Methane Production from Acetate and Associated Methane Fluxes from Anoxic Coastal Sediments

Abstract. The apparent microbial conversion of acetate to methane ranges seasonally from 0.7 to 88 micromoles per liter of whole wet sediment per hour in the top 5 centimeters of methane-producing sediments underlying sulfate-reducing sediments in Cape Lookout Bight, North Carolina. The associated methane flux across the sediment-water interface into overlying waters exhibits the same seasonal pattern. Significant methane production from acetate is observed only in sulfate-depleted sediments in Cape Lookout Bight, North Carolina. The associated methane flux across the sediment-water interface into overlying waters exhibits the same seasonal pattern. Significant methane production from acetate is observed only in sulfate-depleted sediments.

Methane (CH₄) production is known to occur in organic-rich anoxic sediments with low concentrations of nitrate and sulfate (1, 2). This process is the terminal step in the anaerobic decomposition of organic matter (3) and is consequently a major component of both global and local carbon budgets. Microbially mediated methanogenesis has been estimated to account for more than 80 percent of the total global flux to the troposphere (4). Sedimentary methanogenesis results in both the accumulation of CH₄ in the sediment and its transport into the overlying water (5). It is thought that the CH₄ produced in these environments arises from two distinct microbially mediated processes: the fermentation of acetate and the reduction of CO₂ by H₂ (2, 3). The rates of CH₄ production from precursor molecules such as acetate in freshwater sediments have been measured (6, 7), but this has not been done with marine sediments, nor have the values obtained been compared with changes in the in situ CH₄ concentrations or with fluxes of CH₄ out of the sediment being studied.

We report here the first seasonal measurements of microbial CH₄ production rates from acetate in a sediment conducted in association with in situ measurements of fluxes of CH₄ out of the sediment into the overlying water (5). We have found a strong seasonal correlation between these two processes in the sediments of a small coastal basin on the Outer Banks of North Carolina. We have also measured the depth dependency of CH₄ production from acetate and found that significant production occurs only when sulfate is totally depleted.

Our sample site was Cape Lookout Bight, North Carolina, an organic-rich marine basin of approximately 2 km² located 115 km southwest of Cape Hatteras (8). The water column is well oxygenated (> 130 μM O₂) and low in CH₄ (< 5 μM) year-round (5, 9) and is underlain by a fine-grained mud with an organic content ranging from approximately 3 percent (by dry weight) at the surface to 2 percent at a depth of several meters (10). The fine-grained sediment and presumably much of its organic content are derived from the tidal flushing of nearby barrier island lagoons (5). Because nutrient, dissolved gas, and sulfate vertical concentration profiles are reproducible in Cape Lookout sediments from year to year (5, 11), we have combined data from 1976 to 1980 to demonstrate the seasonally dependent production and release of CH₄ from these sediments.

Methane has been observed (5, 8) to be released from Cape Lookout sediments to the overlying water by two processes. The diffusive flux of dissolved CH₄, measured with the use of in situ benthic chambers, was found to have a large summer maximum (Fig. 1A). The quasi-advective flux of CH₄ arising from the release of gas bubbles was found to follow a similar seasonal pattern and to be approximately six times as large as the diffusive flux (Fig. 1A).

We measured the rates of CH₄ and CO₂ production from acetate by using methods described in (12). Briefly, both the whole sediment substrate concentrations (μmole liter⁻¹, where the subscript refers to the volume of whole wet sediment) and the corresponding first-order reaction rate constants (hour⁻¹) were measured periodically, and these parameters were multiplied to obtain apparent production rates (13).

Use of the term "apparent" is based on our measurements of whole sediment acetate concentrations (pool size). The methodology used in this study (see below) allows for determination of total "extractable" pool sizes of acetate; however, this should be distinguished from the "microbiologically available" pool size, which may be less than the total because of the partitioning of acetate between sediment pore water (both free and complexed), adsorption sites on sediment particle surfaces, and microbial cellular material. The actual microbial production rates may therefore be less than the apparent production rates.

In order to measure acetate concentrations, bulk wet sediment samples were basified, freeze-dried, extracted with methanol, derivatized to form methyl esters of the volatile fatty acids, and analyzed by gas chromatography with a hexanoate internal standard. Less than 1 percent of the acetate measured during the summer in Cape Lookout sediments was found to be dissolved in the pore water (14). The remainder was presumably...
bound to the solid phase and also present in cellular material.

We determined the rate constants for CO₂ and CH₄ production from acetate by anaerobically incubating samples at in situ temperatures with tracer concentrations of [1,2-¹⁴C]sodium acetate, 53.5 mCi mmole⁻¹ (New England Nuclear), using a modification of the methods of Christian and Wiebe (15). In situ acetate concentrations ranged from 34 µmole liter⁻¹ in March of 1979 to 660 µmole liter⁻¹ in August; 0.062 to 0.31 mmole of [¹⁴C]acetate were added to each incubating tube containing 3 ml of wet sediment, thereby increasing the total acetate pool by less than 1 percent. During the 5-minute incubation, less than 5 percent of the added label was utilized. Reduction of ¹⁴CO₂ to [¹⁴CH₄ during the incubations was not considered important because of the very low ratio of [¹⁴CH₄]/[¹⁴CO₂ (< 10⁻⁶) at the end of the experiments and because the first-order rate constant for this reaction was less than 0.0001 hour⁻¹ in October 1979 and less than 0.0003 hour⁻¹ in February 1980 (14). Rate constants for acetate conversion to CH₄ were 0.063 and 0.010 hour⁻¹, respectively for these two dates. Based on the experimental conditions described above, we assumed that the rates measured were initial rates; that is, rates were not affected by changes in substrate or end product concentrations.

For seasonal studies of acetate cycling in Cape Lookout Bight sediments, samples were taken over a 5-cm depth interval immediately below the 1 mM sulfate isopleth, that is, at the top of the zone of CH₄ production (16). The apparent production of both CH₄ and CO₂ from acetate showed the same seasonal pattern (Fig. IB) as did the CH₄ flux out of the sediment (Fig. 1A). The production of CO₂ from acetate was approximately five times the production of CH₄. The earlier maxima for the CH₄ fluxes as compared to the maxima for the apparent production rates is most likely due to the earlier occurrence of peak summer air temperature in 1977 and 1978 (~15 July) as compared with 1979 (~15 August).

The depth dependency of CH₄ production from acetate measured on 13 July 1980 is illustrated in Fig. 2A. Maximum production occurred between 10 and 35 cm, where dissolved sulfate was undetectable (<0.2 mM) by gravimetric analysis as BaSO₄. Production of CH₄ in the overlying sulfate-reducing zone between 0 and 5 cm (13 to 25 mM sulfate; Fig. 2) was undetectable (<0.3 µmole liter⁻¹ hour⁻¹).

The respiration index (RI = [¹⁴CO₂ production/¹⁴CO₂ + [¹⁴CH₄ production] from [1,2-¹⁴C]sodium acetate is shown in Fig. 2B. The RI values ranged from 1.00 ± 0.04 between 0 and 5 cm in the sulfate reduction zone to 0.79 ± 0.07 at 30 to 35 cm in the CH₄ production zone. The RI values were invariant within experimental error from 8 to 35 cm, implying that the proportion of the methyl group of acetate oxidized to CO₂ did not vary significantly with depth within the CH₄ production zone at our study site.

Preliminary calculations (17) indicate that more than 50 percent of the observed summertime CH₄ flux (Fig. 1A) can be accounted for by CH₄ production from acetate in the upper 5 cm of CH₄-producing sediments underlying the sulfate reduction zone (Fig. 1B). Further evidence for high rates of methanogenesis down to at least 30 cm at our site is provided by recent studies of the stripping of the dissolved radioactive gas
222Rn from interstitial waters by CH4 bubbles (18). Our data suggest that a significant portion of the CH4 released from Cape Lookout Bight sediments during the summer is derived from the fermentation of acetate to CH4 in the sulfate-depleted sediments in the depth range from 8 to 35 cm.

Francis J. Sansone* Christopher S. Martens
University of North Carolina Marine Science Program, Chapel Hill 27514, and Institute of Marine Sciences, Morehead City, North Carolina 28557

References and Notes
10. C. S. Martens, unpublished data.
13. The sediment porosity decreases from 0.94 to 0.87 cm3 of interstitial water per cubic centimeter of whole wet sediment in the top 25 cm and remains approximately at the latter value to a depth of 50 cm.
15. R. R. Christian and W. J. Wiebe, Limnol. Oceanogr. 23, 328 (1978). Briefly, five to six samples were taken from each experiment with two to three of these used as abiotic ("killed") controls. We obtained samples of cores by inserting 3-ml plastic syringes with cutoff ends; these core samples were transferred to sterile 15-ml Vacutainer tubes flushed with O2-free N2. One milliliter of sterile, degassed, sulfate-free artificial seawater was added to each tube; these tubes were then vortex-mixed and equilibrated for 30 minutes at the in situ temperature. An aqueous solution of labeled acetate (10 to 50 μl) was added anaerobically to each tube; the tubes were incubated for 5 minutes at the in situ temperature and then "killed" with acid formalin. Controls were "killed" before label had been added. Activity was recovered as follows: (i) 14CO2, Chapman-ethanolamine-treated filter paper; (ii) CH4 by the method of A. J. B. Zehnder, B. Huser, and T. D. Brock [Appl. Environ. Microbiol. 37, 897 (1979)]; (iii) labeled particulate (including cellular) material; (iv) labeled water-soluble, but not ether-soluble, material; and (v) labeled ether-soluble material (that is, nonutilized substrate). Typically, 85 to 90 percent of the added label was recovered at the end of each experiment. Only the CH4 and CO2 production data are presented in this report.
16. Preliminary anaerobic sediment incubation experiments by one of us (F.J.S.), using Cape Lookout sediments, indicated immediate net CH4 accumulation only in samples with initial in situ sulfate concentrations less than 1 mM.
17. By numerically integrating the area under the total CH4 flux curve (Fig. 1A) and the apparent acetate turnover to CH4 curve (Fig. 1B) over equal time intervals, one can compare the measured rate of CH4 production in the top 5 cm of the CH4 production zone with the measured flux out of the sediment during the given interval.
18. G. W. Kipphut and C. S. Martens, in preparation. A deficit in the concentration of the dissolved radioactive gas 222Rn between 10 and 30 cm during the summer months results from in situ stripping of this gas from the sediment interstitial waters by CH4 bubbles. This provides direct evidence for CH4 production in, and transport from, this depth interval.
19. We thank G. Kipphut and P. Crill for help in the collection of samples. We also thank the staffs of the University of North Carolina Institute of Marine Sciences and the National Oceanic and Atmospheric Administration National Marine Fisheries Service Laboratory, Beaufort, N.C. We thank C. D. Taylor of Woods Hole Oceanographic Institution and M. B. Goldhaber of the U.S. Geological Survey, Denver, for critical remarks on an early version of this report. Research supported by NSF grants OCE78-09485 and OCE80-09245 (C.S.M.) and OCE78-09507 (F.J.S.) from the Marine Chemistry Program, Oceanography Section.
* Present address: Department of Oceanography, University of Hawaii, Honolulu 96822.