

Coupling of *Methanothermobacter thermautotrophicus* Methane Formation and Growth in Fed-Batch and Continuous Cultures under Different H₂ Gassing Regimens^{∇†}

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In nature, H₂- and CO₂-utilizing methanogenic archaea have to couple the processes of methanogenesis and autotrophic growth under highly variable conditions with respect to the supply and concentration of their energy source, hydrogen. To study the hydrogen-dependent coupling between methanogenesis and growth, *Methanothermobacter thermautotrophicus* was cultured in a fed-batch fermentor and in a chemostat under different 80% H₂–20% CO₂ gassing regimens while we continuously monitored the dissolved hydrogen partial pressures (p_{H_2}). In the fed-batch system, in which the conditions continuously changed the uptake rates by the growing biomass, the organism displayed a complex and yet defined growth behavior, comprising the consecutive lag, exponential, and linear growth phases. It was found that the in situ hydrogen concentration affected the coupling between methanogenesis and growth in at least two respects. (i) The microorganism could adopt two distinct theoretical maximal growth yields ($Y_{\text{CH}_4 \text{ max}}$), notably approximately 3 and 7 g (dry weight) of methane formed mol^{−1}, for growth under low ($p_{\text{H}_2} < 12$ kPa)- and high-hydrogen conditions, respectively. The distinct values can be understood from a theoretical analysis of the process of methanogenesis presented in the supplemental material associated with this study. (ii) The in situ hydrogen concentration affected the “specific maintenance” requirements or, more likely, the degree of proton leakage and proton slippage processes. At low p_{H_2} values, the “specific maintenance” diminished and the specific growth yields approached $Y_{\text{CH}_4 \text{ max}}$, indicating that growth and methanogenesis became fully coupled.

Most methanogenic archaea, including the *Methanothermobacter thermautotrophicus* used in the present study, derive their energy for autotrophic growth from the H₂-dependent reduction of CO₂ into methane. The pathways of methane formation, CO₂ fixation, and ATP synthesis are highly conserved among the different H₂-utilizing (hydrogenotrophic) methanogens (for reviews, see references 5, 6, 9, and 32 and additional information in the supplemental material). Nevertheless, different species display remarkable differences in specific growth yields (Y_{CH_4}), i.e., the amount of biomass formed per mole of methane produced at a given growth condition (Table 1). Y_{CH_4} values can be variable for a given species. Even maximal growth yields ($Y_{\text{CH}_4 \text{ max}}$) seem to differ. $Y_{\text{CH}_4 \text{ max}}$ represents the theoretical maximal growth yield that would be obtained if methanogenesis and growth are fully coupled.

Methanogens have to couple the processes of energy generation (methanogenesis) and biomass formation under highly diverse concentrations of their energy source, hydrogen. In environments such as anaerobic sediments and sewage digestors, hydrogen formed by obligate proton reducers is available at only very low levels (11, 37). In contrast, hydrogen concentrations can be high at sites where methanogens obtain the gas from H₂-producing fermentative microorganisms (29, 37). Un-

der laboratory conditions, the hydrogen availability of the cells depends on the gassing rates applied and the hydrogen-mass transfer capacity of the fermentative devices. In fed-batch systems, dissolved hydrogen partial pressures (p_{H_2}) continuously change over time as the result of increasing consumption rates by a growing biomass. Many authors observed that specific growth yields were relatively low when growth proceeded under hydrogen excess and that yields were highest under conditions of hydrogen limitation (7, 8, 12, 14, 18, 24, 27, 33, 34). Apparently, the degree of coupling between methanogenesis and growth depends on the in situ hydrogen concentration. In these studies, hydrogen-excess and hydrogen-limited conditions were imposed by changing the gassing rates or medium agitation. Unfortunately, with notable exceptions (12, 24), the hydrogen concentrations were not actually measured.

To investigate how methanogenesis and the growth of *M. thermautotrophicus* were coupled, we cultured the organism under a variety of hydrogen gassing regimens, while continuously recording the p_{H_2} value. We did so both in a fed-batch fermentor system, where conditions continuously change, and under the controlled conditions of a chemostat. In the fed-batch system the organism displayed a complex growth behavior comprising different growth phases that were each characterized by the distinct way that specific growth rates, growth yields, and methane-forming activities were interrelated. Both the fed-batch and the chemostat studies substantiated previous suggestions that specific growth yields depended on the dissolved hydrogen partial pressures and increased with decreasing p_{H_2} values. Quite remarkably, our work also suggests that *M. thermautotrophicus* may adopt two different maximal growth yields for growth under low- and high-hydrogen conditions.

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