



## Thermodynamic analysis of *Methanobacterium thermoautotrophicum* <sup>☆</sup>

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### Abstract

A thermodynamic analysis based on the energy efficiencies of the growth of *Methanobacterium thermoautotrophicum* is proposed. By taking into account the thermodynamic and biochemical constraints, the range of possible biomass yields can be determined. One of the main reasons for the low free-energy efficiencies is the high energy requirements of the anabolic processes.

*Keywords:* *Methanobacterium*; Thermodynamics

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### List of symbols

- $Y'_{ij}$  yield coefficient in (C-mol of  $i$  per C-mol of  $j$ )  
 $\Delta_c G_i^\circ$  standard free energy of combustion of  $i$  in (kJ C-mol<sup>-1</sup>)  
 $\Delta_r G^\circ$  standard free energy of the growth reaction in (kJ per mol H<sub>2</sub> consumed)  
 $\Delta_c H_i^\circ$  standard enthalpy of combustion of  $i$  in (kJ C-mol<sup>-1</sup>)  
 $X$  biomass (C-mol)  
 $\gamma_i$  general degree of reduction, Eq. (5)  
 $\eta_G$  free-energy efficiency Eq. (1b)  
 $\eta_H$  enthalpic efficiency, Eq. (1a)

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*Subscripts*

C	carbon source (CO <sub>2</sub> )
D	electron donor (H <sub>2</sub> )
N	nitrogen source (NH <sub>3</sub> )
P	product (CH <sub>4</sub> )
S	substrate
W	water
X	biomass

**1. Introduction**

The transformation of CO<sub>2</sub> and H<sub>2</sub> into CH<sub>4</sub> delivers the energy necessary for growth of *Methanobacterium thermoautotrophicum*. As the substrates for cell synthesis are small molecules (H<sub>2</sub> and CO<sub>2</sub>) there is a large decrease in entropy during anabolic processes. A large amount of heat (10–50 times higher than for other micro-organisms) is therefore produced during the growth process: up to 11 W per gram of biomass in batch cultures [1]. In addition to the unusual exothermicity of the growth process, small biomass yields ( $Y'_{X/CO_2}$ ) of less than 0.1 C-mol mol<sup>-1</sup> are measured. These factors make this microbial system a very interesting object for thermodynamic analysis.

Thermodynamic analysis of microbial systems is a key factor in understanding the dynamics of microbial processes, as a knowledge of the thermodynamic limits of such a process allows the maximal biomass and product yields to be predicted. In this paper we give an analysis of the thermodynamic efficiency of *M. thermoautotrophicum*.

**2. Energetic efficiencies**

One way to analyse a microbial system from the thermodynamic point of view is to use energy efficiencies. Definitions of energy efficiencies have been proposed by several authors [2–4] and discussed recently by von Stockar and Marison [5]. The definitions proposed by Roels [2] is used in the following discussion:

Enthalpic growth efficiency:

$$\eta_H = \frac{Y'_{XIS} \cdot \Delta_c H_X^\circ}{\Delta_c H_S^\circ - Y'_{PIS} \cdot \Delta_c H_P^\circ} \quad (1a)$$

Free-energy growth efficiency:

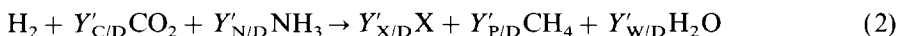
$$\eta_G = \frac{Y'_{XIS} \cdot \Delta_c G_X^\circ}{\Delta_c G_S^\circ - Y'_{PIS} \cdot \Delta_c G_P^\circ} \quad (1b)$$

where X, S and P stand for biomass, substrate, and product, respectively. The physiologically dead state (CO<sub>2</sub>, H<sub>2</sub>O and NH<sub>3</sub>) was taken as the reference state for the enthalpies of combustion ( $\Delta_c H_i^\circ$ ) and free energies of combustion ( $\Delta_c G_i^\circ$ ).

In contrast to most other microbial systems, the carbon and electron sources for *M. thermoautotrophicum* are two different substrates. The carbon source ( $\text{CO}_2$ ) is completely oxidised and therefore has to be reduced during catabolic as well as anabolic processes, which implies energy consumption in both cases. As a consequence, one has to consider the energy (or electron) source in the thermodynamic analysis. The general S, standing for substrate in Eqs. (1) and (2), should then more correctly be denoted by D for “electron donor”.

The second law of thermodynamics implies that the free-energy growth efficiency ( $\eta_G$ ) cannot exceed unity: this is an absolute constraint for the microbial system. However, enthalpic growth efficiency is not bound to an absolute limit and endothermic growth can, in theory, be observed for  $\eta_H > 1$ .

The energy efficiencies can be related directly to the enthalpy and free energy of the growth reaction ( $\Delta_r H^\circ$  and  $\Delta_r G^\circ$ ) by taking into account the energy balances for the specific growth reaction. Thus, for the general stoichiometry for the growth of *M. thermoautotrophicum*



the following energy balances are obtained:

$$\Delta_c H_D^\circ - Y'_{\text{P/D}}\Delta_c H_P^\circ = Y'_{\text{X/D}}\Delta_c H_X^\circ + \Delta_r H^\circ \quad (3a)$$

$$\Delta_c G_D^\circ - Y'_{\text{P/D}}\Delta_c G_P^\circ = Y'_{\text{X/D}}\Delta_c G_X^\circ + \Delta_r G^\circ \quad (3b)$$

Here, the terms for  $\text{CO}_2$ ,  $\text{NH}_3$  and  $\text{H}_2\text{O}$  disappear, as their enthalpy and free-energy of combustion are equal to zero. Combining Eqs. (1a) and (1b) with Eqs. (3a) and (3b), respectively, yields the following relations between  $\eta$  and the enthalpy or free-energy change of the entire growth reaction:

$$\eta_H = \frac{Y'_{\text{X/D}} \cdot \Delta_c H_X^\circ}{\Delta_r H^\circ + Y'_{\text{X/D}} \cdot \Delta_c H_X^\circ} \quad (4a)$$

$$\eta_G = \frac{Y'_{\text{X/D}} \cdot \Delta_c G_X^\circ}{\Delta_r G^\circ + Y'_{\text{X/D}} \cdot \Delta_c G_X^\circ} \quad (4b)$$

Eq. (4b) shows that the free energy of reaction ( $\Delta_r G^\circ$ ) must be zero at maximum efficiency. This means that the growth reaction would be at equilibrium and thus could only proceed at an infinitely slow rate.

### 3. Degree of reduction $\gamma$ balances

The thermodynamic constraint for the metabolism  $\eta_G = 1$  sets the upper limit for the relationship between biomass and product yield. The degree of reduction  $\gamma$  balance determines the biochemical constraint on metabolism and gives the relationship between biomass and product yield. The generalised degree of reduction  $\gamma$  indicates the number of electrons released during complete oxidation of the substance  $i$ . For 1 C-mol of substance  $i$  it is given by

$$\gamma_i = 4 + \text{H}_i - 2\text{O}_i - 3\text{N}_i \quad (5)$$

where  $H_i$ ,  $O_i$  and  $N_i$  indicate the total number of hydrogen, oxygen, and nitrogen atoms, respectively, in substance  $i$  per one atom of carbon.

The degree of reduction  $\gamma$  in the observed system must be conserved, regardless of the kinds of processes going on. On the basis of the growth stoichiometry one can therefore write a degree of reduction balance  $\gamma$  for the system. The degree of reduction  $\gamma$  balance for the growth reaction of *M. thermoautotrophicum* (Eq. (2)) can be written as

$$\gamma_D + \gamma_C Y'_{C/D} + \gamma_N Y'_{N/D} = \gamma_X Y'_{X/D} + \gamma_P Y'_{P/D} + \gamma_W Y'_{W/D} \quad (6)$$

Eq. (6) can be simplified considerably because the degrees of reduction  $\gamma$  for  $\text{CO}_2$ ,  $\text{NH}_3$  and  $\text{H}_2\text{O}$  are zero

$$\gamma_D = \gamma_X Y'_{X/D} + \gamma_P Y'_{P/D} \quad (7)$$

The relationship between  $Y'_{X/D}$  and  $Y'_{P/D}$  can be easily deduced from Eq. (7)

$$Y'_{P/D} = \frac{\gamma_D}{\gamma_P} - \frac{\gamma_X}{\gamma_P} \cdot Y'_{X/D} \quad (8)$$

#### 4. Materials and methods

Energy efficiencies for *M. thermoautotrophicum* were calculated with data obtained during continuous cultures, which were performed in a 2 l bench-scale calorimeter (RC1, Mettler Toledo, Switzerland) at 60°C and 1000 rpm. The bacteria were grown in a mineral medium [6].  $\text{H}_2$  and  $\text{CO}_2$  were provided by the gas stream at 0.5 vvm and at a ratio of 4:1. Biomass was determined using a dry-weight analysis and the off-gas composition was analysed using a gas chromatograph (Shimadzu GC-14B). The dissolved  $\text{H}_2$  concentration was followed continuously with a probe developed in our laboratories [7].

The elemental composition of *M. thermoautotrophicum* determined by Gerhard et al. [6] was  $\text{CH}_{1.58}\text{O}_{0.44}\text{N}_{0.24}$ , with a degree of reduction of 3.98.

#### 5. Thermodynamic and biochemical limits for *M. thermoautotrophicum*

The energy efficiencies and the degree of reduction balance for *M. thermoautotrophicum* are displayed in Fig. 1 as a function of the biomass and the product yield ( $Y'_{X/D}$  and  $Y'_{P/D}$ ). The values for the enthalpies and free energies of combustion ( $\Delta_c H_i^\circ$  and  $\Delta_c G_i^\circ$ ) were taken from Sandler and Orbey [8]. The thick dotted line in Fig. 1 represents the relationship between  $Y'_{X/D}$  and  $Y'_{P/D}$ , as derived from the degree of reduction balance and presented in Eq. (8). It is the absolute biochemical constraint and all data points have to lie on this curve. The plain line in Fig. 1 indicates the relationship between  $Y'_{X/D}$  and  $Y'_{P/D}$ , for which  $\eta_G$  is equal to unity. This is the thermodynamic maximum efficiency at which a microbial process can run and all data points have to lie on or below this curve. The maximum obtainable

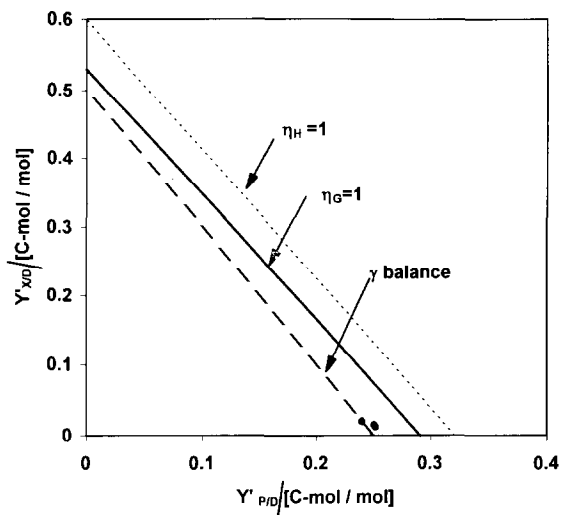


Fig. 1. Thermodynamic and biochemical limits for the growth of *M. thermoautotrophicum*. (—) Theoretical calculation of the free-energy efficiency  $\eta_G = 1$ . (···) Theoretical calculation of the enthalpic efficiency  $\eta_H = 1$ . (---) Theoretical calculation of the degree of reduction balance. (●) Experimental data.

biomass yield ( $Y'_{X/D}^{\max}$ ) is given by the intercept of the degree of reduction balance curve, as there is no intersection between the two constraint lines. Possible biomass yields for the growth reaction of *M. thermoautotrophicum* thus range from 0.0 to 0.5 C-mol per mol of hydrogen consumed.

In addition to the absolute thermodynamic and biochemical limits for *M. thermoautotrophicum*, Fig. 1 shows the curve for  $\eta_H = 1$  (thin dotted line). This curve is always above that of the maximum free-energy efficiency ( $\eta_G = 1$ ), which means that an endothermic growth reaction will never occur in this microbial system. The significant difference between  $\eta_G$  and  $\eta_H$  for the same biomass yield reflects the large difference between  $\Delta_r G^\circ$  and  $\Delta_r H^\circ$  due to the large negative entropy term that accompanies the growth reaction. The entropy decrease occurs because cell synthesis starts with a carbon source ( $\text{CO}_2$ ) containing only one carbon atom.

Table 1 lists the experimental and theoretical efficiencies and biomass yields for *M. thermoautotrophicum*. The free-energy efficiencies calculated with the data obtained from continuous cultures are low compared with the value of 0.6 obtained for many other micro-organisms [5], and lie between 0.18 and 0.23. One reason for this is that the slope of the maximum free-energy efficiency line  $\eta_G = 1$  in Fig. 1 is very low. The intercept of that line, corresponding to the ratio of  $\Delta_c G_D^\circ$  to  $\Delta_c G_X^\circ$ , is small because  $\Delta_c G^\circ$  of the energy donor is much lower than  $\Delta_c G^\circ$  of the biomass. Because of this the maximum possible biomass yield is decreased considerably. The same observation can be made for the degree of reduction balance: the value of the intercept at zero product yield, which represents the biochemical maximum biomass

Table 1

Comparison between real and theoretical maximum biomass yields for the growth of *M. thermoautotrophicum*

	$Y'_{X/D}$ /(C-mol mol <sup>-1</sup> )	$Y'_{X/C}$ /(C-mol mol <sup>-1</sup> )	$\eta_G$
Theoretical maximum	0.250	0.67	1.00
Experimental value	0.015	0.05	0.25

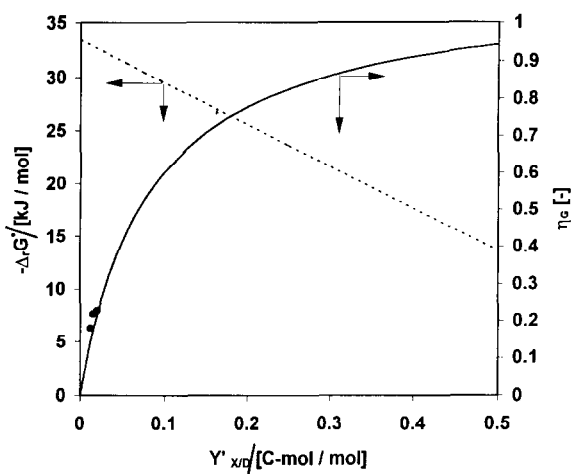


Fig. 2. Energy growth efficiency and free-energy of growth reaction as a function of the biomass yield  $Y'_{X/D}$  for the growth of *M. thermoautotrophicum*. (—) Theoretical calculation of the free-energy efficiency. (····) Theoretical calculation of the free-energy of growth reaction. (●) Experimental data.

and is equal to the relationship between  $\gamma_D$  and  $\gamma_X$ , is again low compared with other micro-organisms.

Thus, because of the low values of  $\Delta_c G_D^\circ$  and  $\gamma$  of the energy donor, the range of possible biomass yields is small for *M. thermoautotrophicum* and a small increase in biomass yield results in a significant drop in the free energy of the growth reaction. The dependency of  $\Delta_r G^\circ$  and  $\eta_G$  on the biomass yield  $Y'_{X/D}$  is shown in Fig. 2. The product yield  $Y'_{P/D}$  in Eq. (3b) was substituted with Eq. (8) for the calculation of  $\Delta_r G^\circ$  as a function of the biomass yield. Similarly, the term  $Y'_{P/D}$  in Eq. (1b) was replaced by Eq. (8) in order to calculate  $\eta_G$  as a function of  $Y'_{X/D}$ . The free energies of reaction for the experimentally determined biomass yields lie between 33.0 and 32.7 kJ per mole of hydrogen consumed. Fig. 2 clearly shows that  $\eta_G$  for *M. thermoautotrophicum* could be much higher without a considerable drop in  $\Delta_r G^\circ$ :  $\eta_G$  increases rapidly up to a biomass yield of 0.1, though the free energy of the growth reaction decreases linearly. The reason for the significant discrepancy between the real and possible free-energy efficiency cannot be explained through the present analysis, but is due to the large amount of free-energy dissipation required for cell

synthesis which, unlike in most other micro-organisms, starts with a one carbon atom compound.

## 6. Conclusions

The low biomass yields measured during the growth of *M. thermoautotrophicum* can partly be explained through thermodynamic analysis. Contrary to most other microbial systems, the energy donor has a low free energy of combustion and degree of reduction, which lowers the maximum possible biomass yield considerably. The fact that the free-energy efficiency of *M. thermoautotrophicum* is still lower than for most other microbial systems, cannot be explained by the present thermodynamic analysis, but reflects the high energy requirements of this bacterium for cell synthesis based on a one carbon atom compound.

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