Earth and Planetary Science Letters, 47 (1980) 345–352

[6]

ANAEROBIC METHANE OXIDATION: RATE DEPTH DISTRIBUTIONS IN SKAN BAY SEDIMENTS

WILLIAM S. REEBURGH

Institute of Marine Science, University of Alaska, Fairbanks, AK 99701 (U.S.A.)

Received September 25, 1979
Revised version received January 12, 1980

A radiotracer method that measures rates of oxidation of methane to carbon dioxide has been applied to anoxic marine sediments. The results confirm the occurrence of anaerobic methane oxidation and agree with model predictions of a zone of intense anaerobic methane oxidation at the base of the sulfate-reducing zone.

1. Introduction

Anaerobic methane oxidation has been suggested as a process occurring in marine sediments [1–3]. The process appears to be a general feature of anoxic marine sediments and has been identified as a major sink for methane [4]. It also accounts for a substantial fraction of sulfate reduction in marine sediments [5]. Fig. 1 [4] schematically summarizes depth distributions of methane (CH_4), total carbon dioxide (ΣCO_2), sulfate (SO_4^{2-}) and the stable carbon isotope ratio of carbon dioxide ($\delta^{13}CO_2$) from a number of anoxic marine sediments. The distributions of all species show slope changes or minima at the same depth, suggesting that most of the methane consumption takes place in a thin subsurface depth interval. The $\delta^{13}CO_2$ minimum appears to be the result of local injection of methane-derived carbon dioxide. Diagenetic models indicate the process occurs at rates in the 1–10 mM/yr range [1,2].

The apparent absence of anaerobic methane oxidation in freshwater (low sulfate) sediments suggests the involvement of sulfate reducers [6].

Anaerobic methane oxidation has been described geochemically by the following net reaction:

$$CH_4 + SO_4^{2-} + 2H^+ \rightarrow H_2S + CO_2 + 2H_2O \qquad (1)$$

This reaction has not been observed directly and organisms capable of mediating it have not been isolated. Laboratory evidence favoring [7] and disputing [8] anaerobic methane oxidation has been presented. Zehnder and Brock [9] reported simultaneous methane production and anaerobic consumption by nine methanogen strains. Since the amounts oxidized were less than 1% of the CH_4 formed, this process is an unlikely explanation for the large CH_4 losses observed in geochemical studies of marine sediments. Panganiban et al. [10] showed that enrichment cultures of organisms from Lake Mendota surface sediments were capable of oxidizing CH_4 anaerobically. These organisms required sulfate, acetate and methane for growth, assimilating acetate and oxidizing methane. Kosiur and Warford [11] added labelled acetate and lactate to Santa Barbara Basin sediments in in-vitro experiments. Changes with time in the specific activity of the $^{14}CH_4$ and $^{14}CO_2$ produced indicated that anaerobic methane oxidation occurred in the 100 μM/yr range. This report describes "quasi-in-situ" measurements of anaerobic methane oxidation and sulfate reduction in Skan Bay sediments. These measurements provide more direct evidence that anaerobic methane oxidation does occur and give rate depth distributions and rates that agree with diagenetic models [1,2].

2. Experimental

These experiments were conducted on sediment cores from Skan Bay (57°37′N, 167°03′W, 65 m), a

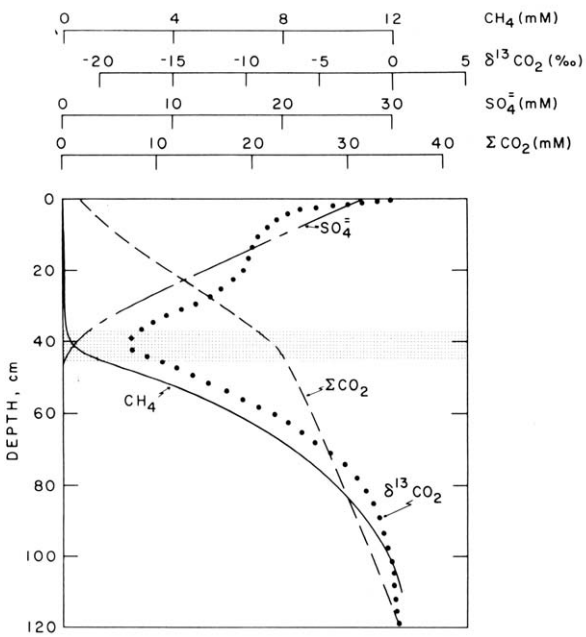

Fig. 1. Schematic diagram showing depth distributions of methane, sulfate, total carbon dioxide and carbon isotope ratio of carbon dioxide in interstitial waters of marine sediments. All distributions show breaks or slope changes in the stippled area, which represents the zone of maximum anaerobic methane oxidation. (From Reeburgh [4] with permission.)

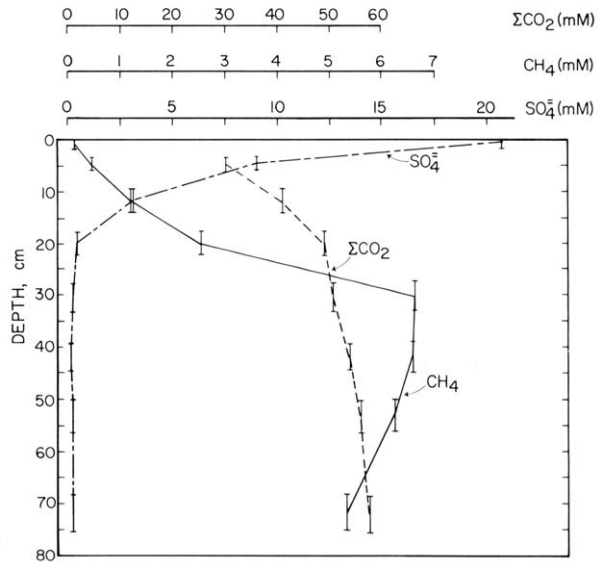

Fig. 2. Measured depth distributions of methane, total carbon dioxide and sulfate in Skan Bay sediments. Vertical bars show the depth interval sampled.

basin with an intermittently anoxic water column on the northwest side of Unalaska Island [12]. Skan Bay has a sill depth of 10 m and a maximum depth of 65 m. The sediments are rich in organic material (kelp fragments) and are anoxic.

Sediments were sampled with a Benthos gravity corer used without a core catcher. Sediment samples were extruded from the cores into squeezers and the interstitial waters were analyzed for CH_4 and ΣCO_2 by gas chromatography [14]. Sulfate samples were collected in pre-weighed bottles containing HCl. Sulfate was separated using ion exchange and evaporation [15] and was analyzed by conductometric titration [16]. The CH_4, ΣCO_2 and SO_4^{2-} depth distributions in Skan Bay sediments are shown in Fig. 2.

Anaerobic methane oxidation rates and sulfate reduction rates were measured using modifications of the technique described by Jørgensen and Tenchel [17]. Solution of $^{14}CH_4$ and $Na_2{}^{35}SO_4$ were injected into selected depth intervals of minimally disturbed sediment cores. Following incubation, the products CO_2 and H_2S were stripped from solution and assayed for radioactivity.

Three separate cores were used in the experiments reported here, one for the determination of CH_4, ΣCO_2 and SO_4^{2-} and one each for the methane oxidation and sulfate reduction tracer experiments. Reeburgh [14] and Mattisof et al. [18] have established that interstitial water distributions in parallel cores from a single station are subject to small lateral variations and much larger vertical variations. Errors introduced by this compromise are expected to be small.

Freshly collected gravity cores were transferred to an experimental core tube for these experiments. The experimental core tube was joined with plastic electrical tape to the top of a sediment core and the sediment was extruded upward into the experimental core tube, overflowing the overlying water and avoiding contact with air. The experimental core tube consisted of segments (6.5 cm I.D. X 2.5 cm) of plastic core liner joined together with plastic electrical tape. Alternate segments of the experimental core tube were equipped with septa of RTV silicone rubber for syringe injection of the tracer solutions. Depth intervals of interest in the sediment core were

positioned within experimental core tube segments for the tracer experiments.

Both tracers were dissolved in a sterile, oxygen-free NaCl solution with a density close to that of the interstitial water to avoid adding electron acceptors and to minimize migration of the added tracer during incubation. $^{14}CO_2$ impurities were removed from the $^{14}CH_4$ used in the methane oxidation rate measurements by admitting the $^{14}CH_4$ to an evacuated manifold and toepler pumping through a trap containing Mallcosorb (20–50 mesh). A portion of $^{14}CH_4$ was transferred to an evacuated reservoir bulb and the tracer solution was formed by admitting the helium-stripped NaCl solution. Aliquots of the $^{14}CH_4$ solution were displaced from the reservoir bulb into Chaney adapter syringes by syringe addition of pure mercury. Mercury added to sediments with the $^{14}CH_4$ tracer solution should be rapidly immobilized by excess sulfide in the sediments [19]. Portions of the $^{14}CH_4$ solution were injected into a carbon dioxide absorbing scintillation cocktail [20] and stripped with helium. Lack of activity in the stripped solution demonstrated that the $^{14}CH_4$ solution was $^{14}CO_2$-free. Fifty microliters of the $^{14}CH_4$ tracer solution (0.15 μCi, 4.25 mCi/mmole, 0.7 mM) were injected into the center of each experimental core segment.

The $Na_2{}^{35}SO_4$ used in the sulfate reduction rate measurements was obtained carrier-free and was dissolved in a similar NaCl solution. Twenty microliters of the $^{35}SO_4^{2-}$ tracer solution (0.1 μCi, 55.5 mCi/mmole, 0.09 mM) were injected into the center of each experimental core segment.

Following incubation in a water bath near in-situ temperatures for 24–36 hours, the experimental core tubes were clamped upright and dismantled by removing the plastic tape and isolating each segment with sheets of stainless steel shim stock. Each labelled core segment was transferred to a helium-flushed Mason jar containing a magnetic stir bar. The jars were capped with rubber gasketed lucite lids fitted with a gas inlet, a gas outlet and a septum modified from Swagelok fittings. The lids were sealed with electrical tape and held in place with threaded metal binding rings. Five milliliters of 2*N* KOH were added to kill the samples and to retain the CO_2 and H_2S formed [21]. The samples were frozen until analysis (4–6 weeks) in the laboratory. The frozen samples were attached to the absorption line, thawed, and a stirrable sediment slurry was formed by adding distilled water through the septum with a syringe. Methane was removed by stripping the basic sediment slurry with helium (100 ml/min) and was trapped as CO_2 after oxidation on a CuO column at 600°C in a series of three LSC vial strippers containing a carbon dioxide-absorbing cocktail [20]. Following removal of the CH_4, the CO_2 was collected by acidifying the slurry and stripping with helium. The CO_2 was trapped in another series of LSC vial strippers. The large quantities of H_2S produced on acidification cause severe quenching; this H_2S was removed in a gas phase trap consisting of $CuSO_4$ dried on Celite. The trapped $^{14}CO_2$ was counted to 1% precision in the LSC vials used for stripping. Quench corrections were determined with external standard ratios and the counts from three stripper vials were summed. The initial activity of the added $^{14}CH_4$ was determined in the laboratory by oxidizing 50 μl aliquots of the $^{14}CH_4$ tracer solution in a circulating combustion and trapping system [21]. The carbon dioxide formed was trapped in phenethylamine and counted in a toluene cocktail. Hydrogen sulfide from the sulfate reduction rate measurements was stripped from an acidified sediment slurry and was trapped as CdS in a series of three LSC vial strippers containing $CdCl_2$ solution. Weighed portions of this CdS was counted in a gas flow proportional counter; a self-absorption curve was prepared using constant specific activity $Cd^{35}S$ obtained by chemical reduction [22] of $^{35}SO_4^{2-}$ and carrier sulfate. The stock $^{35}SO_4^{2-}$ solution activity was measured when the recovered activity was counted, avoiding decay corrections [23].

3. Results and discussion

3.1. CH_4, ΣCO_2 and SO_4^{2-}

The distributions of CH_4, ΣCO_2 and SO_4^{2-} shown in Fig. 2 are similar to those shown schematically in Fig. 1, indicating that Skan Bay is similar to other marine environments. Methane bubbles formed at depths below 17 cm in the cores; decreases in methane concentration at depth and the lack of a distinct low-CH_4 surface zone as shown in Fig. 1 probably reflect the effects of CH_4 ebullition and dissolution after core collection.

3.2. Rate measurements

These tracer experiments are intended to show depth distributions of the rates of anaerobic methane oxidation and sulfate reduction in minimally disturbed sediment cores. Two main points must be addressed in experiments of this type: (1) the tracer added and product formed must be contained in the experimental core segment and recovered completely, and (2) the form and fate of the tracer and products must be known. Equal amounts of tracer were injected into the experimental core segments and identical procedures were used in the addition of the tracer and extraction of the products. Care was taken to insure tracer purity and both were added in small quantities in a biologically passive supporting solution. The tracer solutions in both rate measurements were added to a small volume in the center of a core segment in concentrations that did not exceed ambient concentrations [24]. No attempt was made to homogenize the tracer solution and sediment. The experimental volumes of each core segment were well removed from the effects of sediment smearing along the walls during coring and core transfer. Tracer diffusion distances of less than a centimeter are indicated by characteristic diffusion coefficients and incubation times, so diffusive losses of tracer during incubation and transfer to the Mason jars are not expected to be a problem [25]. By the same reasoning, diffusive inputs of oxygen will be too small to allow aerobic methane oxidation [21] to bias the results. Jørgensen [26] has experimentally confirmed this analysis with $^{35}SO_4^{2-}$. The stripping process in the methane oxidation experiments was conducted in two stages, one with the sediment slurry at high pH to allow removal of the unreacted $^{14}CH_4$ and the other at low pH to recover the product $^{14}CO_2$. The $CuSO_4$ trap removes inorganic and organic sulfides [27], so that the gas collected in an acidic, carbon-containing gas, and must be CO_2. The sediment showed no activity after the low-pH stripping, indicating that the added $^{14}CH_4$ was not converted to a non-volatile form [10]. The sulfate reduction rate measurements can be biased by rapid pyrite formation [28], which would produce lower rates. Skan Bay sediments contain large quantities of acid labile iron sulfides, indicating that conditions are unfavorable for rapid pyrite formation.

It is difficult to perform meaningful poisoned control experiments in studies involving intact undisturbed cores like these. Previous work on sulfate reduction rates has apparently involved no control experiments [17,26]. The main question is how to poison the experimental volume without physical or chemical disruption. One approach to this problem is to immediately dismantle and preserve the experimental core segment, but this precludes incubation and defeats one of the main points of control experiments. Jørgensen [26] has considered tracer measurements of sulfate reduction rates in undisturbed sediments and indicates that tracer methods give absolute rates when points 1 and 2 raised earlier are carefully considered.

A troublesome point is the low recoveries of $^{14}CH_4$, which were about 1% of the amount added. Methane is the only member of this tracer-product system that is not either chemically trapped or non-volatile. Conditions in the stored samples are too basic to permit aerobic oxidation of the $^{14}CH_4$ during storage to bias the results. Several of the lucite Mason jar lids leaked during stripping, so it appears that the unreacted $^{14}CH_4$ was lost during storage or the initial stages of stripping. Subsequent work has involved use of regular metal Mason jar lids and O-seal Swaglock fittings for the gas inlet, outlet and septum.

3.3. Rates and rate depth distributions

Fig. 3 shows measurable anaerobic methane oxidation at all depths in Skan Bay cores with a maximum rate of 3.4 mM/yr in the 24.8- to 27.2-cm depth interval. Both maxima are bracketed by depth intervals over which CH_4, ΣCO_2 and SO_4^{2-} (Fig. 2) show slope changes. The distribution of the anaerobic methane oxidation rate is a mirror image of the $\delta^{13}CO_2$ distribution shown in Fig. 1 and is consistent with the suggested injection of methane-derived CO_2 in a subsurface zone near the bottom of the sulfate-reducing zone. The maximum in the methane oxidation rate and its location are consistent with model predictions and the rate lies within the range predicted [1,2]. Anaerobic methane oxidation rates in sediments are about the same magnitude as aerobic methane oxidation rates reported in water columns of lakes [21].

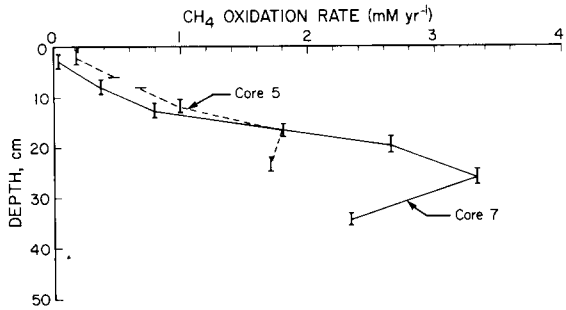

Fig. 3. Depth distribution of anaerobic methane oxidation rate in Skan Bay sediments. Vertical bars show the depth interval studied; horizontal bars show location of lost sample in core 5.

Sulfate reduction rates are shown in Fig. 4. Although there is more scatter in the upper portions of these distributions, the general distributions and rates are similar to other reported results [17,26]. Sulfate must be the principal oxidant in this system [5,29]; sulfate reduction rates are up to a factor of 4 higher than anaerobic methane oxidation rates at the depth of the methane oxidation maximum.

These results show that methane distributions in marine sediments are controlled by intense local anaerobic methane oxidation combined with anaerobic methane oxidation at lower rates throughout the sulfate-reducing zone.

First-order reaction kinetics have been used to describe concentration changes in both methane [3] and sulfate [30] over a range of concentrations in marine sediments. This approach predicts methane oxidation rate depth distributions that parallel the depth distributions of methane concentration. Unless a lower boundary is assigned, this approach predicts high rates of methane consumption at depths where the principal oxidant, sulfate, is depleted. A zone of maximum methane oxidation has been predicted from depth distributions of CH_4, ΣCO_2, SO_4^{2-} and $\delta^{13}CO_2$, and second-order reaction kinetics, i.e. the product of methane and sulfate concentrations, have been suggested as controlling the location of the maximum rate [4]. If equation (1) can be used as a guide, it seems reasonable that the reaction responsible for anaerobic methane oxidation is second- or higher-order overall, and that the reaction kinetics for methane and sulfate are actually pseudo-first order. The methane oxidation rate depth distributions from this study show a maximum bracketed by slope changes in CH_4, ΣCO_2 and SO_4^{2-}, but apparent redistribution of methane leads to no $[CH_4]$ $[SO_4^{2-}]$ maximum. We have considered the location of the anaerobic methane oxidation maximum rate in a mathematical model [31], but more information on the organisms responsible for anaerobic methane oxidation and their substrate requirements will be required to fully resolve this point.

4. Conclusions

Techniques have been developed that permit the use of $^{14}CH_4$ as a tracer in studies of anaerobic methane oxidation in minimally disturbed sediment cores. Preliminary application of these techniques shows that anaerobic methane oxidation occurs throughout the sulfate-reducing zone in anoxic marine sediments, reaching a maximum at the base of

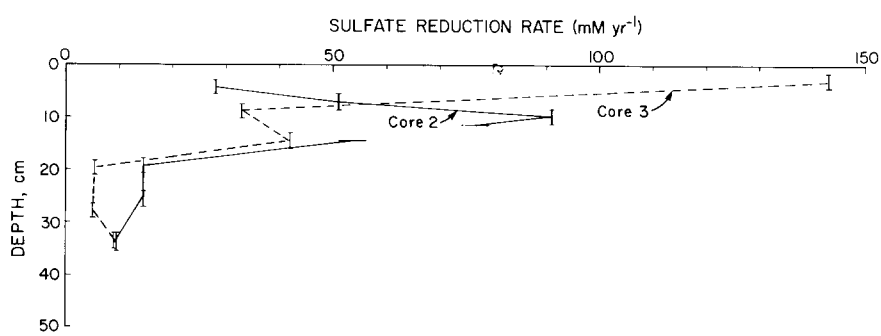

Fig. 4. Depth distribution of sulfate reduction rate in Skan Bay sediments. Vertical bars show the depth interval studied; horizontal bars show location of lost sample in core 2.

the sulfate-reducing zone. The low-methane concentration zone observed at the surface of marine sediments results from the distribution of anaerobic methane oxidation rates in the sulfate-reducing zone. Oxidation at high rates at the base of the zone [1,2,6] consumes the majority of the upward methane flux, while oxidation at lower rates [3,11] removes the small quantities of methane formed in the remainder of the sulfate reducing zone.

"Qausi-in-situ" studies using $^{14}CH_4$ solutions reveal structure not visible in in-vitro studies employing headspace gases. These studies suggest that the search for the organism responsible for anaerobic methane oxidation should be directed toward depth intervals of marine cores chosen on the basis of CH_4, ΣCO_2 and SO_4^{2-} depth distributions.

Acknowledgements

This work was supported by NSF grant OCE 76-08891. The field work was conducted on R/V "Acona", cruise 265. I thank E.J. Brown for his helpful advice during this study and Steve Emerson, Chris Martens and two anonymous reviewers for constructive criticism of versions of the manuscript. Contribution No. 395 from the Institute of Marine Science, University of Alaska, Fairbanks.

Appendix

A. Skan Bay distributions (SKAN 1, 65 m; 24 August 1978)

Δz (cm)	CH_4 (mM)	ΣCO_2 (mM)	SO_4 (mM)
0 – 2.0	0.12	–	20.71
3.5 – 6.0	0.48	29.91	8.07
9.5 – 14.0	1.22	40.40	3.06
17.5 – 22.0	2.55	48.72	0.47
27.5 – 33.0	6.62	50.16	0.29
39.0 – 44.5	6.54	53.56	0.24
50.0 – 56.0	6.19	55.47	0.32
68.0 – 75.0	5.28	57	0.32

B. Methane oxidation rates

Sample	Δz (cm)	CH_4 (mM)	A (dpm)	Δt	Σa_{CO_2} (dpm)	Σa_{CH_4} (dpm)	Rate (mM/yr)
Core 5							
A	0.5 – 3	0.20	3.45×10^5	25.5 hr	968.2	1082	1.93×10^{-1}
B	5.5 – 8	0.65	3.45×10^5	25.5 hr	–	–	–
C	10.5 – 13	1.25	3.45×10^5		792.7	438.7	9.87×10^{-1}
D	15.5 – 18	2.0	3.45×10^5	(2.91×10^{-3} yr)	906.5	223.1	1.81
E	22.0 – 24.8	3.75	3.45×10^5		467.4	235.1	1.74
F	28.7 – 31.5	6.30	3.45×10^5		–	–	–
G	35.5 – 38.5	6.60	3.45×10^5		–	–	–
Core 7							
A	1.5 – 4.3	0.25	3.45×10^5	31.92 hr	198.5	2675	3.95×10^{-2}
B	6.5 – 9.2	0.75	3.45×10^5	31.9 hr	632.9	232.7	3.78×10^{-1}
C	11.5 – 14	1.35	3.45×10^5	(3.64×10^{-3} yr)	739.1	795.8	7.95×10^{-1}
D	18.0 – 21.2	2.50	3.45×10^5		1329.4	7696	2.65
E	24.8 – 27.2	4.95	3.45×10^5		847.1	11 767	3.34
F	33.3 – 35.7	6.60	3.45×10^5		443.8	4279	2.33

C. Sulfate reduction rates

Sample	Δz (cm)	$[SO_4]$ (mM)	a * (dpm)	a/A	Δt	Rate (mM/yr)
Core 2						
A	3.0– 5.5	11.7	176	9.91×10^{-3}	(36.25 hr)	27.9
B	5.5– 8.5	5.5	686	3.87×10^{-2}	(36.25 hr)	51.3
C	8.5–11.5	4.5	1485	8.38×10^{-2}	(4.15×10^{-3} yr)	90.9
D	11.5–14.5	2.7	–	–		–
E	18.0–20.5	0.5	2188	1.23×10^{-1}		14.8
F	24.0–27.5	0.25	4187	2.36×10^{-1}		14.2
G	32.5–35.0	0.25	2813	1.59×10^{-1}		9.58
Core 3						
A	2.0– 4.5	15.2	466	2.63×10^{-2}	(24.50 hr)	143.0
B	7.5–10.0	5.6	294	1.66×10^{-2}	(24.50 hr)	33.3
C	13.0–16.0	2.3	953	5.37×10^{-2}	(2.79×10^{-3} yr)	44.3
D	18.0–21.0	0.6	483	2.47×10^{-2}		5.31
E	26.5–29.0	0.25	1032	5.82×10^{-2}		5.22
F	32.0–35.5	0.25	1953	1.10×10^{-1}		9.86

* a = cpm/g CdS × ΣCdS.
a and A are corrected for self-absorption.

References

1 W.S. Reeburgh, Methane consumption in Cariaco Trench waters and sediments, Earth Planet. Sci. Lett. 28 (1976) 337.
2 R.O. Barnes and E.D. Goldberg, Methane production and consumption in anoxic marine sediments, Geology 4 (1976) 297.
3 C.S. Martens and R.A. Berner, Interstitial water chemistry of Long Island Sound sediments, I. Dissolved gases, Limnol. Oceanogr. 22 (1977) 10.
4 W.S. Reeburgh, A major sink and flux control for methane in marine sediments: Anaerobic consumption, in: The Dynamic Environment of the Ocean Floor, K. Fanning and F.T. Manheim, eds. (D.C. Heath, Lexington, 1979).
5 J.W. Murray, V. Grundmanis and W.M. Smethie, Jr., Interstitial water chemistry in the sediments of Saanich Inlet, Geochim. Cosmochim. Acta 42 (1978) 1011.
6 W.S. Reeburgh and D.T. Heggie, Microbial methane consumption reactions and their effect on methane distributions in freshwater and marine environments, Limnol. Oceanogr. 22 (1977) 1.
7 J.B. Davis and H.F. Yarbrough, Anaerobic oxidation of hydrocarbons by *Desulfovibrio desulfuricans,* Chem. Geol. 1 (1966) 137.
8 Y.I. Sorokin, Ability of sulfate reducing bacteria to utilize methane for the reduction of sulfate to hydrogen sulfide, Dokl. Akad. Nauk SSSR 115 (1957) 816.
M.R. Winfrey and J.G. Zeikus, Effects of sulfate on carbon and electron flow during microbial methanogenesis in freshwater sediments, Appl. Environ. Microbiol. 33 (1977) 275.
R.S. Oremland and B.F. Taylor, Sulfate reduction and methanogenesis in marine sediments, Geochim. Cosmochim. Acta 42 (1978) 209.
9 A.J.B. Zehnder and T.D. Brock, Methane formation and methane oxidation by methanogenic bacteria, J. Bacteriol. 137 (1979) 420.
10 A.T. Panganiban, T.E. Patt, W. Hart and R.S. Hanson, Oxidation of methane in the absence of oxygen in lake water samples, Appl. Environ. Microbiol. 37 (1979) 303.
11 D.R. Kosiur and A.L. Warford, Methane production and oxidation in Santa Barbara Basin sediments, Estuarine Coastal Mar. Sci. 8 (1979) 379.
12 A. Hattori, J.J. Goering and D.W. Boisseau, Ammonium oxidation and its significance in the summer cycling of nitrogen in oxygen depleted Skan Bay, Unalaska Island, Alaska, Mar. Sci. Comm. 4 (1978) 139.
13 W.S. Reeburgh, An improved interstitial water sampler, Limnol. Oceanogr. 12 (1967) 163.
14 W.S. Reeburgh, Determination of gases in sediments. Environ. Sci. Technol. 2 (1967) 573.
15 G.W. Dollman, Determination of sulfate and phosphate in water by an ion exchange-titrimetric method, Environ. Sci. Technol. 2 (1968) 1027.
16 W.S. Reeburgh and M.S. Young, unpublished.
17 B.B. Jørgensen and T. Fenchel, The sulfur cycle of a marine sediment model system, Mar. Biol. 24 (1961) 508.
18 G. Mattisof, O.P. Bricker III, G.R. Holdren, Jr. and P. Kaerk, Spatial and temporal variations in the interstitial

water chemistry of Chesapeake Bay sediments, in: Marine Chemistry in the Coastal Environment, T.M. Church, ed. (American Chemical Society, Washington, D.C., 1975) 343–363.

19 R. Wollast, G. Billen and F.T. Mackenzie, Behavior of mercury in natural systems and its global cycle, in: Ecological Taxicology Research, A.D. McIntyre and C.F. Mills, eds. (Plenum Press, New York, N.Y. 1974) 145–166. These authors report that pure mercury is soluble in aqueous solution to the extent of 56 μg/l (2.8×10^{-7} M). This value can be taken as an upper limit in the oxygen-free tracer solution and is well below the 10^{-4} to 10^{-5} M concentrations necessary for bacteriostasis. Mercury forms extremely insoluble sulfides ($K_{sp} \sim 10^{-50}$), so mobile mercury concentrations in the anoxic experimental core segments can be expected to be less than 1 μg/l (5×10^{-9} M). Mercury is complexed by HS^-; with a time scale of minutes, so we can conclude that neither the $^{14}CH_4$ tracer solution nor the resulting experiments contain enough mercury to cause perturbations.

20 F.H. Woeller, Liquid scintillation counting of $C^{14}O_2$ with phenethylamine, Anal. Biochem. 2 (1961) 508.

21 J.W.M. Rudd. R.D. Hamilton and N.E.R. Campbell, Measurement of microbial oxidation of methane in lake, water, Limnol. Oceanogr. 19 (1974) 519. These authors killed microbial activity by adjusting the pH of their experiments to pH 11. The preserved sediments in this study are 0.1 M in KOH. The effectiveness of this treatment in retaining the products H_2S and CO_2 is shown by inspecting figs. 3–4 (p. 97) and Fig. 4-1 (p. 122) in W. Stumm and J.J. Morgan, Aquatic Chemistry (Wiley-Interscience, New York, N.Y., 1970). These diagrams approximate Skan Bay interstitial water and show that the volatile forms H_2S and CO_2 are less than 0.01% of the total H_2S and total CO_2 at pH 11.

22 C.L. Luke, Determination of total sulfur in rubber, Anal. Chem. 15 (1943) 602.

23 Methane oxidation and sulfate reduction rates were calculated using the following equation:

$$\text{rate} = [C]\ a/A\Delta t$$

where $[C]$ is the concentration of methane or sulfate in mM (from Fig. 3), a is the recovered activity, A is the added or initial activity and Δt is the incubation time. The sulfur isotope fractionation correction of 1.06 [17] was neglected.

24 Ambient sulfate concentrations exceed the tracer solution concentration (0.09 mM) in all cases. Ambient methane concentrations are lower than the tracer solution concentration (0.7 mM) in samples 5A and 7A.

25 The mean square diffusive distance is given by Crank The Mathematics of Diffusion, Oxford, 1956, p. 36) as $2\sqrt{Dt}$, where D is the diffusivity ($\sim 3 \times 10^{-6}$ cm^2/s) and t is the elapsed time (incubation time was 24–36 hours).

26 B.B. Jørgensen, A comparison of methods for the quantification of bacterial sulfate reduction in coastal marine sediments, I. Measurement with radiotracer techniques, Geomicrobiol. J. 1 (1978) 11; II. Calculations from mathematical models, Geomicrobiol. J. (1978) 29; III. Estimation from chemical and bacteriological field data, Geomicrobiol. J. 1 (1978) 49.

27 S.H. Zinder and T.D. Brock, Methane, carbon dioxide and hydrogen sulfide production from the terminal methiol group of methionine by anaerobic lake sediments, Appl. Envir. Microbiol. 35 (1978) 344.

28 R.W. Howarth, Pyrite: its rapid formation in a salt marsh and its importance in ecosystem metabolism, Science 203 (1979) 49.

29 B.B. Jørgensen, The sulfur cycle of a coastal marine sediment (Limfjorden, Denmark), Limnol. Oceanogr. 22 (1977) 814.

30 R.A. Berner, An idealized model of dissolved sulfate distribution in recent sediments, Geochim. Cosmochim. Acta 28 (1964) 1497.

31 D.A. Livingstone and W.S. Reeburgh, unpublished.