell, meaning that neither the Gibbs energy nor the entropy content of the cells changes. Rather, the Gibbs energy change inside the cell is compensated for by a constant intake of nutrients with high chemical potentials and by excreting products of low chemical potentials.

In Eq. 7, the enthalpic part of the ΔG corresponds to the export of entropy in the form of heat exchange. This is because that without volume work

Table 1
Significance of symbols for chemical compounds in the generalized growth stoichiometry (Eq. 9)

Examples of growth	Examples of microorganisms	S	A	P
Oxidative growth	*Kluyveromyces fragilis*	glucose	O_2	–
Reductive growth	*Saccharomyces cerevisiae*	glucose	glucose	CH_3CH_2OH
Methanogenesis utilizing H_2 and CO_2	*Methanobacterium thermoautotrophicum*	CO_2	H_2	CH_4
Methanogenesis utilizing acetate	*Methanosarcina barkeri*	acetate	acetate	CH_4

the overall change in enthalpy corresponds to the heat exchange,

$$\sum_j \Delta_{rj} H \cdot \dot{\xi}_j = \dot{Q} \tag{8}$$

The $T\Delta_{rj}S$ term in Eq. 7 reflects the export of chemical entropy into the environment. Therefore, in order to answer the question posed in the title of this contribution, it will be necessary to compare the $\Delta_{rj}H$ and the $T\Delta_{rj}S$ terms.

This comparison is made in the present review for a number of representative examples of microbial growth. In all of them, a maximum of one major product is formed other than biomass, CO_2 and water. In addition, the growth stoichiometry is constant during the experiment. It is thus possible to lump all metabolic reactions into a single, macrochemical equation that may be written for all examples as follows:

$$\frac{1}{Y_{X/S}} S + Y_{A/X} A + Y_{N/X}\,NH_3 \rightarrow$$

$$X + Y_{P/X} P + Y_{C/X} CO_2 + Y_{W/X} H_2O \tag{9}$$

where S, A, X and P represent the carbon source, the electron acceptor/donor, the biomass and the product, respectively. The identities of the compounds S, A, X and P, in the different examples treated in this text are given in Table 1. For example, oxidative growth of *Kluyveromyces fragilis* on glucose could be represented typically by the following equation.

$$0.293\ C_6H_{12}O_6 + 0.695\ O_2 + 0.150\ NH_3 \rightarrow$$

$$CH_{1.75}O_{0.52}N_{0.15} + 0.758\ CO_2 + 1.110\ H_2O \tag{10}$$

The biomass yield $Y_{X/S}$ in Eq. 9 and the $\Delta_{rj}G$ terms appearing in Eqs. 6 and 7 are linked. This is qualitatively demonstrated in Fig. 2, where the growth stoichiometry (Eq. 9) has been split up into two parallel reactions, representing anabolism and catabolism. The anabolic process is endergonic due to the low entropy content of the biomass it produces, but it can also be slightly exergonic [12]. For efficient growth it is forced to proceed at a high rate by a biochemical coupling to a highly exergonic catabolic process. This ensures that the overall growth process, i.e. the combination of catabolism and anabolism, still has a negative ΔG value, and it thus constantly dissipates Gibbs energy. This situation is known as an energy converter in non-equilibrium thermodynamics [13].

In cases where not much biomass is synthesized per amount of energy substrate, i.e. in cases in which the biomass yield is low, one expects a strongly negative $\Delta_r G_X$ value for the overall growth process that approaches the value for the catabolic process as $Y_{X/S}$ tends to zero. On the other hand, as $Y_{X/S}$ increases, the $\Delta_r G_X$ for the overall growth process will be affected increasingly by the ΔG of anabolism (Fig. 2). The former will thus become less negative if the latter is assumed positive. The theoretical upper limit for the biomass yield is defined by a zero Gibbs energy change of the overall growth process. This, however, would represent growth at thermodynamic equilibrium, and would thus proceed infinitely

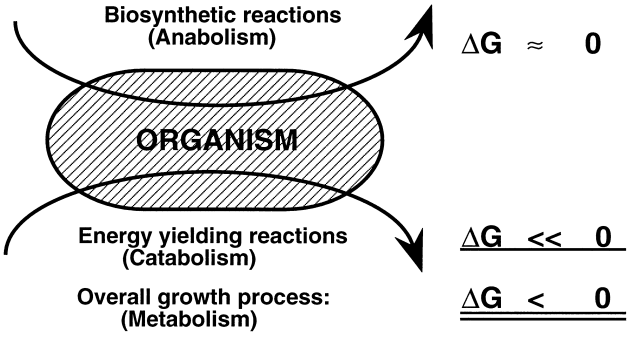

Fig. 2. Coupling of catabolism and anabolism during growth.

slowly. According to Eq. 6 it would neither dissipate Gibbs energy nor generate entropy. Therefore, a high rate of Gibbs energy dissipation leads to high metabolic rates but low biomass yield ($Y_{X/S}$), while a low rate of Gibbs energy dissipation leads in general to the contrary [13].

The quantitative relationship between the Gibbs energy change ($\Delta_r G_X$) and the biomass yield ($Y_{X/S}$) can be derived by splitting the overall growth process, Eq. 9, into two formal parallel reactions:

$$S + Y_A^a A \rightarrow Y_P^a P + Y_C^a CO_2 + Y_W^a H_2O + Y_N^a NH_3 \quad (11a)$$

$$S + Y_A^b A \rightarrow Y_P^b P + Y_C^b CO_2 + Y_W^b H_2O + Y_N^b NH_3 \quad (11b)$$

Reaction 11a represents catabolism, whereas reaction 11b describes a hypothetical degradation of biomass into the products of catabolism. This is an arbitrary, but simple and straightforward way to separate catabolism from biosynthesis, as it is normally not possible to identify the stoichiometry of the correct anabolic process without extensive knowledge of a large amount of biochemical details [14].

Comparing Eqs. 9 and 11 yields

$$\Delta_r G_X = \frac{\Delta_r G_a}{Y_{X/S}} - \Delta_r G_b \quad (12)$$

where $\Delta_r G_X$, $\Delta_r G_a$ and $\Delta_r G_b$ refer to reactions 9, 11a, and 11b, respectively. $\Delta_r G_a$ and $\Delta_r G_b$ may be evaluated based on the Gibbs energies of combustion as shown in Appendix B. They both have large negative values.

Analogously, the correlation between the enthalpy change and the biomass yield is given as

$$\Delta_r H_X = \frac{\Delta_r H_a}{Y_{X/S}} - \Delta_r H_b \quad (13)$$

$\Delta_r H_a$ and $\Delta_r H_b$ may be calculated based on the enthalpies of combustion of pertinent compounds. From the above relations, Eqs. 12 and 13, it appears that the biomass yield can be predicted if $\Delta_r G_X$ (or $\Delta_r H_X$) is known. Once the biomass yield $Y_{X/S}$ is known, it is usually also possible to predict of all the other yield values on the basis of elemental balances. Correlations developed to estimate $\Delta_r G_X$ for microbial growth will be reviewed and discussed later in this paper.

In summary, the entropy generated within growing cells is exported and leads to a decrease of Gibbs energy in the medium. The $\Delta_r H_X$ part in the Gibbs energy change represents the entropy export associated with heat while the $T\Delta_r S_X$ term reflects the entropy export due to the entropy change in the conversion from nutrients to products during growth. The Gibbs energy dissipated per amount of biomass grown, $\Delta_r G_X$, can be regarded as the driving force for growth. Also, it determines the biomass yield. The whole growth stoichiometry can be calculated if either $\Delta_r G_X$ or $\Delta_r H_X$ is known.

3. Experimental techniques and calculation methods

3.1. Enthalpy changes

3.1.1. Calorimetric measurement

Enthalpy changes of microbial growth processes can be determined by calorimetry via measuring continuously the heat exchange between the growth system and the environment. The major three instruments utilized are micro, flow and bench-scale calorimeters [15–18]. In order to obtain quantitatively significant results as opposed to a qualitative 'thermogram', heat measurements must be carried out under realistic biotechnological conditions. This requires some essential techniques: a tight control of all culture parameters, such as pH, pO_2, nutrition concentrations, etc.; a stirred injection vessel; gas and/or liquid perfusion; other analytical techniques for on-/off- line process variables, such as oxygen consumption, carbon dioxide production, biomass production, etc. Although the micro/flow calorimeters have high sensitivity, they do not sufficiently meet these technical requirements. In contrast, bench-scale or reaction calorimeters, after modification, fulfill many of them [18,19].

Since the work pioneered by Cooney et al. [20], great efforts [21–23] have been devoted to the development and the application of bench-scale calorimeters in biotechnology. Bench-scale/reaction calorimeters have traditionally suffered from low measurement sensitivity in comparison to micro-calorimeters, though in the last 10 years many attempts

Table 2
Comparison of detection limits of various calorimetric techniques applied to biotechnological processes

Methods	Volume	Detection limit (± mW/l)	References
Flow microcalorimetry	0.5–2 ml	2	[27]
Standard fermenter[a] (insulation and modeling)	1.5 l	50	[28]
Berghof fermentation calorimeter (BFK)[a]	2 l	20	[25]
Bio-RC1[a]	2 l	50	[19]
Bio-RC1[a] (improved)	2 l	5	[26]

[a]Sampling time: 60 s.

have been made to eliminate this deficiency [24,25]. Since the early 1980s, RC1 reaction calorimeters (Mettler-Toledo, Switzerland) have been adapted and improved such that they are suitable for biotechnology applications. After modification, the Bio-RC1, which has a nominal volume of 2 liters, can be operated as a standard fermenter [18,19]. Recent modifications offer an unprecedentedly high resolution of the calorimetric signals [26]. All the calorimetric work reported in this article was carried out in various versions of the Bio-RC1. A comparison of some recently reported calorimetric techniques in biotechnology is given in Table 2.

3.1.2. Calculation of enthalpy changes

The enthalpy changes of cellular growth processes can also be calculated based on an energy balance. In practice, this will have to be done using standard states, which is designated by a superscript '0' (zero) in the respective symbols, for instance, $\Delta_r H_X^0$. A detailed discussion of energy balance construction and calculation of enthalpy changes of growth processes can be found in von Stockar et al. [11]. This calculation utilizes the enthalpies of combustion or formation of involved substances in the growth process. For organic/inorganic compounds, tabulated data for these latter are readily available. However, the enthalpy of combustion for biomass must be carefully measured by combustion calorimetry. In order to obtain precise and reproducible data, a standardized handling procedure for the determination of elemental composition and enthalpy of combustion of dried biomass must be followed [29]. The difference of the combustion enthalpy between lyophilized and hydrated cells was determined experimentally to be only in the order of 1–2 kJ/C-mol [11]. Elemental compositions and enthalpies of combustion of dried biomass of many different microorganisms have been measured [30], and the average values for a range of microorganisms are given in Table 3. However, the experimental determination of the combustion heat of biomass using bomb calorimetry remains difficult and tedious. An alternative method is to estimate the enthalpy of dried biomass from its elemental composition by correlating $\Delta_C H_X^0$ with degree of reduction γ_X^0. Several such correlations have been proposed (for a review, see [31,32]). According to experimental data from Cordier et al. [31], the formula proposed by Roels [33] offers the best correlation:

$$\Delta_C H_i^0 = -115\gamma_i^0 \quad (14)$$

3.2. Gibbs energy changes

Gibbs energy changes of cellular growth processes

Table 3
The elemental formula, the mass of one C-mol of biomass (M_X), the generalized degree of reduction γ_X, the enthalpy of combustion ($\Delta_C H_X^0$) and the modified enthalpy of combustion ($\Delta_C H_X^*$) of dried biomass of various microorganisms [30]

Organisms	Elemental formula	M_X (g/C-mol)	γ_X	$-\Delta_C H_X^0$ (kJ/C-mol)	$-\Delta_C H_X^*$ (kJ/C-mol)
Average bacteria	$CH_{1.66}O_{0.41}N_{0.21}$	27.76	4.21	521.35	460.29
Average algae	$CH_{1.63}O_{0.44}N_{0.09}$	23.35	4.48	530.08	504.19
Average molds	$CH_{1.71}O_{0.44}N_{0.10}$	24.52	4.55	535.01	506.26
Average yeasts	$CH_{1.65}O_{0.54}N_{0.14}$	26.09	4.17	521.00	481.09
All microorganisms	$CH_{1.66}O_{0.46}N_{0.14}$	25.46	4.31	525.55	485.13

can only be determined from a Gibbs energy balance, which requires the Gibbs energy of combustion (or formation) for the biomass formed during growth. Unfortunately, tabulated values of Gibbs energy of combustion for biomass are not available due to a lack of data on the entropy of biomass.

In 1960s, Morowitz [34] developed an elementary statistical thermodynamic approach to calculate the entropy of biomass. Based on this method, Roels [35] calculated the $\Delta_C G_X^0$ for cells of an average composition ($CH_{1.8}O_{0.5}N_{0.2}$) to be −541.2 kJ/C-mol (Table 4). Later Roels [33] proposed a general correlation between the Gibbs energy of combustion ($\Delta_c G_i^0$) and the degree of reduction (γ_i^0) based on the tabulated values of a large number of organic compounds which can then be used to estimate the $\Delta_C G_X^0$ of biomass:

$$-\Delta_C G_i^0 = 86.6 + 94.4 \cdot \gamma_i^0 \quad (15)$$

The argument of Morowitz was refined and improved by Grosz and Stephanopoulos [36]. And the $\Delta_C G_X^0$ value for the biomass of *Escherichia coli* was estimated to be −558.3 (±5) kJ/C-mol. Battley [37] developed a thermodynamic method to calculate the entropy of biomass by using known thermochemical data of organic compounds and bio-macromolecules. With this method a $\Delta_C G_X^0$ value of −527.6 kJ/C-mol for the biomass of *E. coli* K-12 was obtained.

The entropy can be directly determined using low-temperature calorimetry. The first to apply this technique to microbial biomass were Battley et al. [38]. They measured the heat capacity of lyophilized *Saccharomyces cerevisiae* cells over a temperature range of 10–300 K, and consequently determined the entropy of the dried biomass. Assuming that the entropy of hydration and the residual entropy of biomass are small enough to be neglected, the Gibbs energy of combustion is calculated to be −515.0 kJ/C-mol. More recently, Battley [39] proposed an empirical method to estimate the entropy of the biomass based on the atomic entropies of the atoms comprising the biomass. As he showed, this method gives very good accuracy as compared to the values calculated based on the experimentally determined entropies. The results of the reported work on the entropies and Gibbs energies of combustion of biomass are summarized in Table 4.

As seen in Table 4, the Gibbs energy of combustion for *S. cerevisiae* that was determined using the experimental value of entropy is within 1% of the value estimated with Roels' correlation, or Battley's empirical method. Therefore, it is hypothesized that the Gibbs energy of combustion for other microorganisms may also be estimated by using Roels' correlation or Battley's method without introducing large error in the calculation of Gibbs energy changes. In most cases, the value for average biomass (−541.2 kJ/C-mol) may be used, though some further error might be introduced.

Gibbs energies have been evaluated using standard states in the subsequent treatment, unless otherwise indicated.

Table 4
Comparison of the entropies of biomass and Gibbs energies of combustion of biomass reported in the literature

Methods[a]	Statistical thermo-dynamics	Statistical mechanics	Thermo-dynamics	Low-temperature calorimetry	Roels' correlation	Battley's empirical method
Microorganisms[b]	Average biomass	*E. coli*	*E. coli* K-12	*S. cerevisiae*	*S. cerevisiae*	*S. cerevisiae*
Composition	$CH_{1.8}O_{0.5}N_{0.2}$	$CH_{1.77}O_{0.49}N_{0.24}$	$CH_{1.59}O_{0.374}N_{0.263}$ $P_{0.023}S_{0.006}$	$CH_{1.613}O_{0.557}N_{0.158}$ $P_{0.012}S_{0.003}K_{0.022}$ $Mg_{0.003}Ca_{0.001}$	$CH_{1.613}O_{0.557}N_{0.158}$ $P_{0.012}$ $S_{0.003}K_{0.022}$ $Mg_{0.003}Ca_{0.001}$	$CH_{1.613}O_{0.557}N_{0.158}$ $P_{0.012}$ $S_{0.003}K_{0.022}$ $Mg_{0.003}Ca_{0.001}$
γ_X^0	4.8	4.79	4.998	4.58	4.58	4.58
S^0 (J/K/C-mol)	112.8	13–38	94.4	34.17	48.44	34.69
$-\Delta_C G_X^0$ (kJ/C-mol)[c]	541.2	553–563	527.6	515.0	518.9[d]	515.2
References	[34,35]	[36]	[37]	[38]	[33]	[39]

[a]Methods for determinations of entropies of biomass.
[b]Microorganisms studied for estimation of the absolute entropy and the Gibbs energy of combustion.;
[c]$\Delta_C G_X^0$ calculated according to the reported entropies of biomass.
[d]$\Delta_C G_X^0$ calculated based on Roels' correlation.

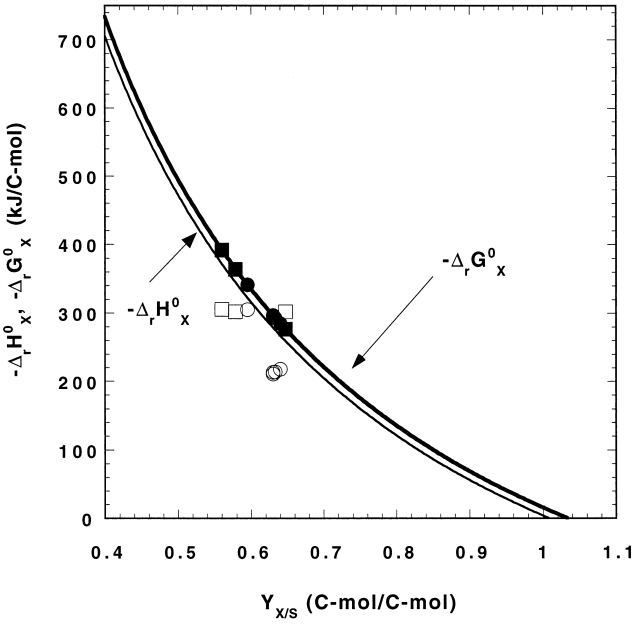

Fig. 3. Gibbs energy dissipation ($\Delta_r G^0_X$) and heat production ($\Delta_r H^0_X$) as functions of biomass yield ($Y_{X/S}$) in aerobic growth on glucose. (Temperature: 303 K. $\Delta_r H^0_a = -467.8$ kJ/C-mol, $\Delta_r G^0_a = -478.7$ kJ/C-mol, $\Delta_r H^0_b = -478.5$ kJ/C-mol, $\Delta_r G^0_b = -482.4$ kJ/C-mol. Open keys: $-\Delta_r H^0_X$; solid keys: $-\Delta_r G^0_X$. Squares: *K. fragilis*; dots: *S. cerevisiae*.)

4. Results

4.1. Enthalpy-driven growth

In Fig. 3, Gibbs energy and enthalpy changes of oxidative growth of typical yeast on glucose, $\Delta_r G^0_X$ and $\Delta_r H^0_X$, are plotted as functions of biomass yield ($Y_{X/S}$), based on Eqs. 12 and 13, respectively. Also presented are the experimental results of oxidative growth of *K. fragilis* and *S. cerevisiae* on glucose [40–43]. The $\Delta_r H^0_X$ values were determined calorimetrically and the $\Delta_r G^0_X$ values were calculated with the measured biomass yields, based on Eq. 12.

The heat production and the Gibbs energy dissipation are predicted to increase following a decrease of biomass yield because less of the generated energy is retained in the biomass. There are two theoretical limiting cases. As $Y_{X/S}$ approaches zero, $\Delta_r H^0_X$ and $\Delta_r G^0_X$ tend to infinity signifying a total conversion of glucose to CO_2 and H_2O. For $\Delta_r G^0_X$ equal to zero, the biomass yield attains a theoretical maximum of 1.03 (see Fig. 3), but the growth rate becomes zero. Therefore, actual growth represents a compromise between a high metabolic rate with zero yield and a zero rate with a maximum yield [44]. As shown in Fig. 3, the actual values of $Y_{X/S}$ measured, and $\Delta_r G^0_X$ calculated, are far from both limits.

Table 5
Heat production ($\Delta_r H^0_X$) and Gibbs energy dissipation ($\Delta_r G^0_X$) of aerobic growth of different microorganism on various substrates (data from [40] except for *S. cerevisiae* from [43])

Organism	Substrate	$Y_{X/S}$ (C-/C-mol)	$-\Delta_r H^0_a$ (kJ/C-mol)	$-\Delta_r G^0_a$ (kJ/C-mol)	$-\Delta_r H^0_X$ (kJ/C-mol)	$-\Delta_r G^0_X$ (kJ/C-mol)
K. fragilis	Glucose	0.578	467.8	478.7	302.0	345.1
K. fragilis	Galactose	0.604	467.3	477.6	334.8	316.2
K. fragilis	Lactose	0.549	470.5	485.5	367.3	409.3
C. lipolytica	Citrate	0.378	327.1	357.8	368.8	483.7
C. lipolytica	Succinate	0.413	373.2	399.8	412.3	505.6
C. lipolytica	Hexadecane	0.567	667.8	649.5	630.5	682.4
C. utilis	Glucose	0.587	467.8	478.7	286.8	352.4
C. utilis	Acetate	0.496	437.2	447.0	437.4	439.1
C. utilis	Glycerol	0.701	553.6	547.6	265.4	318.0
C. utilis[a]	Ethanol	0.522	683.4	659.5	489.8	801.4
C. boidini	Ethanol	0.740	683.4	659.5	410.8	428.5
M. methylotrophus	Methanol	0.552	728.0	693.0	558.8	813.0
C. pseudotropicalis[a]	Glucose	0.557	467.8	478.7	332.1	397.1
S. cerevisiae	Glucose	0.595	467.8	478.7	305.3	321.6
S. cerevisiae	Ethanol	0.597	683.4	659.5	643.3	623.0
S. cerevisiae	Acetate	0.368	437.2	447.0	704.1	731.9

[a]Product formation.

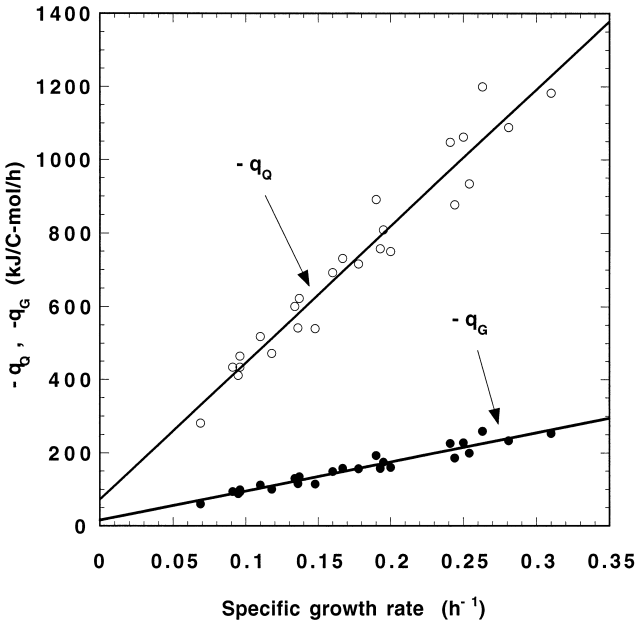

Fig. 4. Specific rates of heat production (q_Q) and Gibbs energy dissipation (q_G) of H_2-limited continuous cultures of *M. thermoautotrophicum* as functions of the specific growth rate. (Temperature: 333 K.)

Another observation is that for all $Y_{X/S}$ values, $\Delta_r H_X^0$ is always close to $\Delta_r G_X^0$, and thus $T\Delta_r S_X^0 \approx 0$. Summarized in Table 5 are similar results from aerobic cultures of microorganisms on various carbon/energy substrates in the Bio-RC1 calorimeter. Here again, the measured enthalpy changes are often close to the calculated Gibbs energy changes. It can be concluded that microorganisms oxidatively grown on organic substances do not feed on *negentropy*. The entropy export occurs almost entirely in form of heat exchange, and the Gibbs energy dissipation during such growth can be observed directly as heat generation.

4.2. Entropy-retarded growth

Growth of *Methanobacterium thermoautotrophicum*, a strictly anaerobic archaebacterium utilizing methanogenesis as the catabolic pathway, has been investigated in continuous cultures [45,46]. Hydrogen, the energy source, is often the growth-limiting substrate because of its poor solubility in water. In this case, the strong influences of temperature and actual concentrations of the involved gaseous compounds have been taken into account in calculation of the Gibbs energies, but not for enthalpy balances, for which these effects can be ignored [46]. The specific rates of heat production and Gibbs energy dissipation, q_Q and q_G, as functions of the specific growth rate, are shown in Fig. 4. Heat production and Gibbs energy dissipation depend on the specific growth rate since as the growth rate decreases, relatively more energy is required for maintenance. Energies required for growth and maintenance can be determined according to the Herbert/Pirt linear equation [46]. In Table 6, the enthalpy change and the Gibbs energy change solely for growth, i.e. without maintenance, $\Delta_r H_X^{min}$ and $\Delta_r G_X^{min}$, and the specific rates of heat production and Gibbs energy dissipation associated with maintenance processes (m_Q and m_G), were calculated and compared to the aerobic growth of yeast in continuous cultures [47]. From Table 6, it is seen that for *M. thermoautotrophicum*, both $\Delta_r H_X^{min}$ and m_Q are more negative than $\Delta_r G_X^{min}$ and m_G, respectively, by a factor of four. Moreover, these thermodynamic parameters are much more negative than the corresponding values obtained from the aerobic growth of yeast (e.g. *K. fragilis* [47]).

In Fig. 5, the theoretical predictions of $\Delta_r H_X^0$ and $\Delta_r G_X$ as functions of the biomass yield ($Y_{X/A}$) are compared with experimental results. Notably, the $(-\Delta_r H_X^0)$ curve is significantly above the $(-\Delta_r G_X)$ curve, signifying very large heat production. This is unusual as compared to either anaerobic or aerobic growth of other microorganisms on organic compounds. The essential reason for the high enthalpy change is the large decrease in chemical entropy during growth, in which 4 mol of H_2 plus 1 mol of CO_2

Table 6
Thermodynamic comparison of anaerobic growth of *M. thermoautotrophicum* with aerobic growth of *K. fragilis* in continuous cultures

Microorganism	$-\Delta_r H_X^{min}$ (kJ/C-mol)	$-\Delta_r G_X^{min}$(kJ/C-mol)	$-m_Q$ (kJ/C-mol)	$-m_G$ (kJ/C-mol)	Reference
M. thermoautotrophicum	3730	798	73	16.4	[46]
K. fragilis (aerobic)	346.6	474.5	0.604	0.869	[47]

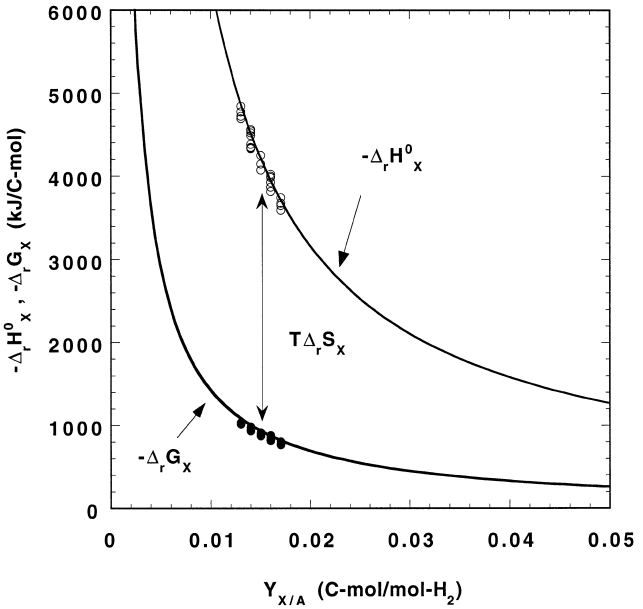

Fig. 5. Gibbs energy dissipation ($\Delta_r G_X$) and heat production ($\Delta_r H^0_X$) as functions of biomass yields ($Y_{X/A}$) in autotrophic methanogenesis. (Temperature: 333 K. $\Delta_r H'_a = -63.00$ kJ/mol-H_2, $\Delta_r G'_a = -14.46$ kJ/mol-H_2, $\Delta_r H'_b = 4.07$ kJ/mol-H_2, $\Delta_r G'_b = -32.70$ kJ/mol-H_2. Open keys: $-\Delta_r H^0_X$; solid keys: $-\Delta_r G_X$.)

are converted to roughly 1 mol of CH_4 and 2 mol of H_2O. For spontaneous growth, the enthalpy change must not only contribute to the driving force for growth, but also overcompensate the retarding effect from the largely negative $\Delta_r S_X$. Growth accompanied by an enormous heat generation, which not only exports the entropy produced within the cells but also compensates the decrease of chemical entropy in the medium, could be called entropy-retarded growth [46]. Here we also see interestingly that *M. thermoautotrophicum* represents an example showing microbial life not only does not feed on *negentropy*, but rather grows in spite of positive entropy (*posentropy*).

4.3. Entropy-driven growth

For ethanol fermentation by *K. fragilis* and *S. cerevisiae*, theoretical prediction curves of $\Delta_r H^0_X$ and $\Delta_r G^0_X$, along with data from the calorimetric experiments [41,43], are plotted (Fig. 6). The biomass yields ($Y_{X/S}$) are much lower in comparison to aerobic growth, and ($-\Delta_r H^0_X$) is lower than ($-\Delta_r G^0_X$) by a factor of three. Phenomenally, much weaker heat production was measured in the calorimeter whereas relatively rapid growth still occurred spontaneously. For anaerobic growth, the $T\Delta_r S^0_X$ introduces a larger difference between the two curves of $\Delta_r H^0_X$ and $\Delta_r G^0_X$ than in aerobic growth (see Fig. 3). About three fourths of the driving force for growth is contributed by the increase of chemical entropy due to the break-up of large molecules (glucose) into smaller molecules (CO_2 and ethanol). In other words, anaerobic growth of yeast indeed feeds on *negentropy*. Another interesting point is that for different yeasts grown on the same substrate, the Gibbs energy dissipated per unit biomass formed ($\Delta_r G^0_X$) varies little. Furthermore, despite a much lower biomass yield for anaerobic growth, $\Delta_r G^0_X$ stays approximately equal to the corresponding value for aerobic growth. This confirms the hypothesis of Roels [33], which will be discussed later in more detail.

4.4. Enthalpy-retarded growth

Despite the fact that endothermic growth does not violate the second law of thermodynamics [32,48], there is doubt about the 'real world' existence of

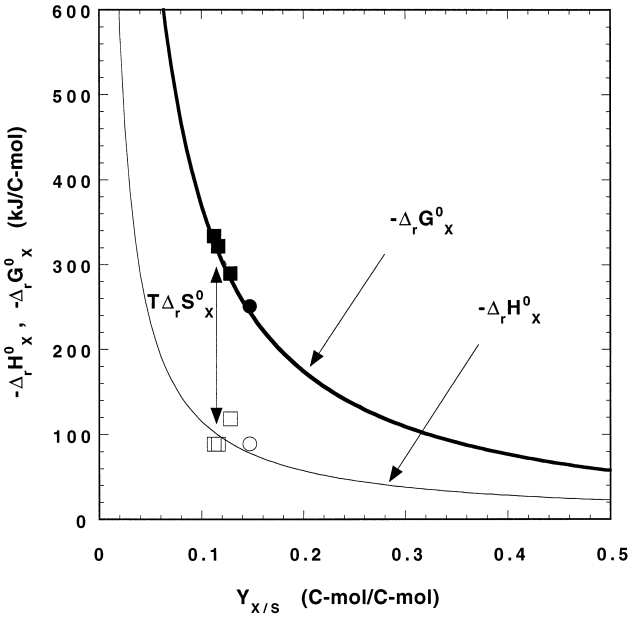

Fig. 6. Plot of Gibbs energy dissipation ($\Delta_r G^0_X$) and heat production ($\Delta_r H^0_X$) versus biomass yield ($Y_{X/S}$) in ethanol fermentation of yeasts. (Temperature: 303 K. $\Delta_r H^0_a = -11.56$ kJ/C-mol, $\Delta_r G^0_a = -39.00$ kJ/C-mol, $\Delta_r H^0_b = -0.273$ kJ/C-mol, $\Delta_r G^0_b = -20.70$ kJ/C-mol. Open keys: $-\Delta_r H^0_X$; solid keys: $-\Delta_r G^0_X$. Squares: *K. fragilis*; dots: *S. cerevisiae*.)

Table 7
Heat absorption and Gibbs energy dissipation of acetoclastic methanogenesis

Strain	$Y_{X/S}$ (C-mol/C-mol)	$-\Delta_r H^0_X$ (a) (kJ/C-mol)	$-\Delta_r H^0_X$ (b) (kJ/C-mol)	$-\Delta_r G^0_X$ (a) (kJ/C-mol)	$-\text{-}\Delta_r G^0_X$ (b) (kJ/C-mol)	$\dot{Q}_{max}$ (c) (mW/l)	References
Ms. thermophila	0.034	−273.9	−221.01	433.5	696.1	144.8	[50,51]
Ms. barkeri MS	0.024	−387.9	−312.9	623.5	995.4	97.3	[50]
Ms. barkeri 227	0.054	−172.6	−139.3	264.7	430.0	44.9	[50,52]
Ms. acetivorans	0.048	−194.1	−156.6	300.6	486.5	57.4	[50]
Mtx. soehngenii	0.025	−372.4	−300.4	597.6	954.7	30.0	[53]
Mtx. concilii	0.023	−404.7	−326.4	651.6	1039.7	105.2	[54]
Mtx. sp. CALS-1	0.022	−423.1	−341.3	682.2	1088.0	106.2	[55]

(a) and (b): thermodynamic parameters calculated without and with taking neutralization into account, respectively; (c) maximum heat absorption rate calculated assuming a cell density of 1 g/l.

such hypothetical life processes [18,49]. Heijnen and van Dijken [14] showed by theoretical calculation that methanogenesis of *Methanobacterium soehngenii* on acetate should absorb heat while growing, though it has not yet been experimentally confirmed. In their calculation, the heat effect of the neutralization for buffering a constant pH, which is required physiologically for growth, has probably been disregarded. If this side reaction is considered, then the heat effect is reduced by ca. 20%, according to the reported values for the heat of neutralization [11]. In such acetoclastic methanogenic growth, acetate is converted to gaseous methane and CO_2. Taking into account the influence of neutralization, theoretical calculations of $\Delta_r H^0_X$ and $\Delta_r G^0_X$ have been done by using the biomass yields ($Y_{X/S}$) of acetoclastic methanogenesis reported in the literature for various strains grown on acetate (Table 7).

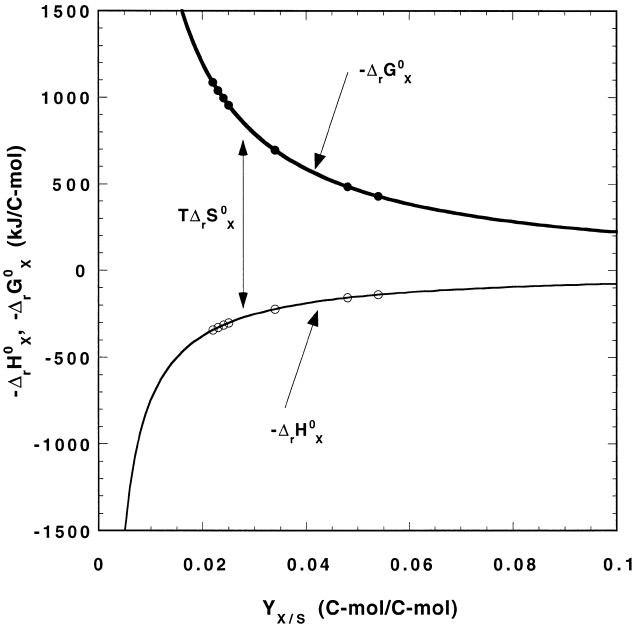

Fig. 7. Heat absorption and Gibbs energy dissipation of acetoclastic methanogenesis. (Temperature: 310 K. $\Delta_r H^0_a = 7.5$ kJ/C-mol, $\Delta_r G^0_a = -24.43$ kJ/C-mol, $\Delta_r H^0_b = -0.362$ kJ/C-mol, $\Delta_r G^0_b = -22.35$ kJ/C-mol. Open keys: $-\Delta_r H^0_X$; solid keys: $-\Delta_r G^0_X$.)

The dependence of $\Delta_r H^0_X$ and $\Delta_r G^0_X$ on the biomass yield ($Y_{X/S}$) is plotted in Fig. 7. For comparison, the data points of $\Delta_r H^0_X$ and $\Delta_r G^0_X$ corresponding to the measured biomass yields in the literature are also given in Fig. 7. Unlike the other cases discussed above, following an increase of biomass yield, Gibbs energy dissipation ($-\Delta_r G^0_X$) decreases while enthalpy exchange ($-\Delta_r H^0_X$) increases. The enthalpy change retards the driving force, for which the increase of chemical entropy overcompensates to make spontaneous growth possible (negative $\Delta_r G^0_X$). Such growth is considered to be enthalpy-retarded: growth is entropy-driven or feeds on *negentropy* to such an extent that it occurs despite the fact that the products are richer in enthalpy than the substrates.

4.5. From enthalpy- to entropy-driven growth

Some growth processes comprise a mixed metabolism in the transition between oxidative and reductive growth. For example, the metabolism of yeast can be switched from purely aerobic (oxidative) toward purely anaerobic (reductive) due to the Pasteur effect (e.g. *K. fragilis*) and/or the Crabtree effect (e.g. *S. cerevisiae*). The overall stoichiometry of such growth can still be described by Eq. 9. If the biomass yields

for oxidative and reductive growth ($Y^{oxi}_{X/S}$ and $Y^{red}_{X/S}$) are used as intrinsic parameters in the mixed metabolism [41], the relationship between heat exchange $\Delta_r H^0_X$, Gibbs energy dissipation $\Delta_r G^0_X$ and biomass yield $Y_{X/S}$ can be formulated as follows

$$\Delta_r G^0_X = \left(\Delta_C G^*_S - \frac{Y^{oxi}_{X/S} - Y_{X/S}}{Y^{oxi}_{X/S} - Y^{red}_{X/S}} \cdot \frac{\gamma_S - \gamma_X \cdot Y^{red}_{X/S}}{\gamma_P} \cdot \Delta_C G^*_P\right) \cdot \frac{1}{Y_{X/S}} - \Delta_C G^*_X \quad (16a)$$

$$\Delta_r H^0_X = \left(\Delta_C H^*_S - \frac{Y^{oxi}_{X/S} - Y_{X/S}}{Y^{oxi}_{X/S} - Y^{red}_{X/S}} \cdot \frac{\gamma_S - \gamma_X \cdot Y^{red}_{X/S}}{\gamma_P} \cdot \Delta_C H^*_P\right) \cdot \frac{1}{Y_{X/S}} - \Delta_C H^*_X \quad (16b)$$

When $Y_{X/S}$ equals $Y^{oxi}_{X/S}$ or $Y^{red}_{X/S}$, the above equations will revert to Eq. 12 and Eq. 13 for oxidative or reductive growth, respectively. The mixed metabolism of *K. fragilis* and *S. cerevisiae* grown on glucose was investigated in continuous cultures [41,42]. The oxygen supply was progressively reduced in relation to the carbon supply forcing the culture to progressively shift from oxidative to reductive metabolism. As a result ethanol production increased while the biomass yield decreased gradually from a higher level typical of oxidative growth ($Y^{oxi}_{X/S}$) to a much lower level characteristic of reductive growth ($Y^{red}_{X/S}$). In Fig. 8, the Gibbs energy dissipation and the enthalpy exchange predicted by Eqs. 16a and 16b, respectively, are shown in comparison to experimental data. The experimental results are in rough agreement with the predictions, though there is a certain degree of scatter attributable to the measurement error. The shift from the oxidative to the reductive growth causes the heat production to decrease from ($-\Delta_r H^{oxi}_X$) (equivalent to ($-\Delta_r G^{oxi}_X$)) to ($-\Delta_r H^{red}_X$) (much smaller than ($-\Delta_r G^{red}_X$)). However, the overall Gibbs energy dissipation per unit biomass formed ($\Delta_r G^0_X$) remains more or less invariant for the $Y_{X/S}$ range of actual growth. Thus, the contribution to the overall driving force by the entropy change ($\Delta_r S^0_X$) increases gradually as the culture is shifted from oxidative to reductive growth.

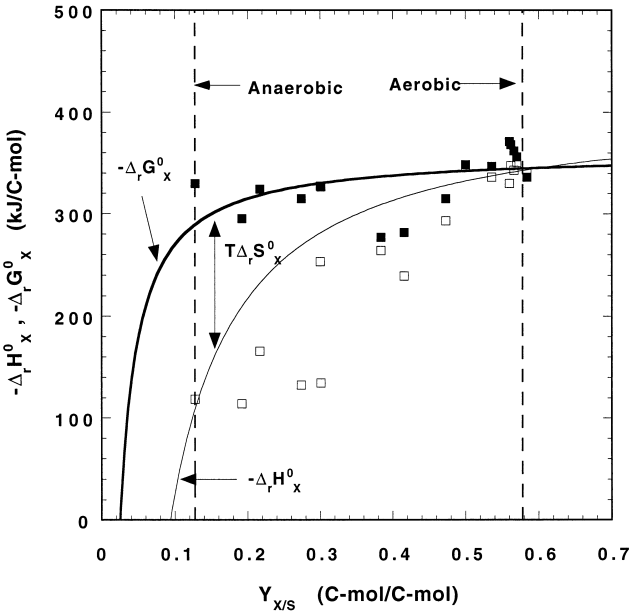

Fig. 8. Gibbs energy dissipation ($\Delta_r G^0_X$) and heat production ($\Delta_r H^0_X$) versus biomass yield ($Y_{X/S}$) in a mixed metabolism of *K. fragilis* grown on glucose. (Open square: $-\Delta_r H^0_X$; solid square: $-\Delta_r G^0_X$.)

5. Correlation between Gibbs energy, enthalpy, entropy changes and growth

5.1. Relationship between Gibbs energy change and growth yield

The yield of biomass on the available substrate ($Y_{X/S}$) is a parameter reflecting the extent of the microbial growth, while the Gibbs energy dissipation $\Delta_r G^0_X$ represents the integral driving force of growth. As shown by Eq. 12, $Y_{X/S}$ and $\Delta_r G^0_X$ can be related quantitatively by a balance such that $Y_{X/S}$ can be predicted from $\Delta_r G^0_X$:

$$Y_{X/S} = \frac{\Delta G^0_a}{\Delta_r G^0_X + \Delta G^0_b} \quad (17)$$

Numerous attempts have been made to establish a correlation between $\Delta_r G^0_X$ and known independent variables in terms of thermodynamic growth efficiencies, and various definitions of efficiency for aerobic and anaerobic have been proposed (see [14,56] for a review). However, each of these definitions has some limitations for use or suffers from intrinsic problems, resulting in difficulties for the prediction

of biomass yield. Therefore such correlations remain of limited use and have to be applied with caution [14].

An alternative approach is to estimate the growth yield directly in terms of the physico-chemical nature of the carbon source. For aerobic growth, Roels [33] proposed a correlation between the biomass yield and the reduction degree of the substrate. More recently, Heijnen et al. [14] developed another correlation scheme, which is based on the hypothesis that the Gibbs energy dissipation per unit of biomass formed is fundamentally determined by the physico-chemical nature of the carbon source and independent of the electron acceptor involved. Calculations based on literature data, covering a wide range of growth substrates and energy metabolism, have shown that within an uncertainty range of 30%, the Gibbs energy dissipation $\Delta_r G_X^0$ can be correlated with the carbon-chain length (C) and the degree of reduction (γ_D) of the electron donor.

For chemotrophic growth without reverse electron transport

$$-\Delta_r G_X^0 = 200 + 18\cdot(6-C)^{1.8} + \exp[\{(3.8-\gamma_D)^2\}^{0.16}\cdot(3.6+0.4\cdot C)] \quad (kJ/C\text{-mol}) \tag{18a}$$

and for chemotrophic growth with reverse electron transport

$$-\Delta_r G_X^0 = 3500 \quad (kJ/C\text{-mol}) \tag{18b}$$

Heijnen's correlation reportedly predicts biomass yield, with an uncertainty of 20%, in the range of 0.01–0.8 for chemotrophic growth. However, this correlation is solely empirical, though the effect from the carbon-chain length of the substrates is taken into account.

5.2. Chemical entropy changes in purely aerobic or purely anaerobic growth

The change of chemical entropy in a microbial growth process determines the relationship of the heat generation to Gibbs energy dissipation and may be expressed as the ratio of $\Delta_r H_X^0$ to $\Delta_r G_X^0$, $(\Delta_r H_X^0/\Delta_r G_X^0)$. Because catabolism is the energy-yielding process for growth, the entropy change of the catabolism is closely related to the entropy change of the overall growth process.

Elimination of $Y_{X/S}$ from Eqs. 12 and 13 yields:

$$\frac{\Delta_r H_X^0}{\Delta_r G_X^0} = \frac{\Delta_r H_a^0}{\Delta_r G_a^0}\cdot\left(1+\frac{\Delta_r G_b^0}{\Delta_r G_X^0}\right)-\frac{\Delta_r H_b^0}{\Delta_r G_X^0} \tag{19}$$

Since for aerobic growth, $\Delta_r H_a^0 \approx \Delta_r G_a^0$ and $\Delta_r H_b^0 \approx \Delta_r G_b^0$, and for anaerobic growth, both $\Delta_r H_b^0$ and $\Delta_r G_b^0$ are much smaller than $\Delta_r G_X^0$, the following equation can be formulated for both cases:

$$\frac{\Delta_r H_X^0}{\Delta_r G_X^0} \approx \frac{\Delta_r H_a^0}{\Delta_r G_a^0} = 1 + T\cdot\frac{\Delta_r S_a^0}{\Delta_r G_a^0} \tag{20}$$

The entropy change, or the $(\Delta_r H_X^0/\Delta_r G_X^0)$ of growth, can be estimated by the corresponding value of the relevant catabolic process which is easy to determine from the tabulated data. Results obtained from calorimetric experiments and from calculations on anaerobic growth from literature data [57] are plotted according to the above relationship, Eq. 20

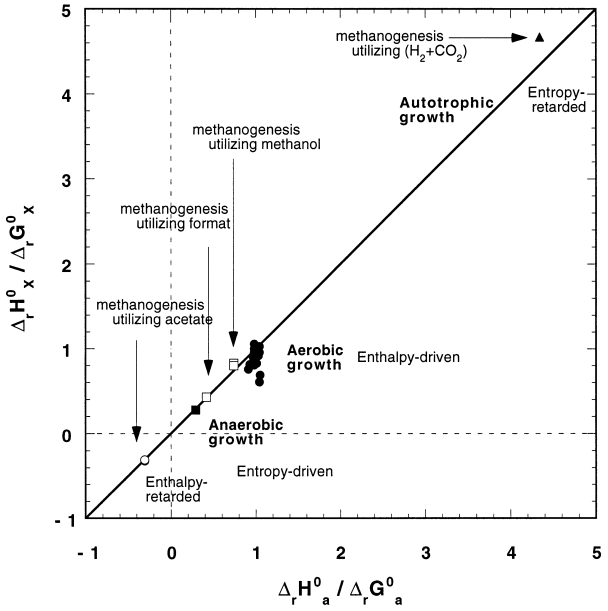

Fig. 9. Plot of $(\Delta_r H_X^0/\Delta_r G_X^0)$ against $(\Delta_r H_a^0/\Delta_r G_a^0)$ for aerobic and anaerobic growth. (Open keys: $\Delta_r H_X^0$ were calculated based on enthalpy balance; solid keys: $\Delta_r H_X^0$ were measured calorimetrically.)

(Fig. 9). It can be seen that this relationship fits the experimental data quite well.

Aerobic growth on organic substrates is entropy-neutral, enthalpy-driven growth, i.e. $\Delta_r H_X^0 \approx \Delta_r G_X^0$. Thus data points of $(\Delta_r H_X^0/\Delta_r G_X^0)$ for aerobic growth are distributed around the point (1,1). In contrast, for anaerobic growth on organic substrates, entropy increases considerably and the entropy change becomes the main contribution to $\Delta_r G_X^0$. In this case, $(\Delta_r H_X^0/\Delta_r G_X^0)$ is smaller than one. An extreme example is enthalpy-retarded growth, e.g. methanogenesis on acetate. In such growth, the increase of entropy is sufficiently large to overcome a small enthalpic increase (positive value of $\Delta_r H_X^0$), leading to an overall negative value of $\Delta_r G_X^0$. In this case, $(\Delta_r H_X^0/\Delta_r G_X^0)$ is negative. An example at the other extreme is entropy-retarded growth, e.g. autotrophic methanogenesis on H_2 plus CO_2. Here growth involves a substantial decrease of chemical entropy, and thus, a huge amount of heat must be liberated to overcompensate the retarding effect from the change in chemical entropy. In this case, $(\Delta_r H_X^0/\Delta_r G_X^0)$ is much larger than one.

Another interesting point from Fig. 9 is that different electron donors of microbial growth processes will result in dramatic differences in the change of chemical entropy. Methanogenesis on various substrates H_2, methanol, formate and acetate, yields a ratio of $\Delta_r H_X^0$ to $\Delta_r G_X^0$ that varies from 4.6 to −0.3, which demonstrates the increasing contribution of the chemical entropy change to the driving force of growth. The growth varies from strongly exothermic, to almost athermic, and to theoretically even endothermic.

5.3. Chemical entropy changes in growth with mixed oxido-reductive metabolism

Based on the assumption that Gibbs energy dissipation per unit biomass formed in aerobic or anaerobic growth on the same substrate remains approximately the same, i.e. $\Delta_r G_X^{oxi} \approx \Delta_r G_X^{red}$, one may express Eqs. 16a and 16b in terms of $\Delta_r G_X^{oxi}$, $\Delta_r G_X^{red}$, $\Delta_r H_X^{oxi}$ and $\Delta_r H_X^{red}$. Consequently, the ratio of enthalpy change to Gibbs energy change in the mixed metabolism can be expressed as the following:

$$\frac{\Delta_r H_X^0}{\Delta_r G_X^0} = \left[\frac{\Delta_r H_X^{red}}{\Delta_r G_X^{red}} + \left(\frac{Y_{X/S}^{oxi}}{Y_{X/S}^{oxi} - Y_{X/S}^{red}}\right)\cdot\left(\frac{\Delta_r H_X^{oxi}}{\Delta_r G_X^{oxi}} - \frac{\Delta_r H_X^{red}}{\Delta_r G_X^{red}}\right)\right] - \left(\frac{Y_{X/S}^{oxi}\cdot Y_{X/S}^{red}}{Y_{X/S}^{oxi} - Y_{X/S}^{red}}\right)\cdot\left(\frac{\Delta_r H_X^{oxi}}{\Delta_r G_X^{oxi}} - \frac{\Delta_r H_X^{red}}{\Delta_r G_X^{red}}\right)\cdot\left(\frac{1}{Y_{X/S}}\right) \quad (21)$$

For aerobic and anaerobic growth, it has been demonstrated that

$$\frac{\Delta_r H_X^{oxi}}{\Delta_r G_X^{oxi}} \approx \frac{\Delta_r H_a^{oxi}}{\Delta_r G_a^{oxi}} = R^{oxi} \quad (22a)$$

and

$$\frac{\Delta_r H_X^{red}}{\Delta_r G_X^{red}} \approx \frac{\Delta_r H_a^{red}}{\Delta_r G_a^{red}} = R^{red} \quad (22b)$$

Here again, the biomass yields for oxidative ($Y_{X/S}^{oxi}$) and reductive ($Y_{X/S}^{red}$) growth are used as intrinsic parameters for a mixed metabolic process. Therefore, $(\Delta_r H_X^0/\Delta_r G_X^0)$ can be correlated to the $(\Delta_r H_a^0/\Delta_r G_a^0)$ of catabolic processes for both oxidative and reductive growth in the following manner:

$$\frac{\Delta_r H_X^0}{\Delta_r G_X^0} = \left[R^{red} + \left(\frac{Y_{X/S}^{oxi}}{Y_{X/S}^{oxi} - Y_{X/S}^{red}}\right)\cdot(R^{oxi} - R^{red})\right] - \left(\frac{Y_{X/S}^{oxi}\cdot Y_{X/S}^{red}}{Y_{X/S}^{oxi} - Y_{X/S}^{red}}\right)\cdot(R^{oxi} - R^{red})\cdot\left(\frac{1}{Y_{X/S}}\right) \quad (23)$$

This equation can be used to estimate the change of chemical entropy during mixed metabolism. For purely aerobic or purely anaerobic growth, $Y_{X/S}$ equals $Y_{X/S}^{oxi}$ or $Y_{X/S}^{red}$, respectively, and thus, Eq. 23 reverts to Eq. 22a or Eq. 22b, respectively. From Eq. 23 it can be seen that $(\Delta_r H_X^0/\Delta_r G_X^0)$ decreases linearly from one extreme value for oxidative growth (R^{oxi}) to the other for reductive growth (R^{red}) in relation to the decrease of the overall biomass yield ($Y_{X/S}$). This implies that the change of chemical entropy plays an increasing role in driving growth. Eq. 23 has been verified with experimental results from continuous cultures of *K. fragilis* on glucose [41] that were shifted gradually from oxidative to reductive growth (Fig. 10).

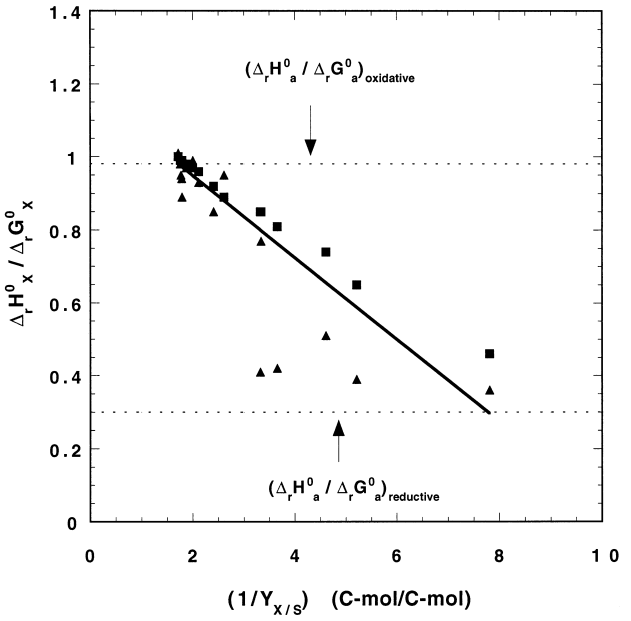

Fig. 10. Plot of $(\Delta_r H^0_X/\Delta_r G^0_X)$ against $(1/Y_{X/S})$ for growth of *K. fragilis* with a mixed metabolism. (Solid line: predicted $\Delta_r H^0_X/\Delta_r G^0_X$); dotted line: $(\Delta_r H^0_X/\Delta_r G^0_X)$ of oxidative or reductive growth. Squares: $\Delta_r H^0_X$ calculated by energy balances; triangles: $\Delta_r H^0_X$ measured by calorimetry.)

6. Discussion and conclusions

Microbial metabolism consists of irreversible processes and therefore generates entropy continuously within the cell. The living cell maintains itself however in a state of constant entropy by exporting the internally generated entropy to the environment. This may occur by generating metabolic products of higher entropies than the nutrients, a process that was described as "life feeds on negative entropy" by Schrödinger. With this statement, however, Schrödinger disregarded heat generation as a mechanism for entropy export [58] and "was misleading by interpreting the feeding too literally by emphasizing the ordered structures of nutrients as the source of negative entropy" [6].

The export of the internally generated entropy in these forms of both 'negative entropy' and heat reflects itself in a decrease, or *dissipation*, of Gibbs energy in the environment. The amount of Gibbs energy dissipated per C-mol of new biomass grown, $\Delta_r G^0_X$, is the key parameter for understanding the thermodynamics of microbial growth. On the one hand, it is linked to the rate of metabolism and therefore regarded as the driving force for growth. On the other, the Gibbs energy balance shows that $\Delta_r G^0_X$ also determines the biomass yield $Y_{X/S}$. The higher the value of $\Delta_r G^0_X$ is, the lower the biomass yield ($Y_{X/S}$) will be. The $\Delta_r G^0_X$ value found for an actual growth process represents a compromise between a very high negative value offering a large driving force for growth but a biomass yield approaching zero, and a very low value corresponding to a zero growth rate but a maximum biomass yield. The Gibbs energy dissipation per biomass formed, $\Delta_r G^0_X$, is thus a fundamental variable rooted in the biology of microbial growth. Under favorable conditions, most microbial strains dissipate Gibbs energy between 300 and 800 kJ/C-mol, and the actual values appear to depend on the biochemical difficulty with respect to synthesizing biomass from given carbon and energy sources [59]. The Gibbs energy dissipation can therefore be correlated with, and thus approximately predicted from the physico-chemical nature of these sources.

The Gibbs energy change $\Delta_r G^0_X$ in the environment of the cell contains an enthalpic part ($\Delta_r H^0_X$) and an entropic part ($-T\Delta_r S^0_X$). The former reflects the export of internally generated entropy in the form of heat, whereas the latter represents the entropy export contributed by the change of chemical entropy of a growth process, and thus corresponding to Schrödinger's 'negative entropy'. The importance of the latter as a means of entropy export and Gibbs energy dissipation has been assessed for a number of microbial cultures of widely different nature by comparing calorimetrically measured $\Delta_r H^0_X$ with $\Delta_r G^0_X$ values. This may also be done based on a simple calculation, since it has been demonstrated both theoretically and experimentally that the ratio of enthalpic change to Gibbs energy dissipation in microbial growth is approximately equal to the corresponding ratio of the relevant catabolic process.

Contributions of enthalpic and entropic changes to the driving force found in this study for different cases are schematically illustrated in Fig. 11. It shows that microbial growth by no means always feeds on negative entropy, but that there exist entropy-driven, entropy-neutral and entropy-retarded growth. Aerobic growth, for instance, is entropy-neutral in that the sum of the entropies of all nutrients is approximately equal to the entropy sum of all products of

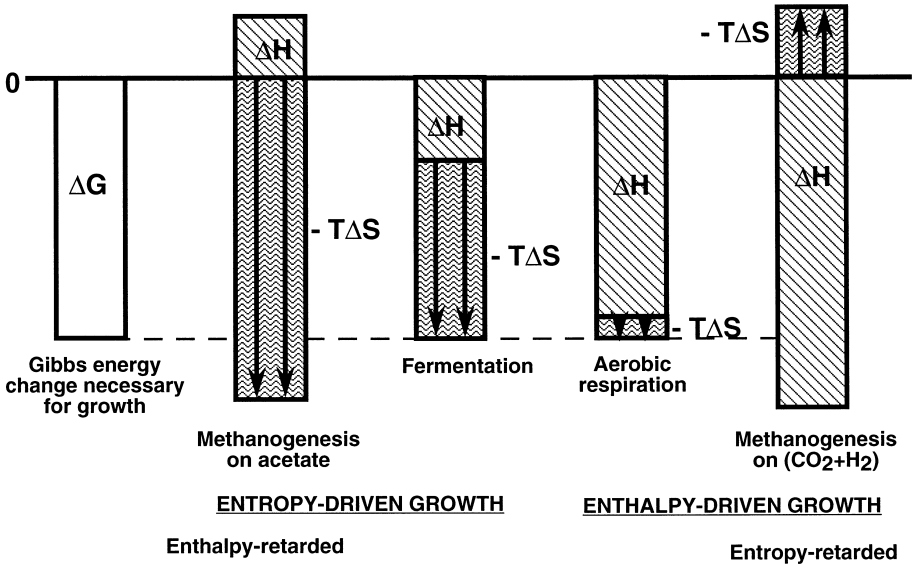

Fig. 11. Schematic of enthalpic and entropic contributions to the driving force of microbial growth.

metabolism. The entire Gibbs energy dissipation comes from heat generation, such that the former may be measured directly in a calorimeter. Anaerobic reductive growth does indeed produce a positive change of chemical entropy in the environment and thus feeds on negative entropy (left part of Fig. 11). But cases exist where the cells grow despite of the fact that they reduce the chemical entropy of their environment (right part of Fig. 11, entropy-retarded growth). The cases shown at the very left and right extremes of Fig. 11 demonstrate that either a negative chemical entropy change or a positive enthalpy change may retard the export of the internal entropy production of growth. These retardant terms have to be overcompensated by the other terms, either by a largely negative enthalpy change or by a largely positive chemical entropy change, to make spontaneous growth possible. The existence of enthalpy-retarded growth has been indicated thus far only by calculations. The enthalpic retarding effect would result in heat uptake during growth and would serve as an extreme example for "life feeds on negentropy". However, the existence of endothermic microbial life still awaits direct evidence from calorimetric measurements.

7. List of symbols

C_p	heat capacity (kJ/mol/K)
G	Gibbs energy (kJ)
$\Delta_{rj}G$	Gibbs energy change of reaction j [kJ/(C)-mol]
$\Delta_r G_a^0$	standard Gibbs energy change of catabolic reaction per C-mol carbon substrate converted (kJ/C-mol)
$\Delta_r G_a'$	Gibbs energy change of catabolic reaction per mol energy substrate (H_2) converted in autotrophic methanogenesis, as defined in Eq. B8a (kJ/mol)
$\Delta_r G_b^0$	standard Gibbs energy change of degradation of biomass to the products of catabolism per C-mol biomass converted (kJ/C-mol)
$\Delta_r G_b'$	Gibbs energy change of degradation of biomass to the product CH_4 of catabolism and CO_2 per C-mol biomass converted in autotrophic methanogenesis, as defined in Eq. B8b (kJ/C-mol)
$\Delta_r G_X$	Gibbs energy change of an overall growth process per C-mol biomass formed (kJ/C-mol)
$\Delta_r G_X^0$	standard Gibbs energy change of an overall growth process per C-mol biomass formed (kJ/C-mol)
$\Delta_C G_i^0$	standard Gibbs energy of combustion of chemical species i using CO_2 (g), H_2O (l) and N_2 (g) as reference states [kJ/(C)-mol]
$\Delta_C G_i^*$	modified Gibbs energy of combustion of chemical species i using CO_2 (g), H_2O (l) and NH_3 (aq) as reference states (kJ/(C)-mol)
$\Delta_C G_X^0$	standard Gibbs energy of combustion of biomass using CO_2 (g), H_2O (l) and N_2 (g) as reference states (kJ/C-mol)
$\Delta_C G_X^*$	Gibbs energy of combustion of biomass using CO_2 (g), H_2O (l) and NH_3 (aq) as reference states (kJ/C-mol)
H	enthalpy (kJ/mol)
$\overline{h}_i$	partial molar enthalpy of chemical species i (kJ/mol)

$\overline{h}_{e,i}$ partial molar enthalpy of the exchanged chemical species i under the conditions of the system boundary (kJ/mol)
$\Delta_{rj}H$ enthalpy change of reaction j [kJ/(C)-mol]
Δ_rH_X enthalpy change of an overall growth process per C-mol biomass formed [kJ/C-mol]
$\Delta_rH_X^0$ standard enthalpy change of an overall growth process per C-mol biomass formed [kJ/C-mol]
$\Delta_CH_i^0$ standard enthalpy of combustion of chemical species i [kJ/(C)-mol]
$\Delta_CH_X^*$ modified enthalpy of combustion of biomass using CO_2(g), H_2O (l) and NH_3 (aq) as reference states (kJ/C-mol)
m_G Gibbs energy dissipation rate associated with the maintenance process (kJ/C-mol/h)
m_Q heat production rate associated with the maintenance process (kJ/C-mol/h)
n number of moles
$\dot{n}_{e,i}$ exchange rate of chemical species i (mol/s)
q_G specific Gibbs energy dissipation rate (kJ/C-mol/h)
q_Q specific heat production rate (kJ/C-mol/h)
$\dot{Q}$ rate of heat exchange (kJ/s)
R ratio of enthalpy change to Gibbs energy dissipation
S entropy (kJ/K)
S_i^0 standard molar entropy of chemical species i [J/K/(C)-mol]
$\dot{S}_{prod}$ rate of internal entropy production due to the irreversible processes inside the system (kJ/K/s)
$\bar{s}_i$ partial molar entropy of chemical species i (kJ/K/mol)
$\bar{s}_{e,i}$ partial molar entropy of the exchanged chemical species i under the conditions of the system boundary (kJ/K/mol)
$\Delta_{rj}S$ entropy change of reaction j [kJ/K/(C)-mol]
Δ_rS_X entropy change of an overall growth process per C-mol biomass formed [kJ/K/C-mol]
$\Delta_rS_X^0$ standard entropy change of an overall growth process per C-mol biomass formed [kJ/K/C-mol]
t time (s)
T temperature (K)
U internal energy (kJ/mol)
V volume (l)
$\dot{W}$ rate of work exchange between the system and the environment (kJ/s)
X biomass concentration (g/l) or (C-mol/l)
$Y_{i/j}$ yield coefficient in (C)-mol of chemical species i per (C)-mol of chemical species j
Y_i^a yield coefficient in (C)-mol of substance i per C-mol of carbon source S converted in catabolic reaction, as represented by Eq. 11a
Y_i^b yield coefficient in (C)-mol of substance i per C-mol of biomass X converted in the degradation reaction, as represented by Eq. 11b
γ_i^0 degree of reduction of chemical species i using CO_2 (g), H_2O (l) and N_2 (g) as reference states
γ_i degree of reduction of chemical species i using CO_2 (g), H_2O (l) and NH_3 (aq) as reference states. For any substance i with a general formula $C_cH_hO_oN_n$, $\gamma_i^0 = 4c+h-2o-3n$; $\gamma_i = 4c+h-2o$
$\dot{\xi}_j$ rate of j-th chemical reaction (mol/s)
ν_{ij} stoichiometric coefficient of substance i in j-th chemical reaction
μ_i chemical potential of chemical species i (kJ/mol)
$\mu_{e,i}$ chemical potential of the exchanged chemical species i under the conditions of the system boundary (kJ/mol)
RC1 reaction calorimeter of Mettler-Toledo AG (Switzerland)
Bio-RC1 reaction calorimeter of Mettler-Toledo AG modified for biological process operation by EPFL (Switzerland)

8. Subscripts and superscripts

A electron acceptor/donor
a catabolic reaction
b degradation of biomass to the products of catabolism
C product CO_2
G Gibbs energy
e exchange
i referring to chemical species i
int internal
j referring to j-th process
min referring to the enthalpy or Gibbs energy change solely for growth (without maintenance)
N nitrogen source (NH_3)
O oxygen
oxi oxidative growth
P product other than biomass, CO_2, and H_2O
prod production
Q heat
r reaction
red reductive growth
S carbon source
W water
X biomass
0 referring to standard state

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Appendix A. Derivation of entropy and enthalpy balances (Eqs. 2 and 3)

For simplification, 1 C-mol of cellular biomass defines the system boundary, and we assume that cells

exchange chemical entities with the environment through one site only, the cell surface. From Eq. 1 one obtains

$$T\frac{\mathrm{d}S}{\mathrm{d}t} = \dot{Q} + T\sum_i \bar{s}_{\mathrm{e,i}}\cdot\dot{n}_{\mathrm{e,i}} + T\dot{S}_{\mathrm{Prod}} \tag{A1}$$

The entropy of the system is related to temperature (T) and pressure (P) of the system and to the number of moles of the various species (n_{i}) in the specified thermodynamic state within the system. Therefore, a change in entropy is given by a total differential as follows:

$$\frac{\mathrm{d}S}{\mathrm{d}t} = \left(\frac{\partial S}{\partial T}\right)_{\mathrm{P}}\cdot\frac{\mathrm{d}T}{\mathrm{d}t} + \left(\frac{\partial S}{\partial P}\right)_{\mathrm{T}}\cdot\frac{\mathrm{d}P}{\mathrm{d}t} + \sum_i \bar{s}_{\mathrm{i}}\cdot\frac{\mathrm{d}n_{\mathrm{i}}}{\mathrm{d}t} \tag{A2}$$

Since

$$\mathrm{d}H = T\mathrm{d}S + V\mathrm{d}P \tag{A3}$$

$$C_{\mathrm{P}} = \left(\frac{\partial H}{\partial T}\right)_{\mathrm{P}} \tag{A4}$$

one has

$$\left(\frac{\partial S}{\partial T}\right)_{\mathrm{P}} = \frac{C_{\mathrm{P}}}{T} \tag{A5}$$

and from the Maxwell relations, one readily obtains that

$$\left(\frac{\partial S}{\partial P}\right)_{\mathrm{T}} = -\left(\frac{\partial V}{\partial T}\right)_{\mathrm{P}} \tag{A6}$$

The molar balance of the system can be described as the following

$$\frac{\mathrm{d}n_{\mathrm{i}}}{\mathrm{d}t} = \dot{n}_{\mathrm{e,i}} + \sum_j \nu_{\mathrm{ij}}\cdot\dot{\xi}_{\mathrm{j}} \tag{A7}$$

By substituting Eq. A5, A6 and A7 into Eq. A2, and combining with Eq. A1, the general entropy balance for a non-steady open system can be derived as the following

$$C_{\mathrm{P}}\frac{\mathrm{d}T}{\mathrm{d}t} - T\left(\frac{\partial V}{\partial T}\right)_{\mathrm{P}}\cdot\frac{\mathrm{d}P}{\mathrm{d}t} + T\cdot\sum_i \bar{s}_{\mathrm{i}}\sum_j \nu_{\mathrm{ij}}\cdot\dot{\xi}_{\mathrm{j}} = \dot{Q} + T\cdot\sum_i (\bar{s}_{\mathrm{e,i}} - \bar{s}_{\mathrm{i}})\cdot\dot{n}_{\mathrm{e,i}} + T\dot{S}_{\mathrm{Prod}} \tag{2}$$

According to the first law of thermodynamics, the balance for internal energy U may be written as

$$\frac{\mathrm{d}U}{\mathrm{d}t} = \dot{Q} + \dot{W} - P\cdot\frac{\mathrm{d}V}{\mathrm{d}t} + \sum_i \bar{h}_{\mathrm{e,i}}\cdot\dot{n}_{\mathrm{e,i}} \tag{A8}$$

By substitution of U with H according to the definition of enthalpy, $H \equiv U + PV$, one obtains

$$\frac{\mathrm{d}H}{\mathrm{d}t} = \dot{Q} + \dot{W} + V\cdot\frac{\mathrm{d}P}{\mathrm{d}t} + \sum_i \bar{h}_{\mathrm{e,i}}\cdot\dot{n}_{\mathrm{e,i}} \tag{A9}$$

Analogous to Eq. A2, a total differential of the enthalpy can be written as

$$\frac{\mathrm{d}H}{\mathrm{d}t} = \left(\frac{\partial H}{\partial T}\right)_{\mathrm{P}}\cdot\frac{\mathrm{d}T}{\mathrm{d}t} + \left(\frac{\partial H}{\partial P}\right)_{\mathrm{T}}\cdot\frac{\mathrm{d}P}{\mathrm{d}t} + \sum_i \bar{h}_{\mathrm{i}}\cdot\frac{\mathrm{d}n_{\mathrm{i}}}{\mathrm{d}t} \tag{A10}$$

According to Eqs. A3 and A6, one derives

$$\left(\frac{\partial H}{\partial P}\right)_{\mathrm{T}} = -T\left(\frac{\partial V}{\partial T}\right)_{\mathrm{P}} + V \tag{A11}$$

Inserting Eqs. A4 and A11 into Eq. A10 and combining Eq. A9 to eliminate ($\mathrm{d}H/\mathrm{d}t$), one obtains

$$C_{\mathrm{P}}\frac{\mathrm{d}T}{\mathrm{d}t} - T\left(\frac{\partial V}{\partial T}\right)_{\mathrm{P}}\cdot\frac{dP}{dt} + \sum_i \bar{h}_{\mathrm{i}}\cdot\frac{\mathrm{d}n_{\mathrm{i}}}{\mathrm{d}t} = \dot{Q} + \dot{W} + \sum_i \bar{h}_{\mathrm{e,i}}\cdot\dot{n}_{\mathrm{e,i}} \tag{A12}$$

Substitution of Eq. A7 into Eq. A12 yields the general enthalpy balance for a non-steady open system

$$C_{\mathrm{P}}\frac{\mathrm{d}T}{\mathrm{d}t} - T\left(\frac{\partial V}{\partial T}\right)_{\mathrm{P}}\cdot\frac{\mathrm{d}P}{\mathrm{d}t} + \sum_i \bar{h}_{\mathrm{i}}\sum_j \nu_{\mathrm{ij}}\cdot\dot{\xi}_{\mathrm{j}} = \dot{Q} + \dot{W} + \sum_i (\bar{h}_{\mathrm{e,i}} - \bar{h}_{\mathrm{i}})\cdot\dot{n}_{\mathrm{e,i}} \tag{3}$$

Appendix B. Calculation of Gibbs energy changes of microbial growth processes based on the Gibbs energies of combustion of relevant substances

For a catabolic process represented by Eq. 11a, the Gibbs energy change can be expressed as the following:

$$\Delta_{\mathrm{r}}G_a^0 = \Delta_{\mathrm{C}}G_{\mathrm{S}}^* + Y_{\mathrm{A}}^{\mathrm{a}}\cdot\Delta_{\mathrm{C}}G_{\mathrm{A}}^* - Y_{\mathrm{P}}^{\mathrm{a}}\cdot\Delta_{\mathrm{C}}G_{\mathrm{P}}^* \tag{B1}$$

where $\Delta_{\mathrm{C}}G_{\mathrm{S}}^*$, $\Delta_{\mathrm{C}}G_{\mathrm{A}}^*$ and $\Delta_{\mathrm{C}}G_{\mathrm{P}}^*$ are the modified Gibbs

energies of combustion of carbon source S, electron acceptor/donor A and product P respectively, using CO_2 (g), H_2O (l) and NH_3 (aq) as reference states. The yields in this reaction are constrained by a reduction degree balance and a carbon balance as follows

$$\gamma_S + Y_A^a \cdot \gamma_A = Y_P^a \cdot \gamma_P \quad \text{(B2a)}$$

$$Y_P^a + Y_C^a = 1 \quad \text{(B2b)}$$

For most microbial growth processes, in fact, even more constraints or simplifications can be applied to the stoichiometry of growth. Now we consider these for the four cases given in Table 1.

For oxidative growth, electron acceptor A is O_2, and $\Delta_C G_A^* = 0$. Since there is no product P other than CO_2, the last term relevant to P in Eq. B1 vanishes, and Eq. B1 can be simplified as the following:

$$\Delta_r G_a^0 = \Delta_C G_S^* \quad \text{(B3a)}$$

Analogously, for the biomass degradation process, Eq. 11b, Gibbs energy change can be calculated based on the following equation:

$$\Delta_r G_b^0 = \Delta_C G_X^* \quad \text{(B3b)}$$

For reductive growth and methanogenesis utilizing acetate, the carbon/energy source S and the electron acceptor A are the same substance. Since $\Delta_r G_a^0$ is expressed as the Gibbs energy change per C-mol S converted, the term pertaining to A does not need to be included in Eq. 11a. Thus, for these cases, the terms in Eqs. B1 and B2a relevant to electron acceptor A vanish. By combining Eqs. B1 and B2a, we can readily obtain that

$$\Delta_r G_a^0 = \Delta_C G_S^* - \frac{\gamma_S}{\gamma_P} \cdot \Delta_C G_P^* \quad \text{(B4a)}$$

Analogously, for the biomass degradation process, Eq. 11b, $\Delta_r G_b^0$ can be evaluated as the following

$$\Delta_r G_b^0 = \Delta_C G_X^* - \frac{\gamma_X}{\gamma_P} \cdot \Delta_C G_P^* \quad \text{(B4b)}$$

For autotrophic methanogenesis, $S = CO_2$, $A = H_2$, and $Y_P^a = 1$, as shown by the carbon balance. Thus, using the degree of reduction balance for calculating Y_A^a and combining this with Eq. B1, we have

$$\Delta_r G_a^0 = \Delta_C G_S^* - \Delta_C G_P^* + \frac{\gamma_P - \gamma_S}{\gamma_A} \cdot \Delta_C G_A^* \quad \text{(B5a)}$$

Analogously we can obtain

$$\Delta_r G_b^0 = \Delta_C G_X^* - \Delta_C G_P^* + \frac{\gamma_P - \gamma_X}{\gamma_A} \cdot \Delta_C G_A^* \quad \text{(B5b)}$$

Consequently, the Gibbs energy change of the overall growth process, which is represented by Eq. 9, can be calculated by combining $\Delta_r G_a^0$, $\Delta_r G_b^0$ and growth yield $Y_{X/S}$, as the following

$$\Delta_r G_X^0 = \frac{\Delta_r G_a^0}{Y_{X/S}} - \Delta_r G_b^0 \quad \text{(12)}$$

In the case of methanogenesis utilizing (H_2+CO_2), the energy source is electron donor H_2. It is often the growth-limiting substrate due to its poor solubility in the medium, and thus, the biomass yield is often expressed with respect to H_2 (A) [45,46].

Combining the carbon balance with the reduction degree balance, and using $\gamma_S = 0$, we can correlate $Y_{X/S}$ with $Y_{X/A}$ as the following

$$\frac{1}{Y_{X/S}} = \left(1 - \frac{\gamma_X}{\gamma_P}\right) + \frac{\gamma_A}{\gamma_P} \cdot \frac{1}{Y_{X/A}} \quad \text{(B6)}$$

Substituting Eq. B6 into Eq. 12, and combining this with Eqs. B5a and Eq. B5b, we can prove that $\Delta_r G_X$ can also be correlated to $Y_{X/A}$ as follows

$$\Delta_r G_X = \frac{\Delta_r G_a'}{Y_{X/A}} - \Delta_r G_b' \quad \text{(B7)}$$

where

$$\Delta_r G_a' = \Delta_C G_A^* + \frac{\gamma_A}{\gamma_P} \cdot (\Delta_C G_S^* - \Delta_C G_P^*) \quad \text{(B8a)}$$

$$\Delta_r G_b' = (\Delta_C G_X^* - \Delta_C G_S^*) + \frac{\gamma_X}{\gamma_P} \cdot (\Delta_C G_S^* - \Delta_C G_P^*) \quad \text{(B8b)}$$

Eq. B7 is the equation that was used for generating the plots of Fig. 5 in this contribution. In doing this, the influences of the actual culture temperature and the actual concentrations of the involved gaseous compounds have been taken into account rather than simply using the common standard states [46].

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