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Endothermic microbial growth. A calorimetric investigation of an extreme case of entropy-driven microbial growth*

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Abstract: Life is almost always associated with the generation of heat. Thus far, all chemotrophic life forms that have been studied in calorimeters were found to be exothermic. Certain literature reports have even cast doubt on the existence of endothermic growth, even though thermodynamic principles do not rule it out. The present report describes the first experiments demonstrating the actual existence of chemotrophic life forms that take up heat rather than produce it.

HEAT GENERATION AND THE DRIVING FORCE FOR MICROBIAL GROWTH

Heat exchange between living cells and their environment is a universal phenomenon. Life is almost always associated with the generation of heat. This is already evident in expressions in everyday language: We tend to refer to the "warmth" of living things as opposed to the "cold inanimate world". Although from a scientific point of view there is no reason why organisms could not absorb heat and thus cool down their environment [1], the actual existence of endothermic chemotrophic growth has been considered unlikely [2–4] in the scientific literature. More recently, Heijnen and van Dijken [5] have, however, predicted on the basis of a theoretical energy balance that acetotrophic methanogenesis could be a net heat-uptake process.

The driving force for microbial growth is the change of Gibbs energy occuring in the growth medium as a result of microbial metabolism. Growing microorganisms consume high Gibbs energy foodstuffs and release waste products of low Gibbs energy. It can be shown that the decrease of Gibbs energy resulting from this exchange of metabolites reflects the total rate of entropy production generated by all the irreversible processes that make life and growth possible:

$$-T\dot{S}_{prod} + \sum_i \mu_i \cdot \dot{n}_i = 0 \qquad (1)$$

On average, the products must have a lower Gibbs energy than the substrates, even though one of the "products" of metabolism is new biomass.

The driving force for growth is thus the dissipation of Gibbs energy and may be described as a ΔG for the overall growth reaction. It must be negative for growth to occur. The heat release of the growth reaction depends on the respective ΔH value. Although, as already pointed out, there is no thermodynamic constraint that would prevent ΔH from being positive, it is negative in an overwhelming majority of cases and thus contributes to the driving force according to

$$\Delta_r G = \Delta_r H - T\Delta_r S \qquad (2)$$

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In the case of aerobic, respiratory growth $\Delta_r H$ makes up practically 100% of the driving force and $T\Delta_r S$ is close to zero. This could be called enthalpy-driven, entropy-neutral growth [6]. In autotrophic methanogenesis $T\Delta_r S$ is even negative and must be compensated by an enthalpy change of a very large negative value. This growth form is therefore extremely exothermic and could be called "entropy retarded" [6].

In fermentative growth, no electron acceptor is involved, and the dissipation of Gibbs energy is brought about by a degradation of one substrates molecule into several smaller molecules. In such reactions, the entropy increase provides a substantial part of the driving force, and the rate of heat generation of such microorganisms is quite low.

The theoretical analysis of the acetotrophic methanogenesis, which has been predicted to be endothermic [5], shows that this type of metabolism is accompanied by a particularly large positive entropy change, resulting from the degradation of acetate into two gaseous molecules, CH_4 and CO_2 [6]. The very large $T\Delta S$ term makes the ΔG negative despite the fact that the process yields products (CH_4 and CO_2) that contain more energy than the substrate, thus rendering ΔH positive. This extremely entropy-driven form of microbial growth could be called "enthalpy retarded" [6]. The experimental work described in this contribution was aimed at demonstrating that this form of microbial growth is endothermic by direct calorimetry.

EXPERIMENTAL

Acetotrophic methanogenesis of *Methanosarcina barkeri* is an anaerobic growth process using acetic acid as carbon and energy source and producing both methane and CO_2. The experiments were carried out in a modified bench-scale reaction calorimeter (Bio-RC1) of a very high sensitivity (5 mW/L) [7]. This calorimeter functions as a normal laboratory fermentor of 2 liters working volume, in which all cultivation parameters such as pressure, temperature, pH, nutrient concentrations and so on can be tightly controlled, and thus, cells can grow under defined biological conditions [3,7]. In this work, the temperature was kept at 37 °C and pH at 6.8. The culture was followed by measuring heat evolution, the biomass formation, the acetic acid consumption, and the product (CH_4 and CO_2) generation during the growth of *M. barkeri*.

RESULTS

After a lag phase, growth was visible from the rise of dry biomass concentration, which was measured off-line by gravimetry. The heat evolution rate, however, decreased continuously from zero into negative values. It paralleled perfectly the rates of CH_4 and CO_2 evolution and also the appearance of dry biomass. By plotting the cumulative amounts of biomass, CH_4 and CO_2 formed against the cumulative amount of acetate consumption approximately linear correlations were obtained, the slopes of which indicate the respective yields (Fig. 1). The biomass yield was determined to be 0.043 C-mol biomass formation per C-mol acetate consumption, which was very close to the reported values in the literature [8]. The CH_4 and CO_2 yields were 0.49 and 0.52 C-mol, respectively. The heat yield (i.e., the enthalpy change per C-mol or gram biomass formed) was determined to be +145 kJ/C-mol (+5.2 kJ/g). This value was in general agreement with the calculated value based on an enthalpy balance of the growth process in which the gaseous state was taken as the final state for the products, CH_4 and CO_2. Full details of this experiment are published elsewhere [9].

DISCUSSION AND CONCLUSION

In the calorimetric experiment, the system boundary under study was the culture vessel. Thus, the measured heat also included the vaporization of volatile products, CH_4 and CO_2. Therefore, the question arises whether the result would be the same for the actual growth process where the cell surface is the sys-

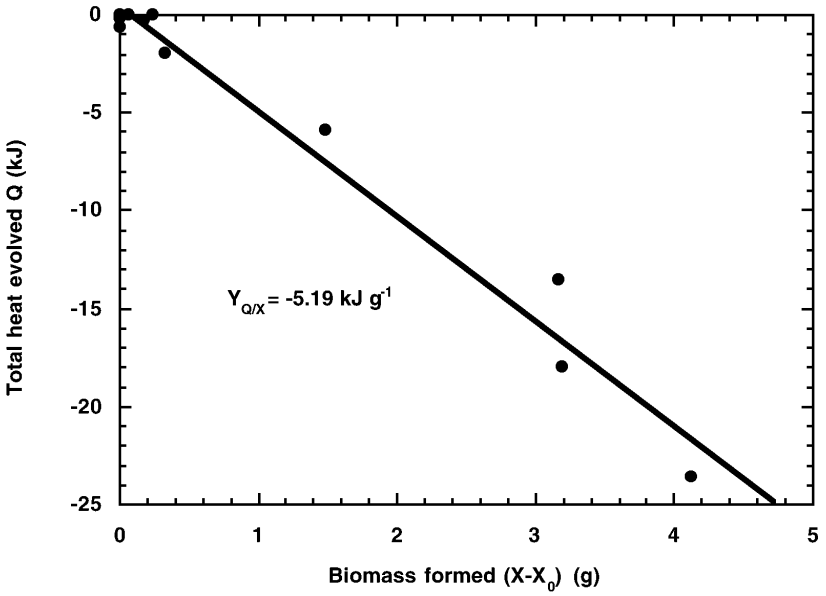

Fig. 1 Determination of heat yield for acetotrophic methanogenesis by *M. barkeri*. Reprinted with permission [8].

tem boundary, and which might or might not include the evaporation, depending on what one considers the physiologically correct state of the end products to be.

Since this experiment cannot be done in a calorimeter, the effects must be estimated based on a calculation. This may be done by comparing the ΔH values for the catabolic or the energy-yielding process alone, as anabolism hardly contributes anything to the ΔH or ΔG, due to the low biomass yield [6,10]. The values for ΔH^0_{cat} and ΔG^0_{cat} evaluated using the cell surface as the system boundary and assuming various states in which the products might be released are reported in Table 1. Process 1 is the reference case. Since the standard states assumed for the products are those at which they left the calorimeter, Process 1 corresponds to the calorimetric measurement.

It turns out that the evaporation of CH_4 and CO_2 is indeed a major reason for the heat uptake during growth. If both methane and carbon dioxide could be released from the cell in the aqueous state (Process 3), the metabolism of growth would be slightly exothermic. However, the Gibbs energy change of such a process would be so close to zero that growth at a nonzero rate would be virtually impossible. An analysis of a large number of published growth stoichiometries has shown that even the most "growth-efficient" organisms dissipate a minimum of about 300 kJ of Gibbs energy per C-mol biomass grown [5,11]. The ΔG^0_{cat} of Process 3 would thus allow a maximum biomass yield of half of what we observed. Also, the rate of making Gibbs energy available for microorganisms is limited by a maximum rate of electron transport in the catabolic energy production. According to a thermodynamic correlation for maximum specific growth rate (μ^{max}) proposed by Heijnen [11], μ^{max} of the growth reflecting Process 3 is estimated to be nearly zero, which implies that from the point of view of bioenergetics, the $\Delta_r G^0_{cat}$ of conversion from acetate to aqueous CH_4 and HCO_3^- is too low to drive any realistic anabolic processes, or the growth would be infinitesimally slow.

Judging from the literature [5,12,13], the most physiologically probable states are the ones indicated in Process 2 rather than the ones defined by Process 3. Thus, if one assumes that the carbon dioxide stays in the aqueous phase as biocarbonate ions, but that methane is evaporated immediately upon its production by the cell, growth would still take up heat, although only about a third of what was experimentally measured (Table 1). However, the Gibbs energy change of this growth process is sufficiently negative to support growth.

Table 1 ΔH^0_{cat} and ΔG^0_{cat} for catabolism assuming different standard states.

Catabolic process	ΔH^0_{cat}(kJ mol^{-1})	ΔG^0_{cat}(kJ mol^{-1})
1) $Ac^-(aq) + H^+(aq) \rightarrow CH_4(g) + CO_2(g)$	+16.9	–35.9
2) $Ac^-(aq) + H_2O(l) \rightarrow CH_4(g) + HCO_3^-(aq)$	+5.0	–31.0
3) $Ac^-(aq) + H_2O(l) \rightarrow CH_4(aq) + HCO_3^-(aq)$	–9.0	–14.7

We therefore conclude that growth is only possible if at least CH_4 is evacuated from the cells by evaporation. This is a major reason for the observed heat uptake during growth, but the evaporation also generates the entropy increase necessary for providing a reasonable driving force for growth.

The corresponding enthalpy change must, therefore, also be counted as part of the catabolism, and the growth process must be viewed as endothermic.

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