

TABLE 3. Growth properties of *Methanothermobacter thermautotrophicus* in the fed-batch fermentor^a

| Culture condition | | | Exponential phase | | | | Linear phase | |
|----------------------------------|-------------|------|-------------------------------|---|--|---|----------------------|--|
| v_{in} (ml min ⁻¹) | Vol (liter) | Mode | μ_{ex} (h ⁻¹) | $q_{CH_4\max}$ (mol g ⁻¹ h ⁻¹) | Δq_{CH_4} (mol g ⁻¹ h ⁻¹) | $Y_{CH_4\max}$ (g mol ⁻¹ CH ₄) | $p_{H_2\ lin}$ (kPa) | $Y_{CH_4\ lin}$ (g mol ⁻¹ CH ₄) |
| 107 | 2.5 | b | 0.25 | 0.167 | 0.079 | 3.15 | 1 | 3.00 |
| 214 | 2.5 | a | 0.22 | 0.144 | 0.070 | 3.15 | 1 | 3.20 |
| | | b | 0.24 | 0.127 | 0.034 | 7.05 | 4 | 2.20 |
| | | b | 0.25 | 0.161 | 0.037 | 6.75 | 4 | 2.00 |
| 428 | 2.5 | a | 0.23 | 0.174 | 0.034 | 6.70 | 6 | 1.70 |
| | | b | 0.23 | 0.177 | 0.035 | 6.70 | 29 | 1.65 |
| | 2.0 | b | 0.22 | 0.178 | 0.033 | 6.70 | 49 | 1.55 |
| | 1.5 | b | 0.22 | 0.188 | 0.033 | 6.70 | 59 | 1.40 |

^a *M. thermautotrophicus* was cultured at the indicated gassing rates and culture volumes. Abbreviations: v_{in} , gassing rate (80% H₂-20% CO₂ [vol/vol]); μ_{ex} , specific growth rate during exponential growth; $q_{CH_4\max}$, maximal specific methane-forming activity in the exponential phase; Δq_{CH_4} , difference between the maximal and minimal specific methane-forming activity in the exponential phase; $Y_{CH_4\max}$, theoretical maximal growth yield; $p_{H_2\ lin}$, dissolved hydrogen partial pressure during linear growth; $Y_{CH_4\ lin}$, specific growth yield during linear growth. Modes: a, one impeller mounted; b, two impellers mounted. $Y_{CH_4\max}$ and $Y_{CH_4\ lin}$ were calculated as specified in the text and in the supplemental material (1).

time, the slope representing the specific growth rate during the exponential phase (μ_{ex}) (see also the supplemental material [1]). Deviation from linearity was always seen in the period preceding the exponential phase (lag period). Here, perfect straight lines were obtained, if the $\ln(OD_{600})$ values were plotted against the squared times (Fig. 3A, inset). As outlined in the supplemental material, this implies that the specific growth during the lag phase increases linearly in time (Fig. 1B and 2B). Also, the specific methane-forming activity increased linearly in time during this period (Fig. 1B and 2B). In fact, a plot of the q_{CH_4} values against the corresponding μ values showed a direct proportional relationship between both parameters, the slope representing the reciprocal of the specific growth yield (Y_{CH_4}), which now was constant (Fig. 1C, 2C, and 3A). The physiological meaning of these findings is that, after inoculation, cells adapt to the new growth condition by the uniform and concerted acceleration of their specific growth rate and specific methane-forming activity in a way that Y_{CH_4} remains fixed.

The specific growth rates became maximal at the entry of the exponential stage and remained constant throughout the particular stage (Fig. 1B, 2B, and 3A). The values did not significantly differ among the various gassing regimens (0.22 to 0.25 h⁻¹; doubling times of 2.8 to 3.1 h) (Table 3). However, the q_{CH_4} was not constant. Specific methane-forming activities initially increased to a maximum and declined thereafter. The decline occurred concomitant with the decrease in the dissolved hydrogen partial pressures, resulting from the increasing hydrogen uptake rates by the growing biomass (Fig. 1 and 2 and Table 3). Apparently, the physiology of the cells continuously changed, even at a constant growth rate.

After exponential growth, the cells entered the linear phase. During this stage, the specific growth rates and specific methane-forming activities, calculated by using the equations S.5 and S.6, respectively, in the supplemental material(1), decreased in parallel (Fig. 1B and 2B). Moreover, q_{CH_4} -versus- μ plots showed the direct proportional relationship as expected from equation S.8 (Fig. 1C, 2C, and 3B). The slope of the plots, which equals the reciprocal of the specific growth yield (equation S.8), varied with the dissolved hydrogen partial pressure

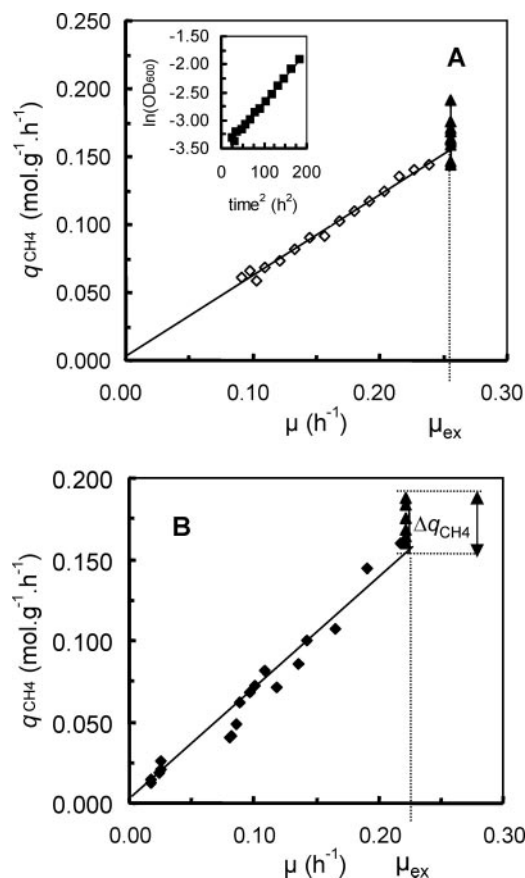


FIG. 3. Relationship between the specific rates of methanogenesis and specific growth rates during the lag (A), linear (B), and exponential growth phases. *M. thermautotrophicus* was cultured in 2.5 (A) and 1.5 (B) liters of mineral medium at a constant gassing rate of 428 ml min⁻¹ in 80% H₂-20% CO₂ (vol/vol). Measurements started 5 h (A) and 8 h (B) after adjustment of the gassing rates. In the inset of Fig. 3A, the $\ln(OD_{600})$ is plotted versus the squared time after inoculation. Lag (\diamond), exponential (\blacktriangle), and linear (\blacklozenge) growth stages are as indicated. μ_{ex} , specific growth rate during the exponential phase; Δq_{CH_4} , change between the maximal and minimal specific activities during the exponential phase.