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Bacterial ethane formation from reduced, ethylated sulfur compounds in anoxic sediments

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Abstract—Trace levels of ethane were produced biologically in anoxic sediment slurries from five chemically different aquatic environments. Gases from these locations displayed biogenic characteristics, having ^{12}C-enriched values of $\delta^{13}CH_4$ (−62 to −86‰), $\delta^{13}C_2H_6$ (−35 to −55‰) and high ratios (720 to 140,000) of $CH_4/[C_2H_6 + C_3H_8]$. Endogenous production of ethane by slurries was inhibited by autoclaving or by addition of the inhibitor of methanogenic bacteria, 2-bromoethanesulfonic acid (BES). Ethane formation was stimulated markedly by ethanethiol (ESH), and, to a lesser extent, by diethylsulfide (DES). Formation of methane and ethane in ESH- or DES-amended slurries was blocked by BES. Experiments showed that ethionine (or an analogous compound) could be a precursor of ESH. Ethylamine or ethanol additions to slurries caused only a minor stimulation of ethane formation. Similarly, propanethiol additions resulted in only a minor enhancement of propane formation. Cell suspensions of a methyltrophic methanogen produced traces of ethane when incubated in the presence of DES, although the organism did not grow on this compound. These results indicate that methanogenic bacteria produce ethane from the traces of ethylated sulfur compounds present in recent sediments. Preliminary estimates of stable carbon isotope fractionation associated with sediment methane formation from dimethylsulfide was about 40‰, while ethane formation from DES and ESH was only 4. 6 and 6.5‰, respectively.

INTRODUCTION

THE ASSOCIATION OF petroleum deposits with thermocatalytic (thermogenic) natural gases, but not with microbially-derived natural gases, serves as the basis for a geochemical means of petroleum exploration. The ability to distinguish between these two types of natural gases has, therefore, considerable economic importance. The major component of both types of natural gases is methane, which can have either a microbial or thermogenic origin. However, "significant" quantities of higher gaseous alkanes (*e.g.*, > ~1% of total) like ethane and propane are thought to be derived solely from thermogenic reactions (BERNARD *et al.*, 1978; SCHOELL, 1983). The traces (*e.g.*, < ~0.1%) of C_{2+} alkanes which are commonly detected in microbial natural gases (BERNARD *et al.*, 1978; HAMMOND, 1974; HUNT, 1974; WHELAN *et al.*, 1980; VOGEL *et al.*, 1982) are thought to be products associated with anaerobic bacterial decay (DAVIS and SQUIRES, 1954; VOGEL *et al.*, 1982; GOLLAKOTA and JAYALAKSHMI, 1983).

Despite the fact that ethane is a minor component of thermogenic gases and a trace component of microbial gases, relatively little is known about its mechanism(s) of formation by either process. Such information could be exploited to devise better criteria for distinguishing between these two major categories of natural gases. For example, values of $\delta^{13}C_2H_6$ may prove to be a useful parameter for ultimate identification of the gas formation process (JENDEN and KAPLAN, 1986). OREMLAND (1981) found that certain methanogenic bacteria in anoxic, intertidal sediments formed traces of ethane, and that an ethylated analogue of coenzyme M, namely ethylthioethanesulfonic acid (ethyl-S-CoM), stimulated this ethanogenesis. It was suggested that ethyl-S-CoM or some similar compounds existed freely in sediments, and that ethane was ultimately derived from these molecules.

Recently, KIENE *et al.* (1986) reported that methylated reduced sulfur compounds (*e.g.*, methanethiol, dimethylsulfide, dimethyldisulfide) were metabolised to methane by methanogenic bacteria in sediments and that a pure culture was isolated which could grow on dimethylsulfide. As an extension of this work, it was hypothesized that ethylated forms of these compounds, namely ethanethiol (ESH) and diethylsulfide (DES), could be the precursor molecules from which certain methanogenic bacteria present in anoxic sediments form traces of ethane. We now present evidence that this process is possible, based on the observation that addition of either of these two compounds to sediments consistently stimulated ethanogenic activity, and that such activity was abolished by a specific inhibitor of methanogenic bacteria. Preliminary results indicated only a small carbon isotopic fractionation may be associated with ethane formation from these compounds.

METHODS AND MATERIALS

Study sites and sampling

Anoxic sediments and associated gases were collected from five chemically-different aquatic environments. These environments included: 1) The littoral zone of Big Soda Lake, Nevada (salinity = 27‰; pH = 9.8; sulfate = 58 mM), which had a large component of malodorous decomposing cyanobacteria and detectable levels of DMS and MeSH (KIENE *et al.*, 1986; OREMLAND, 1983). For these reasons, this site was most intensively studied; 2) The pelagic zone of Mono Lake, California (salinity = 90‰; pH = 9.9; sulfate = 130 mM; OREMLAND *et al.*, 1987); 3) An estuarine saltmarsh in South San Francisco Bay (salinity = 20‰; pH = 8.1; sulfate = 18 mM; OREMLAND *et al.*, 1982); 4) The littoral zone of freshwater (sulfate = <1 mM) Searsville Lake, California (SMITH and OREMLAND, 1983); and 5) A lotic pool of Hot Creek, California (MARINER and WILEY, 1974; OREMLAND, 1983b), a geothermal, freshwater stream (sediment temperatures = 30–70°C). Gases were collected from shallow sedi-

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ments by hand stirring the soft upper 0.5 m and collecting the released bubbles with the aid of a large funnel attached to an inverted, water-filled 4 liter bottle. When all the water was displaced by the gases, the bottle was sealed with a black rubber stopper, analysed for hydrocarbon content (see below) and stored at −20°C (to prevent any subsequent bacterial activity from altering the original isotopic composition) until stable isotopic analyses of carbon and hydrogen in methane and carbon in ethane were performed (see below). Gases collected from the pelagic sediments of Mono Lake represent hydrocarbons extracted from cores as reported elsewhere (OREMLAND *et al.*, 1987). Sediments from this site were recovered from the upper 30 cm by use of an Eckman dredge.

Preparation of sediment slurries

Sediments were taken from the sites in completely filled Mason jars (to avoid exposure to air) and stored at room temperature (22°C), or in the case of Mono Lake sediments, at 10°C. Experiments were commenced usually within 24 h of collection. In cases of repeated experiments with Big Soda Lake sediments (the most studied materials) storage for as long as six weeks before initiating experiments sometimes occurred. Sediments were homogenized under N_2 with an equal volume of water collected from the respective site, and dispensed (volume = 10, 15 or 25 ml) into serum vials (37 or 63 ml) as described elsewhere (OREMLAND and POLCIN, 1982; KIENE *et al.*, 1986). The bottles were crimp-sealed with black, butyl-rubber stoppers which were previously treated with boiling 0.1 N NaOH to remove volatile organics. Substrates added to the slurries included: ethanethiol (ESH), diethylsulfide (DES), propanethiol (PSH), dimethylsulfide (DMS), trimethylamine (TMA), ethylamine (EAM), methanol (MeOH), ethionine, ethanol (EtOH) and/or the methanogenic inhibitor 2-bromoethanesulfonic acid (BES; GUNSALUS *et al.*, 1978). Substrate addition was achieved by syringe injection from anaerobic stock solutions or as additions as crystals (final concentrations are indicated in the text). All volatile organosulfur compounds, BES and alkylated amines were obtained from Aldrich Chemical Co. (purity > 99%). All other chemicals were of standard reagent grade (denatured EtOH purity = 95%). Heat-killed controls were autoclaved at 250 Kpa and 121°C for 30 min. Unless indicated otherwise, slurries were incubated under a N_2 atmosphere at room temperature or, in the case of the Hot Creek samples, at 30°C. Sediments incubated under H_2 developed negative pressures caused by bacterial H_2 consumption and were restored to ambient pressure by syringe (OREMLAND and POLCIN, 1982). Incubations were conducted in the dark with constant rotary shaking (150 rpm). Headspace analyses of C_1 to C_3 hydrocarbons and ESH were made by flame ionization gas chromatography as described elsewhere (OREMLAND, 1981; KIENE *et al.*, 1986; OREMLAND *et al.*, 1987). Detection limits (nmol in headspace/liter sediment slurry) were: methane (1), ethane (5), propane (5).

Stable isotopic fractionation during sediment slurry incubations

Sediment slurries were prepared as described above using material from Big Soda Lake. In order to produce sufficient ethane for stable isotopic analysis, it was necessary to "scale up" the quantity of slurry used. Thus, 1 liter of homogenate was placed in 2.2 liter flasks and sealed under He. DES (10 mM), ESH (2 mM), DMS (10 mM) or no additions were made to 4 flasks. The DMS condition was chosen in order to compare the fractionation associated with ethanogenesis with that of methane formation from an analogous compound. The flasks were incubated at 22°C with reciprocal shaking (100 rpm). When ethane production leveled off after 27 days incubation, the entire gas phases were transferred to 1 liter conical flasks and sealed with black rubber stoppers. In the case of ethanogenesis, only a small quantity (< 0.3%) of the ethylated compound was converted to ethane. However, in the case of DMS conversion to methane, a significant quantity was reacted (approx. 17% as determined from methane produced) before enhanced methane production over the high endogenous rate was apparent. The DMS part of experiment was repeated using a much more dilute ratio (1:15) of sediment:water in order to reduce the contribution of endogenous methane formation. Collected gases from the above experiments were analysed for $\delta^{13}CH_4$ and $\delta^{13}C_2H_6$ as described below.

Experiments with a methanogenic bacterium

A pure culture of a methylotrophic metanogen capable of growth on DMS was tested for its ability to grow on DES. A mineral salts medium (OREMLAND, 1981; KIENE *et al.*, 1986) was distributed into test tubes with 10 mM DES as substrate. Growth was followed by measuring headspace hydrocarbons and the ability of the culture to be successfully transferred. In an experiment with washed cell suspensions, a DMS-containing sterile culture tube was inoculated with log-phase cells. After 2 weeks incubation, the cell density had reached between 10^7 to 10^8 cell ml^{-1} (approximately 15–20 ug protein ml^{-1}). Three ml of the culture were placed in each of 3 centrifuge tubes, spun down (6,000 × g for 10 min.), decanted and resuspended in 2.5 ml of mineral salts medium. The tubes were given the following substrate conditions: 1) DMS plus DES (10 mM each), 2) DES alone (10 mM), and 3) boiled for 10 min. with 10 mM DES added after cooling. All manipulations were made in an anaerobic glove bag (Coy Laboratory Products, Ann Arbor, Mi). The tubes (vol = 14 ml) were capped with black butyl rubber stoppers and flushed with 4:1 N_2:CO_2 (oxygen free) for 15 min. Levels of CH_4, C_2H_4 and C_2H_6 in the tubes were measured over the following 6 days.

Stable isotopic analyses

The basic analytical procedures used in determination of $^{13}C/^{12}C$ and D/H ratios in the samples obtained in this study have been published previously (COLEMAN *et al.*, 1982; FABER and STAHL, 1983; WHITICAR *et al.*, 1986; OREMLAND *et al.*, 1987). Results of mass spectrometric analyses are reported in the usual delta notation:

$$dR = \left[\frac{(\text{Ra/Rb sample})}{(\text{Ra/Rb standard})} - 1\right] \times 1000$$

where Ra/Rb is the $^{13}C/^{12}C$ or D/H ratios relative to the PDB standard for carbon and the SMOW standard for hydrogen, respectively. Because of the small quantity of ethane in the samples relative to methane (approx. 10^{-4} or higher), the presence of CO_2 in the samples and, in the case of the slurry experiments, the presence of volatile carbon precursors (*e.g.*, DES, ESH, DMS) it was necessary to devise a system to separate the ethane from these associated gases. This was achieved by initial addition of KOH to the sample bottle (to lower the PCO_2), followed by flushing the sample out of the bottles with high purity He. The exiting gases were passed over water traps (dry ice + propanol) and into a stainless steel column (20 cm × 0.3 cm) held in liquid N_2. The trap allowed most of the methane to pass through while it retained ethane and carbon dioxide. The untrapped methane was analysed for $^{13}C/^{12}C$ and D/H as reported elsewhere (OREMLAND *et al.*, 1987). The trapping column was subsequently warmed and the exiting gases were switched into a temperature-programmed gas chromatograph having a Poropak column (FABER and STAHL, 1983). Following chromatographic separation, the ethane was combusted in a CuO oven (880°C) and the product carbon dioxide and water were separated cryogenically. A minimum of 50 μl ethane was required for analysis. Precision, reproducibility and blanks of the system were checked using gas standards of known isotopic composition. This was performed at concentrations similar to those found in the samples. The uncertainty of the $^{13}C/^{12}C$ ratios in the samples having the lowest quantities of ethane was about ±1‰, while it was about ± 0.3‰ at higher concentrations (>100 μl). The analyses of the precursor substrates DES, DMS and ESH were prepared on a separate line and analytical precision was ±0.1‰ for $^{13}C/^{12}C$ and ±0.4‰ for D/H.

RESULTS

Characteristics of hydrocarbons from sampling sites

Methane was the most abundant hydrocarbon present in the gases at all five sampling sites, ranging from 25 to 65% by volume of the collected bubbles and 1.3 mM for the interstitial gases of Mono Lake (Table 1). No significant quantities of ethylene or propylene were detected at any of the

Table 1: Abundance and stable isotopic composition of C_1 - C_3 alkanes collected from sampling sites.

SITE[a]	CH_4 (%)	$\delta^{13}CH_4$ (o/oo)	δDCH_4 (o/oo)	C_2H_6 (%)	$\delta^{13}C_2H_6$ (o/oo)	C_3H_8 (%)	$\frac{C1}{C2+C3}$
SVL	50	-66	-353	0.00031	-55	0.00012	119,048
SFB	65	-63	-331	0.00018	ND[c]	0.00050	95,588
BSL	25	-79	-531	0.00018	-50	BD[d]	138,888
HC	28	-62	-400	0.00053	-35	0.00035	31,818
ML[b]	1.3 mM	-86	-280	1.3 uM	ND	0.5 uM	722

[a] SVL = Searsville Lake; SFB = San Francisco Bay; BSL = Big Soda Lake; HC = Hot Creek; ML = Mono Lake.
[b] Mono Lake data from Oremland et al., (1987)
[c] ND = not determined
[d] BD = below detection limits

sites (<0.00001%); however all sites had traces of ethane and, with the exception of Big Soda Lake, propane. Ratios of $CH_4/[C_2H_6 + C_3H_8]$ ranged from 720 to 140,000. Values of $\delta^{13}CH_4$ and δDCH_4 at all five sites were strongly depleted in ^{13}C and deuterium, respectively. Values of $\delta^{13}C_2H_6$ were obtained for 3 sites and ranged from −35‰ (Hot Creek) to −55‰ (Searsville Lake). Values of $\delta^{13}CO_2$ (or carbonate) were: Searsville Lake (−18‰), San Francisco Bay (−12‰), Big Soda Lake (−16‰), Hot Creek (−5‰), Mono Lake (−1‰).

Production of hydrocarbons during sediment slurry incubations

Sediment slurries continuously produced methane and ethane over the course of 6–12 week incubations. A typical 3-week time course for the production of these gases is shown for an experiment with Big Soda Lake sediments (Fig. 1). Endogenous methane formation was strongly inhibited by BES, and was slightly stimulated by 10 mM DES (Fig. 1A). In contrast, addition of DES caused a 10-fold stimulation of the endogenous rate of ethane formation, while BES caused a transient inhibition of ethanogenesis (Fig. 1B). In another experiment, endogenous ethanogenesis displayed a similar enhanced response to both 10 mM DES and 1 mM ESH (Fig. 2). Controls with BES plus DES or autoclaved sediments plus DES did not produce ethane. Final levels of methane (μmoles) present in experimental flasks after 21 days incubation were similar: no additions = 28.8 ± 0.7; DES = 29.8 ± 1.4; ESH = 25.5 ± 0.5. Autoclaved and BES-inhibited controls did not form significant methane (<0.09 and 0.13 μmoles, respectively).

The effect of DES or ESH additions upon methane and ethane formation from the five sites is summarized in Table 2. The data represent final values achieved after gas production ceased during prolonged incubations. The amount of methane and ethane formed endogenously by these sediments was quite variable and differed by as much as 20-fold between sites, or as much as 3-fold at the same site (*e.g.*, Big Soda Lake). Nonetheless, all of the sediment types responded to DES or ESH by enhanced ethane levels over the unsupplemented slurries, with little noticeable influence on the amount of methane formed. Ten-fold increases in the quantity of DES or ESH added to sediments resulted in higher levels of ethane recovered; however, ethane increases were less than a factor of ten, possibly due to adsorption of the precursor sulfur compounds (KIENE *et al.*, 1986). Extremely high levels of ESH (100 mM) added to Big Soda Lake sediments had an inhibitory effect on both methane and ethane evolution. The highest yield of ethane formed from ESH was ~0.6% for the 1 mM addition to Big Soda Lake sediments. The final methane:ethane ratio in these ESH-amended sediments was 38:1. In contrast, the ratio was 2300:1 for unamended sediments. Levels of ethylene formed in these experiments were usually less than 10% of the ethane values. Only addition of BES

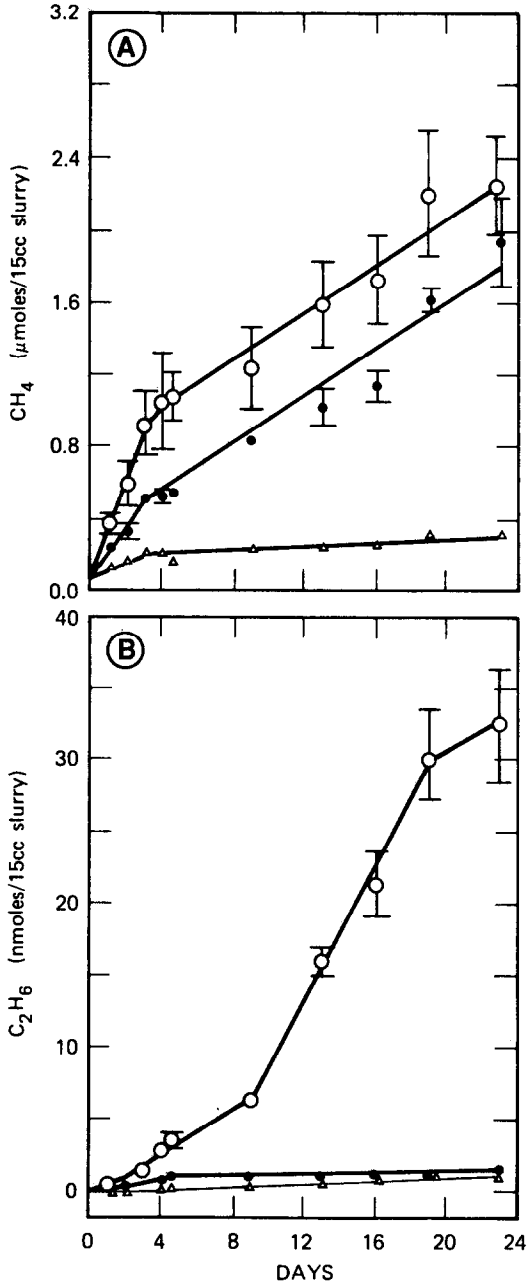

FIG. 1. Production of methane (A) and ethane (B) during incubation of sediment slurries from Big Soda Lake. Symbols: no additions (●), with 10 mM DES (○), with 40 mM BES (△). Symbols represent the mean of 3 samples and bars indicate ±1 standard deviation. Absence of bars indicates error was less than symbol.

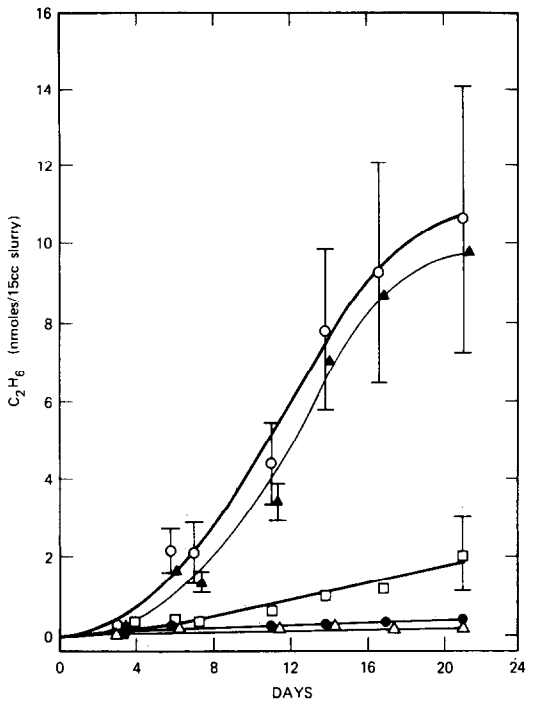

FIG. 2. Production of ethane during incubation of Big Soda Lake sediment slurries. Symbols: No additions (□), with 10 mM DES (○), with 1 mM ESH (▲), with 10 mM DES and 40 mM BES (●), and autoclaved with 10 mM DES (△). Symbols represent the mean of three samples and bars indicate ±1 standard deviation. Absence of bars indicates error was smaller than symbols.

Table 2: Levels of methane and ethane formed by sediment slurries from five aquatic environments after prolonged incubation.

SITE[a]	ADDITION	CONC'N (mM)	METHANE[b] (umol/L)		ETHANE[b] (nmol/L)	
BSL-A	None	-	1040	(134)	20	(2)
	DES	0.2	910	(82)	18	(1)
	DES	1.0	910	(27)	24	(2)
	DES	10.0	1100	(209)	130	(6)
	DES + BES	10.0 + 40.0	67	(7)	5	(1)
BSL-B	None	-	306	(68)	135	(12)
	ESH	1.0	215	(49)	5680	(111)
	ESH	10.0	294	(14)	13760	(163)
	ESH	100.0	81	(136)	169	(30)
SVL	None	-	4580	(258)	53	(17)
	DES	0.1	4264	(484)	99	(10)
	DES	1.0	4350	(70)	248	(14)
	DES	10.0	3950	(244)	281	(13)
	ESH	0.1	4840	(374)	80	(10)
	ESH	1.0	4900	(477)	177	(29)
	ESH	10.0	4110	(705)	377	(102)
	ESH + BES	1.0 + 40.0	6	(1)	14	(3)
	BES	40.0	6	(1)	12	(0)
	H_2	-	19224	(2032)	129	(23)
SFB	None	-	4830	(515)	25	(3)
	DES	0.1	4860	(217)	23	(2)
	DES	1.0	4590	(177)	79	(2)
	DES	10.0	5540	(611)	1931	(1682)
	ESH	0.1	3950	(306)	182	(8)
	ESH	1.0	5500	(1066)	720	(308)
	ESH	10.0	3360	(273)	3700	(1352)
ML	None	-	160	(16)	73	(5)
	ESH	1.0	220	(20)	2037	(295)
	DES	1.0	180	(19)	573	(35)
HC	None	-	6660	(945)	13	(3)
	DES	1.0	6810	(253)	377	(47)
	ESH	1.0	5870	(253)	107	(5)
	BES	40.0	200	(7)	5	(2)

a- Key to sites and incubation times: BSL-A = Big Soda Lake, 44 days; BSL-B = Big Soda Lake, 64 days (separate experiment); SVL = Searsville Lake, 87 days; SFB = San Francisco Bay, 110 days; ML = Mono Lake, 127 days; HC = Hot Creek, 127 days.

b- Values represent the mean of 3 samples and parenthesis indicate 1 standard deviation.

resulted in a noticeable accumulation of ethylene (data not shown).

Addition of BES to Searsville Lake sediments inhibited endogenous methane and ethane production, as well as the enhanced formation from ESH (Table 2). Similar results were obtained with the more extensively studied Big Soda Lake sediments (Figs. 1, 2; Tables 2, 5). BES also inhibited such activity in Hot Creek (Table 2). San Francisco Bay and Mono Lake were not tested for sensitivity to BES in this study, but inhibition of methanogenesis by BES in these sediments was reported previously (OREMLAND *et al.*, 1982; KIENE *et al.*, 1986).

Incubation under a H_2 atmosphere markedly stimulated methane production by sediments from Searsville Lake (Table 2), San Francisco Bay (Table 3), and Big Soda Lake (Table 4). Production of ethane from Searsville Lake sediments was also stimulated (2.4-fold) by H_2. Big Soda Lake sediments sometimes demonstrated enhanced ethane formation with H_2 whereas sometimes the opposite occurred. For example, in one experiment, sediments formed 3.2-fold less ethane under H_2 than under N_2, and H_2 lowered ethane formation from ESH by a factor of 9 (Table 4). However, in another experiment, H_2 enhanced ethane formation from ESH by a factor of 43 (not shown). Hydrogen inhibited production of ethane in San Francisco Bay saltmarsh sediments (Table 3).

Addition of 13 μM ethionine to sediments resulted in the production of ESH (Fig. 3). Higher levels of ethionine (130 μM) caused a 6-fold increase in the quantity of ESH evolved. No ESH was evolved from unamended sediments or from autoclaved sediments with 13 μM ethionine.

Ethylated compounds other than those of sulfur were tested for their ability to stimulate ethane formation. In the case of ethylamine (EAM), sediments from San Francisco Bay responded to 1.0 mM EAM with only a modest amount of enhanced ethane formation (no stimulation occurred with 0.1 mM EAM) (Table 3). Incubation with 10.0 mM EAM did not enhance ethane formation over the 1.0 mM addition. Sediments incubated with H_2 + EAM did not form ethane. No stimulation of ethane formation was noted when Big Soda Lake sediments were incubated with 10.0 mM EAM.

Table 3: Influence of ethylamine on the formation of methane and ethane by sediment slurries.

SITE[a]	ADDITION	CONC'N (mM)	METHANE[b] (umol/L)		ETHANE[b] (nmol/L)	
SFB	None	-	644	(132)	11	(2)
	EAM	0.1	640	(112)	12	(0)
	EAM	1.0	1020	(216)	28	(2)
	EAM	10.0	688	(108)	23	(0)
	H_2	-	14480	(960)	0	(0)
	EAM + H_2	10.0	11600	(5520)	0	(0)
BSL	None	-	306	(68)	135	(12)
	EAM	10.0	287	(79)	111	(9)

a San Francisco Bay = SFB; incubated for 63 days; Big Soda Lake = BSL, 64 days incubation.

b Values represent the mean of three samples and parenthesis indicate 1 standaqrd deviation. Expressed as umol or nmol formed per liter of sediment slurry.

Table 4: Levels of methane and ethane formed by sediment slurries from Big Soda Lake.

ADDITION	CONC'N (mM)	METHANE[a] (umol/L)		ETHANE[a] (nmol/L)	
None	-	3592	(252)	220	(10)
ESH	1.0	3356	(520)	5680	(2000)
BES	40.0	52	(5)	76	(0)
ESH + BES	1.0 + 40.0	152	(4)	84	(5)
H_2	-	28800	(1360)	68	(72)
H_2 + BES	40.0	816	(56)	39	(14)
ESH + H_2	1.0	27160	(1040)	664	(284)

[a] Values represent the mean of 3 samples and parenthesis indicate 1 standard deviation. Expressed as umol or nmol per liter of sediment slurry. Incubation time = 62 days.

Addition of ethanol (EtOH) resulted in an enhanced production of methane when added at the 1.0 and 10.0 mM levels to Searsville Lake and San Francisco Bay sediments (Table 5). No enhanced methane was observed with 1.0 mM EtOH in Big Soda Lake sediments. An apparently enhanced ethane formation occurred with 1.0 mM EtOH in sediments from Big Soda Lake and by 10.0 mM additions to Searsville Lake and San Francisco Bay sediments. No stimulation of ethane formation was noted at the 0.1 or 1.0 mM additions to these last two environments.

In contrast to the above results with ethylated compounds, all three classes of methylated compounds markedly stimulated methanogenesis in Big Soda Lake sediments. Incubation with DMS (10 mM), MeSH (4 mM), MeOH (10 mM) or TMA (10 mM) were carried out over 19 days. The mean levels of methane formed by triplicate samples (± std. dev.) were (μmoles/L): no additions, 107 (4); trimethylamine, 10,432 (315); methanol, 4,891 (107), methanethiol, 563 (38), and dimethylsulfide, 1,749 (1,380).

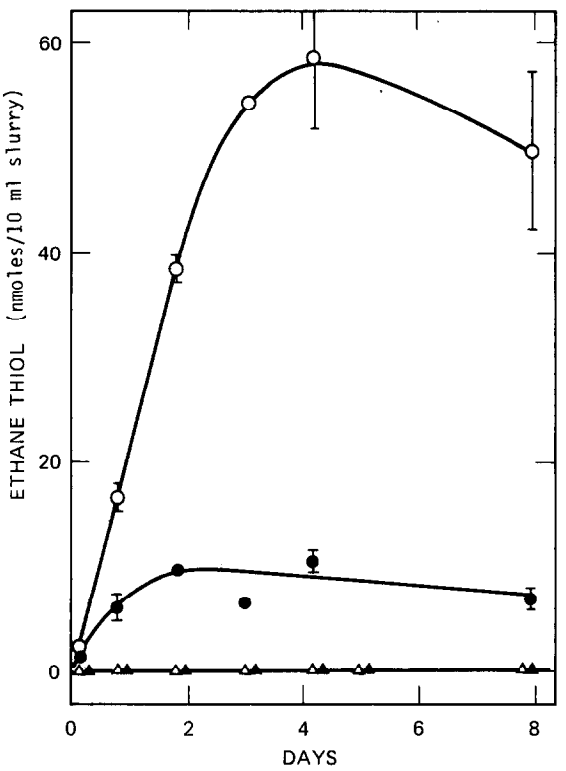

FIG. 3. Formation of ESH during incubation of sediment slurries from San Francisco Bay. Symbols: No additions (△), with 13 μM ethionine (●), with 130 μM ethionine (○), autoclaved with 130 μM ethionine (▲). Symbols represent the average of two samples and bars indicate range of values. Absence of bars indicates spread was smaller than symbol size.

Table 5: Levels of methane and ethane formed by sediment slurries incubated with ethanol.

SITE[a]	EtOH ADDED (mM)	METHANE[b] (umol/L)		ETHANE[b] (nmol/L)	
SVL	None	3393	(101)	23.5	(1.6)
	0.1	3189	(103)	23.5	(1.6)
	1.0	4175	(810)	22.4	(0.9)
	10.0	10770	(1235)	57.3	(1.8)
SFB	None	355	(74)	5.2	(0.9)
	0.1	386	(59)	4.7	(0.0)
	1.0	484	(22)	4.2	(0.9)
	10.0	1343	(91)	10.4	(1.8)
BSL	None	3632	(226)	3.1	(0.0)
	1.0	3224	(197)	19.5	(1.8)

[a] SVL = Searsville Lake (25 days); SFB = San Francisco Bay (22 days); BSL = Big Soda Lake (21 days).
[b] Results represent the mean of three samples and parenthesis indicate one standard deviation.

Sediments from Big Soda Lake were also examined for their ability to produce propane (Table 6). Only a very small quantity of propane was formed by unamended sediments, and there was a large variation amongst the samples. Additions of PSH caused a slight increase in the amount of propane evolved; however, the experimental variability between 0.1, 1.0 and 10.0 mM PSH was so great that it cannot be stated that there was an enhanced formation over the endogenous content. There was, however, slightly more propane in the 1.0 and 10.0 mM sediments than those which were autoclaved with 0.1 mM PSH. Incubation of sediments with 0.1 mM PSH under H_2 did not result in enhanced propane formation, even though methane and ethane formation were stimulated. Similarly, incubation under H_2 at 1.0 mM PSH did not enhance propane formation (not shown). In addition, we were unable to detect clearly enhanced propane formation from various levels of PSH added to sediments from San Francisco Bay or Searsville Lake (data not shown).

Stable isotopic composition of methane and ethane formed during incubation of sediments.

Big Soda Lake sediment slurries produced methane and ethane during the course of a 27 day incubation (Table 7).

Table 6: Levels of methane, ethane and propane formed by Big Soda Lake sediment slurries.

ADDITION	CONC'N (mM)	METHANE[a] (umol/L)		ETHANE[a] (nmol/L)		PROPANE[a] (nmol/L)	
None	-	7440	(240)	7	(2)	11	(10)
PSH	0.1	7720	(1200)	8	(2)	16	(8)
PSH	1.0	7280	(240)	8	(2)	23	(6)
PSH	10.0	4640	(1960)	6	(1)	31	(8)
PSH + H_2	0.1	34320	(1200)	48	(6)	14	(5)
Autocl.+ PSH	0.1	1960	-	6	-	8	-

[a] Values represent the mean of 3 samples and parenthesis indicate 1 standard deviation. Autoclaved samples were single determinations. Expressed as umol or nmol per liter of sediment slurry. Incubation time = 93 days.

Table 7: Stable isotope fractionation of DMS, DES and ESH by incubated Big Soda Lake sediment slurries.

Addition	Conc'n (mM)	δ^{13}C-Cmpd (o/oo)	δD-Cmpd (o/oo)	CH_4 (umol/l)	C_2H_6 (nmol/l)	$\delta^{13}CH_4$ (o/oo)	δDCH_4 (o/oo)	$\delta^{13}C_2H_6$ (o/oo)
experiment 1: concentrated slurries incubated 27 days								
None	-	-	-	2450	0.15	-94.9	-352.5	ND[a]
DMS	10	-34.8	-112	4870	0.01	-84.8	-404.7	-32.3
DES	10	-32.4	-147	2180	1.48	-77.8	-361.5	-37.0
ESH	2	-34.6	-112	700	6.62	-85.1	-371.9	-41.1
experiment 2: dilute slurries incubated 4-5 days								
None[b]	-	-	-	4	ND	BDL[d]	BDL	ND
DMS[b]	10	-34.8	-112	186	ND	-76.1	-226.7	ND
DMS[c]	10	-34.8	-112	659	ND	-76.8	-201.9	ND

[a]ND = not determined; [b]incubated 4 days; [c]incubated 5 days [d]BDL = below detection limit

ESH and DES enhanced ethane formation by sediments (10 to 50-fold), while DMS stimulated methanogenesis by two-fold. The $\delta^{13}CH_4$ determined for the DMS flask was −84.8‰; however, this value represents equal contributions from the endogenously formed methane ($\delta^{13}CH_4$ = −94.9‰) with that produced from DMS. Because the DMS-formed methane contributed only 50% of the methane produced, it's $\delta^{13}CH_4$ should be −74.7‰. This yields a carbon isotopic enrichment factor of 40.1‰ relative to the DMS (−34.8). This enrichment factor for methane formed from DMS was confirmed when the experiment was repeated with dilute sediment slurries in which there was no significant contribution from endogenous methane formation (Table 7). In two experiments, carbon isotopic enrichment was 41.3 and 42.0‰ relative to the DMS (−34.8‰). Fractionation of hydrogen associated with DMS metabolism resulted in a deuterium depletion of 52‰ in the first experiment, and 167.1 to 191.9‰ in the second experiment. These results were consistent with the strongly deuterium-depleted values expected for methane formation from methylated compounds (WHITICAR *et al.*, 1986). Ethane formed by endogenous sources was minor (<10%) and could be ignored when calculating fractionation associated with ESH and DES. However, in the first experiment, ^{12}C-enrichments of only 4.6 and 6.5‰ were observed for ethane formation from DES (−32.4‰) and ESH (−34.6‰), respectively (Table 7).

Experiments with a methyltrophic methanogen

No growth occurred when this organism was inoculated into medium with DES instead of DMS. After 18 days incubation, only a small quantity of methane was present (<0.2 μmol), probably caused by carry-over of DMS from the inoculum. However, a steady production of traces of ethane was observed, reaching 1.6 nmol after 21 days. The culture could not be transferred successfully. In contrast, a culture incubated with 10 mM DMS formed methane (32 μmoles in 18 days) and had less ethane present (0.25 nmol) in the headspace.

In the experiments with washed cells, those incubated with DES formed a small quantity of methane and traces of ethane, but no appreciable ethylene (Fig. 4). By contrast, cells incubated with DMS plus DES formed about 18-fold more methane and produced ethane at a slower rate. Final levels of ethane in the DMS plus DES and the DES tubes were equivalent. Traces of ethylene were evolved in the tube containing DMS plus DES. Heat-killed cells with DES did not form significant levels of either methane or ethane; however, traces of ethylene accumulated in the headspace.

DISCUSSION

Characteristics of collected gases

The methane collected from the five sites was clearly of biogenic origin (Table 1). Values of $\delta^{13}CH_4$ were enriched in ^{12}C (range = −62 to −86‰) and were highly depleted in deuterium (range = −280 to −531‰) which is characteristic of bacterially-formed natural gases (*e.g.*, BERNARD *et al.*, 1978; SCHOELL, 1983; WHITICAR *et al.*, 1986). In addition, ratios of $CH_4/[C_2H_6 + C_3H_8]$ were all in the range expected for biogenic natural gas, with 4 out of five sample having values >30,000. Only Mono Lake pelagic sediments had a markedly lower value (720), although this value too is considered characteristic of biogenic gases (BERNARD *et al.*, 1978).

All of the locations had traces of ethane present, which suggests that this gas was also of biogenic origin. The $\delta^{13}C_2H_6$ values were enriched in ^{12}C by a similar magnitude relative to the $\delta^{13}CO_2$ values. Thus, Searsville Lake had an enrichment

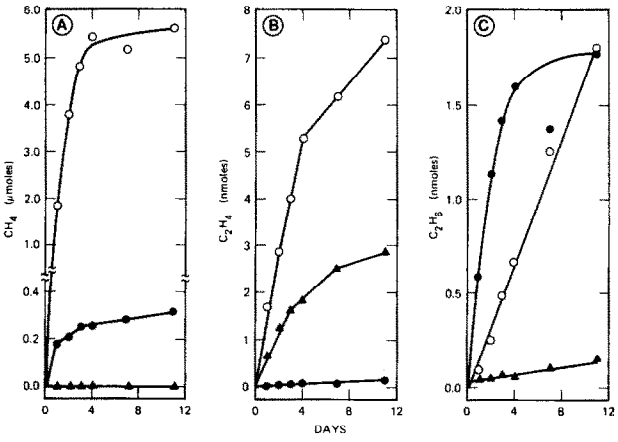

FIG. 4. Formation of methane (A), ethylene (B), and ethane (C) during incubation of cell suspensions of a methylotrophic methanogen. Symbols: with 10 mM DMS plus 10 mM DES (O), with 10 mM DES (●), boiled cells with 10 mM DES (▲).

of −37‰, Big Soda Lake had −34‰, and Hot Creek had −30‰. If ethane were derived from bacterial processes, then a progressive enrichment in ^{12}C should occur with the fixation of carbon dioxide into organic matter followed by its decomposition to ethane. The ^{12}C enrichment associated with the fixation of inorganic carbon into plants is about −15 per mil (CRAIG, 1953). Thus, the values we observed for ethane indicate a further enrichment (−15 to −22‰) than what we would expect from photosynthetic activity alone. In comparison, the enrichments for methane relative to carbon dioxide (or carbonate for Mono Lake) were much greater and more variable, ranging from −48‰ (Searsville Lake) to −85‰ (Mono Lake). We would expect less of a carbon fractionation factor to be associated with bacterial ethane formation than methanogenesis because of the involvement of a two-carbon precursor rather than only one carbon atom. The variability of the $\delta^{13}CH_4$ values could reflect seasonality (MARTENS *et al.*, 1986) as well as diversity in the isotopic content of the precursors (BLAIR *et al.*, 1987).

Interpretation of $\delta^{13}C_2H_6$ data with respect to assigning a thermogenic or microbial origin is restricted by the paucity of $\delta^{13}C_2H_6$ data in the literature. Values of $\delta^{13}C_2H_6$ present in the anaerobic bottom waters of Big Soda Lake were −27‰, and this was thought to be of microbial origin (OREMLAND and DES MARAIS, 1983). JENDEN and KAPLAN (1986) reported $\delta^{13}C_2H_6$ values of −26.6 to −34.5‰ for natural gases which appeared to have a mixture of microbial and thermogenic components. Therefore, our samples were enriched in ^{12}C relative to these reports, which would also suggest a microbial source.

Biological experiments

Incubated sediment slurries evolved both methane and ethane and evolution of both gases was blocked by the methanogenic inhibitor BES (Fig. 1; Table 2). Because BES is a specific inhibitor of methanogenic bacteria (OREMLAND and CAPONE, 1988), these results indicate that methanogens are involved in the formation not only of methane but of ethane as well. Similar results were reported previously for intertidal, estuarine sediments (OREMLAND, 1981). The endogenous production of ethane was stimulated by DES or ESH, and this production was also blocked by BES (Fig. 2; Tables 2, 4) as well as by autoclaving (not shown). These results demonstrate that evolution of ethane from DES or ESH is a biological process carried out by methanogenic bacteria present in the sediments. The fact that cell suspensions of an obligately methylotrophic methanogen were capable of evolving traces of ethane from DES further reinforces this conclusion (Fig. 4).

Sediments from all five aquatic environments responded to additions of DES or ESH by demonstrating enhanced ethane formation over the unsupplemented controls (Table 2). Also, more ethane was recovered upon addition of greater quantities of these compounds to the sediments. In general, addition of DES or ESH did not significantly effect evolution of methane (Tables 2, 4). Only in the case of addition of 100 mM ESH to Big Soda Lake sediments was there an inhibitory effect on both methane and ethane formation (Table 2). KIENE *et al.* (1986) reported that addition of methylated, reduced sulfur compounds (*e.g.*, dimethylsulfide, methane thiol, dimethyldisulfide) stimulated methanogenesis in sediments from some of the same locations tested in these ethane investigations (*e.g.*, Big Soda Lake, Mono Lake, San Francisco Bay and Searsville Lake) and that an inhibitory effect on methanogenesis was also observed at high concentrations of these compounds. Therefore, there are many similarities between formation of methane and ethane from methylated and ethylated reduced sulfur compounds, respectively. We would expect that this similarity also extends to the biochemistry of ethane formation in these diverse sediments.

Differences were also apparent between these various sediment types with regard to certain aspects of methane and ethane evolution. Clearly, the quantities of these gases evolved per unit of sediment were highly variable (Table 2). In part, this variability could be a reflection in the large differences in sulfate (and intensity of sulfate-reduction) present in these environments, which ranged from <1 mM (Searsville Lake) to 130 mM (Mono Lake).

Another obvious difference was the way in which sediments responded to H_2. Incubation of slurries under H_2 always stimulated methane formation (Tables 1, 2, 4, 6) and ethane formation was enhanced as well in Searsville Lake (Table 2). This observation agrees with previous ones made with San Francisco Bay intertidal sediments (OREMLAND, 1981). However, H_2 inhibited ethane formation in San Francisco Bay saltmarsh sediments (Table 3), while Big Soda Lake sediments responded with either inhibited (Table 4) or enhanced ethane formation (not shown). These contradictory results can be best explained by differences in the methanogenic flora. Thus, in the cases of stimulation, incubation under H_2 may have promoted the growth of H_2-utilizing, methylotrophic methanogens (*e.g.*, like *Methanosarcina barkerii*) which carried out the conversion of DES or ESH to ethane. However, in the case of H_2 inhibition, these transformations may have been carried out by obligate methylotrophs which do not use H_2 (SOWERS and FERRY, 1983; KIENE *et al.*, 1986). Under these circumstances, addition of H_2 would have enriched for H_2-oxidizing methanogens (which do not use methylated compounds) at the expense of the obligate methylotrophs.

The conversion efficiencies of DES or ESH to ethane by the sediments were low (<0.3%). In contrast, conversion of methylated sulfur compounds to methane by sediments ran as high as 63% (KIENE *et al.*, 1986). Growth of the methylotrophic methanogen on DMS resulted in stoichiometric conversion to CH_4, CO_2 and H_2S *via* an MeSH intermediate (KIENE *et al.*, 1986). This organism was unable to grow upon DES and cell suspensions formed only traces of ethane from the compound (Fig. 4). These results suggest that methanogens are incapable of growth upon either ESH or DES, and that their conversion of these substances to ethane is a consequence of co-metabolism, whereby the organism obtains its energy from oxidation of another compound. This process is illustrated by the results of the cell suspensions held in DMS + DES, in which some growth (as measured by significant quantities of methane formation) occurred while only traces of ethane were evolved (Fig. 4). Thus, although ethyl-

ated analogues of coenzyme M can be reduced by the CH_3-S-CoM reductase system (GUNSALUS *et al.*, 1978), it is unlikely that these compounds can assume importance in cellular metabolism.

It is relevant that EAM did not influence either methane or ethane evolution (Table 3). This observation agrees with that of FAHLBUSCH *et al.* (1983) who reported that *Methanosarcina barkeri* metabolizes only the methyl groups of dimethylethylamine. The stimulation of methanogenesis by EtOH (Table 5) is to be expected in anoxic sediments because this compound is quickly oxidized to acetate and H_2 (CULBERTSON *et al.*, 1988), which would then be available to the methanogens. A small stimulation of ethane formation was observed with EtOH, but in general this effect occurred only at the 10 mM applications. The stimulation could have been due to increased H_2 pools caused from EtOH oxidation or perhaps from contaminants (it was "denatured" alcohol). The enhanced ethane formation was, therefore, sufficiently different from that observed with ESH to suggest that EtOH is not a likely precursor for ethane. Thus, we can conclude that even though various methylated one-carbon compounds stimulated methanogenesis in our experiments (*e.g.*, MeOH, TMA, DMS, etc.), only the ethylated, reduced sulfur compounds were reduced to ethane. However, we were unable to detect a clear stimulation of propane formation with PSH from any of the environments tested (*e.g.*, Table 6). GUNSALUS *et al.* (1978) reported that the CH_3-S-CoM reductase of *Methanobacterium thermoautotrophicum* could reductively cleave ethylated, but not propylated forms of CoM. Our results indicate that neither the methanogens nor PSH are involved in the capacity of these sediments to form traces of propane.

These results bring to question whether DES and ESH are naturally-occurring compounds in sediments and from what molecules are they derived. Ethane thiol is present in certain natural gases (THOMPSON *et al.*, 1955) and in rabbit urine (see REID, 1958). This compound was recently identified in marine sediments (MOPPER and TAYLOR, 1986) from which it may enter the atmosphere (JORGENSEN and OKHOLM-HANSEN, 1985). Diethylsulfide was reported (but not quantified) to be emitted from an anaerobic lagoon receiving dairy manure (RASMUSSEN, 1974). These compounds appear to be present in sediments at levels much lower than DMS or MeSH (JORGENSEN and OKHOLM-HANSEN, 1985; MOPPER and TAYLOR, 1986). Although DES cannot be detected with procedures for identifying thiols, preliminary results found ESH at micromolar levels, with much of the compound bound to the sediments (R. KIENE, unpublished data). If the analogy to methanogensis holds, then DES and/or ESH should be derived from microbial metabolism of larger, ethylated macromolecules (*e.g.*, WOLFE, 1971; MAH *et al.*, 1977; OREMLAND, 1988). Methane thiol and DMS in sediments are apparently formed by metabolism of the larger sulfur compounds dimethylsulfoniopropionate and methionine by anaerobes other than methanogens (KIENE and VISSCHER, 1987). Therefore, the observation that ESH was formed during incubation of ethionine-amended sediments, and that autoclaving eliminated this activity (Fig. 3) argues that the analogy holds for ethylated compounds as well. Formation of ESH from ethionine was also reported in soils (BANWART and BREMNER, 1975). These results do not mean that ethionine is the precursor of ESH (or DES), but rather they merely indicate that the analogy is tractable.

In the experiments with sediment slurries, ethylene accumulated only when BES was added to block methanogenesis. It has been shown that BES addition to autoclaved sediments will result in the evolution of ethylene (OREMLAND, 1981). This result indicates that the gas arises *via* a chemical degradation of small quantities of BES (for a discussion, see OREMLAND and CAPONE, 1988), which is unrelated to its role as an intermediate in ethanogenesis. Non-biological evolution of traces of ethylene from various compounds has been reported in soils (SUTHERLAND and COOK, 1980). Ethylene was evolved during incubation of heat-killed cells (Fig. 4B), which would indicate that the DES present was also degrading chemically to traces of ethylene. The lack of ethylene in the headspaces of the live cells plus DES could be construed to indicate removal by the bacteria, although such activity was not previously observed (OREMLAND, 1981). Recently, BELAY and DANIELS (1987) reported that methanogens are capable of evolving traces of ethylene as well as ethane and acetylene from pollutant halogenated hydrocarbons. Thus, the question of ethylene is complex, and it appears to arise as an artifact of BES or DES additions, as well as perhaps the metabolic activities of the methanogens themselves.

Finally, a distinction must be made with regard to the biological *vs.* geochemical significance of DES and ESH as ethane precursors. The biological significance is obviously inconsequential, because ethane is only a minor product and does not appear to sustain growth. In geochemical terms, however, the fact that ethane can clearly have a biological origin is of considerable significance. For example, the presence of traces of ethane in natural gases need not be attributed to a mixture of a large quantity of microbial methane with a smaller thermogenic component because the ethane can also have a microbial origin. The large variation in the extent of ethanogenic activity we observed in our sediment slurry incubations (Table 2), suggests that there can be a wide range of trace ethane content possible in microbial natural gases. Presumably this variability would be a function of the amount of potential precursor compounds (*e.g.*, DES or ESH) initially present in the sediments.

Stable isotope fractionation

Methane formation from DMS resulted in about a 40‰ enrichment in with respect to carbon and about a 56‰ enrichment with respect to hydrogen (Table 7). We have observed a carbon enrichment of 44‰ for pure cultures grown on DMS (OREMLAND and WHITICAR, unpublished data). By contrast, only a small carbon fractionation occurred for ethane formation from DES (4.6‰) and ESH (6.5‰). Because these molecules contain two carbon atoms, we would expect that the fractionation should be half of that observed for methane from DMS, or about −20‰. This result is even more perplexing because of the ^{12}C-enriched values of $\delta^{13}C_2H_6$ we observed in the gases we collected (Table 1). Several explanations are possible for this less-than-anticipated fraction-

ation: 1) There are aspects of the chemistry of DES and ESH which differ from DMS and tend to obscure any biological fractionation. Because MeSH and DMS pools readily interchange in sediments (KIENE and VISSCHER, 1987), it probably holds true for DES and ESH as well. However, whereas much of the DMS is converted to methane, only a small portion of the DES or ESH is converted to ethane. Exchange of an intermediate (MeSH with DMS; ESH with DES) with the large pool of added reactant would tend to obscure isotopic fractionation effects. This could be most extensive when there was a large pool of reactants (DES or ESH) relative to the small amount of product (ethane) formed; 2) The biological site of reductive de-ethylation is different from that of reductive de-methylation; 3) Some fractionation also occurs during the bacterial attack of the larger ethylated compounds (*i.e.*, ethionine), which results in ^{12}C-enriched ethane in biogenic gases: and, 4) all of the above factors are involved.

CONCLUSIONS

1. Ethane formation in recent sediments is a process carried out by methanogenic bacteria.
2. Ethanethiol and diethylsulfide are the likely substrates for ethane formation by methanogens, although neither compound supports growth. These compounds probably originate from bacterial metabolism of larger ethylated compounds.
3. Only a small ^{12}C-enrichment was associated with ethanogenesis, despite the fact that ^{12}C-enriched values of $\delta^{13}C_2H_6$ are observed in recent sediments.

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REFERENCES

BANWART W. L. and BREMNER J. M. (1975) Formation of volatile sulfur compounds by microbial decomposition of sulfur-containing amino acids in soils. *Soil Biol. Biochem.* **7**, 359–364.

BELAY N. and DANIELS L. (1987) Production of ethane, ethylene and acetylene from halogenated hydrocarbons by methanogenic bacteria. *Appl. Environ. Microbiol.* **53**, 1604–1610.

BERNARD B. B., BROOKS J. M. and SACKETT W. M. (1978) Light hydrocarbons in recent Texas continental shelf and slope sediments. *J. Geophys. Res.* **83**, 4053–4061.

BLAIR N. E., MARTENS C. S. and DES MARAIS D. J. (1987) Natural abundances of carbon isotopes in acetate from a coastal marine sediment. *Science* **236**, 66–68.

COLEMAN M. L., SHEPARD T. J., DURHAM J. J., ROUSE J. E. and MOORE G. R. (1982) Reduction of water with zinc for hydrogen isotope analysis. *Anal. Chem.* **54**, 993–995.

CRAIG H. (1953) The geochemistry of stable carbon isotopes. *Geochim. Cosmochim. Acta* **3**, 53–92.

CULBERTSON C. W., STROHMAIER F. E. and OREMLAND R. S. (1988) Acetylene as a substrate in the development of primordial bacterial communities. *Origins of Life and Evolution of the Biosphere* (in press).

DAVIS J. B. and SQUIRES R. M. (1954) Detection of microbially produced hydrocarbons other than methane. *Science* **119**, 381–382.

FABER E. and STAHL W. (1983) Analytical procedure and results of an isotope geochemical survey in an area of the British North Sea. In *Petroleum Geochemistry and Exploration of Europe* (ed. J. BROOKS), pp. 51–63. Blackwell Sci. Publ., London.

FAHLBUSCH K., HIPPE H. and GOTTSCHALK G. (1983) Formation of ethylamine and methane from dimethylethylamine by *Methanosarcina barkeri*. *FEMS Microbiol. Lett.* **19**, 103–104.

GOLLAKOTA K. G. and JAYALAKSHMI B. (1983) Biogas (natural gas?) production by anaerobic digestion of oil cake by a mixed culture isolated from cow dung. *Biochem. Biophys. Res. Comm.* **110**, 32–35.

GUNSALUS R. A., ROEMESSER J. A. and WOLFE R. S. (1978) Preparation of coenzyme M analogues and their activity in the methyl coenzyme M reductase system of *Methanobacterium thermoautotrophicum*. *Biochemistry* **17**, 2374–2377.

HAMMOND D. E. (1974) Dissolved gases in Cariaco Tranch sediments: anaerobic diagenesis. In *Natural Gases in Marine Sediments* (ed. I. R. KAPLAN), pp. 71–90. Plenum Publishing Corp., New York.

HUNT J. M. (1974) Hydrocarbon geochemistry of the Black Sea. In *The Black Sea—Geology, Chemistry and Biology* (eds. E. T. DEGENS and D. A. ROSS), pp. 449–504. American Assoc. Petroleum Geologists, Tulsa, Okla.

JENDEN P. D. and KAPLAN I. R. (1986) Composition of microbial gases from the Middle America Trench and Scripps Submarine Canyon: implications for the origin of natural gas. *Appl. Geochem.* **1**, 631–646.

JORGENSEN B. B. and OKHOLM-HANSEN B. (1985) Emissions of biogenic sulfur gases from a Danish estuary. *Atmospheric Environ.* **19**, 1737–1749.

KIENE R. P., OREMLAND R. S., CATENA A., MILLER L. G. and CAPONE D. G. (1986) Metabolism of reduced methylated sulfur compounds in anaerobic sediments and by a pure culture of an estuarine methanogen. *Appl. Environ. Microbiol.* **52**, 1037–1045.

KIENE R. P. and VISSCHER P. T. (1987) Production and fate of methylated sulfur compounds from methionine and dimethylsulfoniopropionate in anoxic salt marsh sediments. *Appl. Environ. Microbiol.* **53**, 2426–2434.

MAH R. A., WARD D. M., BARESI L. and GLASS T. (1977) Biogenesis of methane. *Ann. Rev. Microbiology* **31**, 309–341.

MARINER R. H. and WILEY L. M. (1974) Geochemistry of thermal waters in Long Valley, Mono County, California. *J. Geophys. Res.* **81**, 792–800.

MARTENS C. S., BLAIR N. E., GREEN C. D. and DES MARAIS D. J. (1986) Seasonal variations in the stable carbon isotopic signature of biogenic methane in a coastal sediment. *Science* **233**, 1300–1303.

MOPPER K. and TAYLOR B. F. (1986) Thiols in coastal marine sediments. In *Organic Marine Geochemistry* (ed. M. SOHN), pp. 324–339. Amer. Chemical Soc. Sympos. Ser. 305, Washington, D.C.

OREMLAND R. S. (1981) Microbial formation of ethane in anoxic estuarine sediments. *Appl. Environ. Microbial.* **42**, 122–129.

OREMLAND R. S. (1983a) Hydrogen metabolism by decomposing cyanobacterial aggregates in Big Soda Lake, Nevada. *Appl. Environ. Microbiol.* **45**, 1519–1525.

OREMLAND R. S. (1983b) Methane in association with seismic activity. *Eos* **64**, 410.

OREMLAND R. S. (1988) The biogeochemistry of methanogenic bacteria. In *Biology of Anaerobic Microorganisms* (ed. A. J. ZEHNDER), pp. 641–702. J. Wiley & Sons, New York.

OREMLAND R. S. and DES MARAIS D. J. (1983) Distribution, abundance and carbon isotopic composition of gaseous hydrocarbons in Big Soda Lake, Nevada: An alkaline, meromictic lake. *Geochim. Cosmochim. Acta* **47**, 2107–2114.

OREMLAND R. S. and POLCIN S. P. (1982) Methanogenesis and sulfate reduction: competitive and non-competitive substrates in estuarine sediments. *Appl. Environ. Microbiol.* **44**, 1270–1276.

OREMLAND R. S. and CAPONE D. G. (1988) Use of "specific" inhibitors in biogeochemistry and microbial ecology. In *Advances in Microbial Ecology* **10** (ed. K. C. MARSHALL), pp. 285–383. Plenum, N.Y.

OREMLAND R. S., MARSH L. and POLCIN S. (1982) Methane production and simultaneous sulfate reduction in anoxic saltmarsh sediments. *Nature (London)* **292**, 143–145.

OREMLAND R. S., MILLER L. G. and WHITICAR M. J. (1987) Sources and flux of natural gases from Mono Lake, California. *Geochim. Cosmochim. Acta* **51**, 2915–2929.

RASMUSSEN R. A. (1974) Emission of biogenic hydrogen sulfide. *Tellus* **26**, 254–260.

REID E. E. (1958) *Organic Chemistry of Bivalent Sulfur*, Vol. I, Chemical Publishing Co., N.Y.

SCHOELL M. (1983) Genetic characterization of natural gases. *Amer. Assoc. Petrol. Geol. Bull.* **67**, 2225–2238.

SMITH R. L. and OREMLAND R. S. (1983) Anaerobic oxalate degradation: Widespread natural occurrence in aquatic sediments. *Appl. Environ. Microbiol.* **46**, 106–113.

SOWERS K. R. and FERRY J. G. (1983) Isolation and characterization of a methylotrophic marine methanogen, *Methanococcoides methylutens*, gen. nov., sp. nov. *Appl. Environ. Microbiol.* **45**, 684–690.

SUTHERLAND J. B. and COOK R. J. (1980) Effects of chemical and heat treatments on ethylene production in soil. *Soil Biol. Biochem.* **12**, 357–362.

THOMPSON C. J., COLEMAN H. J., RALL H. T. and SMITH H. M. (1955) Separation of sulfur compounds from petroleum. *Anal. Chem.* **27**, 175–185.

VOGEL T. M., OREMLAND R. S. and KVENVOLDEN K. A. (1982) Low-temperature formation of hydrocarbon gases in San Francisco Bay sediment. *Chem. Geol.* **37**, 289–298.

WHELAN J. K., HUNT J. M. and BERMAN J. (1980) Volatile C_1–C_7 organic compounds in surface sediments from Walvis Bay. *Geochim. Cosmochim. Acta* **44**, 1767–1785.

WHITICAR M. J., FABER E. and SCHOELL M. (1986) Biogenic methane formation in marine and freshwater environments: CO_2 reduction *vs.* acetate fermentation-isotope evidence. *Geochim. Cosmochim. Acta* **50**, 693–709.

WOLFE R. S. (1971) Microbial formation of methane. In *Advances in Microbial Physiology*, Vol. 6 (eds. A. H. ROSE and J. F. WILKINSON), pp. 107–146. Academic Press, New York.