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Minimum Threshold for Hydrogen Metabolism in Methanogenic Bacteria

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Methanogenic isolates did not consume hydrogen below partial pressures of 6.5 Pa. Thus, in contrast to a previous report, results from pure-culture studies do not invalidate the threshold model for methane production from hydrogen in sediments.

The development of an appropriate model for the distribution of sulfate reduction and methane production is an important aspect of modeling decomposition processes in aquatic sediments. Two conceptual models have been proposed to describe the partitioning of hydrogen metabolism between sulfate-reducing bacteria (SRB) and methanogenic bacteria (MB).

The Michaelis-Menten model states that SRB and MB consume hydrogen according to simple Michaelis-Menten kinetics (6, 10). In this model, the V_{max} and K_m of the SRB and MB populations determine the relative importance of sulfate reduction and methane production with hydrogen. At hydrogen concentrations more than 100-fold greater than those in sediments, the Michaelis-Menten model does accurately describe the competition for hydrogen in mixtures of MB and SRB cultures (6, 10) and in sediments (7). However, the Michaelis-Menten model does not accurately predict hydrogen uptake in sediments at in situ hydrogen concentrations. For example, in freshwater sediments supplemented with sulfate, the MB had a V_{max} for hydrogen that was twice that in the SRB (Table 4 of reference 7). According to the Michaelis-Menten model (Fig. 6 of reference 10), the MB should have consumed nearly half of the hydrogen produced in the sediments. However, there was no detectable methane production. The potential for methane production in the sediments was only expressed when the hydrogen partial pressure was artifically increased, either by the addition of hydrogen or by the inhibition of sulfate reduction with molybdate (7).

An alternative model for hydrogen competition is the threshold model, which states that SRB outcompete MB by maintaining the hydrogen partial pressure below a minimum threshold necessary for methane production (7). In accordance with the observations described above and other studies (1, 2, 9, 11), the threshold model predicts that regardless of the V_{max} for methane production, there will be no methane production from hydrogen at in situ hydrogen concentrations when the size (V_{max}) of the SRB population is not sulfate limited.

Proponents of the Michaelis-Menten model have discounted the threshold model by implying that pure cultures of MB do not have a minimum threshold for hydrogen (10). However, the hydrogen threshold was not specifically investigated in that study. Even though physiological studies on sediment populations are preferred over pure-culture studies for describing sediment processes, the lack of a threshold in pure cultures would necessitate a reevaluation of the threshold model. Therefore, hydrogen uptake by pure cultures of MB was investigated further.

Methanobacterium formicicum JF-1, *Methanobacterium bryantii* M.o.H., and *Methanospirillum hungatei* JF-1 were obtained from the culture collection of J. G. Ferry (Virginia Polytechnic Institute and State University, Blacksburg). The organisms were grown in the medium that is used to maintain these organisms. The medium contained (in grams per liter of deionized water) the following: NH_4Cl, 1.44; K_2HPO_4, 1.13; KH_2PO_4, 1.13; NaCl, 0.45; Na_2CO_3, 3.0; $MgSO_4 \cdot 7H_2O$, 0.09; $CaCl_2 \cdot 2H_2O$, 0.06; yeast extract (Difco Laboratories, Detroit, Mich.), 0.5; Trypticase (BBL Microbiology Systems, Cockeysville, Md.), 0.5; $Fe(NH_4)_2(SO_4)_2$, 0.01; cysteine hydrochloride, 0.27; $Na_2S \cdot 9H_2O$, 0.27; and resazurin, 0.001. Trace element (8) and vitamin solutions (3) were added at a final concentration of 1% (vol/vol). $NaCH_3COO \cdot 3H_2O$ (final concentration, 2.5 g/liter) was included in the medium for *M. bryantii* and *M. hungatei*. The medium was prepared by standard anaerobic techniques (3).

The cultures (11 ml) were grown under 230 kPa of hydrogen-carbon dioxide (80:20) in anaerobic pressure tubes (3) and incubated horizontally at 39°C on a reciprocating shaker. The absorbance of the cultures was read at 660 nm in a Bausch & Lomb Spectronic 20. The headspace of cultures with an absorbance of 0.2 to 0.3 was flushed and pressurized (230 kPa) with nitrogen-carbon dioxide (80:20). Hydrogen was added to provide 0.5 to 2.0 kPa of hydrogen in the culture headspace, and hydrogen was monitored over time. Hydrogen was quantified by gas chromatography with a thermal conductivity detector (8) at an oven temperature of 110°C.

M. formicicum metabolized hydrogen down to ca. 7 Pa, but hydrogen was not further metabolized with extended incubation (Fig. 1). This incubation period at the threshold partial pressure was more than sufficient to ensure that in the absence of hydrogen uptake, the hydrogen partial pressure in the headspace and dissolved hydrogen were in equilibrium. With the addition of more hydrogen, the organism repeatedly consumed hydrogen down to the threshold of ca. 7 Pa. *M. formicicum* cultures that were twice as dense had a similar threshold. The other organisms studied also could not metabolize hydrogen below a minimum threshold. The mean threshold ± standard deviation for five cultures of each organism were as follows: *M. formicicum*, 6.5 ± 0.6 Pa; *M. bryantii*, 6.9 ± 1.5 Pa; and *M. hungatei*, 9.5 ± 1.3 Pa. Five methanogenic isolates from the rumen could not metabolize hydrogen below 7.7 to 12 Pa (8).

The results demonstrate that under standard culture con-

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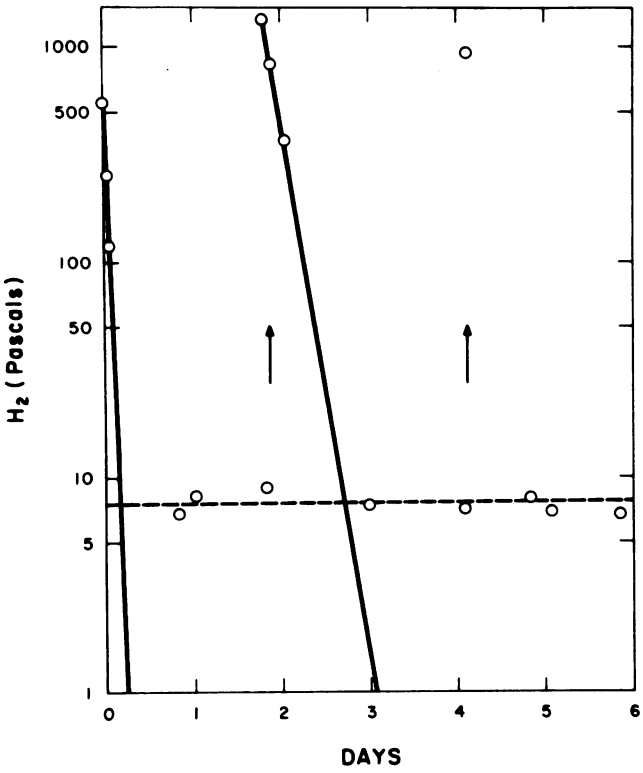

FIG. 1. Hydrogen uptake by *M. formicicum*. The hydrogen-carbon dioxide gas phase was replaced with nitrogen-carbon dioxide, and hydrogen was added at time zero and at the times indicated by the arrows. The solid lines represent the hydrogen uptake expected if there were no threshold for hydrogen metabolism. The dashed line represents the mean hydrogen threshold.

ditions, pure cultures of MB do not consume hydrogen below a minimum concentration. The threshold was probably not observed in a previous study (10) because uptake was not measured at low enough hydrogen concentrations. Aerobic hydrogen-oxidizing bacteria also have a minimum threshold for hydrogen uptake (4), suggesting that hydrogen thresholds are a common characteristic of hydrogen-oxidizing bacteria.

The threshold for hydrogen uptake in pure cultures of MB is at least sixfold higher than the hydrogen partial pressure in methanogenic sediments (7). Similarly, culturable aerobic hydrogen-oxidizing bacteria cannot metabolize hydrogen down to the partial pressures in aerobic soils (4). Unsuitable culture conditions might prevent pure cultures of MB from metabolizing hydrogen to lower concentrations. Alternatively, the isolation of MB at high hydrogen concentrations might not select for the MB that metabolize hydrogen at low concentrations in sediments (5). The hydrogen threshold for methane production in sediments is considered to be the hydrogen concentration at which the energy yield from methane production is insufficient to support the process (7).

In summary, this study indicates that the previous criticism of the threshold model for hydrogen uptake in sediments is unwarranted, as MB in pure cultures also have a minimum threshold for hydrogen metabolism. The threshold model is preferred over the Michaelis-Menten model, as the latter is inconsistent with results in sediments.

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