

Production and Consumption of H₂ during Growth of *Methanosarcina* spp. on Acetate

DEREK R. LOVLEY†* AND JAMES G. FERRY

Department of Anaerobic Microbiology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Received 2 July 1984/Accepted 17 October 1984

***Methanosarcina* sp. strain TM-1 and *Methanosarcina acetivorans* produced and consumed H₂ to maintain H₂ partial pressures of 16 to 92 Pa in closed cultures during growth on acetate. Strain TM-1 produced H₂ continuously when H₂ was continuously removed from the culture. The potential physiological significance of H₂ in acetate metabolism to methane is discussed.**

Methanosarcina barkeri, *Methanosarcina* sp. strain TM-1, and *Methanosarcina acetivorans* synthesize hydrogenase during growth with acetate as the sole substrate (1, 5). Since these organisms consume H₂ with the reduction of carbon dioxide or methanol (1, 16), synthesis of hydrogenase could be constitutive and unrelated to growth on acetate. However, H₂ appears to interact with electron carriers involved in acetate metabolism, since H₂ inhibits methanogenesis from acetate, even in organisms that do not metabolize H₂-CO₂ to methane (10, 16). This study investigated H₂ production and consumption during acetate metabolism to methane in growing cultures of strain TM-1 and *M. acetivorans*, two methanogens that cannot grow on H₂-CO₂ (11, 16).

The media previously used for cultivation of the organisms (4, 11), which contained minerals, vitamins, yeast extract, and trypticase, were modified with the addition of the following to final concentrations (in grams per liter): NaHCO₃, 2.5; and NaCH₃COO · 3H₂O, 13.6. The gas phase was N₂-CO₂ (50:50). *M. acetivorans* was cultured in 11-ml volumes in anaerobic pressure tubes sealed with butyl rubber stoppers and aluminum crimps (Bellco Glass, Inc.). Strain TM-1 was cultured in 110-ml volumes in 160-ml serum bottles which were sealed as described above. The cultures were incubated in a horizontal position with slight agitation on a wrist action shaker at 50 and 40°C for strain TM-1 and *M. acetivorans*, respectively. H₂ and CH₄ were quantified by gas chromatography with a thermal conductivity detector (3) with an oven temperature of 110°C. Culture absorbance of *M. acetivorans* was read directly in a Bausch & Lomb Spectronic 20. Clumping of strain TM-1 prevented accurate absorbance measurements.

Strain TM-1 and *M. acetivorans* produced H₂ during growth on acetate (Fig. 1). H₂ increased with the initiation of growth and methane production, reached a maximum, and then declined. The H₂ partial pressure in cultures of strain TM-1 was typically higher than in cultures of *M. acetivorans* (Table 1).

When the H₂ partial pressure in cultures was perturbed by flushing H₂ out with N₂-CO₂ (50:50) or by adding H₂, the initial H₂ partial pressure was reestablished with H₂ production or consumption (Fig. 2). The addition of 2-bromoethanesulfonic acid (500 μM [final concentration]) inhibited methane production in strain TM-1 and inhibited H₂ consumption and production in experiments similar to those in Fig. 2.

Methane production in *M. acetivorans* could not be consistently inhibited by the addition of 2-bromoethanesulfonic acid.

A 10-liter culture of strain TM-1 was grown at constant pH (6.0) and acetate concentration (50 mM) in a pH auxostat with a substrate reservoir of 10 M acetic acid (9). The culture was continuously sparged with N₂, and the rates of H₂ and methane production were calculated from the flow rate and composition of the gas exiting the fermentor. H₂ was produced over time at a rate that was in constant proportion to the rate of methane production, except immediately after inoculation (Fig. 3). A similar experiment was not performed with *M. acetivorans*.

The results indicate that the hydrogenases of *M. acetivorans* and strain TM-1 are active during acetate metabolism to methane. In nongastrointestinal methanogenic environments, H₂-utilizing methanogens maintain the H₂ partial pressure at 1 to 7 Pa (2, 8). Batch cultures (this study) or acetate-limited continuous cultures (unpublished data) of strain TM-1 and *M. acetivorans* have a constant net production of H₂ at these partial pressures. Therefore, hydrogenase(s) may function primarily to vent excess reducing equivalents as H₂ during growth on acetate in sediments and digestors. In a closed culture system, with no other H₂-consuming organisms to maintain low H₂ partial pressures, H₂ is produced until a partial pressure is reached above which H₂ is consumed. The H₂ partial pressure in these cultures may be regulated by the redox state of electron carriers involved in acetate metabolism.

Net rates of H₂ production and consumption were less than 1% of the rates of methane production. Although reducing equivalents are generated from the oxidative cleavage of acetate to methyl-coenzyme M and CO₂, they must be used to reductively demethylate methyl-coenzyme M and thus recycle coenzyme M for further acetate metabolism (10, 15). Net H₂ production could result from net reducing potential generated from the oxidation of the methyl group

TABLE 1. H₂ partial pressures during methane production from methyl substrates

Organism	H ₂ partial pressure (Pa) ^a on:		
	Acetate	Methanol	Trimethylamine
<i>Methanosarcina</i> sp. strain TM-1	67-92	90-160	80-130
<i>Methanosarcina acetivorans</i>	16-68	8-20	14-33

^a Range of H₂ partial pressures for more than 10 cultures each on acetate and more than 5 culture each on the other substrates.

* Corresponding author.

† Present address: Mail Stop 432, U.S. Geological Survey, Reston, VA 22092.

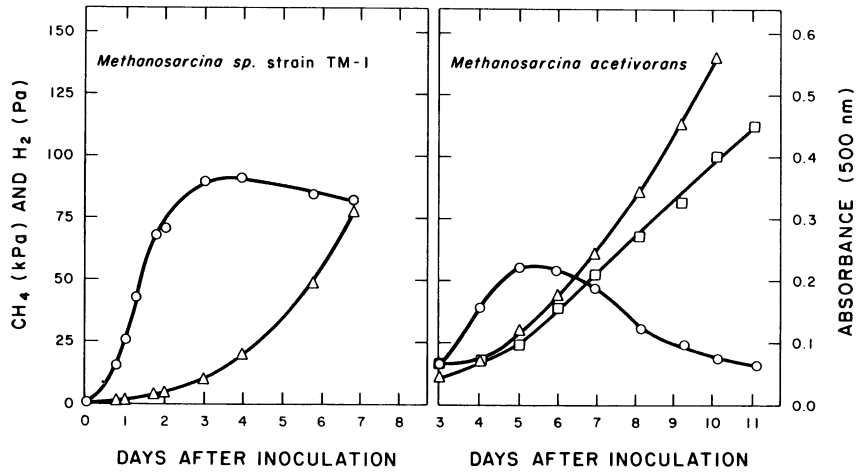


FIG. 1. H₂ and methane during growth in closed cultures. Symbols: Δ , methane; \circ , H₂; \square , culture absorbance.

of acetate or other organic compounds (10, 13). Net H₂ consumption could result from the anabolic fixation of CO₂ (14). If these processes are the sources for net production or consumption of reducing potential, then the net rates of H₂

production or consumption should be low in comparison to methane production; the rates of acetate oxidation and anabolic CO₂ fixation are much lower than the rates of acetate metabolism to methane (10, 14-16).

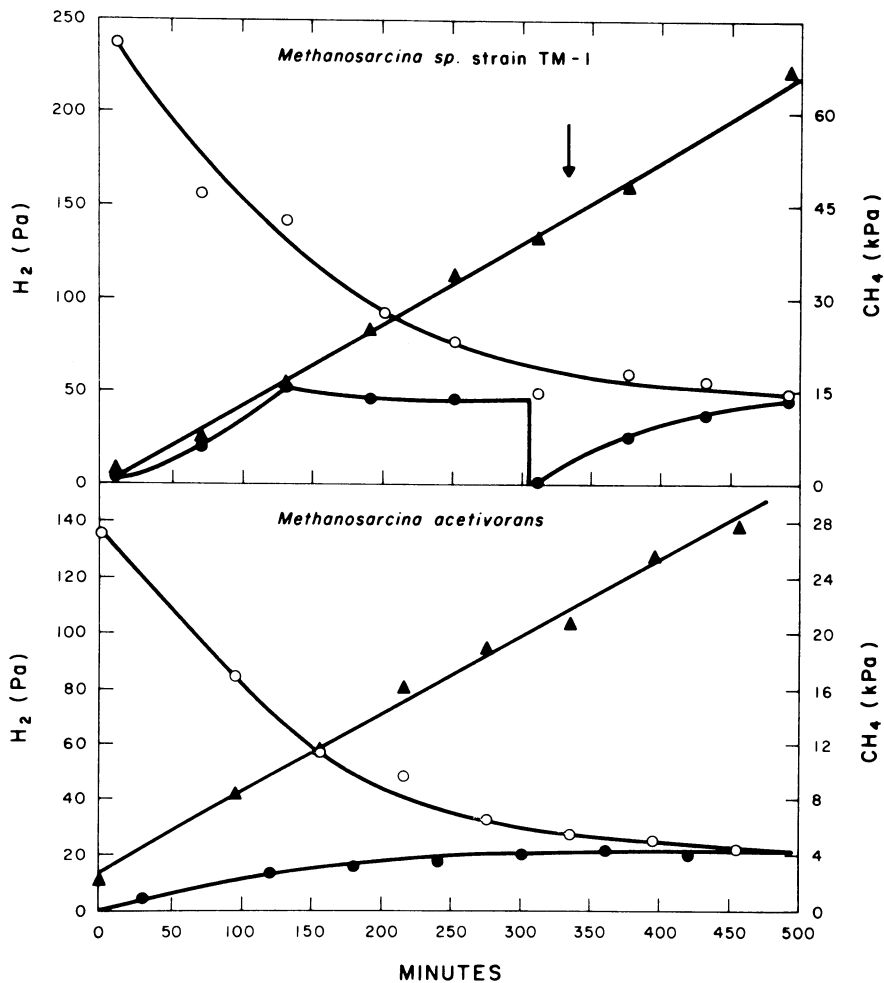


FIG. 2. H₂ production and consumption during methane production from acetate. Cultures in closed vessels were flushed with N₂-CO₂ (50:50) (●) or H₂ was added (○) at time zero. The culture of *Methanosarcina sp. strain TM-1* was also flushed with N₂-CO₂ at 305 min as denoted by the arrow. Also shown is the time course for methane production (▲).

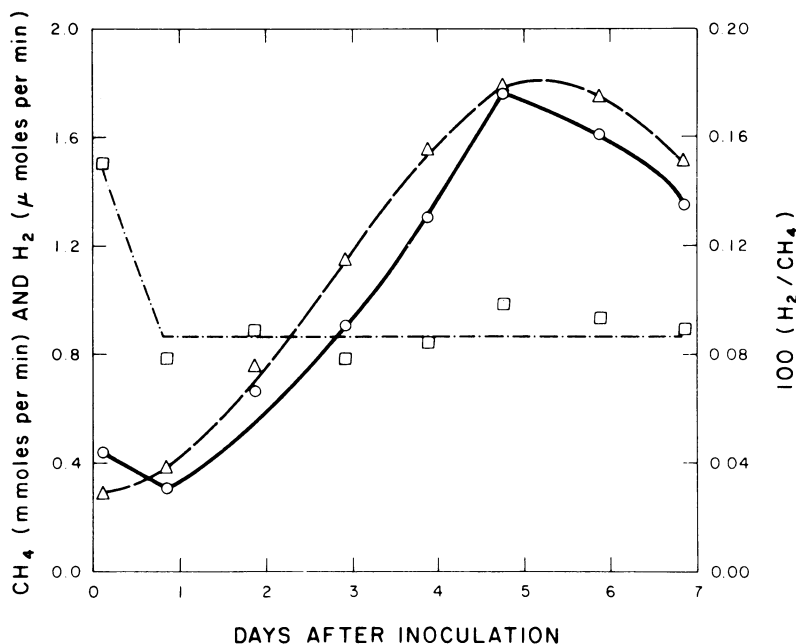


FIG. 3. H₂ and methane production by *Methanosarcina* sp. strain TM-1 during growth on acetate in a pH auxostat culture flushed with N₂. Symbols: ○, H₂ production; △, methane production; □, ratio of H₂ to methane production expressed as a percentage.

Although the net rates of H₂ production and consumption are low during acetate metabolism, the possibility exists that H₂ could be simultaneously produced and consumed rapidly with no net change in the H₂ concentration. Reducing equivalents from the oxidative cleavage of acetate could be used to generate H₂ within the cytoplasm. After diffusion across the cell membrane, H₂ could be oxidized on the outside of the membrane with the reducing equivalents used for the reduction of methyl-coenzyme M on the cytoplasmic side of the membrane. This H₂-cycling mechanism would be similar to the mechanism proposed for the generation of a proton gradient to drive ATP synthesis in sulfate-reducing bacteria (7).

M. acetivorans and strain TM-1 produced H₂ during methanogenesis when methanol (75 mM) or trimethylamine (50 mM) was substituted for acetate in the medium (Table 1). *Methanococcoides methylutens*, a methanogen able to use only methylamines and methanol as methanogenic substrates (12), produced H₂ (H₂ partial pressure, 3 to 6 Pa) during growth on trimethylamine. After the completion of the studies reported here, unpublished results were communicated that *Methanosarcina barkeri* produces "traces" of H₂ during growth on acetate or methanol (6). *Methanotherix soehngenii*, a methanogen that exclusively uses acetate as a substrate (14), contains hydrogenase (15). These findings suggest that H₂ production during the metabolism of methyl substrates is common among methanogens, but the physiological role has not been determined.

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