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Microbial Formation of Ethane in Anoxic Estuarine Sediments

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Estuarine sediment slurries produced methane and traces of ethane when incubated under hydrogen. Formation of methane occurred over a broad temperature range with an optimum above 65°C. Ethane formation had a temperature optimum at 40°C. Formation of these two gases was inhibited by air, autoclaving, incubation at 4 and 80°C, and by the methanogenic inhibitor, 2-bromoethanesulfonic acid. Ethane production was stimulated by addition of ethylthioethanesulfonic acid, and production from ethylthioethanesulfonic acid was blocked by 2-bromoethanesulfonic acid. A highly purified enrichment culture of a methanogenic bacterium obtained from sediments produced traces of ethane from ethylthioethanesulfonic acid. These results indicate that the small quantities of ethane found in anaerobic sediments can be formed by certain methanogenic bacteria.

Biogenic rather than thermogenic processes have been implicated as the source of C_2 hydrocarbons in certain geologically recent sediments (4, 16, 18, 21, 40; B. B. Bernard, Ph.D. thesis, Texas A&M University, College Station). Little is known, however, about the microbes or their biosynthetic pathways for C_2 hydrocarbon formation within these environments. However, in plants (see review by Lieberman [23]), macroalgae (39), fungi (26, 38), and certain soil bacteria (33) ethylene is produced in trace quantities, usually from a methionine precursor (32, 42). In addition, microbial formation of traces of ethane, acetylene, and ethylene were reported during incubation of sewage or enrichment cultures derived from fecal matter (8). Recently, the production of ethane and ethylene was observed during the course of a 7-month incubation of San Francisco Bay sediments (T. M. Vogel, R. S. Oremland, and K. A. Kvenvolden, Abstr. Annu. Meet. Pacific Div. Am. Assoc. Advancement Sci., p. 33; T. M. Vogel, R. S. Oremland, and K. A. Kvenvolden, submitted for publication). This study tests one possible biosynthetic pathway that may account for the presence of traces of ethane in recent sediments.

Coenzyme M (CoM) is a low-molecular-weight cofactor (37) found only in methanogenic bacteria (2). Some methanogens require CoM for growth and must transport the compound from the environment into their cells, whereas other species of methanogens are relatively impermeable (3) and obtain CoM by biosynthesis. CoM exists in methanogens as mercaptoethanesulfonic acid (HS-CoM), methylthioethanesulfonic acid (CH_3-S-CoM), and 2,2'-dithiodiethanesulfonic acid. CoM is involved in the terminal methylation and reduction reactions of one-carbon units to methane and functions as a carrier of reduced C_1 units (13, 15, 27, 35, 37). Gunsalus et al. (14) demonstrated that the methyl CoM reductase enzyme complex present in cell-free extracts of *Methanobacterium thermoautotrophicum* evolve ethane when provided with ethylthioethanesulfonic acid (ethyl-S-CoM), a structural analog of CH_3-S-CoM. Furthermore, Balch and Wolfe (3) found that ethyl-S-CoM satisfies the growth requirement of *Methanobacterium ruminantium* for CoM. *M. ruminantium* normally obtains CoM from rumen fluid in which the compound is present due to cell lysis of methanogens (36). Because methanogenic bacteria are present in recent, anaerobic marine sediments (25, 29) this study was undertaken to determine whether ethyl-S-CoM can serve as a precursor for ethane biosynthesis. In this communication I present evidence, based on incubations of sediment slurries and bacterial cultures, that ethane can be of a microbial origin and arises from the activity of methanogenic bacteria.

(Portions of this work were presented at the 1980 Annual Meeting of the American Society for Microbiology, Miami, Fla. [abstr. no. I118, p. 104].)

MATERIALS AND METHODS

Preparation of sediment slurries. Sediments were collected from an anaerobic intertidal mudflat in San Francisco Bay at Palo Alto, Calif. The mean concentrations of C_1 and C_2 hydrocarbons in the upper 15 cm of sediment were (per liter of wet sediment): CH_4, 68 μmol; C_2H_6, 4.6 nmol; and C_2H_4, 4.3 nmol (Vogel et al. 1980; Abstr. Annu. Meet. Pacific Div. Am.

Assoc. Advancement Sci., p. 33; Vogel et al., submitted for publication). Sediments from the upper 30 cm of the mudflat were collected in a suction core, brought to the laboratory, and processed within 1 h of collection. The core (sediment volume, ≅400 cm^3) was homogenized in a Waring blender with an equal volume of San Francisco Bay water (salinity, 18 to 22 g/liter) for 5 min under a flow of N_2. The resulting homogenate was pipetted (25 ml) into Erlenmeyer flasks (total volume, ≅142 ml) containing 50 ml of bay water. Flasks were sealed under N_2 with recessed black rubber stoppers. Control flasks were either incubated under air or autoclaved (15 lb/in^2 for 30 min), cooled, and sealed under N_2 or H_2. All anaerobic flasks were flushed for 7 min (flow, ≅100 ml/min) with high-purity N_2 or H_2 (Linde Gas Co.) to remove traces of hydrocarbons and air. Flasks were incubated at 20°C (unless stated otherwise) in the dark with constant rotary shaking (150 rpm). Flasks incubated under H_2 developed negative pressures due to consumption of the gas by sulfate reducers (31; Polcin and Oremland, unpublished data), and H_2 consumption was followed by allowing flasks to draw up H_2 from H_2-filled glass syringes. Hydrocarbons in the gas phases of flasks were sampled with 0.5-ml Glaspak syringes (Becton-Dickenson Co.; 25 gauge needles). To avoid cross-contamination, new and separate syringes were used for each flask during every experiment. Substrates and inhibitors were added from stock solutions (at a final pH of 7) either just before addition of homogenate or after sealing (syringe injection) but before final flushing with H_2 or N_2. Substrate and inhibitor concentrations are reported for a liquid phase of 65 ml (total slurry volume, ≅75 ml). All CoM derivatives were injected from stock solutions held under N_2. Compounds added were: HS-CoM, Na salt (Pierce Chemical Co.), 3.5×10^{-3} M; CH_3-S-CoM, NH_4^+ salt, 3.5×10^{-3} M; ethyl-S-CoM, NH_4^+ salt, 7×10^{-5} to 3.5×10^{-3} M; 2-bromoethanesulfonic acid (BES; Aldrich Chemical Co.), 7×10^{-4} M to 7×10^{-3} M. Cysteine-hydrochloride and $Na_2S \cdot 9H_2O$ (7×10^{-4} M, each) were added to serve as a reducing agent where indicated.

Preparation of CoM compounds. CH_3-S-CoM and ethyl-S-CoM were synthesized by the method of Gunsalus et al. (14) by reacting CH_3I and CH_3CH_2I, respectively, with commercially obtained HS-CoM dissolved in concentrated NH_4OH. After rotary evaporation, 95% acetone–5% distilled water was added to the crystal, and the resulting precipitate was filtered, acetone washed, filter dried, and stored under N_2 until needed. Twice-crystallized ethyl-S-CoM was kindly provided by R. S. Wolfe and was used where indicated.

Organisms and media. Media were prepared by using techniques for culture of strict anaerobes (5, 17). Media (10 ml) were dispensed into test tubes (18 by 150 mm; Bellco Biological Glassware) and sealed with butyl rubber, recessed stoppers (no. 1; A. H. Thomas Co.) under N_2 (sulfate reducers) or N_2-CO_2 (4:1; methanogens). Transfers (0.5 ml) were made with sterile Glaspak syringes flushed with N_2 or N_2-CO_2. After innoculation, the gas phase of the methanogen cultures was changed to H_2-CO_2 (4:1) by flushing for 5 min with a sterile, cotton-filled gassing syringe attached to a hot copper column (O_2 scrubber; flow, ≅120 ml/min). Sterile, N_2-flushed Glaspak syringes were used to sample the headspace of the cultures for hydrocarbons. All flame sterilizations of needles and stoppers were done with the H_2 flame from a gassing syringe (to avoid trace contamination with hydrocarbons in the bunsen or alcohol lamp flames).

Desulfovibrio desulfuricans ATCC 7757 and *D. aestuarii* ATCC 14822 were cultured in the lactate-yeast extract-sulfate medium of Mara and Williams (24) supplemented with resazurin (0.02%, wt/vol) as a redox indicator. Ethyl-S-CoM (2×10^{-3} M) was filter sterilized (0.22-μm pore size, Millex; Millipore Corp.) before addition to the autoclaved medium. *D. aestuarii* media also contained NaCl (2.5%, wt/vol).

Methanobacterium bryantii (strain MoH; Balch et al. [1]) was kindly provided by J. G. Ferry. The organism was grown on medium containing the following (per 960 ml of distilled water): K_2HPO_2, 0.225 g; KH_2PO_4, 0.225 g; $(NH_4)_2SO_4$, 0.225 g; NaCl, 0.45 g; $MgSO_4 \cdot 7H_2O$, 0.09 g; $CaCl_2 \cdot 2H_2O$, 0.06 g; tryptone, 2 g; yeast extract, 2 g; fatty acid mixture (6), 0.3 ml; DL-methylbutyric acid, 2 ml; trace elements solution (41), 18 ml; vitamin solution (41), 20 ml; hemin, 0.001 g; Na_2CO_3, 0.4 g; cysteine-hydrochloride, 0.25 g; $Na_2S \cdot 9H_2O$, 0.25 g; resazurin, 0.002 g; ethyl-S-CoM, 0.357 g corresponding to 2×10^{-3} M. *M. bryantii* was also cultured in media without cysteine-hydrochloride but with twice the concentration of $Na_2S \cdot 9H_2O$. The pH was 7.2 ± 0.2.

An enrichment culture of a methanogenic bacterium was obtained from a sediment slurry containing ethyl-S-CoM (7×10^{-3} M) under H_2 that actively produced CH_4 and traces of C_2H_6. The medium differed from that used for *M. bryantii* in that cysteine-hydrochloride, tryptone, and yeast extract were eliminated, and NaCl and $MgSO_4 \cdot 7H_2O$ were increased to 20 and 0.5 g, respectively. $Na_2S \cdot 9H_2O$ (0.25 g) was the sole reducing agent. Ethyl-S-CoM was added at 2×10^{-3} M, but the enrichment was also cultured in media containing HS-CoM (2×10^{-3} or 1×10^{-4} M). Optical densities of the culture were followed with a Bausch & Lomb Spectronic 21 spectrophotometer set at 660 nm (path length, 18 mm). Cultures were treated with tetracycline (~6 μg/ml) to reduce the levels of nonmethanogenic contaminant bacteria (12). The culture was examined by phase contrast and epifluorescence microscopy (American Optical cluster no. 2073; λ, 380 to 430 nm). Presumptive identification of methanogens was made by the persistence of green fluorescence, indicative of the presence of factor 420 (9, 10, 28).

Determinations of hydrocarbons. A Hewlett-Packard model 5730A gas chromatograph equipped with a flame ionization detector was used to measure C_1 and C_2 hydrocarbons. Injected samples (500 μl; Glaspak) were separated on a Porapak Q column (183 by 0.064 cm) attached in series to a Porapak S column (244 by 0.64 cm). The carrier gas was helium (ultrahigh purity; Linde Co.), the column flow was 30 ml/min, and the temperature was 50°C. This procedure achieved discrete separations of CH_4, C_2H_4, C_2H_2, and C_2H_6 within 6 min. The limit of detection of these gases was about 1.5 pmol per 500-μl injection (about 0.2 nmol $flask^{-1}$). Hydrocarbon concentrations are presented as the total present in the headspace of flasks

(~68 ml) or culture tubes (~15 ml). Corrections were not made for dissolved gases because the amounts in solution were calculated to be minor and did not alter results. When Bunsen coefficients (22) were applied to the equations of Flett et al. (11) it was estimated that the amounts of CH_4, C_2H_6, and C_2H_4 in solution were only 3, 4.5, and 10%, respectively, of the observed gas-phase concentrations.

RESULTS

Sediment slurry experiments. Sediment slurries incubated under H_2 produced CH_4, C_2H_6, and C_2H_4 and consumed H_2 (Table 1). Production of all three hydrocarbons was stimulated by an H_2 atmosphere. Addition of ethyl-S-CoM to slurries stimulated the production of C_2H_6 (12-fold) and C_2H_4 (2-fold) under H_2 (Table 1). The levels of C_2H_4 and C_2H_6 produced (3.5×10^{-9} and 18×10^{-9} mol, respectively) were much less than the amount of ethyl-S-CoM added (2.5×10^{-4} mol). Addition of HS-CoM or CH_3-S-CoM to slurries did not stimulate the production of C_2H_4 or C_2H_6 when compared with flasks incubated under H_2 without CoM amendments (Table 1).

In another experiment, the production of CH_4, C_2H_4, and C_2H_6 and uptake of H_2 as a function of time are shown for flasks incubated with ethyl-S-CoM (Fig. 1). Uptake of H_2 and evolution of C_2H_4 preceded CH_4 and C_2H_6 formation. Production of both C_2H_4 and C_2H_6 was stimulated by 10-fold increases in the concentration of ethyl-S-CoM (7×10^{-5}, 7×10^{-4}, and 7×10^{-3} M) under H_2. A saturation effect occurred for C_2H_6 at 7×10^{-3} M ethyl-S-CoM (incubation time, 17 days). Ethane levels (three flasks ± 1 standard deviation) were 4.4 ± 1.1, 9.1 ± 1.8, 11.9 ± 2.1, and 11.7 ± 3.8 nmol $flask^{-1}$ at ethyl-S-CoM concentrations of 0, 7×10^{-5}, 7×10^{-4}, and 7×10^{-3} M, respectively. By contrast, C_2H_4 did not exhibit a saturation effect, and levels increased with higher ethyl-S-CoM concentrations (1.7 ± 1.6, 1.3 ± 0.3, 4.8 ± 1.1, and 23.5 ± 3.4 nmol $flask^{-1}$ at 0, 7×10^{-5}, 7×10^{-4}, and 7×10^{-3} M ethyl-S-CoM, respectively). There was

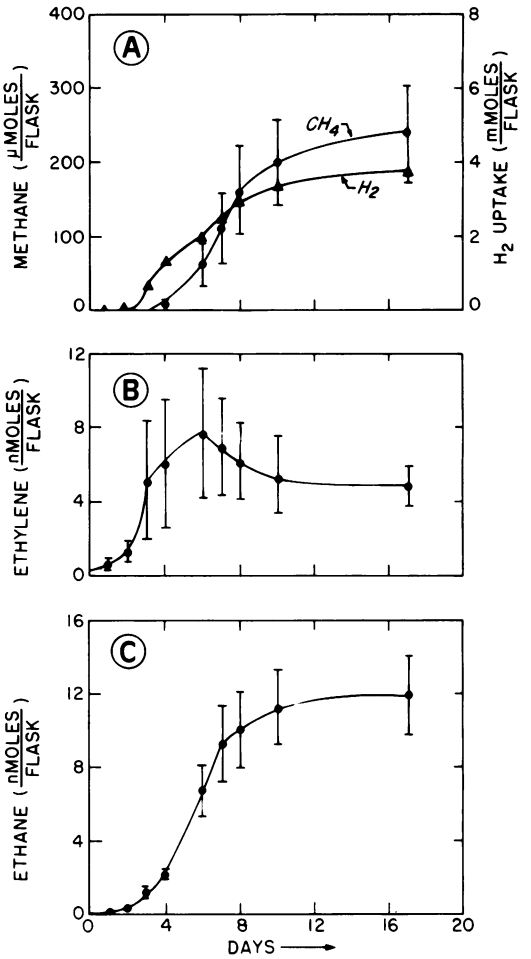

FIG. 1. *Gaseous metabolism of sediment slurries incubated under H_2 with ethyl-S-CoM (7×10^{-4} M, corresponding to 5×10^{-5} mol $flask^{-1}$). Results represent the mean ± 1 standard deviation of three experimental flasks. (A) Formation of methane (●) and uptake of H_2 (▲); the standard deviations for H_2 uptake were too small to be plotted. (B) Formation of C_2H_4. (C) Formation of C_2H_6.*

TABLE 1. *Final concentrations of CH_4, C_2H_4, and C_2H_6 and total uptake of H_2 for sediment slurries incubated for 39 days*[a]

Atmosphere	Amendments	CH_4 (μmol)	C_2H_4 (nmol)	C_2H_6 (nmol)	H_2 uptake (mmol)
N_2	None	0.26 (0)	0.2 (0.2)	0.5 (0.0)	
H_2	None	317 (199)	1.7 (0.1)	1.2 (0.7)	6.1 (0.7)
H_2	H-S-CoM	333 (134)	2.0 (1.0)	0.8 (0.3)	6.7 (0.2)
H_2	CH_3-S-CoM	404 (72)	1.9 (0.5)	1.2 (0.4)	6.9 (0.3)
H_2	Ethyl-S-CoM	310 (92)	3.5 (0.5)	18.0 (5.0)	7.3 (1.9)

[a] Values represent mean (1 standard deviation) per flask of three experimental flasks. Concentrations of HS-CoM, CH_3-S-CoM, and ethyl-S-CoM were 3.5×10^{-3} M (2.5×10^{-4} mol $flask^{-1}$).

a strong positive correlation between CH_4 and C_2H_6 for the flasks incubated under H_2 with or without ethyl-S-CoM. The relationship between the two gases had an exponential characteristic of the form $CH_4 = M(C_2H_6)^B$, and high correlation coefficients were observed ($r = 0.94$ to 0.97).

A small but discernable amount of C_2H_6 was produced under an H_2 atmosphere without amendments of ethyl-S-CoM (Fig. 2). BES, an inhibitor of methanogenic bacteria (14), totally blocked the production of C_2H_6 (with and without ethyl-S-CoM; Fig. 2) and of CH_4, but not H_2 uptake (data not shown). Ethane production had a temperature optimum at 40°C (with or without ethyl-S-CoM), whereas CH_4 had an optimum above 65°C (Fig. 3). Production of both CH_4 and C_2H_6 was inhibited by incubation at 4°C (Fig. 3), as was uptake of H_2 (mean ± 1 standard deviation uptake by 7 days at 18°C, 2,013 ± 395 μmol versus 80 ± 51 μmol at 4°C). In another experiment, CH_4 and C_2H_6 formation and H_2 uptake were inhibited by incubation at 80°C (Table 2). In addition, no noticeable formation of CH_4 or C_2H_6 occurred when sediments were incubated under air or were autoclaved. By contrast, C_2H_4 formation (from ethyl-S-CoM) was not stopped by incubation under air or by autoclaving, and BES did not block C_2H_4 formation from ethyl-S-CoM. Incubation at 80°C caused C_2H_4 release from the rubber stoppers, thereby masking the effect on the sediment slurries (this did not occur at temperatures below 70°C).

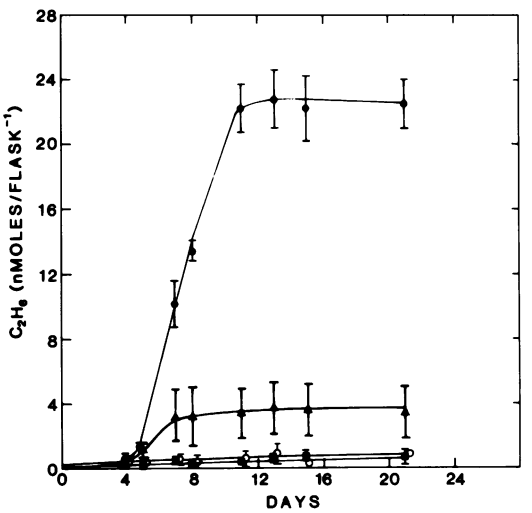

FIG. 2. *Formation of C_2H_6 by sediment slurries incubated under H_2. Points indicate the mean ± 1 standard deviation (bars) of three flasks with no additions (▲); amended with ethyl-S-CoM, 2.1 × 10^{-3} M (●); inhibited by BES (■), 2.8 × 10^{-3} M; or amended with ethyl-S-CoM and inhibited by BES (○). CH_4 production was also inhibited by BES (not shown). Flasks incubated under N_2 with or without ethyl-S-CoM did not produce C_2H_6. After 21 days of incubation under N_2, levels of C_2H_6 were 0.45 ± 0.41 and 0.48 ± 0.17 nmol flask^{-1} for unamended and ethyl-S-CoM amended flasks, respectively (mean of three flasks ± 1 standard deviation).*

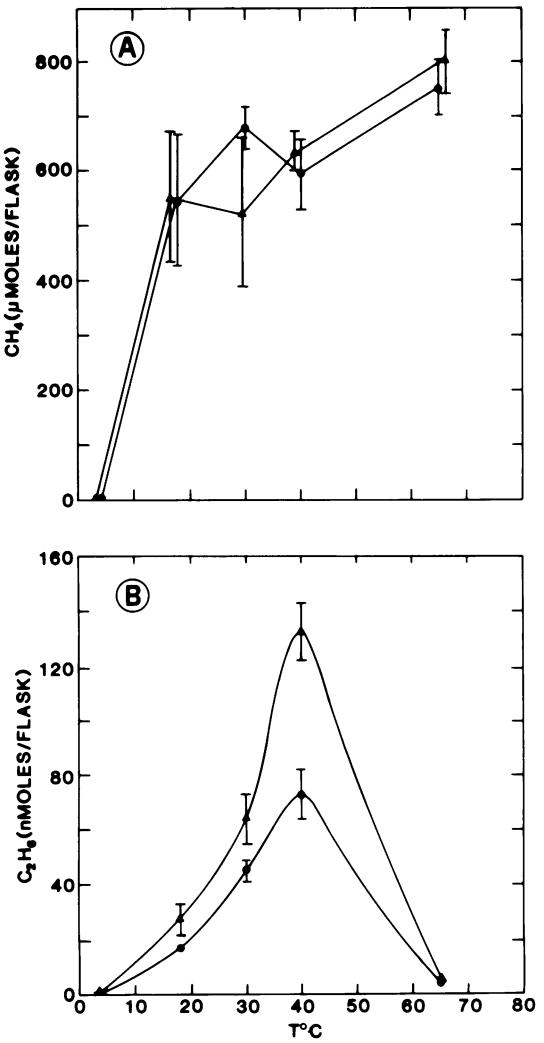

FIG. 3. *Concentrations of CH_4 (A) and C_2H_6 (B) after sediment slurries were incubated for 7 days at the temperatures indicated. Results represent the mean ± 1 standard deviation (bars) of three flasks amended with ethyl-S-CoM (▲) at 7 × 10^{-4} M or with no amendments (●). All flasks contained a cysteine-sulfide reducing agent. Significant C_2H_4 production was not observed.*

Experiments with bacterial cultures. An enrichment culture was obtained from a sediment slurry that had produced CH_4 and traces of C_2H_6. Two sequential transfers of the culture were made after 3 or 4 days in the enrichment

TABLE 2. *Levels of CH_4 and C_2H_6 and uptake of H_2 for flasks incubated at either 20 or 80°C*[a]

Amendment	Temp (°C)	CH_4 level (μmol)	C_2H_6 level (nmol)	H_2 uptake (mmol)
None	20	475	1.10	3.12
	80[b]	0.56	0.75	0
Ethyl-S-CoM	20	418	26.8	2.58
	80[b]	0.61	0.95	0

[a] Flasks were incubated for 10 days under H_2 with or without ethyl-S-CoM (2.5×10^{-3} M). Figures represent level or uptake per flask.

[b] Flasks incubated at 80°C maintained positive pressures (about 1.3 atm) due to the higher temperature. No loss of pressure was observed.

medium during which time CH_4 and traces of C_2H_6 continued to be produced. With the third sequential transfer, sediment particles were diluted out and bacterial growth could be followed by turbidity (absorbancy at 660 nm).

The enrichment produced CH_4 and traces of C_2H_6 as a consequence of bacterial growth in medium containing ethyl-S-CoM (Fig. 4). Ethylene production was not observed, and the final ratio of CH_4 to C_2H_6 was about 10^4:1. Formation of CH_4 and C_2H_6 and bacterial growth were totally blocked either by filter sterilization of the syringe inoculum or by inclusion of BES in the medium (Fig. 4). Microscopic examination after tetracycline treatment and serial dilution (10^{-4}) revealed the presence of only coccoid-shaped

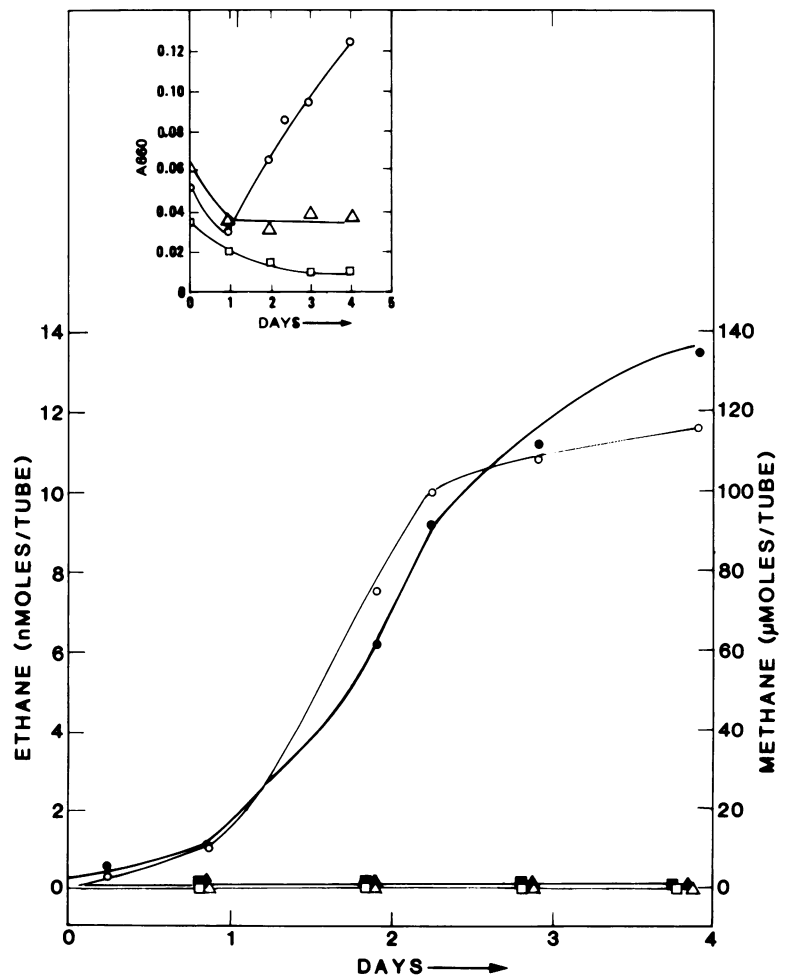

FIG. 4. *Concentrations of CH_4 and C_2H_6 in enrichment cultures containing 2×10^{-3} M (20 μmol per tube) ethyl-S-CoM. Experimental tube, CH_4 (○) and C_2H_6 (●); filter-sterilized inoculum control, CH_4 (□) and C_2H_6 (■); BES (4×10^{-3} M), CH_4 (△) and C_2H_6 (▲). Insert shows growth (Absorbance at 660 nm) in experimental (○), filter-sterilized (□), and BES-inhibited (△) tubes.*

cells (diameter, ~1.3 to 2.5 μm). The cells fluoresced green when examined by epifluorescence microscopy, suggesting that the organisms were methanogenic bacteria. Transfer of the enrichment to a medium in which ethyl-S-CoM was replaced by HS-CoM (2×10^{-3} or 1×10^{-4} M) caused a cessation of C_2H_6 formation, but not CH_4 formation. After several transfers in HS-CoM medium, the enrichment was transferred to a medium containing the twice-crystallized ethyl-S-CoM. Ethane production resumed as observed previously and continued to do so after several repeated transfers.

No production of CH_4, C_2H_4, or C_2H_6 occurred when *D. desulfuricans* or *D. aestuarii* were grown in the lactate-yeast extract-sulfate medium containing ethyl-S-CoM. In addition, C_2H_6 production by *M. bryantii* was never observed, even after several transfers in media containing ethyl-S-CoM with or without cysteine. *M. bryantii* grew and produced CH_4 in all of the media tested.

DISCUSSION

This report demonstrates that the small quantities of C_2H_6 found in recent anaerobic sediments can arise from a microbial reaction carried out, at least in part, by certain methanogenic bacteria. Evidence for microbial participation was the observed inhibition of C_2H_6 formation at biologically extreme temperatures (4°C and above 65°C) and by the presence of an optimum temperature (Fig. 3B, Table 2). Temperature optima and inhibition at extremes are common features of microbial systems and have been observed for bacterial methane formation in these experiments (Fig. 3A) and in lake sediments (43), 44). Methane formation was inhibited at 80°C (Table 1) but was not inhibited at 65°C (Fig. 3A). Presumably the sediment harbors some thermotolerant or thermophilic methanogens capable of forming CH_4, but not C_2H_6, at 65°C.

Evidence that methanogenic bacteria were involved in the formation of C_2H_6 was the common inhibition of CH_4 and C_2H_6 evolution by air and BES, both known inhibitors of methanogenesis (14, 34). In addition, CH_4 and C_2H_6 production were closely correlated during sediment slurry incubations (Fig. 1), and the production of both gases was stimulated by H_2 (Table 1, Fig. 2), an energy source common to most methanogenic bacteria (1). Finally, a methanogenic enrichment culture was recovered from sediments which could cleave ethyl-S-CoM to yield C_2H_6 (Fig. 4).

Production of C_2H_6 was not observed when *M. bryantii* or the sulfate reducers were grown in the presence of ethyl-S-CoM. This indicates that C_2H_6 production from ethyl-S-CoM may be confined to certain species of methanogens. Methanogens have different permeabilities to CoM (3), and the enrichment which we cultured is probably highly permeable (future work will be done to characterize the isolate). The C_2H_6 produced from ethyl-S-CoM arises from cleavage of the ethyl group since neither HS-CoM nor CH_3-S-CoM could stimulate C_2H_6 production by sediments (Table 1), and C_2H_6 was not produced by the enrichment culture grown with HS-CoM. Since ethyl-S-CoM stimulates C_2H_6 production in sediments and by the enrichment culture, the molecule (or an analogous compound) may be the precursor for biogenic C_2H_6 formation in anaerobic sediments.

The behavior displayed by C_2H_4 during these experiments is more difficult to explain than that of CH_4 or C_2H_6. Ethylene production was stimulated by addition of ethyl-S-CoM to sediment slurries (Fig. 1, Table 1), but formation was not blocked by autoclaving the sediments before addition. This indicates a chemical cleavage of some of the ethyl-S-CoM to yield C_2H_4. Ethylene can be produced by various microorganisms (26, 32, 33, 38), and it has been suggested that the gas is reduced to C_2H_6 or C_2H_5SH by chemical or biological reactions occurring in sediments (19, 40). Since C_2H_4 inhibits CH_4 formation (30), it is possible that methanogens remove C_2H_4 by reducing it with H_2 to form C_2H_6. However, a clear stimulation of C_2H_6 production was not observed when C_2H_4 was added to the gas phase of methanogenic enrichment cultures obtained from tropical marine sediments (Oremland, Ph.D. thesis, University of Miami, Miami, Fla., 1976) or when ultrahigh-purity C_2H_4 (0.22 to 2.2% by volume; Matheson Gas Co.) was added to the gas phases of H_2-incubated San Francisco Bay sediment slurries.

The variations found in the composition of natural gases with respect to their relative alkane content forms part of a basis for determining their origin. In general, natural gases having high $CH_4/(C_2H_6 + C_3H_8)$ ratios are thought to be of biogenic origin, whereas low ratios signal a thermogenic origin and may indicate the presence of oil and natural gas deposits (Bernard, Ph.D. thesis). Use of the $CH_4/(C_2H_6 + C_3H_8)$ ratio has its limitations, however, especially in regions where the total gaseous hydrocarbon content is low (21) and may be derived both from biogenic and thermogenic sources. Mechanisms for possible low-temperature C_2H_6 formation in recent anaerobic sediments are usually speculative (16, 19, 40). The results presented in this paper indicate that methanogenic bacteria produce traces of C_2H_6 from an ethyl-S-CoM

precursor. A search of recent, anaerobic sediments for CoM compounds and their derivatives (or structural analogs) would therefore be of great interest to microbial ecologists and organic geochemists.

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