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The definition of energetic growth efficiencies for aerobic and anaerobic microbial growth and their determination by calorimetry and by other means [1]

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Abstract

Several possible definitions for energetic growth efficiency are reviewed. Although definitions based on a simple comparison of inflowing and outflowing energy suffer from shortcomings, they are the only ones that can be related to the respective energy dissipation in a unique way, even in cases with complex growth stoichiometry. It is therefore possible to determine the enthalpic growth efficiency in a very simple way by calorimetry. In completely aerobic growth, the result is also virtually equal to the free energy efficiency. In mixed respiro-fermentative growth, the two efficiencies are different and the free energy efficiency must be computed based on detailed knowledge of the growth stoichiometry. In aerobic, mixed, and anaerobic growth of *Kluyveromyces fragilis* yeast, the free energy efficiency remained constant at about 60%.

LIST OF SYMBOLS

Ds_0	product of dilution rate and initial substrate concentration: feed rate of carbon substrate in C-mol s^{-1} m^{-3}
$k_La c^*$	product of volumetric mass transfer coefficient and oxygen saturation concentration: feed rate of oxygen in mol s^{-1} m^{-3}
$\Delta_r H^\ominus$	standard enthalpy of the growth reaction in kJ per C-mol
$\Delta H_a^\ominus$	standard enthalpy of reaction (3a) in kJ per C-mol^{-1}
$\Delta H_b^\ominus$	standard enthalpy of reaction (3b) in kJ per C-mol
$\Delta_c H_i^\ominus$	modified combustion enthalpy of i in kJ per C-mol
$\Delta_r G^\ominus$	standard Gibbs energy of the growth reaction in kJ per C-mol
$\Delta G_a^\ominus$	standard Gibbs energy of reaction (3a) in kJ per C-mol
$\Delta G_b^\ominus$	standard Gibbs energy of reaction (3b) in kJ per C-mol
$\Delta_c G_i^\ominus$	modified Gibbs energy of combustion of i in kJ per C-mol
r_s	rate of substrate consumption in C-mol s^{-1} m^{-3}

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Y_i stoichiometric coefficient in C-mol per C-mol or mol per C-mol
$Y_{x/s}$ Biomass yield in $g\,g^{-1}$ or C-mol per C-mol
η_H enthalpic growth efficiency
η_G Free energy growth efficiency

INTRODUCTION

Isothermal reaction calorimetry has been developed mainly for chemical reactions [1, 2], but its application to biological processes has also been proposed and explored to some extent [3, 4]. It has been suggested that isothermal reaction calorimetry could be a valuable tool for monitoring the biological process, for obtaining an on-line assessment of the main metabolic pathways and events occurring in a cellular culture, and as a basis for computer-assisted process control [5–8].

A point of more fundamental interest is the question whether the heat released by a growing culture tells us anything about the deep physio-chemical nature of the energy transformations and dissipations linked to the growth processes of living beings. In chemotrophic growth, heat could be interpreted as the part of the chemical energy assimilated by the cellular culture that has not been retained in either the fabric of the emerging biomass or in the form of products, but is instead dissipated in the form of heat. In this way, it may represent an energetic "inefficiency" of growth which could be measured quantitatively by calorimetry.

The aim of this contribution is to study whether meaningful energetic growth efficiences can be determined from direct calorimetric measurements. To this effect, the relationships between various possible measures of energetic growth efficiency and energy dissipation will be reviewed. Because these relationships provide the link between heat dissipation measurements and efficiency, they will also be used to compare the various definitions for energetic growth efficiency.

The most suitable definition will then be applied to actual microbial cultures. In order to study the energetic growth efficiency as a function of differential energy metabolisms, this report not only focuses on aerobic, fully respiratory growth, but also includes anaerobic and mixed respiro-fermentative experiments, in which respiration could be decreased in a controlled way to low values by forcing the culture progressively towards a more reductive metabolism.

VARIOUS MEASURES FOR THE ENERGETIC GROWTH EFFICIENCY AND THEIR RELATIONSHIP TO THE DISSIPATION OF ENERGY

Though the definition of energetic efficiencies for completely aerobic microbial growth is quite straightforward, problems arise for growth with product formation such as in anaerobiosis. Several authors have accounted for the energy contained in the fermentation products by proposing different definitions of the energetic growth efficiency.

The relationship between several efficiency definitions and the dissipation of free energy has recently been developed by Heijnen et al. [9] by decomposing the growth stoichiometry into four generalized "half-reactions" yielding and consuming electrons. Similar relations will be derived here, but using a much simpler approach.

Thermodynamic growth efficiency by Battley [10]

The early version of the thermodynamic growth efficiency proposed by Battley is defined in terms of enthalpy and free energy

$$\eta_H^{\mathrm{Bat}} = \frac{\Delta H_{\mathrm{NC}}^{\ominus} - \Delta H_{\mathrm{C}}^{\ominus}}{\Delta H_{\mathrm{NC}}^{\ominus}} \tag{1a}$$

$$\eta_G^{\mathrm{Bat}} = \frac{\Delta G_{\mathrm{NC}}^{\ominus} - \Delta G_{\mathrm{C}}^{\ominus}}{\Delta G_{\mathrm{NC}}^{\ominus}} \tag{1b}$$

where the subscripts NC and C refer to the so-called "non-conservative" reaction, i.e. the one that would ensue without any biomass formation, and, respectively, to the "conservative", i.e. the actual growth reaction.

A direct, simple relationship between η^{Bat} and the energy dissipation accompanying growth can be developed by assuming a general microbial growth stoichiometry for a reasonably simple case

$$\frac{1}{Y_{\mathrm{x/s}}}\mathrm{S} + Y_{\mathrm{E}}\mathrm{E} + Y_{\mathrm{ND}}\mathrm{ND} \rightarrow \mathrm{X} + Y_{\mathrm{SOX}}\mathrm{SOX} + Y_{\mathrm{ER}}\mathrm{ER} + Y_{\mathrm{W}}\mathrm{H_2O} \tag{2}$$

In this equation, S is the carbon substrate, while X denotes a C-mol of biomass. The other symbols stand for chemical compounds with a generalized functionality as defined in Table 1. Thus, E is usually an electron acceptor, which is used by the microbial catabolism to oxidize S,

TABLE 1

Significance of symbols for chemical compounds in the generalized growth stoichiometry, eqn. (2)

Symbol	Significance	Examples		
		Respiration	Ethanol fermentation	Methanogenesis
S	Carbon source	Carbohydrate	Carbohydrate	CO_2
E	Electron acceptor/donor	O_2	Carbohydrate	H_2
ND	Nitrogen donor	NH_3	NH_3	NH_3
X	Biomass	Biomass	Biomass	Biomass
SOX	Oxidized/red. carbon	CO_2	CO_2	CH_4
ER	Reduced/oxidized E	H_2O	EtOH	H_2O

usually into CO_2, denoted by SOX, and is itself reduced to ER in the process. Due to its generalized form, eqn. (2) also represents a number of microbial processes other than aerobic respiration. In anaerobic fermentations, for example, the carbon substrate also serves as electron acceptor, the net result being a disproportionation of S into SOX (CO_2) and ER, e.g. ethanol.

Equation (2) may be split into two redox equations

$$S + Y_{E1}E \rightarrow Y_{c1}SOX + Y_{R1}ER + Y_{w1}H_2O + Y_{N1}ND \quad (3a)$$

$$X + Y_{E2}E \rightarrow Y_{c2}SOX + Y_{R2}ER + Y_{w1}H_2O + Y_{N2}ND \quad (3b)$$

Equation (3a) may be interpreted as the energy-yielding catabolic reaction. In the form proposed here, however, eqn. (3b) does not represent the inverse of anabolism. It rather reflects the degradation of biomass into the products of the energy-yielding reaction. Both reactions (3a) and (3b) usually have a unique stoichiometry that can be established by element balancing. Examples for the stoichiometry of eqn. (3b), which is the more complex of the two, appear in Table 2.

In Battley's terminology, eqns. (2) and (3a) are the conservative and the non-conservative reactions, respectively. Therefore, his definition for η translates into

$$\eta_H^{Bat} = \frac{\frac{1}{Y_{x/s}}\Delta H_a^{\ominus} - \Delta_r H^{\ominus}}{\frac{1}{Y_{x/s}}\Delta H_a^{\ominus}} \quad (4)$$

TABLE 2

Stoichiometry for eqn. (3b) for three types of microbial catabolism

1. Aerobic respiration

$$CH_{x_1}O_{x_2}N_{x_3} + \frac{\gamma_x}{4}O_2 \rightarrow CO_2 + \delta_x H_2O + x_3NH_3$$

2. Anaerobic fermentation

$$CH_{x_1}O_{x_2}N_{x_3} \rightarrow \left(1 - \frac{\gamma_x}{\gamma_r}\right)CO_2 + \frac{\gamma_x}{\partial_r}CH_{r_1}O_{r_2} + \left(\delta_x - \frac{\gamma_x}{\gamma_r}\frac{r_1}{2}\right)H_2O + x_3HN_3$$

3. Methanogenesis from CO_2 and H_2

$$CH_{x_1}O_{x_2}N_{x_3} + (2 - \tfrac{1}{2}x_1 + x_2 - \tfrac{3}{2}x_3)H_2 \rightarrow CH_4 + x_2H_2O + x_3NH_3$$

Key: γ_x is the degree of reduction of dry biomass, defined as $\gamma_x = 4 + x_1 - 2x_2 - 3x_2$; γ_r is the degree of reduction of ER, defined as $\gamma_r = 4 + r_1 - 2r_2$; δ_x is defined as $\delta_x = 1/2x_1 - 3/2x_3$. The stoichiometry for eqn. (3a) is obtained by substituting $CH_{s_1}O_{s_2}N_{s_3}$, γ_S, and δ_S, for, respectively, $CH_{x_1}O_{x_2}N_{x_3}$, γ_x and δ_x.

Here, the subscripts r, a and b denote reactions (2), (3a) and (3b), respectively. However, application of Hess' law yields

$$\frac{1}{Y_{x/s}}\Delta H_a^{\ominus} = \Delta_r H^{\ominus} + \Delta H_b^{\ominus} \tag{5}$$

Combining eqns. (4) and (5) yields the following dependence of η on the enthalpy and the free energy change of the entire growth reaction

$$\eta_H^{Bat} = \frac{\Delta H_b^{\ominus}}{\Delta_r H^{\ominus} + \Delta H_b^{\ominus}} \tag{6a}$$

$$\eta_G^{Bat} = \frac{\Delta G_b^{\ominus}}{\Delta_r G^{\ominus} + \Delta G_b^{\ominus}} \tag{6b}$$

The negative value of $\Delta_r H^{\ominus}$ may be interpreted as the part of the chemical energy contained in the substrate that was not conserved, but "dissipated" into heat. It may be measured in a calorimeter as the heat released per C-mol of biomass growth. The negative value of $\Delta_r G^{\ominus}$ is the amount of free energy dissipated per unit biomass. Equation (6) postulates a very simple relationship between η^{Bat} and these dissipations. When no biomass is grown, the dissipation of enthalpy and free energy per unit biomass becomes infinity and the energetic efficiency is zero. If, however, growth is so efficient as to reduce the need for energy dissipation to zero, the energetic efficiency tends towards unity.

It is obvious by virtue of the second law of thermodynamics that $\Delta_r G^{\ominus}$ cannot become positive. Because it is inconceivable that the decomposition of biomass into simple molecules according to eqn. (3b) is endergonic, η_G^{Bat} cannot exceed unity. There is no such constraint on η_H^{Bat}. However eqn. (6) shows that η_H^{Bat} may be directly observed by calorimetry.

Energy-transducer-type efficiencies

Several authors have proposed that cellular metabolism be compared to an energy transducer and the efficiency be defined as the inverse ratio of the power released by the energy-yielding, or "input" reaction to the power needed to synthesize the cells ("output" reaction) [11, 12]. This type of definition has been discussed in detail by Gnaiger [13], who also showed that it is very suitable for assessing the efficiency of mitochondrial ATP production.

For whole cells, however, it often seems impossible to define the biochemically correct energy-yielding reaction and to dissociate it from the true anabolic process [14]. The values of η unfortunately appear to depend considerably on the exact split of the overall stoichiometry into energy-yielding and anabolic reactions. The definition of the energy-transducing

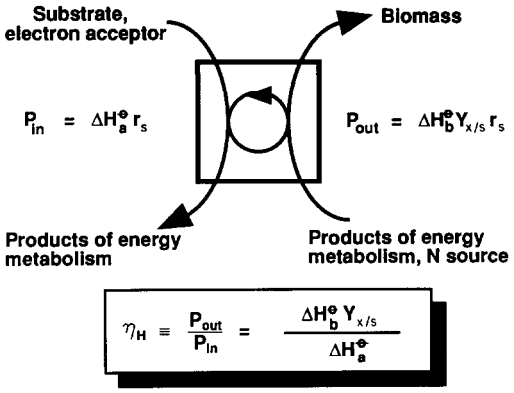

Fig. 1. Definition of an energy transducer efficiency.

efficiency would thus probably best be based on a formal split such as the one indicated on Fig. 1. If eqn. (2) is adopted as a general growth stoichiometry, the energy-yielding reaction could be taken as eqn. (3a) and the formal anabolic process as the reverse of eqn. (3b). The energy transducer efficiency would then be defined as

$$\eta_H^{\text{etr}} \equiv \frac{P_{\text{out}}}{P_{\text{in}}} = \frac{-r_s Y_{x/s}(-\Delta H_b^{\ominus})}{r_s \Delta H_a^{\ominus}} = Y_{x/s} \frac{\Delta H_b^{\ominus}}{\Delta H_a^{\ominus}} \tag{7}$$

A similar definition can be proposed for η_G^{etr}. Combining this with eqn. (5) again yields eqn. (6). It may be concluded that this type of definition is identical to Battley's for the relatively simple stoichiometries covered by eqn. (2). Therefore, all the remarks made with respect to calorimetry and the upper limit of η^{Bat} also apply to η^{etr}.

In–out efficiencies

Another way to define the energetic growth efficiency is to compare the power flowing into the cells in the form of chemical energy contained in all the substrates, with the power produced in the form of chemical energy contained in the biomass. The efficiency is defined as the ratio of the latter to the former (see Fig. 2).

This yields a very similar definition to the η^{etr} for aerobic microbial growth without fermentation products. For cases where products are formed, Roels [15] has suggested that the energy contained in them be subtracted from the energy contained in the substrates because the chemical energy of the products is not available for growth. Therefore, for a growth reaction with general stoichiometry

$$\frac{1}{Y_{x/s}} S + \sum Y_{Si} S_i \rightarrow X + \sum Y_{Pi} P_i \tag{8}$$

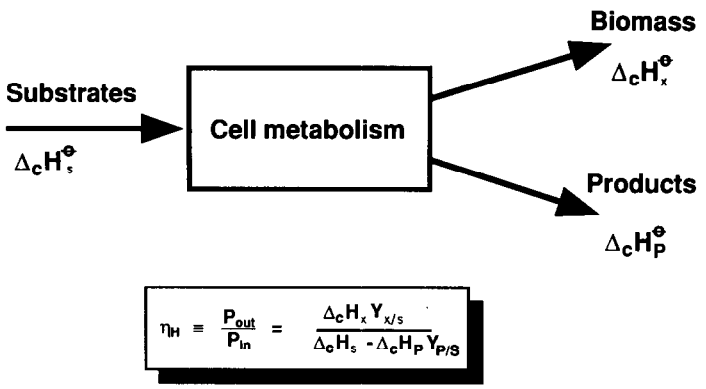

Fig. 2. Definition of input–output-type energetic growth efficiencies.

the growth efficiency is defined as

$$\eta_H^{i/o} = \frac{\Delta_c H_x^\ominus}{\frac{1}{Y_{x/s}}\Delta_c H_s^\ominus + \sum Y_{Si}\Delta_c H_{Si}^\ominus - \sum Y_{Pi}\Delta_c H_{Pi}^\ominus} \tag{9a}$$

$$\eta_G^{i/o} = \frac{\Delta_c G_x^\ominus}{\frac{1}{Y_{x/s}}\Delta_c G_s^\ominus + \sum Y_{Si}\Delta_c G_{Si}^\ominus - \sum Y_{Pi}\Delta_c G_{Pi}^\ominus} \tag{9b}$$

where $\Delta_c H_i^\ominus$ is the enthalpic content of i, usually taken as the heat of combustion. In order to align definition (9) with η^{etr}, at least for aerobic respiration, it is proposed that eqns. (3a) and (3b) be used with $E = O_2$, $SOX = CO_2$ and $ER = H_2O$ as the combustion stoichiometry for the definition of all heats of combustion ($\Delta_c H_i^\ominus$) and free energies of combustion ($\Delta_c G_i^\ominus$.) In these modified enthalpies of combustion the nitrogen would not be oxidized to N_2, as in the usual definition, but to the nitrogen donor actually employed by the culture, e.g. NH_3.

An energy balance for eqn. (8) yields

$$\frac{1}{Y_{x/s}}\Delta_c H_s^\ominus + \sum Y_{Si}\Delta H_{Si}^\ominus - \sum Y_{Pi}\Delta_c H_{Pi}^\ominus = \Delta_r H^\ominus + \Delta_c H_x^\ominus \tag{10}$$

Substituting the denominator of eqn. (9) and repeating the derivation for free energy yields

$$\eta_H^{i/o} = \frac{\Delta_c H_x^\ominus}{\Delta_r H^\ominus + \Delta_c H_x^\ominus} \tag{11a}$$

$$\eta_G^{i/o} = \frac{\Delta_c G_x^\ominus}{\Delta_r G^\ominus + \Delta_c G_x^\ominus} \tag{11b}$$

For purely aerobic growth, the catabolic or non-conservative reaction has

the same stoichiometry as the combustion serving for the definition of $\Delta_c H_x^\ominus$ and $\Delta_c G_x^\ominus$. Therefore, eqns. (6) and (11) become identical. For other catabolic reactions, $\Delta H_b^\ominus$ and $\Delta_c G_b^\ominus$ change and reflect the energy change of the oxidation or reduction of 1 C-mol of biomass by the electron acceptor/donor actually employed by the catabolic reaction, whereas the evaluation of $\eta^{i/o}$ by eqn. (11) continues to be based on the constant energies of combustion of biomass.

The ratio of the actual to the highest thermodynamically possible biomass yield

Recently, Heijnen et al. [9] proposed the following definition for η

$$\eta^{Hei} = Y_{x/s}/(Y_{x/s})_{max} \tag{12}$$

The authors showed that for simple growth reactions, η^{Bat} is equivalent to η^{Hei}.

Assuming, again, the stoichiometry of eqn. (2), the enthalpy balance, eqn. (5), can be reformulated in terms of free energy and solved for the actual biomass yield $Y_{x/s}$

$$Y_{x/s} = \frac{\Delta G_a^\ominus}{\Delta_r G^\ominus + \Delta G_b^\ominus} \tag{13}$$

Because the thermodynamic maximum for $Y_{x/s}$ is given by setting $\Delta_r G^\ominus = 0$, η^{Hei} is found to be related to the actual free energy dissipation as

$$\eta_G^{Hei} = \frac{\Delta G_b^\ominus}{\Delta_r G^\ominus + \Delta G_b^\ominus} \tag{14}$$

which is identical to eqn. (6).

Comparison of the different definitions for growth efficiencies

It appears that for the simple generalized growth stoichiometry described by eqn. (2), all of the analyzed definitions for η are equivalent, except for $\eta^{i/o}$, which will only yield identical values for purely aerobic growth. All these efficiencies are related to the respective energy dissipation in a very simple way, which is given by eqn. (11) for $\eta^{i/o}$ and by eqn. (6) for the other definitions.

One clear advantage of η^{Bat}, η^{etr} and η^{Hei} is that their Gibbs versions reflect directly the ratio of the actual biomass yield to the maximum biomass yield, as indicated by eqn. (12). Also, they do not depend on the choice of a reference state, because only energy differences appear in their definitions and in eqns. (6) and (14). As has been pointed out by Heijnen and van Dijken [14], this is not so for input–output-type efficiencies, which are affected by the reference state chosen for measuring the energy content

of the involved compounds. Indeed, by defining $\Delta_c H_i^{\ominus}$ with respect to the usual molecular state for nitrogen instead of NH_3, $\eta^{i/o}$ would differ slightly from the other definitions even for purely aerobic growth.

The various definitions for η may readily be converted into each other by combining eqns. (6), (11) and (12), and by eliminating $\Delta_r G^{\ominus}$

$$\eta_H^{Bat} = \eta_H^{etr} = \eta_H^{Hei} = \frac{\eta_H^{i/o}\Delta H_b^{\ominus}}{(1-\eta_H^{i/o})\Delta_c H_x^{\ominus} + \eta_H^{i/o}\Delta H_b^{\ominus}} \tag{15a}$$

$$\eta_G^{Bat} = \eta_G^{etr} = \eta_G^{hei} = \frac{Y_{x/s}}{(Y_{x/s})_{max}} = \frac{\eta_G^{i/o}\Delta G_b^{\ominus}}{(1-\eta_G^{i/o})\Delta_c G_x^{\ominus} + \eta_G^{i/o}\Delta G_b^{\ominus}} \tag{15b}$$

Extension of efficiency definitions for multiple electron acceptors

Thus far, the relationship between various definitions of η and the dissipation of energy has been derived for microbial growth involving a maximum of one electron acceptor (see eqn. (2)). In the experiments discussed in the experimental sections of this paper, yeast cultures were forced to grow on various mixtures of respiro-fermentative metabolisms in order to study the growth efficiency as a function of energy metabolism. Such cultures, therefore, use both O_2 and glucose as electron acceptors simultaneously, and produce a mixture of water and ethanol as reduced products. In cases with several ER products, eqns. (3a) and (3b) no longer have a uniquely defined stoichiometry and the formulation of the energy-yielding reaction becomes arbitrary. For an anaerobic yeast fermentation yielding ethanol and glycerol simultaneously, Battley [10] arbitrarily defined the production of ethanol from glucose as the non-conservative, i.e. energy-generating, reaction and did not consider a hypothetical energy gain from glycerol production. Heijnen et al. [9], however, advocate an averaging technique based on the actual electron distribution into the various ER products. None of them discuss how to account for multiple electron acceptors E.

The suggested averaging procedure [9] would mean replacing ER in eqns. (2) and (3) by a sum of various products, the exact distribution of which depends on the particular growth conditions employed. A comparison of growth efficiencies for various mixtures of aerobic and anaerobic metabolism on the basis of eqns. (6) or (14) therefore requires the experimental determination of the detailed stoichiometry for each experimental point, because $\Delta H_b^{\ominus}$ and $\Delta G_b^{\ominus}$ depend on the actual mixture of reduced electron acceptors.

However, $\Delta_c H_x^{\ominus}$ and $\Delta_c G_x^{\ominus}$ in eqn. (11) are constant. In experiments with varying energy metabolism, $\eta^{i/o}$ is therefore easier to use than other definitions. By the same token, $\eta^{i/o}$ reflects more directly the energy dissipation in that it will remain constant for a constant $\Delta_r H^{\ominus}$ or $\Delta_r G^{\ominus}$, even with different energy metabolisms. This is why $\eta^{i/o}$ will be used in the

subsequent analysis despite the shortcomings it displays, as discussed above.

MATERIALS AND METHODS

The calorimetric data used for determining the energetic growth efficiency were obtained by von Stockar and Birou [6]. These experiments were performed in modified isothermal reaction calorimeters (BSC 81, Ciba-Geigy, Basel, Switzerland). Such calorimeters have a useful culture volume of 1.6 l and may be operated in exactly the same way as normal laboratory bioreactors. The calorimetric measuring principle applied in these bench-scale calorimeters has been described in two reviews [4, 5].

Kluyveromyces fragilis NRRL 1109 was grown continuously both aerobically and anaerobically in a standard medium containing initially 15 g l^{-1} of glucose [6]. The cultures were aerated with a stream of 3 nl min^{-1} of air. In continuous culture, the metabolism was gradually forced from a purely respiratory to mixed respiro-fermentative metabolism by replacing part of this air stream with nitrogen, while the supply rate of glucose, i.e. the dilution rate, was kept constant at about 0.2 h^{-1}. Each experimental point reported for continuous cultures was obtained at steady state.

RESULTS

Aerobic growth

Figure 3 displays the result of a calculation of the dissipation of enthalpy and free energy as a function of the C-molar biomass yield $Y_{x/s}$ [16]. The

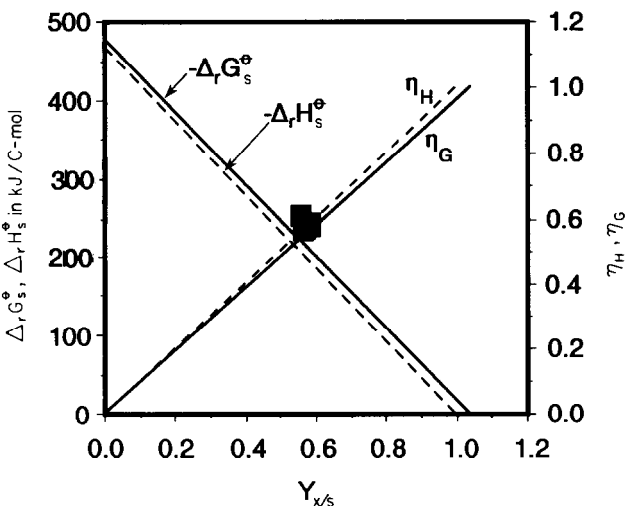

Fig. 3. Energy dissipations and energetic growth efficiency as a function of the C-molar biomass yield for fully respiratory growth of *K. fragilis*: ——, theoretical calculations pertaining to free energy; – – –, theoretical calculations pertaining to enthalpy; ■, η_H measured by calorimetry. Reprinted with permission from ref. 16.

growth stoichiometry was determined as a function of $Y_{x/s}$ according to eqn. (2) for pure respiratory growth, and the modified enthalpies and free energies of combustion for a C-mol of glucose and of biomass were calculated according to values given by Sandler and Orbey [17], Cordier et al. [18], and Gurakan et al. [19].

In order to linearize the plots in Fig. 3, the values of the enthalpy and free energy change per C-mole of glucose ($\Delta_r H_s^\ominus$ and $\Delta_r G_s^\ominus$) were plotted, rather than per unit of biomass grown ($\Delta_r H^\ominus$ and $\Delta_r G^\ominus$). At zero biomass yield, the intercepts of the dissipation lines simply correspond to the enthalpy and free energy of combustion of one C-mole of glucose. With increasing $Y_{x/s}$, the enthalpy and free energy changes of the overall growth reaction decrease because some of the energy initially contained in the glucose is now retained in the biomass. Also shown are $\eta_H^{i/o}$ and $\eta_G^{i/o}$, computed according to their definition, eqn. (9).

As can be seen in Fig. 3, the values for the heat production $-\Delta_r H_s^\ominus$ nearly coincide with the free energy dissipation $-\Delta_r G_s^\ominus$, independent of the value of $Y_{x/s}$. It appears that $T\Delta_r S^\ominus$, separating the two lines, is so small that it can be neglected compared with either $\Delta_r H$ or $\Delta_r G$, except for biomass yields close to unity. For the same reason, η_H and η_G also nearly coincide. It can be concluded that for purely respirative, aerobic growth, the free energy dissipation can be measured directly in a calorimeter and used to compute a good estimate of the energetic growth efficiency, either by eqn. (11a) or by eqn. (11b).

Energetic growth efficiencies were computed from continuous culture data of *K. fragilis* [6], both from measured biomass yields and eqn. (9), and from direct calorimetry data. The points appearing on Fig. 3 indicate the two values on the x and y axes, respectively. As can be seen, both η_H and η_G amount to about 0.6 in all experiments. It is therefore obvious that the energetic growth efficiency is far removed from its thermodynamic maximum value of 1, and also that the biomass yield could be much higher.

A similar conclusion was also reached in a calorimetric study involving purely aerobic batch experiments of various yeasts and bacteria grown on a variety of substrates (ref. 20). In addition to various mono- and dihexoses, the choice of substrates included citrate, succinate, acetate, glycerol, methanol, and hexadecane. Strong aeration and agitation was always provided in order to maintain the oxygen saturation of the aqueous phase above 50%. According to the wide variation of the energetic content of the substrates used biomass yields ranged from 0.29 to 0.99 g g^{-1}. Nevertheless, the calorimetrically measured energetic efficiencies stayed at values of around 0.4 and 0.6.

It may be concluded that energetic efficiencies in fully aerobic microbial growth reflect a compromise between two possible extremes. At one end of the scale, η values could theoretically approach unity and therefore enable the culture to grow with nearly the highest biomass yield that is thermo-

dynamically possible, but this could only be realized at the price of very low free energy dissipations and, hence, of growth rates tending to zero.

However, low energetic efficiencies would afford high dissipations and thus a very high growth rate, but at the cost of a low biomass yield. In practice, aerobically growing microorganisms appear to dissipate about 40% of the available energy in order to sustain a reasonable growth rate, and this dissipation can be seen directly in a calorimeter.

Growth with mixed respiro-fermentative metabolism

In order to force a continuous culture of *Kluyveromyces fragilis* yeast to shift progressively towards fermentative metabolism, the oxygen supply was progressively reduced as compared to the carbon supply. As shown in Fig. 4, lowering the ratio of oxygen to carbon supply first resulted in a reduction of the unused dissolved oxygen tension (pO_2) in the broth, until it dropped to limiting values near zero. Decreasing the supply ratio further forced the culture to degrade part of the glucose by a fermentative pathway, thereby giving rise to an increasing amount of ethanol production. In parallel, the biomass yield decreased gradually from high levels typical for respiration to a much lower level characteristic of anaerobic fermentation.

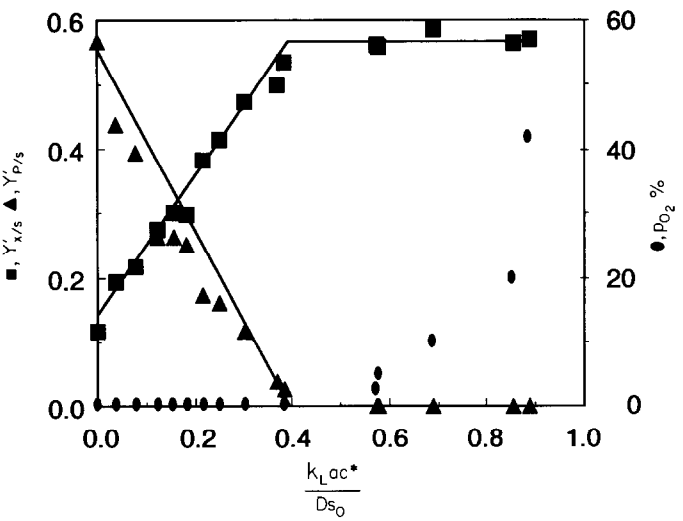

Fig. 4. Effect of limiting the relative oxygen to carbon supply ($k_La c^*/Ds_0$) on the growth stoichiometry of *K. fragilis*: ●, dissolved oxygen concentration (%); ■, C-molar biomass yield; ▲, C-molar ethanol yield.

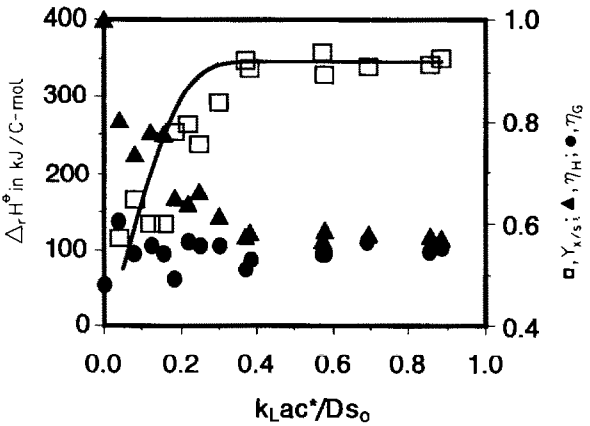

Fig. 5. Effect of limiting the relative oxygen to carbon supply ($k_L ac^*/Ds_0$) on measured heat dissipation $\Delta_r H^\ominus$ (□), on η_H (▲) and on η_G (●). Recalculated from ref. 6.

The effect of this shift on the heat dissipation and on η is shown in Fig. 5. As soon as fermentation sets in, $\Delta_r H$ decreases and reaches almost immeasurably low levels for purely anaerobic growth. In accordance with eqn. (11a), the enthalpic efficiency tends towards unity.

Also shown on Fig. 5 is $\eta_G^{i/o}$, as calculated from the biomass yield by means of the free energy equivalent of eqn. (9). Despite the shift in metabolism, the free energy efficiency remains at 0.6.

Anaerobic growth

The reason why $\eta_H^{i/o}$ is so different from $\eta_G^{i/o}$ in anaerobic growth is clearly visible on Fig. 6, which represents a plot of the energy dissipations as a function of C-molar biomass yield, similar to Fig. 3. In this case, the enthalpy and the free energy change associated with the overall growth reaction is so small that the positive $T\Delta_r S^\ominus$ term introduces a very large difference between the two. Because $\Delta_r G^\ominus$ is more negative than $\Delta_r H^\ominus$ by a factor of 3–4, rapid growth will spontaneously occur but it will dissipate very little heat. Anaerobic ethanol fermentation obviously does not release much chemical energy, as demonstrated by the low heat generation, but microorganisms mainly use as the driving force for growth the large entropy increase resulting from the decomposition of glucose into the small molecules CO_2 and ethanol. This process is an illustration that life can indeed feed on "negentropy", as was once implied by Schroedinger [21] in a famous statement.

Because $\Delta_r H^\ominus$ becomes so small for anaerobic growth, $\eta_H^{i/o}$ is close to unity and bears no real meaning. It is necessary to evaluate $\eta_G^{i/o}$ as a function of $Y_{x/s}$ using its definition, eqn. (9), which yields a curved line on Fig. 6. Two

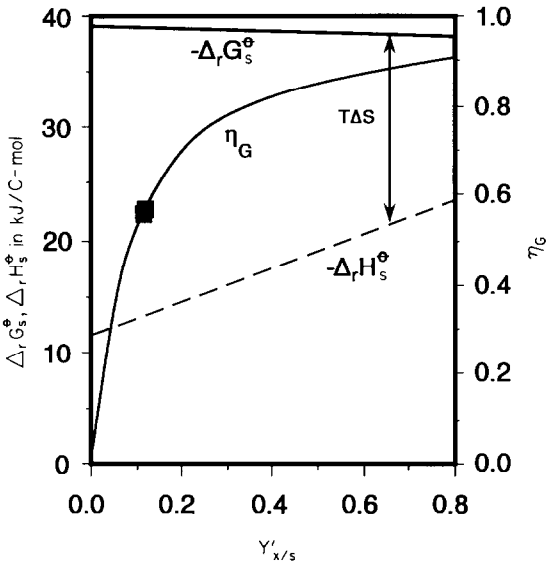

Fig. 6. Energy dissipation and free energy growth efficiency for anaerobic growth of *K. fragilis* as a function of the C-molar biomass yield: ——, theoretical calculations pertaining to free energy; – – –, theoretical calculation of enthalpy change; ■, η_G as determined from the measured value of the biomass yield. Reprinted with permission from ref. 16.

values of η_G based on actually measured yields are also given. They demonstrate that η_G remains at 60% despite relatively low biomass yields.

CONCLUSIONS

The various types of energetic growth efficiencies considered in this paper are all related in a very simple way to either "dissipation" of chemical energy into heat or free energy dissipation per unit biomass grown. They become zero if no biomass is formed and unity if the biomass yield is so high that no dissipation occurs. The definitions proposed by Battley and Heijnen and some of those based on the energy transducer concept are equivalent. Definitions based on a simple comparison of the amount of energy entering and leaving the cells are different and suffer from the fact that their values depend on the arbitrary choice of a reference for measuring energy. However, they are the only definition that can be related to the respective energy dissipation in a unique way even in cases with complex growth stoichiometry. The enthalpic growth efficiency of this type can therefore be determined directly from calorimetry. Even when several metabolisms are used simultaneously by the microbial culture, a detailed knowledge of the growth stoichiometry is not required.

In aerobic growth the heat dissipation and the free energy dissipation are virtually equal so that the free energy efficiency practically coincides with the enthalpic efficiency. In this case the free energy efficiency, which is the

more fundamental of the two, can be determined directly by a calorimetric heat dissipation measurement. In anaerobic growth, the dissipations are vastly different, and calorimetry only indicates the enthalpic efficiency. The free energy efficiency must then be calculated from its definition based on an exact determination of the growth stoichiometry.

Free energy efficiencies $\eta_G^{i/o}$ were found to amount to about 60% in aerobic growth of a variety of different microbial strains. In *K. fragilis* yeast, $\eta_G^{i/o}$ is independent of the extent of fermentation in cultures with mixed oxido-reductive metabolism, and it retains the same efficiency of 60% even when grown completely anaerobically. The free energy dissipation per unit biomass grown is thus constant for a given type of substrate. This confirms the work of Heijnen and van Dijken [13], who developed a universal correlation for $\Delta_r G^{\ominus}$ depending only on the nature of the carbon substrate and the chemical energy source.

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