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# Calorimetry and energetic efficiencies in aerobic and anaerobic microbial growth

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***Abstract***

A simple definition of the energetic growth efficiency both in terms of enthalpy and free energy is given and discussed. In cultures growing by respiratory catabolism, these efficiencies may conveniently be observed on-line by isothermal reaction calorimetry. If the fermentative part of catabolism is important, the free energy growth efficiency must be evaluated based on biomass yield measurements. In continuous cultures of two yeasts, growing by respiration, by pure fermentation, and on a mixed catabolism the efficiency was always between 0.5 and 0.6 despite widely varying growth yields.

## I. INTRODUCTION

The concept of energetic growth efficiency has been widely used to analyze and to explain observed variations in microbial growth yields. Indeed, different chemotrophic microbial strains growing on various carbon and energy sources exhibit growth yields $Y'_{x/s}$ ranging from a few percent to well above 0.5 C-mol/C-mol even if optimal growth conditions are employed in each single case. Based on the implicit paradigm that the reasons for such variations are ultimately to be found in the thermodynamics of growth processes, a number of attempts were made to correlate microbial yield data in terms of efficiencies as a basis for their prediction (refs. 1-3).

Since energetic growth efficiencies represent a measure of the energy retained in the biomass, they ought to be related in some way to the heat dissipated during the growth process, which measures the energy available in the carbon and energy substrates that was not retained in either biomass or products. The aim of this contribution is to explore in how far meaningful energetic growth efficiency values can be extracted from direct calorimetry in fully aerobic, mixed respiratory and fermentative, and completely anaerobic growth.

## 2. DEFINITIONS OF ENERGETIC GROWTH EFFICIENCIES

Energetic growth efficiencies have been defined in many different ways based both on enthalpy and free energy (refs. 1-4). One of the simplest definitions which may easily be applied to both completely aerobic catabolism and also to moderately or completely anaerobic cases where product formation occurs, has been suggested by Roels (ref. 4). Formulated in terms of enthalpy and slightly modified for the purpose of this work it reads:

$$\eta_H \equiv \frac{\Delta_c{*}H^\circ_x \cdot Y'_{x/s}}{\Delta_c H^\circ_s - Y'_{p/s} \cdot \Delta_c H^\circ_p} \qquad (1)$$

In terms of free energy, $\eta$ is defined as:

$$\eta_G \equiv \frac{\Delta_c{*}G^\circ_x \cdot Y'_{x/s}}{\Delta_c G^\circ_s - Y'_{p/s} \cdot \Delta_c G^\circ_p} \qquad (2)$$

For completely aerobic growth, when no organic product is formed and $Y'_{p/s} = 0$, these definitions can be regarded as so called "energy converter" efficiencies. In analogy to the characterization of the performance of motors, energy converter efficiencies represent the ratio of the energy "output" per unit time produced by an output reaction such as anabolism, and the energy "input" per unit time by catabolism. If complete oxidation of all of the carbon and energy substrate into $CO_2$ and $H_2O$ is assumed to be the input reaction and the formation of cells from this oxidized state is defined as the output reaction, $\Delta_c H^\circ_s \cdot r_s$ would represent the power input and $\Delta_c{*}H^\circ_x \cdot Y'_{x/s}\, r_S$ the "output power". $\Delta_c{*}H^\circ_i$ represents the standard enthalpy of combustion of i to $CO_2$, $H_2O$ and $NH_3$. Assuming no product formation, the ratio of these two expressions yield $\eta_H$ as defined in Eq. (1), whereas a similar ratio formulated in terms of G, yields $\eta_G$.

Although concepts such as energy converter efficiencies appear to assign physical meanings to $\eta$ values, interpretations based thereon must be subject to great caution, as Heijnen and van Dijken pointed out in a recent review (ref. 1). It is obvious that the definitions of catabolic and anabolic reactions underlying Eqs. (1) and (2) must be regarded as highly schematic from a biochemical point of view.

This is even more true in cases where fermentation products are formed. Roels (ref. 4) suggested subtracting the energy contained in these products from the one contained in the C-substrate since the former is not available for growth. Eqs (1) and (2) in this general form thus do not correspond to any input and output reactions any more.

The energetic growth efficiencies as defined by Eqs. (1) and (2) however, remain useful due to their simple relationship with the biomass yield. They may be shown to be related in an unequivocal way to the respective energy dissipations.

An enthalpy balance yields (refs. 5-6)

$$Y_{Q/X} = \Delta_c{*}H_X^o - \frac{\Delta_c H_S^o - Y'_{P/S}\,\Delta_c H_P^o}{Y'_{X/S}} \qquad (3)$$

where $Y_{Q/X} = -\Delta_r H_X^o$, the energy dissipated in the form of heat per C-mol biomass grown. Dividing this by the first right hand term and solving for $\eta_H$ yields

$$\eta_H = \frac{\Delta_c{*}H_X^o}{\Delta_c{*}H_X^o - Y_{Q/X}} \qquad (4)$$

The more fundamental parameter is $\eta_G$. A similar treatment yields

$$\eta_G = \frac{\Delta_c{*}G_X^o}{\Delta_c{*}G_X^o + \Delta_r G_X^o} \qquad (5)$$

Heijnen and van Dijken (ref. 1) succeeded in developping a universal correlation for $\Delta_r G_X^o$ for many different types of chemotrophic growth processes. They concluded that this parameter, which corresponds to the negative free energy dissipation per C-mol of growth, fundamentally determines the biomass yields. According to Eqs (4) and (5), it also determines $\eta$. The energetic growth efficiencies as defined by Eqs (1) and (2) thus represent a dimensionless measure of the respective energy dissipation. They range from 0 for no biomass yield (infinite dissipation) to 1 for a growth process being so efficient that it does not dissipate any energy.

## 3. MATERIALS AND METHODS

Calorimetric chemostat experiments were performed in modified isothermal reaction calorimeters of different types (BC8 81, CIBA-GEIGY, Basel, Switzerland and RC1, Mettler AG, Greifensee, Switzerland). Such calorimeters have a useful culture volume of 1,6 liters and may 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 (refs. 5-6).

*Kluyveromyces fragilis* NRRL 1109 was grown continuously both aerobically and anaerobically in a standard medium containing initially 15 g/l of glucose (ref. 7). *Saccharomyces cerevisiae* CBS 426 was cultivated on 20 g/l of glucose using dilution rates ranging from 0.05 $h^{-1}$ to 0.35 $h^{-1}$.

## 4. RESULTS

### 4.1 Aerobic growth of *K. fragilis*

Figure 1 demonstrates that $\eta_H$ and $\eta_G$ are virtually equal in aerobic growth, whatever their actual values are. The reason for this lies in the near-equality of $\Delta_r H^o$ and $\Delta_r G^o$, which have been calculated for fully aerobic growth of *K. fragilis* as a function of the biomass yield and plotted as broken and solid lines, respectively in Figure 1. At zero biomass yield, the intercepts of these two 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 decreases because some of the energy is retained in the biomass. Except for $Y'_{X/S}$ close to unity, the $T\Delta_r S^o$ value separating the two lines is almost negligeable compared with either $\Delta_r G^o$ or $\Delta_r H^o$. Hence, $\Delta_r G^o$ may quite accurately be measured in a calorimeter, by recording the heat dissipated per C-mole of glucose consumed, which corresponds of course to the negative value of $\Delta_r H^o$. For the same reason $\eta_G$ and $\eta_H$, which were computed as a function of $Y'_{X/S}$ by means of Equation (1) and (2), also nearly coincide.

Energetic growth efficiencies were computed from continuous culture data of *K. fragilis* (ref. 7) both from measured biomass yields and Equation (1), and from direct calorimetry data. The shaded points appearing on

Figure 1 indicate the two values on the x on the y axes, respectively. As may be seen, both $\eta_H$ and $\eta_G$ amount to about 0.6 in all experiments.

### 4.2 Oxydo-reductive growth of *S. cerevisiae*

In order to induce the different mixes between respiratory and fermentative catabolism during continuous cultures of *S. cerevisiae*, nitrogen supply was limited in addition to the carbon supply. By feeding nitrogen at a constant flow rate but varying the flow rate of the carbon containing medium, the N/C supply rates were reduced from 0.167 to 0.032 (g/g) and from 0.067 to 0.020 in two sets of experiments. Lowering the N/C supply progressively first resulted in a decreasing steady state concentration of $NH_4^+$, as shown in Figure 2, until it became limiting. Decreasing the N/C ratio in the feed further forced the culture to reduce its biomass yield correspondingly, either by fermenting an ever increasing part of the glucose supply or by oxidizing it by decoupled respiration. Figure 2 clearly shows the decrease in $Y'_{X/S}$ and the onset of fermentation, reflected in an increasing ethanol yield.

Figure 3 shows the effect of the N/C ratio on the heat dissipated per C-mole biomass growth $Y'_{X/S}$ and on $\eta_H$ and $\eta_G$. As limiting N concentrations are approached, more heat is dissipated although no ethanol is formed yet. In this region, $Y'_{X/S}$ is reduced by uncoupled respiration which is clearly reflected by the decreasing $\eta_H$ as calculated from calorimetry data using Eq. (4). As soon as fermentative metabolism sets in, $Y'_{Q/X}$ decreases and $\eta_H$ increases again. In this progressively "anaerobic" or fermentative domain $\Delta_r H^o$ and $\Delta_r G^o$ are starting to diverge. Therefore, $\eta_G$ has to be computed from $Y'_{X/S}$ data based on Eq. (2). It agrees well with calorimetric $\eta_H$ values in the respiratory region, but tends to a value of about 0.5 in the oxido-reductive metabolism. The slight reduction also results from partially decoupled respiration.

### 4.3 Anaerobic growth

In purely fermentative growth, the enthalpy and the free energy change associated with the overall growth reaction are so small that the positive $T\Delta_r S^o$ term introduces a very large difference between the two (Fig. 4). Since $\Delta_r G^o$ is a factor of 3 to 4 more negative than $\Delta_r H^o$, rapid growth can spontaneously occur but it will dissipate only very little heat. Energetic efficiencies computed from calorimetry using Eq. (4) are thus close to unity and have no real meaning. It is thus necessary to evaluate $\eta_G$ from biomass yields using Eq. (2), a plot of which appears in Figure 4 for growth of *K. fragilis* on glucose. Two values of $\eta_G$ based on actually measured yields are also given. They demonstrate that $\eta_G$ remains at about 60% despite relatively low biomass yields.

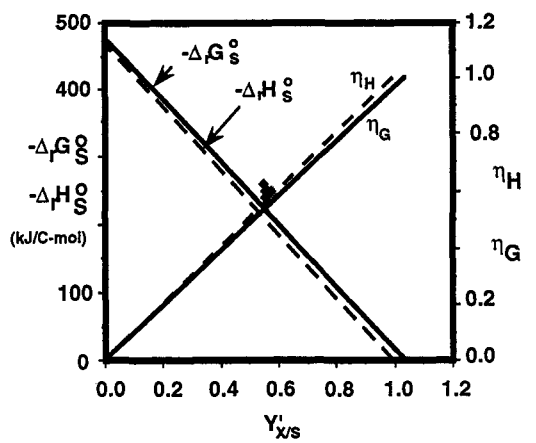


Figure 1. Standard enthalpy change, enthalpic growth efficiency (broken lines), free energy change and free energy growth efficiency (solid lines) for aerobic growth of *K. fragilis* yeast as a function of biomass yield. The shaded points represent calorimetrically measured enthalpic growth efficiencies.

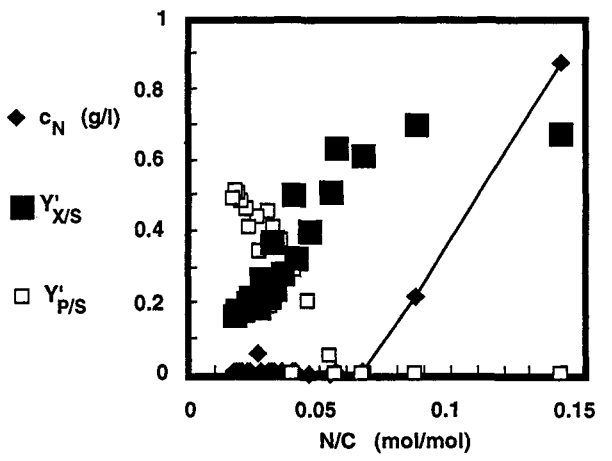


Figure 2. Free ammonia concentration (◆), C-molar biomass yield (■), and C-molar ethanol yield ( □ ) in dually nitrogen/carbon limited chemostat cultures of *S. cerevisiae*.

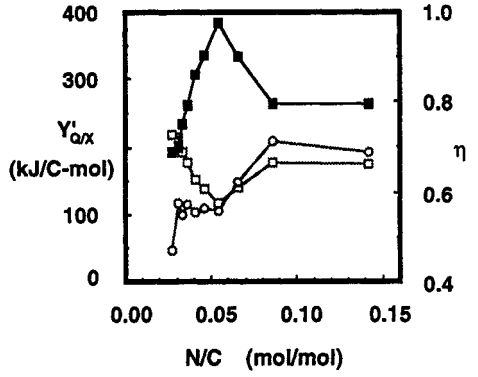


Figure 3. Energetic growth efficiencies for growth of *S.cerevisiae* according to a mixed oxido-reductive catabolism. ■ = $Y'_{Q/X}$; □ = $\eta_H$; O = $\eta_G$.

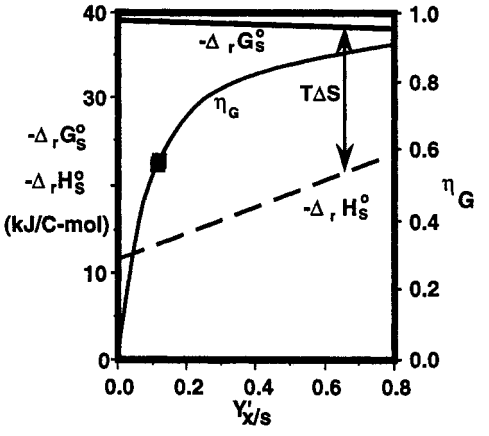


Figure 4. Standard enthalpy and free energy change and free energy growth efficiency during fermentative growth of *K. fragilis* as a function of biomass yield. ■ = Energetic growth efficiency as calculated from measured biomass yields.

## 5. CONCLUSIONS

Yeast growth on glucose according to various proportions of respiratory and fermentative catabolism always occurs with a similar energetic efficiency $\eta_G$ of around 50-60%. This would be expected if growth yields are fundamentally determined by a free energy dissipation per unit biomass which is constant for a given type of carbon source (ref. 1).

In respiratory growth, the $T\Delta_rS^o$ term is so small in comparison with either $\Delta_rG^o$ or $\Delta_rH^o$, that the enthalpy and the free energy efficiencies are virtually the same. They then can conveniently be determined on-line from direct calorimetry. This is not the case any more as soon as an important fraction of the metabolism is fermentative, for which the energetic growth efficiency must be computed from a free energy balance. Calorimetry remains, however, a useful tool in microbial energetics for detecting such features as decoupled respiratory growth.

## LIST OF SYMBOLS

| | |
|---|---|
| $\Delta_cH_i^o$ | Standard enthalpy of combustion of i, kJ/C-mol |
| $\Delta_c{*}H_i^o$ | Modified standard enthalpy of combustion, yielding N in the form of $NH_3$, kJ/C-mol |
| $\Delta_rH_i^o$ | Standard enthalpy change of the growth reaction per C-mol of i, kJ/C-mol |
| $\Delta_c{*}G_i^o$ | Analogous quantities to the above three, but defined for G |
| $\Delta_cG_i^o$ | " |
| $\Delta_rG_i^o$ | " |
| $r_s$ | rate of substrate consumption, C-mol $s^{-1}$ $m^{-3}$ |
| $Y_{i/j}$ | C-molar stoichometric coefficient or yield of i per mol of j |
| $\eta H$ | Enthalpic growth efficiency |
| $\eta G$ | Free energy growth efficiency |

Subscripts

| | |
|---|---|
| S | Carbon and energy substrate |
| P | Ethanol |
| Q | Heat |
| X | Biomass |

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