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Production of Ethane, Ethylene, and Acetylene from Halogenated Hydrocarbons by Methanogenic Bacteria

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Several methanogenic bacteria were shown to produce ethane, ethylene, and acetylene when exposed to the halogenated hydrocarbons bromoethane, dibromo- or dichloroethane, and 1,2-dibromoethylene, respectively. They also produced ethylene when exposed to the coenzyme M analog and specific methanogenic inhibitor bromoethanesulfonic acid. The production of these gases from halogenated hydrocarbons has a variety of implications concerning microbial ecology, agriculture, and toxic waste treatment. All halogenated aliphatic compounds tested were inhibitory to methanogens. *Methanococcus thermolithotrophicus*, *Methanococcus deltae*, and *Methanobacterium thermoautotrophicum* ΔH and Marburg were completely inhibited by 7 μM 1,2-dibromoethane and, to various degrees, by 51 to 1,084 μM 1,2-dichloroethane, 1,2-dibromoethylene, 1,2-dichloroethylene, and trichloroethylene. In general, the brominated compounds were more inhibitory. The two *Methanococcus* species were fully inhibited by 1 μM bromoethanesulfonic acid, whereas both *Methanobacterium* strains were only partly inhibited by 2,124 μM. Coenzyme M protected cells from bromoethanesulfonic acid but not from any of the other inhibitors.

The methane-producing bacteria (methanogens) (3, 19) produce methane as an end product of their ATP-generating metabolism by using a narrow range of possible substrates; most methanogens can use H_2-CO_2 or formate, and a few use acetate or methanol. These strict anaerobes are found in aquatic sediments, anaerobic sewage digestors, and a variety of other O_2-free environments, where they participate in the decomposition of organic matter. Two gases, CO_2 and CH_4, are their major products. Ecological observations have suggested that these organisms might produce low levels of 2-carbon gases in some environments, but there have been no demonstrations of this ability in pure culture (33, 34).

The problem of environmental contamination by toxic and carcinogenic halogenated 2-carbon hydrocarbons (HHs) is widespread (2, 20, 22, 26, 29, 37). Of 114 organic chemicals established as priority "polutants" by the U.S. Environmental Protection Agency, 31 are halogenated aliphatic compounds (29). Contamination of groundwater, surface water, soil, and air occurs. However, the effectiveness and widespread use of these compounds as solvents and synthetic intermediates makes a major reduction in their use unlikely in the near future. Techniques for the treatment of contaminated water (21, 30) include the drilling of new wells, aeration, and activated carbon treatment; but HHs still exist and must ultimately be released into the environment (e.g., air stripping into the atmosphere). Biological decomposition is an attractive alternative, especially in anaerobic environments. Results of recent work by McCarty and co-workers (9, 12, 41) have demonstrated that these compounds are degraded by methanogenic mixed cultures. We report here our work on the metabolism of several HHs by pure cultures of methanogens to produce ethane, ethylene, and acetylene and HH inhibition of methanogens. Although generally considered by microbiologists as a useful inhibitor for a variety of methanogen studies (11, 23, 33, 39, 45), bromoethanesulfonate (BES) is actually a modified HH and was included in the study.

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MATERIALS AND METHODS

Organisms. *Methanococcus thermolithotrophicus* (24) was a gift from K. O. Stetter. *Methanobacterium thermoautotrophicum* ΔH (44) was a gift from J. Winter, and *Methanobacterium thermoautotrophicum* Marburg (15) was provided by G. Fuchs. *Methanococcus deltae* ΔLH (17) was obtained from J. Reeve.

Medium, growth, and suspension conditions. Cells were grown on H_2-CO_2 by procedures described previously (4, 18). *Methanococcus thermolithotrophicus* was grown at 62°C in a medium described previously (18), except that NH_4Cl and $CaCl_2 \cdot 2H_2O$ were used at concentrations of 8.41 and 0.24 mM, respectively. Both *Methanobacterium thermoautotrophicum* strains were grown at 62°C in a medium described previously (18), except that the mixture of trace elements was that described above for *Methanococcus thermolithotrophicus*; vitamin mix, as described for *Methanococcus deltae* (36), was included for the growth of strain Marburg. *Methanococcus deltae* was grown at 37°C in a medium described previously (36). The organisms were cultivated in 5-ml volumes in anaerobic serum tubes (2048-00150; Bellco Glass, Inc., Vineland, N.J.) or in 55-ml amounts in 250-ml serum bottles (223950; Wheaton Industries, Millville, N.J.).

Stock solutions of halogenated compounds (except 2-bromoethanesulfonic acid, which was dissolved in water) were made in 95% ethanol. The stock solutions were filter sterilized into sealed sterile tubes by using filter units (pore size, 0.22 μm; Millex-GV; Millipore Corp., Bedford, Mass.) and made anaerobic as described previously (18) before they were added to culture tubes. Ethanol, always at a final concentration of 0.33% in the culture tubes, including controls, had no effect on the growth of any of the methanogens, except for strain ΔH, the growth rate of which was slightly

TABLE 1. Production of 2-carbon gases by mid-logarithmic-phase cultures of methanogens incubated with various halogenated hydrocarbons

HH[a]	Gas produced	nmol of gas produced/tube (no. of h of incubation) by:			
		Methanobacterium thermoautotrophicum ΔH	*Methanococcus deltae* ΔLH	*Methanococcus thermolithotrophicus*	Control[b]
Bromoethane	Ethane	41 (149)	51 (72)	111 (23)	0[c] (152)
Bromoethane sulfonate	Ethylene	334 (107)	50 (230)	18 (334)	0[c] (190)
1,2-Dibromoethane	Ethylene	290 (110)	87 (47)	126 (70)	17 (110)
1,2-Dichloroethane	Ethylene	20 (70)	24 (154)	94 (50)	0[c] (113)
1,2-Dichloroethylene	None	0[c] (154)	0[c] (154)	ND[d]	0[c] (154)
1,2-Dibromoethylene	Acetylene	98 (158)	38 (150)	45 (70)	11 (158)

[a] The following concentrations were used for certain strains: bromoethane for *Methanococcus thermolithotrophicus* and strain ΔLH, 60 μM; bromoethane for strain ΔH, 237 μM; BES for all organisms, 107 μM; dibromoethane for strains ΔH and ΔLH, 213 μM; dibromoethane for *Methanococcus thermolithotrophicus*, 108 μM; dichloroethane for *Methanococcus thermolithotrophicus*, 1,405 μM; dichloroethane for the other species, 157 μM; 1,2-dichloroethylene for all species, 1,000 μM; 1,2-dibromoethylene for strain ΔH and *Methanococcus thermolithotrophicus*, 1,000 μM; 1,2-dibromoethylene for strain ΔLH, 510 μM.

[b] Strain ΔH cells were inactivated with 5% Formalin. HH concentrations were the same as for the corresponding live strain ΔH cells (see footnote *a*).

[c] A value of 0 means that <5 nmol was produced.

[d] ND, Not determined.

(10 to 15%) inhibited. For experiments on 2-carbon gas production, cells were grown to the mid-logarithmic stage (A_{600} of 0.3 to 0.6) before HHs were added. For inhibition studies, a 10% inoculum was added to the medium from a mid-logarithmic stage culture. Cultures were incubated in the dark in a gyratory shaker at 150 rpm. The A_{600} for the cultures was read in a spectrophotometer (Spectronic 20; Bausch & Lomb, Inc., Rochester, N.Y.). All data represent average values from duplicate tubes or bottles.

Experiments designed to examine the effect of cell concentrations on the rate of gas formation from HHs were conducted with *Methanococcus thermolithotrophicus*. Cells grown to the mid-logarithmic stage were centrifuged and washed twice with medium lacking NH_4Cl, $CaCl_2 \cdot 2H_2O$, and KCl and then suspended in this modified medium at a concentration that was 3.75-fold higher than that in the growth medium. Dilutions were made into serum tubes containing a total of 5.1 ml of modified medium and cells, resulting in a protein concentration of 55, 102, or 173 μg of protein per ml. Bromoethane was then added to a level of 60 μM, and the tubes were incubated and analyzed as described above. Protein was determined by using the assay described by Bradford (14).

Chemicals. The HHs used were reagent grade. BES and 2-mercaptoethanesulfonic acid (coenzyme M [CoM]) were purchased from Sigma Chemical Co., St. Louis, Mo. 1,2-Dichloroethane, 1,2-dibromoethane, monobromoethane, *cis*- and *trans*-dibromoethylene, *trans*-dichloroethylene, *cis*- and *trans*-dichloroethylene, and trichloroethylene were purchased from Aldrich Chemical Co., Milwaukee, Wis. Premixed H_2-CO_2 (80:20; vol/vol) was purchased from Air Products, Allentown, Pa.

Gas chromatographic analysis. Methane, ethane, ethylene, and acetylene were measured by using a gas chromatograph (GC-8A; Shimadzu) equipped with a flame ionization detector and a column (2 m by 3 mm [outer diameter]) packed with Porapack Q (Supelco, Bellefonte, Pa.) that was maintained at 70°C. For dichloroethane analysis, culture samples were removed in 2.5-ml amounts and extracted with an equal volume of methylene chloride by thoroughly vortexing the solution. A 1.0-μl sample of the methylene chloride phase was injected into the same gas chromatograph equipped with a column (2 m by 3 mm [outer diameter]) packed with Graphpac GC (Alltech Associates, Deerfield, Ill.) that was maintained at 70°C.

RESULTS

Production of ethane, ethylene, and acetylene. When cultures of several methanogens were grown with H_2-CO_2 to the mid-logarithmic stage (A_{600} of 0.3 to 0.6) and the medium was then supplemented with HHs, production of gases other than CH_4 occurred (Table 1). The HH levels used here were chosen from preliminary experiments to give near maximal 2-carbon gas production. Monobromo-substituted ethane produced ethane, and ethane substituted with either a halogen and sulfonic acid group (BES) or two halogen molecules produced ethylene. Dibrominated ethylene produced acetylene (ethylene was produced concurrently at ≤6 nmol per tube), but dichlorinated ethylene did not produce any 2-carbon gas in these experiments. The amount of 2-carbon gas produced depended on both the organism and HHs (Table 1).

The effect of HH concentration on 2-carbon gas produc-

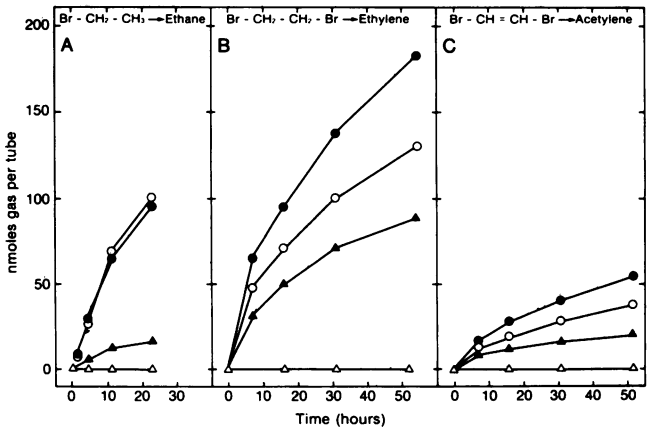


FIG. 1. Effects of HH concentration on the production of 2-carbon gases by mid-logarithmic-phase cultures of methanogens. (A) Ethane production by *Methanococcus thermolithotrophicus* in the presence of various concentrations of bromoethane. Symbols: ○, 119 μM; ●, 60 μM; ▲, 24 μM; △, control. (B) Ethylene production by *Methanobacterium thermoautotrophicum* ΔH in the presence of various concentrations of dibromoethane. Symbols: ●, 213 μM; ○, 108 μM; ▲, 23 μM; △, control. (C) Acetylene production by *Methanobacterium thermoautotrophicum* ΔH in the presence of various concentrations of dibromoethylene. Symbols: ●, 1,000 μM; ○, 509 μM; ▲, 210 μM; △, control.

tion and methanogenesis was examined. Higher HH levels resulted in increased 2-carbon gas production (Fig. 1). In some cases, however, methanogenesis was inhibited at the HH concentrations needed for near maximal levels of 2-carbon gas formation. This point is illustrated in Fig. 2A, with higher acetylene levels produced at 2,000 μM dibromoethylene, although methanogenesis was inhibited at this concentration; at 50 μM, methanogenesis was only partly inhibited, but acetylene was formed at very low levels. An example of a HH (BES) which allowed methane production at a normal rate concurrent with ethylene production is shown in Fig. 2B.

The effect of cell concentration on the rate of ethane formation from monobromoethane was examined with cells of *Methanococcus thermolithotrophicus* suspended in a medium lacking several components to prevent cell growth during the experiment. Ethane was produced at 5.26, 2.86, and 1.19 nmol h^{-1} $tube^{-1}$ in tubes containing 173, 102, and 55 μg of protein ml^{-1}, respectively.

The disappearance of a HH was observed concurrently with the appearance of a 2-carbon gas (Fig. 3). When cells of *Methanobacterium thermoautotrophicum* Marburg were exposed to 100 μM dichloroethane, 2.3 μmol of the substrate was consumed and 0.9 μmol of ethylene was produced per bottle at the end of 85 h. The autoclaved controls showed little decrease of the substrate and no ethylene production. Because dichloroethane degradation probably proceeds via an intermediate, some of the material should be in this form, which could account for the less than 100% conversion to ethylene. Substrate depletion concurrent with ethylene formation was also observed with dibromoethane, although in this case there was significant, but low (12%), chemical degradation in controls incubated with air (data not shown). These results are in agreement with results of previous studies on mixed cultures in which ^{14}C-labeled HH yielded labeled ethylene (16). We conclude that the HHs are converted into 2-carbon gases.

A variety of control experiments were also conducted. When the HHs were added to uninoculated media, insignificant amounts (≤14 nmol per tube) of the relevant gases were produced. Because nonenzymatic dehalogenation reactions can be mediated by killed cell components (28, 29, 32), to establish that the 2-carbon gas formation observed was not a result of such reactions, control experiments with inactivated cells were conducted. Cultures at the same cell density as test cultures were inactivated in several ways before they were incubated with the HHs. Cells of strains ΔH and ΔLH and *Methanococcus thermolithotrophicus* treated with 5% Formalin produced gases at levels similar to those observed in uninoculated controls when incubated with each HH; the highest levels of gas were produced in strain ΔH controls (Table 1). When cells of strain Marburg were autoclaved under a gas phase of N_2-CO_2 (80:20; vol/vol) and then incubated in the presence of 1,2-dichloroethane under H_2-CO_2 (80:20, vol/vol), no ethylene was produced (Fig. 3). Cultures of *Methanococcus thermolithotrophicus* and strain ΔH also produced no gases from bromoethane or 1,2-dibromoethane when incubated in the presence of air. Viable cell cultures containing ethanol alone produced ≤5 nmol of 2-carbon gas per tube.

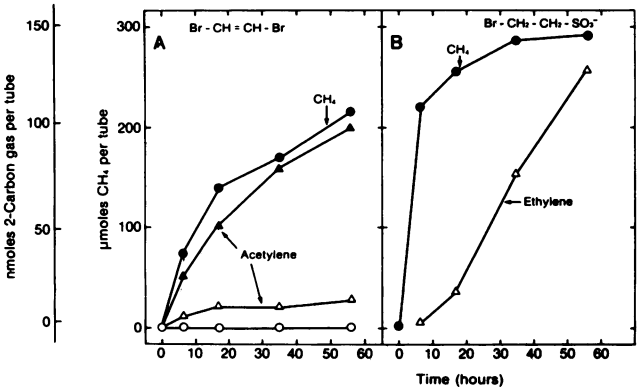


FIG. 2. Methanogenesis and 2-carbon gas production by mid-logarithmic-phase cultures of methanogens. (A) Methane and acetylene production by *Methanobacterium thermoautotrophicum* ΔH in the presence of dibromoethylene. Symbols: ●, CH_4 in 50 μM dibromoethylene tubes; △, acetylene in 50 μM dibromoethylene tubes; ○, CH_4 in 2,100 μM dibromoethylene tubes; ▲, acetylene in 2,100 μM tubes. (B) Methane and ethylene production by *Methanobacterium thermoautotrophicum* ΔH in the presence of 107 μM bromoethanesulfonate. Symbols: ●, CH_4; △, ethylene.

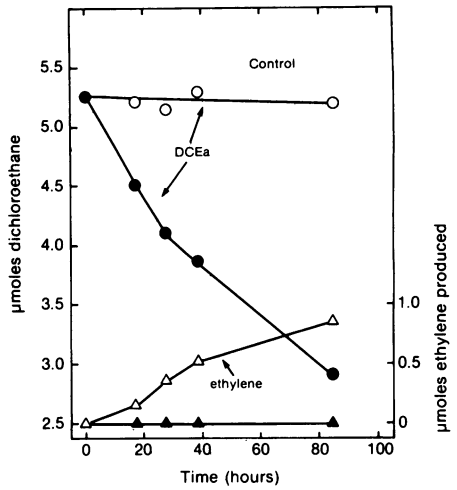


FIG. 3. Production of ethylene (micromoles per bottle) concurrently with consumption of dichloroethane (DCEa) by mid-logarithmic-phase cultures of *Methanobacterium thermoautotrophicum* Marburg. Symbols: ○, dichloroethane in control cultures (autoclaved before the addition of dichloroethane); ●, dichloroethane in test cultures; △, ethylene in test cultures; ▲, ethylene in control cultures.

Gas production in the absence of H_2 was also examined. *Methanobacterium thermoautotrophicum* Marburg cells incubated under a N_2-CO_2 (80:20; vol/vol) atmosphere in the presence of 120 μM dichloroethane produced no ethylene after 54 h, whereas cells under a H_2-CO_2 (80:20; vol/vol) gas phase produced 36 nmol of ethylene per tube.

Inhibition of methanogens by HHs. All HHs tested were inhibitory to growth of methanogenic bacteria (Table 2). Based on results of preliminary experiments, two inhibitor concentrations were chosen: one which gave only partial inhibition and a higher one which gave more complete inhibition. There was a great variation in sensitivity, depending on the organism and the HH tested. Dibromoethane was the most potent, resulting in complete inhibition of all organisms at a concentration of 7 μM. Inhibition by *trans*-dichloroethylene of *Methanobacterium thermoautotrophicum* ΔH was complete at 270 μM, but 1,081 μM only slightly inhibited *Methanococcus thermolithotrophicus*. The same result as that with *trans*-dichloroethylene was ob-

TABLE 2. Inhibitory effect of halogenated ethanes or ethylenes on growth of methanogens[a]

Inhibitor	*Methanococcus thermolithotrophicus*		*Methanococcus deltae* ΔLH[b]		*Methanobacterium thermoautotrophicum* Marburg		*Methanobacterium thermoautotrophicum* ΔH	
	Inhibitor concn (μM)	A_{600}[c]	Inhibitor concn (μM)	A_{600}[c]	Inhibitor concn (μM)	A_{600}[c]	Inhibitor concn (μM)	A_{600}[c]
None		0.70		0.90		0.83		0.68
Monobromoethane	11.8	0.22	23.6	0.35	11.8	0.83	59.6	0.22
	23.6	0.02	59.6	0.04	59.6	0.08	118.9	0.07
Dibromoethane	1.0	0.66	1.0	0.03	1.1	0.21	1.1	0.62
	6.0	0.03			7.2	0.05	7.2	0.09
Dichloroethane	542.0	0.62	534.0	0.26	542.0	0.33	135.0	0.44
	1,084.0	0.12	1,068.0	0.07	1,084.0	0.07	271.0	0.18
Dibromoethylene	51.0	0.37	50.0	0.63	51.0	0.75	14.2	0.52
	153.0	0.05	151.0	0.11	153.0	0.08	51.0	0.08
Dichloroethylene	541.0	0.71	270.0	0.36	541.0	0.41	135.0	0.30
	1,081.0	0.57	541.0	0.10	1,082.0	0.18	270.0	0.09
Trichloroethylene	135.0	0.72	135.0	0.40	539.0	0.65	135.0	0.23
	539.0	0.06	270.0	0.08	1,078.0	0.07	270.0	0.05
BES	1.0	0.04	0.6	0.16	2,124.0	0.61	1,081.0	0.31
			1.0	0.07			2,124.0	0.22

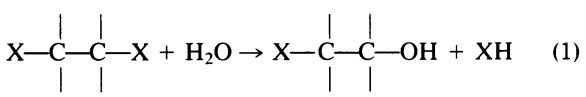
[a] All cultures were started with a 10% inoculation into tubes containing 5 ml of medium, with a resulting beginning A_{600} of 0.06 to 0.13.
[b] Incubated without shaking for the first 12 h.
[c] The A_{600} readings given are those values attained at the time that control cultures with no inhibitor reached the late logarithmic or early stationary phase; the incubation periods were 8 h for *Methanococcus thermolithotrophicus*, 23 h for strain ΔLH, 35 h for strain Marburg, and 43 h for strain ΔH.

served when the *trans*- and *cis*-mixture was added to cultures (data not shown). In general, methanogens were more sensitive to brominated hydrocarbons than to the chlorinated analogs; chlorinated hydrocarbons were added at 3- to 180-fold greater concentrations than their brominated analogs to achieve equivalent effects. Furthermore, methanogens showed a much greater sensitivity to the brominated derivative of ethane than to the corresponding ethylene. Concentrations of 1 to 7 μM dibromoethane, compared with 51 to 153 μM dibromoethylene, were needed to observe complete inhibition. A major difference in BES sensitivity was observed between the *Methanococcus* species and *Methanobacterium* species, with the former being >2,000-fold more susceptible. All HHs caused both increased lags and lower absorbances. Methane production was affected concurrently and roughly proportionally with growth inhibition (data not shown).

Growth inhibition by BES in methanogenic bacteria is known to be reversed by CoM addition (6, 38, 39). This possibility was examined with several HHs. CoM and inhibitory levels of HH were added simultaneously into the growth medium before inoculation with *Methanococcus thermolithotrophicus* at a CoM to inhibitor ratio of about 20:1. Reversal of growth inhibition was observed only in cells exposed to BES and not in those exposed to other HHs. The time course of inhibition of cells grown with BES or dibromoethane is shown in Fig. 4. When *Methanococcus thermolithotrophicus* was exposed to BES at time zero, inhibition occurred even when CoM was added 1 h later at a CoM to BES ratio of about 20:1. The beginning of recovery took more than 8 h in both cases, but recovery was slightly earlier when CoM was present. When CoM was added at the same ratio at time zero, however, growth was not significantly inhibited by BES when added either originally or 1 h later.

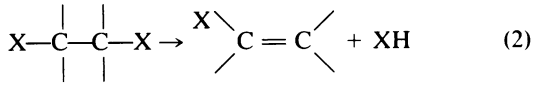
DISCUSSION

The biochemical basis for production of the 2-carbon gases observed here is not yet clear, but a variety of enzymatic reactions may participate in the dehalogenation of HHs by methanogenic bacteria or other anaerobes. A hydrolysis reaction (25) in which a halogenated alkane is converted to an alcohol and halide is described in the following equation:



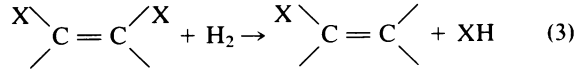
(1)

A dehydrohalogenation and elimination reaction, known in organic chemistry to be base catalyzed (31), can also be a participant in the series of reactions that degrade HH (equation 2):



(2)

Reductive dehalogenation (equation 3) is likely to be involved in degradation of some halogenated species; results of studies on mixed cultures have suggested that this reaction is used in several instances (7, 9, 12, 16, 27, 41).



(3)

Evaluation of potential pathways of HH degradation sug-

gests that the reactions shown in equations 2 and 3 can act alone or in concert, depending on the substrate. For example, conversion of bromoethane can occur via reductive dehalogenation (equation 3) because ethane was the product; if equation 2 were used, ethylene would be the product. The possible involvement of equation 1 is unlikely because with monobromoethane, ethanol would be an intermediate; control experiments with ethanol showed insignificant ethane production, (<5 nmol per tube), supporting a one-step reductive dehalogenation mechanism. The experimentally observed conversion of 1,2-dihaloethane to ethylene may involve both equations 2 and 3, because two reductive dehalogenations would yield ethane and two dehydrohalogenations would yield acetylene. A reversal of this sequence of reactions, reduction followed by dehydrohalogenation, is unlikely because the intermediate monobromoethane was shown (Table 1) to result in ethane formation. Also, cells of strain Marburg incubated in the absence of hydrogen produced no ethylene (or ethane) from dichloroethane, suggesting that reductive dehalogenation may be the major mechanism in the formation of ethylene from this HH. Further experiments with a variety of substrates and analysis for intermediates are needed to fully describe the observed conversions of HHs into 2-carbon gases.

Both ethylene and acetylene are known inhibitors of methanogenesis (35, 40). The levels required for inhibition (1 to 5%) are somewhat higher than the levels of production demonstrated in this study.

Our data indicate that all halogenated ethanes and ethylenes are inhibitory to methanogens, with the brominated derivatives having more potent effects. With the exception of dibromoethane, the HHs described here, when present at the levels used by Bouwer and McCarty (9, 12), do not inhibit the methanogens that we examined. Thus, this will not be a problem in treating these compounds in a dilute aqueous environment. However, in environments in which concentrated HHs exist, inhibition would most likely occur (e.g., a toxic site in Florida discharges water with both 24 μM trichloroethylene and 19 μM dichloroethylene [43]; closer to the source, this level would be much higher).

Inhibition by BES in *Methanococcus thermolithotrophicus* was prevented by excess CoM only when it was added before or with BES (Fig. 4), an observation that is consistent with the findings of others with *Methanobrevibacter ruminantium* and *Methanospirillum hungatei* (5, 6, 39). This suggests that CoM competed with BES in entering the cell; when BES was present alone, it could enter and preload the cell with inhibitor and prevent the protective effect of CoM, even if CoM was added afterward. CoM did not protect cells from inhibition by other HHs when added concurrently at a 20- to 28-fold excess, suggesting that CoM cannot prevent the entry of the HHs other than BES or that it cannot prevent the action of the inhibitor once it is inside the cells, or both. Except for BES, all the HH we have discussed here are uncharged molecules that are lipophilic in nature; because of this, they can freely enter the cells through the membranes, whereas both BES and CoM, which have charged sulfonic acid groups, must proceed via some transport mechanism; indeed, Balch and Wolfe (6) have described a transport system for CoM.

Also shown in Fig. 4 is the ability of *Methanococcus thermolithotrophicus* to recover from BES inhibition independent of any CoM addition; the cells also showed normal growth on transfer into BES-containing medium, thus indicating adaptation. *Methanospirillum hungatei* and *Methanococcus deltae* had similar responses to BES (N. Belay,

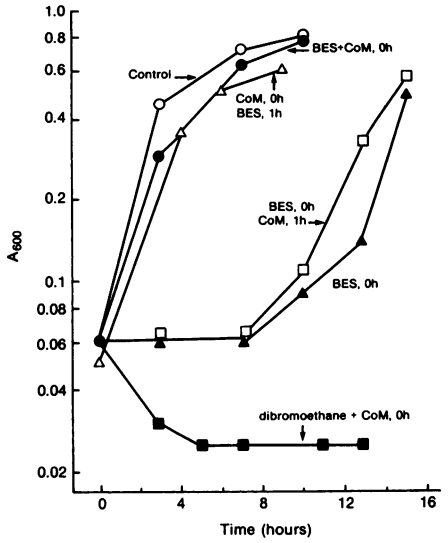


FIG. 4. Effects of BES, dibromoethane, and CoM on the growth of *Methanococcus thermolithotrophicus*. The cultures were started with a 10% inoculation into tubes containing 5 ml of medium. Symbols: ○, control with no additions; ●, 5.0 μM BES and 92 μM CoM both added at 0 h; □, 5.0 μM BES added at 0 h, 92 μM CoM added at 1 h; ▲, 5.1 μM BES added at 0 h, no CoM added; △, 92 μM CoM added at 0 h, 5.0 μM BES added at 1 h; ■, 5.6 μM dibromoethane and 160 μM CoM both added at 0 h.

unpublished data)., which is consistent with observations by Smith and Mah (39) with *Methanosarcina barkeri*. A similar phenomenon was not observed when the other HHs were used as inhibitors; growth recovery from partly inhibitory levels sometimes occurred, but cells were inhibited again on transfer into HH-containing medium. Adaptation to BES did not impart resistance to the other inhibitors.

The production of ethane, ethylene, and acetylene by methanogens is significant for several reasons. Methanogens in pure culture were previously known to produce a 2-carbon gas only via nitrogenase when acetylene was reduced to ethylene (8). Results of ecological experiments (33, 34, 42) have suggested that methanogens might produce ethane; we verified this by establishing, for the first time, that ethane, ethylene, and acetylene are produced by pure cultures. Results of this study clarify these ecological observations, but it is not clear what the precursors for these gases might be in nature or if methanogens are the only bacteria that are responsible. In another ecological context, ethylene is a growth promoter and regulator in plants (1), and in some environments it can have an agricultural impact, e.g., in a rice paddy in which halogenated pesticides, methanogens, and plants are in close association.

One of the more important aspects of our results is the degradation of toxic and hazardous wastes. Both 1- and 2-carbon HHs are thought not to be degraded well aerobically (9, 13, 29), but results of previous experiments with mixed culture systems have demonstrated bioconversions of several of these compounds under anaerobic conditions (7, 9, 10, 12, 13, 16, 27, 41). In particular, Bouwer and McCarty (9, 12) and Vogel and McCarty (41) have reported that 1,1,1-trichloroethane, 1,2-dichloroethane, trichloroethylene, 1,2-dibromoethane, and tetrachloroethylene are metabolized by anaerobic methanogenic mixed culture systems. Our results and those of mixed culture experiments described above suggest that although not all compounds react rapidly,

with the proper choice of organism several HHs might be effectively treated to yield harmless gases and salts under anaerobic conditions. Anaerobic environments would be particularly suited for HH detoxification, because much soil is largely anaerobic below a few centimeters, and groundwater has little O_2 available unless it is very nutrient poor. Sewage or waste treatment facilities most often have an anerobic portion. Lagoons used for simple waste treatments are often almost entirely anaerobic. Anaerobic degradation of HH, however, has not been carefully studied with respect to the organisms involved, including their adaptability, capabilities, and enzymes, nor has the potential for mixed culture treatment been fully explored. Indeed, anaerobes other than methanogens may carry out similar reactions.

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