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Inhibition of Methanogenesis in Marine Sediments by Acetylene and Ethylene: Validity of the Acetylene Reduction Assay for Anaerobic Microcosms

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Methanogenesis was irreversibly inhibited in sediments by concentrations of acetylene employed in nitrogen fixation assays (1 to 20%, vol/vol). Ethylene, but not ethane, also stopped methane production, and the inhibition was reversed by gassing with hydrogen.

The acetylene reduction technique is widely used for field and laboratory estimations of nitrogenase activity (6). It was recently reported that some species of methane-oxidizing bacteria may be inhibited by acetylene and also oxidize ethylene (3), thereby complicating field assays (5). We now wish to draw attention to a further problem when the assay is used to measure nitrogen fixation in anaerobic microcosms, a problem due to the inhibition of methanogenesis by acetylene.

Sediments associated with communities of the sea-grass *Thalassia testudinum* possess methanogenic activity (R. S. Oremland and B. F. Taylor, Abstr. Annu. Meet. Am. Soc. Microbiol., 1975, N8, p. 185). The sediments from these marine angiosperm communities contained about 2% (dry weight) of organic carbon and 50 to 60% (by weight) water. Sediments were homogenized, in a Waring blender, with oxygen-free seawater. The homogenate was pipetted, in 20-ml portions, into 50-ml Erlenmeyer flasks, which were gassed with hydrogen or argon and then sealed with recessed butyl rubber stoppers. Oxygen was excluded during manipulations by the techniques described for the cultivation of strict anaerobes (7). Acetylene, ethylene, or ethane was injected to the desired concentration into the gas phase, and chloroform (0.05%, vol/vol), an inhibitor of methanogenic bacteria (1), was added to some flasks. The flasks were incubated at 26 C in the dark with reciprocal shaking. Hydrocarbons were determined, in the gas phase, by flame ionization chromatography with a stainless-steel column (6 feet by 1/8 inch, outer diameter [ca. 182.9 by 0.38 cm]) containing porapak R (100 to 120 mesh) at 75 C with nitrogen as carrier gas (flow rate, 25 ml/min). Hydrogen was detected with a gas partitioner (thermal conductivity) using two aluminum columns in series containing, respectively, a molecular sieve (13 feet by 3/16 inch, outer diameter [ca. 396 by 0.5 cm]) and hexamethyl-phosphoramide (6 feet by 3/16 inch, outer diameter). The columns were operated at 35 C with argon (60 ml/min) as carrier gas.

Methane production was inhibited by 1.25 to 20% (vol/vol) acetylene and did not resume when the flasks were gassed with hydrogen (Fig. 1). Lower concentrations of acetylene (0.13 and 0.01%) merely decreased the rate of methanogenesis. Ethylene was inhibitory at concentrations of 5 and 20%, but not at 1.25%, and the inhibition was relieved by subsequent gassing for 5 min with hydrogen at the lower but not the higher concentration (Fig. 2). The reversal of C_2H_4 inhibition by gassing was presumably related to the extent of elimination of C_2H_4 from the inhibited flasks, because gassing for 10 min with H_2 reversed inhibition caused by 20% C_2H_4. Ethane (1.25 to 20%) had no effect on the rate of methane production. Methanogenesis in control flasks was unaffected by the injection of 0.5 ml of air either at zero time or during the exponential phase of methane production. The sediment homogenates were therefore presumably well buffered against oxygen, probably by sulfide and oxygen-consuming microbes, and the observed inhibitions were due to the unsaturated C_2 hydrocarbons and not oxygen contamination. It is also relevant that unsaturated fatty acids inhibit methanogenesis (10).

A transient accumulation of hydrogen was observed in systems containing 20% acetylene in argon, whereas in sediments inhibited with both acetylene and $CHCl_3$ hydrogen accumulated and did not disappear (Table 1). The production of hydrogen in sediments inhibited with C_2H_2 is similar to the effects observed with

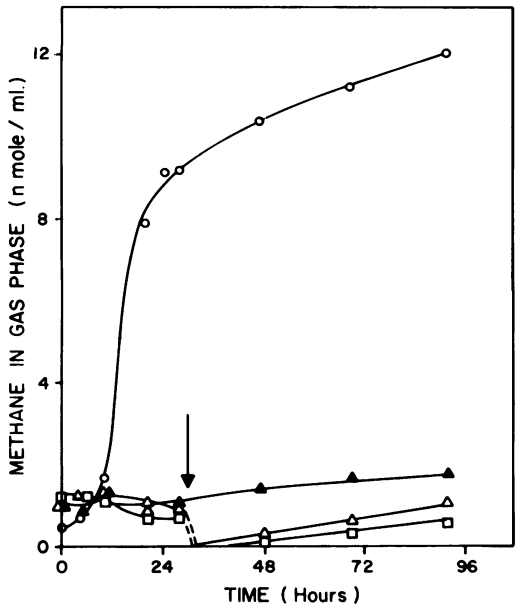

FIG. 1. *Irreversible inhibition of methanogenesis by 1.25% (vol/vol) C_2H_2. The Erlenmeyer flasks contained 20 ml of sediment homogenate (dry weight, about 8 g) incubated at 26 C in the dark under hydrogen. Symbols: ○, uninhibited control; ▲, 1.25% C_2H_2 ungassed; △, 1.25% C_2H_2 gassed at 30 h with H_2 (arrow); □, 1.25% C_2H_2 and 0.05% (vol/vol) $CHCl_3$ gassed at 30 h with H_2. Gassing with H_2 removed 99% of the C_2H_2 from the gas phase.*

chloromethanes (1), although $CHCl_3$ did not always cause hydrogen accumulation in our experiments. The eventual disappearance of hydrogen in the acetylene-inhibited homogenates is due to its consumption by other anaerobes (Oremland and Taylor, Abstr. Annu. Meet. Am. Soc. Microbiol., 1975, N8, p. 185) and possibly its reduction of acetylene. Ethane production was not detected in flasks containing non-inhibitory levels of ethylene, and at inhibitory concentrations the chromatographic peak for ethylene obscured that for ethane. However, systems inhibited with acetylene produced ethylene, and its rate of production was lower in flasks containing both acetylene and $CHCl_3$ (Table 2). There appears to be a correlation between hydrogen consumption and ethylene production in the results shown in Tables 1 and 2, but the system is too complex to reach any firm conclusions. $CHCl_3$ may have retarded the reduction of acetylene by the methanogenic enzymatic systems, but it could also have inhibited a hydrogen-dependent nitrogenase activity of either methanogens or other anaerobes (e.g., clostridia containing B_{12} enzymes). Further work is needed to resolve this situation, particularly since it is still not known if methanogenic bacteria fix nitrogen (9). Pine and Barker (8) demonstrated $^{15}N_2$ incorporation by *Methanobacterium omelianskii*, but subsequent investigations employing the acetylene reduction technique on the S organism and *Methanobacte-*

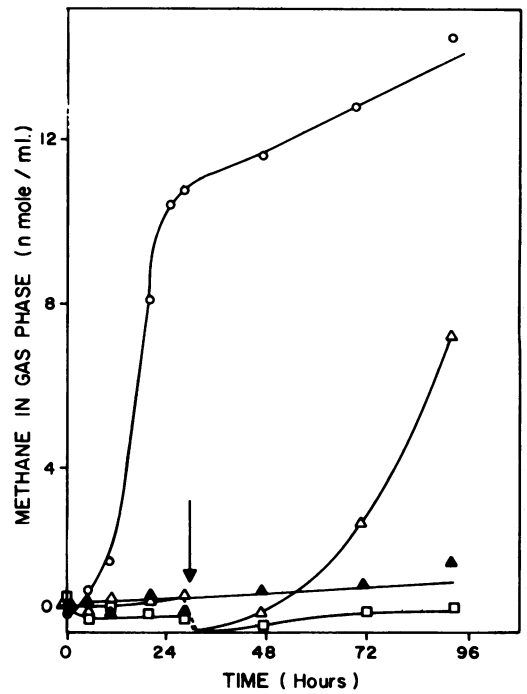

FIG. 2. *Reversible inhibition of methanogenesis by 5% (vol/vol) C_2H_4. The Erlenmeyer flasks contained 20 ml of homogenized sediment (dry weight, about 8 g) incubated at 26 C in the dark under hydrogen. Symbols: ○, uninhibited control ▲, 5% C_2H_4 ungassed; △, 5% C_2H_4 gassed at 30 h with H_2 (arrow); □, 5% C_2H_4 and 0.05% (vol/vol) $CHCl_3$ gassed at 30 h with H_2. Gassing with H_2 removed 98% of the C_2H_4 from the gas phase.*

TABLE 1. *Hydrogen concentrations in the gas phases of uninhibited and inhibited sediment systems*[a]

Time (h)	Hydrogen concn (nmol/ml)[b]		
	No inhibitor	20% C_2H_2	20% C_2H_2 + 0.05% $CHCl_3$
0	0	0	0
5	26	26	18
29	0	40	160
52	0	17	166
78	0	0	120

[a] The Erlenmeyer flasks contained about 8 g (dry weight) of sediment and were sealed under argon.

[b] The limit for the detection of H_2 was about 6 nmol/ml.

TABLE 2. *Methane and ethylene concentrations in the gas phases of uninhibited and inhibited sediment systems*[a]

Time (h)	Methane concn (no inhibitor) (nmol/ml)[b]	Ethylene concn (nmol/ml)[c]	
		20% C_2H_2	20% C_2H_2 + 0.05% $CHCl_3$
0	0.80	0.30	0.36
6	0.86	0.54	0.46
21	2.62	1.22	0.76
29	5.38	1.09	0.86
43	9.34	1.97	0.98
68	10.34	3.17	0.90

[a] The Erlenmeyer flasks contained about 8 g (dry weight) of sediment and were sealed under argon.

[b] Ethylene was not produced in the absence of acetylene.

[c] Methane was not produced by inhibited systems.

rium MoH failed to specify which organism possesses nitrogenase (M. P. Bryant, personal communication), perhaps because acetylene inhibited the latter organism.

It is apparent, from the above results, that acetylene inhibits methanogenesis at concentrations used in the nitrogenase assay, but it is not clear if this causes an over- or underestimate of sediment nitrogen fixation rates. Hardy et al. (6) in their development of the acetylene reduction assay noted that acetylene markedly decreased methane formation by rumen contents and Elleway et al. (4) confirmed this observation. Neither group of workers, however, questioned the validity of the acetylene reduction assay for nitrogen fixation by anaerobic microbial communities. Even if methanogenic bacteria do not fix nitrogen, they probably play a symbiotic role in nitrogen fixation by anaerobic microbial communities, and, since *Clostridium pasturianum* is also adversely affected by acetylene (2), the acetylene reduction assay must be checked with the ^{15}N method when studying anaerobic microcosms. Work is underway to further define the site and nature of the inhibition of methanogenesis by acetylene and ethylene.

ADDENDUM

Since this communication was submitted for publication, Raimbault (11) reported the inhibition by acetylene of methane evolution from anaerobic paddy soils and the acetylene inhibition of methanogenesis and growth of a strain of *Methanosarcina* isolated from paddy soil.

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