Field and laboratory studies of methane oxidation in an anoxic marine sediment: Evidence for a methanogen–sulfate reducer consortium

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Abstract. Field and laboratory studies of anoxic sediments from Cape Lookout Bight, North Carolina, suggest that anaerobic methane oxidation is mediated by a consortium of methanogenic and sulfate-reducing bacteria. A seasonal survey of methane oxidation and CO₂ reduction rates indicates that methane production was confined to sulfate-depleted sediments at all times of year, while methane oxidation occurred in two modes. In the summer, methane oxidation was confined to sulfate-depleted sediments and occurred at rates lower than those of CO₂ reduction. In the winter, net methane oxidation occurred in an interval at the base of the sulfate-containing zone. Sediment incubation experiments suggest both methanogens and sulfate reducers were responsible for the observed methane oxidation. In one incubation experiment both modes of oxidation were partially inhibited by 2-bromoethanesulfonic acid (a specific inhibitor of methanogens). This evidence, along with the apparent confinement of methane oxidation to sulfate-depleted sediments in the summer, indicates that methanogenic bacteria are involved in methane oxidation. In a second incubation experiment, net methane oxidation was induced by adding sulfate to homogenized methanogenic sediments, suggesting that sulfate reducers also play a role in the process. We hypothesize that methanogens oxidize methane and produce hydrogen via a reversal of CO₂ reduction. The hydrogen is efficiently removed and maintained at low concentrations by sulfate reducers. Pore water H₂ concentrations in the sediment incubation experiments (while net methane oxidation was occurring) were low enough that methanogenic bacteria could derive sufficient energy for growth from the oxidation of methane. The methanogen-sulfate reducer consortium is consistent not only with the results of this study, but may also be a feasible mechanism for previously documented anaerobic methane oxidation in both freshwater and marine environments.

Introduction

Despite the large reservoir of methane stored in marine sediments, the ocean contributes only about 2% of the global flux of methane to the atmosphere [Cicerone and Orernland, 1988]. Anaerobic methane oxidation provides a nearly quantitative sink for upwardly diffusing methane in many marine sediments. Reeburgh and Alperin [1988] estimated that anaerobic methane oxidation consumes a quantity of methane equivalent to 5-20% of the global atmospheric flux. To understand the factors that control this methane sink, it is important to determine the mechanism by which the process occurs. Although anaerobic methane oxidation has been documented in a number of environments, the mechanism remains unclear because the responsible organism has not been identified.

Three independent lines of evidence suggest the occurrence of net methane consumption in anoxic environments: models of methane concentration profiles, stable isotope distributions, and radiotracer measurements. Methane profiles in many marine sediments show low concentrations throughout the upper portion of the sulfate-reducing zone, rising rapidly to high concentrations in sulfate-depleted sediments. Advection-diffusion-reaction models indicate that these "concave up" distributions can only be accounted for by net consumption of methane [Barnes and Goldberg, 1976; Martens and Berner, 1977]. Similar model studies indicate that anaerobic methane oxidation also occurs in anoxic water columns [Reeburgh, 1976; Scranton, 1988]. Anaerobic methane oxidation is also supported by the presence of 13C-enriched methane [Orernland and DesMarais, 1983; Whiticar and Faber, 1986; Alperin et al., 1988] and 13C-depleted CO₂ [Reeburgh, 1982; N. E. Blair and R. C. Aller, Anaerobic methane oxidation on the Amazon Shelf, submitted to Geochirnica et Cosmochirnica Acta, 1994] in depth intervals where net methane oxidation is thought to occur. The isotopically "heavy" methane and "light" CO₂ are consistent with kinetic fractionation that would result from bacterial conversion of methane to carbon dioxide. Further evidence of methane oxidation is provided by studies using radiotracers to directly measure rates of anaerobic methane oxidation. Conversion of 14CH₄ to 14CO₂ has been shown to occur in the anoxic waters of the Cariaco Trench [Ward et al., 1987], Saanich Inlet [Ward et al., 1989], and the Black Sea [Reeburgh et al., 1991]. Rate-depth
distributions from marine sediments indicate that peak rates of anaerobic methane oxidation generally occur near the sulfate-methane transition zone [Reeburgh, 1980; Devol, 1983; Iversen and Blackburn, 1981; Iversen and Jørgensen, 1985].

Despite the sizable body of geochemical evidence indicating that methane is oxidized in anoxic marine systems, neither the bacteria which mediate the oxidation nor the terminal electron acceptor for the process is known. Likely mediators of methane oxidation include sulfate reducers (see (1)) or methanogens (see (2)), using sulfate or water, respectively, as an electron acceptor:

\[
\begin{align*}
\text{CH}_4 + \text{SO}_4^{2-} & \rightarrow \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}, \\
\text{CH}_4 + 2\text{H}_2\text{O} & \rightarrow \text{CO}_2 + 4\text{H}_2.
\end{align*}
\]

Circumstantial evidence suggests that sulfate-reducing bacteria may play an important role in methane oxidation. Martens and Berner [1977] calculated that methane oxidation with sulfate (see (1)) is thermodynamically favorable (\(\Delta G = -25 \text{ kJ mol}^{-1}\)) at typical in situ conditions. Additionally, several radiotracer studies have measured a subsurface maximum in sulfate reduction rates coincident with the zone of anaerobic methane oxidation [Devol and Ahmed, 1981; Alperin and Reeburgh, 1985; Iversen and Jørgensen, 1985], suggesting that sulfate acts as the terminal electron acceptor for methane oxidation. Pure cultures of sulfate reducers can carry out net methane oxidation while growing on another substrate [Davis and Yarborough, 1966].

However, Iversen [1984] showed that methane oxidation rates in pure cultures of four species of sulfate-reducing bacteria (growing on pyruvate or ethanol) ranged from only 0.01% to 0.2% of the sulfate reduction rate. Given this small fraction, it is unlikely that sulfate reducers alone can account for environmental methane oxidation rates ranging from 15 to ~100% of sulfate reduction rates [Devol, 1983; Alperin and Reeburgh, 1985; Iversen and Jørgensen, 1985]. No pure cultures of sulfate reducers have been found capable of growth with methane as the sole substrate [Sorokin, 1957; Iversen, 1984]. Additionally, molybdate and tungstate (specific inhibitors of sulfate reduction) appear to have no effect on anaerobic methane oxidation [Alperin and Reeburgh, 1985; Iversen et al., 1987; Sandbeck, 1987].

There is also circumstantial evidence to suggest that methanogenic bacteria play a role in methane oxidation. Radiotracer studies show that pure cultures of methanogens can oxidize methane anaerobically (though the rate of oxidation represents only a small fraction of the concurrent methane production rate [Zehnder and Brock, 1979]). Methane oxidation of this type (with rates at a fraction of methane production rates) has been observed in freshwater sediments and attributed to methanogens [Zehnder and Brock, 1980]. Net methane oxidation using water (see (2)) as the terminal electron acceptor would be thermodynamically feasible at very low hydrogen or \(\text{CO}_2\) concentrations or at very high methane concentrations [Zehnder and Brock, 1979].

However, the ability to bring about net consumption of methane has not been demonstrated in methanogens. Additionally, though methane oxidation in pure cultures of methanogens and lake sediments was affected by the methanogen-specific inhibitor 2-bromoethanesulfonic acid (BES) [Zehnder and Brock, 1979, 1980], no consistent effect has been observed in marine sediments [Alperin and Reeburgh, 1985; Sandbeck, 1987].

The existing data are consistent with the four following hypotheses regarding the mechanism of anaerobic methane oxidation: (1) strains of sulfate reducers present in marine sediments (and not yet tested in the laboratory) are able to grow on methane as the sole energy source; (2) sedimentary conditions make net oxidation of methane by methanogens thermodynamically favorable; (3) a consortium of anaerobic bacteria is necessary to achieve the net oxidation observed in field studies; or (4) an unidentified organism is responsible for anaerobic methane oxidation.

We sought to determine the mechanism responsible for methane oxidation in the anoxic sediments of Cape Lookout Bight, North Carolina. These sediments experience dramatic seasonal changes in temperature and rates of metabolic processes, which allow for study of methane oxidation under a variety of conditions. We monitored the seasonal changes in distributions of sulfate and methane and in rates of \(\text{CO}_2\) reduction and anaerobic methane oxidation to investigate the relationship between methane-oxidizing, methane-producing, and sulfate-reducing bacteria. We also conducted laboratory experiments designed to ascertain how methane oxidation is affected by temperature and sulfate. Finally, we amended both sulfate-containing and sulfate-depleted sediments with BES to investigate the potential involvement of methanogenic bacteria in methane oxidation.

Our field measurements show that methane oxidation occurs throughout the year in methanogenic sediments, at a fraction of concurrent methane production rates. We hypothesize that methanogens are responsible for this mode of oxidation. In the winter, net methane consumption occurs in an interval at the base of the sulfate-reducing zone. Our laboratory studies indicate that net methane oxidation can be induced by adding sulfate to methanogenic Cape Lookout Bight sediments. The evidence from field and laboratory studies suggests that a consortium of methanogenic and sulfate-reducing bacteria is responsible for the wintertime net methane oxidation in Cape Lookout sediments.

**Methods**

**Study Site**

Sediment for field and laboratory studies was collected from station A-1 in Cape Lookout Bight, North Carolina, a 10-m deep barrier island lagoon located 70 km southwest of Cape Hatteras. Sediment accumulates in Cape Lookout at \(\sim 10 \text{ cm yr}^{-1}\) [Chanton et al., 1983; Canuel et al., 1990]. The large flux of organic carbon to the seabed (\(165 \pm 20 \text{ mol m}^{-2}\text{yr}^{-1}\), [Martens et al., 1992]) fuels high rates of terminal metabolism, resulting in oxygen depletion within a few millimeters of the sediment-water interface [Canuel, 1992].

The sediment temperature increases from \(< 6^\circ\text{C}\) in the winter to \(> 28^\circ\text{C}\) in the summer, driving substantial seasonal variations in rates of organic matter remineralization [Klump and Martens, 1989]. The seasonal changes in sulfate reduction rates cause the depth of sulfate depletion to oscillate vertically between \(\sim 10 \text{ cm}\) in the summer and \(> 25 \text{ cm}\) in the winter [Crill and Martens, 1987; Klump and Martens, 1989]. About 70% of organic matter remineralization occurs via sulfate reduction, the remainder through methane production [Martens and Klump, 1984].

Unlike the typical concave up methane profiles found in most anoxic marine sediments [Reeburgh and Heggie, 1977], methane
concentrations in Cape Lookout increase approximately linearly through the sulfate-containing zone, from near zero at the sediment-water interface to in situ saturation at the depth of sulfate depletion. Below this horizon, methane bubbles form [Crill and Martens, 1986], leading to ebullition during the summer months [Martens and Klump, 1980]. Bubble transport through the upper portion of the sediment column makes it difficult to apply advection-diffusion-reaction models to methane profiles at this site.

Sample Collection

Sediment samples were collected by SCUBA diver using 50 cm x 9 cm OD plexiglass core tubes. Cores were kept in the dark at in situ temperature until subsectioning, which generally occurred within 6 hours of collection. Sediment for depth profiles was obtained by extruding the core upward at 3-cm intervals and sampling through two side-by-side 2.4-cm holes centered 1.5 cm below the top. The plunger of a cut off 30-mL plastic syringe was inserted through the 2.4-cm hole a few millimeters into the sediment and held fixed, while the syringe barrel was pushed horizontally into the sediment. The syringe was then removed (holding the plunger fixed relative to the barrel) and fitted with a Teflon nozzle that facilitated filling of centrifuge tubes for pore water extraction and "rate syringes" (described below) for radiotracer rate measurements.

Laboratory Studies

Laboratory experiments were conducted using a syringe-like apparatus, consisting of a 10-cm ID glass barrel and a solid polyvinyl chloride (PVC) piston [Alperin et al., 1992]. A stopcock at one end allowed for periodic removal of samples or addition of aqueous solutions. The sediments could be mixed, following addition of solutions, with a Teflon plate fixed to a rod that passed through the piston. This design enabled us to conduct anaerobic, headspace-free sediment incubations with periodic sampling over timescales of several months. The absence of a headspace was necessary to avoid loss of dissolved gases from the pore water via aqueous gas exchange.

Each vessel was filled with ~2L of Cape Lookout sediment. Sediment from the desired depth interval of several cores was pooled and homogenized in a nitrogen-flushed plastic bag. The incubation vessel was filled by removing the PVC piston, flushing the glass barrel with nitrogen, and transferring the sediment so as to avoid air contact and exclude bubbles. The piston was replaced so as to prevent inclusion of any headspace. Samples were removed and anoxic solutions added through the stopcock using a 60-mL plastic catheter-tip syringe, fitted with a 10-cm nozzle. This allowed us to sample sediment several inches from the stopcock. The void created upon removing the sampling syringe was eliminated by advancing the piston.

Sediment Incubation experiment. In the sediment incubation experiment, sulfate-containing surface sediment (homogenized 0 to 3-cm interval) was incubated at 22°C for 120 days. The data set created by weekly sampling over the long incubation period essentially constitutes a time-based analog of the depth-based biogeochemical zonation in marine sediments. The resultant profiles enabled us to observe changes in methane oxidation during the progression from sulfate reduction to methanogenesis.

Sulfate/temperature manipulation experiment. The sulfate/temperature manipulation experiment was designed to ascertain the effects of sediment temperature and sulfate content on methane oxidation. Homogenized sediment collected during the summer from the 15 to 25-cm depth interval (sulfate-depleted) was divided between three vessels and incubated for 3 weeks at 26°C. After the initial 3 weeks, each vessel received one of the following treatments: (1) temperature lowered to 10°C and sediments amended with 50 mL of anoxic (boiled and bubbled with O2-free Argon) sodium sulfate/34% NaCl solution (to achieve a final porewater sulfate concentration of 10.0 mM); (2) temperature lowered to 10°C and sediments amended with 50 mL of anoxic 34% NaCl solution (as a control); (3) temperature held at 26°C and sediments amended with 50 mL of anoxic sodium sulfate/34% NaCl solution (to achieve a final porewater sulfate concentration of about 5 mM).

Following treatment, the syringes were allowed to equilibrate for 4 days, and sampling was resumed. Pore water H2 concentrations were determined in this experiment, in addition to the usual suite of concentration and rate measurements.

Inhibition experiment. As a test for the possible involvement of methanogenic bacteria in methane oxidation, we measured methane oxidation, production, and sulfate reduction rates in samples treated with BES. BES is an analog of coenzyme M [Gunsalus et al., 1978], an enzyme cofactor thought to be present only in methanogens [Balch and Wolfe, 1979]; hence the inhibitory effect of BES is considered specific to these bacteria.

The concentration range of BES employed (0-60 mM) was intended to result in partial inhibition of methanogen-mediated processes; previous dose-response experiments indicated that 120 mM BES completely inhibits CO2 reduction and methane oxidation in Cape Lookout Bight sediments. It must be noted that this concentration range is as much as 1000-fold higher than that typically used in pure culture work. Methanogens in environmental settings often require such high concentrations of BES for inhibition [King, 1984; Zinder et al., 1984, Oremland et al., 1988]. The necessity for high doses may be due to membrane impermeability in some strains [Oremland and Capone, 1988] or to sorption or other sequestering of BES in a sedimentary setting. High concentrations of BES could alter the biochemistry of microbes other than the target population (i.e., fermenters or sulfate reducers). We monitored sulfate reduction rates in the sulfate-containing samples to determine if nonspecific inhibition had occurred. Presumably, alteration of the normal metabolic activity of either fermenters or terminal bacteria would lead to observable effects on sulfate reduction rates.

The inhibition experiment used methanogenic sediments which had been incubated in two vessels. One vessel was amended with anoxic sulfate solution (as described previously for the sulfate/temperature manipulation experiment), and the other was left untreated. Sediments were transferred into glass rate syringes (50 for each treatment) and allowed to sit at incubation temperature (18°C) for 2 days. Each was then injected with 25 μL of concentrated anoxic BES solution to achieve the desired final concentration. The samples were allowed to equilibrate for 3 days before rates measurements were made.

Analyses and Measurements

Pore water concentrations. Pore water for sulfate and ΣCO2 analyses was obtained by centrifugation of whole sediment. Sediment was transferred to argon-flushed 30-mL Teflon centrifuge tubes, taking care that the tubes were filled to the top.
Porewater was extracted by centrifuging for 15 min at 6000 g. The supernatant was collected in a glass syringe with a Teflon plunger and stainless steel needle and then filtered through a 0.2-

μm filter; the first milliliter of filtrate was discarded.

Pore water for \( \Sigma \text{CO}_2 \) analysis was transferred to glass vials closed by means of a screw cap and Teflon-coated septum fitted with a movable glass rod. The rod was used to displace any headspace in the vial, thus preventing loss of CO\(_2\) gas into the headspace. Samples were analyzed using the inorganic carbon channel of a Shimadzu TOC-5000 (CO\(_2\) quantified using a nondispersive infrared detector). Precision of the analysis was generally ± 2%.

For sulfate analyses, 2 mL of pore water was transferred directly to a glass vial containing 200 μL of 10% vol/vol Ultrace HCl and bubbled with argon for 5 min. This treatment removed volatile sulfur compounds (predominantly H\(_2\)S), eliminating interference from sulfide oxidation. Measured concentrations were later corrected for the volume change caused by the acid addition. Samples were diluted (1:10 or 1:100) and analyzed on a Dionex 2010i ion chromatograph. For low level sulfate determinations, samples were passed through a Dionex ONGUARD-Ag pretreatment cartridge which removed chloride from the porewater. Sulfate could be detected at 5 μM; analytical precision was generally ± 2% for concentrations above 1.0 mM and ± 10% below 1.0 mM.

**Dissolved gas concentrations.** Methane concentrations were determined using a headspace equilibration technique [Alperin and Reeburgh, 1985]. Whole sediment was rapidly extruded from a 3-mL rate syringe into a tared 20-mL serum vial (containing 3.0 mL of 0.1 N NaOH to terminate further bacterial activity) which was quickly sealed with a silicone stopper. The sediment was generally exposed to air for <2 s, minimizing potential loss of methane. The vial was shaken to equilibrate the pore water methane between aqueous and gas phases in the vials. A gas-tight syringe was used to remove 25 μL of headspace, which was analyzed for methane concentration by gas chromatography with flame ionization detection (GC/FID). Standards were prepared by injecting known quantities of pure methane into sealed, 20-mL serum vials containing 3.0 mL of 0.1 N NaOH and 2.5 mL distilled water. The whole sediment methane concentrations that result from the headspace equilibration method were corrected to pore water concentration by taking sediment porosity into account. Analytical precision of the GC method was better than ± 1%. Measurements of replicate sediment samples had a precision of about ± 4% for concentrations less than saturation, and often ± 10% or more for super-saturated sediments (due to methane bubble inclusion).

Pore water H\(_2\) concentrations were determined by a modification of the method described by Lovley and Goodwin [1988]. Whole sediment (10 mL) was transferred anaerobically from 60-mL catheter-tip syringes to nitrogen-flushed 20-mL glass serum vials. The vials were sealed with black, butyl rubber stoppers and incubated upside down, without shaking (preliminary studies with Cape Lookout Bight sediments indicate that shaking may result in erroneous H\(_2\) concentration measurements). This method requires that H\(_2\) partial pressure in the gas phase reach equilibrium with the steady state H\(_2\) concentration in the porewater. Note that this method assumes homogeneity within the sample. Only thoroughly mixed samples from the incubation vessels were used for H\(_2\) determinations, reducing the likelihood of gradients within the sample; however, the possibility of microenvironments cannot be ruled out.

We used a time series to determine when samples had reached aqueous gas equilibrium. For each concentration measurement, five replicate vials were prepared and one was analyzed for H\(_2\) concentration every few days. When the headspace concentrations in replicate vials had not changed over a period of several days, we assumed that equilibrium had been reached, and the remainder of the samples were analyzed. To obtain a headspace sample, a 3-mL plastic syringe flushed with H\(_2\)-free (hopcalite treated [Haruta and Sano, 1981]) nitrogen was used to inject 2.5 mL of H\(_2\)-free distilled water into a sample vial and then remove 2.5 mL of headspace gas. The H\(_2\) partial pressure in the sample was determined by direct injection into an RGA-3 reduction gas analyzer (Trace Analytical, Menlo Park, California). The analysis is based on GC separation of H\(_2\) and quantification by a mercuric oxide reduction detector. Gas phase partial pressures were converted to pore water concentrations using H\(_2\) solubility data corrected for temperature and salinity [Crozier and Yamamoto, 1974]. Because of the large gas to aqueous partition coefficient for H\(_2\), the detection limit is equivalent to 0.005 nM.

Working standards were prepared by diluting an N\(_2\)-H\(_2\) mix with air and storing under pressure in gas cylinders. These standards were calibrated against primary standards prepared by static dilution of pure hydrogen with hopcalite-treated nitrogen. Blanks were determined by carrying hopcalite-treated nitrogen through the analysis, and they were always below the detection limit. Accuracy of the analysis was estimated to be ± 2% (by comparing standards prepared by independent dilutions of different hydrogen mixtures). Analytical precision was about ± 1%, while measurements on replicate sediment samples had a precision of about ± 5%.

**Radiotracer rate measurements.** Previously described radiotracer methods were used to determine rates of methane oxidation [Reeburgh, 1980], CO\(_2\) reduction [Crill and Martens, 1986], and sulfate reduction [Alberi et al., 1994]. Whole sediment for rate determinations was transferred from 60-mL catheter-tip syringes to 3.0-mL glass tubes equipped with a plunger (rate syringes). The tubes were filled to overflowing (ensuring no headspace) and stoppered with one-hole black rubber stoppers. The holes in the stoppers had been previously filled with silicone caulkling to serve as septa. The sediments were amended with 25 μL of \(^{13}\)CH\(_4\) (gaseous, 5x10\(^5\) cpm), NaH\(^{14}\)CO\(_3\) (aqueous, 5x10\(^5\) cpm) or H\(_2\)^{35}\)SO\(_4\) (aqueous, 1.5x10\(^5\) cpm) via line injection through the septa. Samples were maintained at the appropriate temperature in the dark for the duration of the incubation (generally 1-2 days). Methane oxidation and CO\(_2\) reduction rate samples were "killed" by contact with NaOH solution, while sulfate reduction incubations were terminated by freezing the rate syringes.

Rates were calculated using the following equation:

$$\text{Rate} = \frac{[C] \cdot a \cdot A}{t}$$

where [C] is the reaction concentration (methane, CO\(_2\), or sulfate); a and A are the reacted and added activities of tracer, respectively; t is the incubation time, and α is the isotope fractionation factor associated with the process. We used fractionation factors of 1.12 for CO\(_2\) reduction [Blair et al., 1993], 1.02 for methane oxidation [Alperin et al., 1988], and 1.045 for sulfate reduction [Jorgensen, 1978]. Note that \(^{13}\)C/\(^{12}\)C fractionation is double that for \(^{13}\)C/\(^{12}\)C. In general, >90% of added tracer was recovered in methane oxidation and CO\(_2\)
reduction incubations. Time course experiments using $^{14}$CH$_4$ and $^{14}$CO$_2$ indicated tracer turnover was linear with time (over the incubation timescale). Analysis of replicate sediment samples had a precision of about ± 10% for methane oxidation rates and about ± 5% for CO$_2$ and sulfate reduction rate measurements.

Autoclaved samples amended with $^{14}$CH$_4$ yielded no measurable $^{14}$CO$_2$. Zero-time-killed controls were routinely conducted for methane oxidation measurements and never yielded $^{14}$CO$_2$ upon analysis. GC/FID analysis of the tracer methane did not detect ethane or other nonmethane hydrocarbons.

**Results**

**Field Studies**

Typical rate and concentration profiles for Cape Lookout sediments are shown in Figures 1 and 2. During the summer months, sulfate is generally depleted by ~8 cm, while it penetrates to >18 cm in the winter. At all times of year, methane concentrations show an approximately linear increase with depth. This may indicate that methane concentration profiles in Cape Lookout Bight are dominated by rapid diffusive and bubble transport of methane into the sulfate zone, whereas the concave up distributions typical of coastal marine sediments appear to be controlled predominantly by methane consumption. Methane bubbles are present below the sulfate depletion depth and give rise to the somewhat erratic profiles.

Rate profiles from the summer months (Figures 1a-1c) indicate that both methane oxidation and CO$_2$ reduction are confined to sediments that contain <1.0 mM sulfate (hereafter referred to as "sulfate-depleted" sediments). The lack of methane oxidation in the sulfate-containing zone is probably not due to methane limitation, as methane concentrations are moderate to high throughout the upper portion of the sediment column (Figures 1d-1f). Replicate cores obtained on July 16, 1990, illustrate the

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**Figure 1.** Summer depth profiles from Cape Lookout Bight sediments. (a-c) Methane oxidation and CO$_2$ reduction rates. (d-f) Pore water methane and sulfate concentrations. Horizontal dashed line indicates the depth at which sulfate concentration equals 1.0 mM. Error bars represent 1 standard deviation about the mean of triplicate (Figures 1a and 1d) or duplicate (Figures 1b and 1e) samples.
sensitivity of methane oxidation to the presence of sulfate. Two cores contained sulfate to a depth of 7 cm (Figure 1e), while sulfate penetrated to 13 cm in a third (Figure 1f). Correspondingly, methane oxidation and CO$_2$ reduction occurred immediately below 7 cm in the first two cores (Figure 1b) but were limited to depths >13 cm in the third (Figure 1c). The ratio of methane oxidation rate to concurrent CO$_2$ reduction rate (MOR/CRR) within the methanogenic portion of the sediment column was generally 0.1 to 0.2.

As in the summer, methane oxidation in the winter occurred below the sulfate depletion depth, at a fraction of the methane production rate. However, the winter profiles also reveal methane oxidation occurring at the base of the sulfate-reducing zone (Figures 2a-2c). Several lines of evidence indicate that methane is not produced in the sulfate-containing zone and that the oxidation represents net consumption of methane. First, the January 31, 1993, profile (Figure 2c) shows CO$_2$ reduction did not occur within the sulfate-containing zone (in fact, in all field and laboratory measurements, CO$_2$ reduction did not occur at appreciable rates in the presence of sulfate). Second, $^{14}$CH$_3$COOH is not converted to $^{14}$CH$_4$ in sulfate-containing Cape Lookout Bight sediments [Sansone and Martens, 1981; D. B. Albert et al., manuscript in preparation, 1994]. Third, there is no evidence of methane production from noncompetitive substrates [Alperin et al., 1992]. The net methane oxidation appears to be limited to depths >10 cm, despite the presence of methane in the upper 10 cm of the sediment column (Figures 2d-2f).

**Laboratory Studies**

**Sediment incubation experiment.** The results of the sediment incubation experiment are summarized in Figure 3. The sulfate concentration decreased during the initial 42 days (from 19 mM to -0.01 mM), while the methane concentration remained relatively constant at ~0.2 mM (Figure 3a). Following sulfate

Figure 2. Winter depth profiles from Cape Lookout Bight sediments. (a-c) Methane oxidation and CO$_2$ reduction rates. (d-f) Pore water methane and sulfate concentrations. Horizontal dashed line indicates the depth at which sulfate concentration equals 1.0 mM. Error bars represent 1 standard deviation about the mean of triplicate samples. CO$_2$ reduction rates were not measured for the 1990 or 1991 samples.
depletion, the methane concentration increased to 1.6 mM, before bubble formation resulted in degassing and a decrease in pore water concentration. Neither methane oxidation nor CO$_2$ reduction occurred while sulfate was present (Figure 3b), despite 0.2 mM methane available as a substrate for methane oxidation. Following sulfate depletion, methane oxidation and CO$_2$ reduction rates increased, peaked, and fell. However, methane oxidation rates increased more gradually than CO$_2$ reduction rates following sulfate depletion from < 0.01 to 4.1 (Figure 3c). The H$_2$ concentration dropped to 1.82 ± 0.04 nM (Figure 4d), consistent with the work of Conrad et al. [1987] and Westermann [1994], who observed that steady state H$_2$ concentrations in methane producing sediments decrease at lower temperatures.

Cooling and adding sulfate to the sediment resulted in net consumption of methane. While CO$_2$ reduction rates (Figure 4b) dropped to very low levels, methane oxidation (Figure 4a) continued to occur at a slowed rate (~15% of the rate before treatment) so that the MOR/CRR ratio rose to 2.7 (Figure 4c). Note that net methane oxidation resulted from the considerable drop in CO$_2$ reduction rates rather than an increase in methane oxidation rates. Cooling the sediment and adding sulfate resulted in a pore water H$_2$ concentration (0.16 ± 0.02 nM) considerably lower than cooling alone. The results of this experiment appear analogous to the winter conditions in Cape Lookout Bight: low temperatures and penetration of sulfate into methanogenic sediments give rise to net consumption of methane.

Inhibition experiment. Methane oxidation in sulfate-depleted sediments was considerably more sensitive to BES than was CO$_2$ reduction (Figure 5a). At 60 mM BES, methane oxidation was 76% inhibited relative to only 13% for CO$_2$ reduction. This result is consistent with the findings of Zehnder and Brock [1980], who showed that methane oxidation in methanogenic sediments is more sensitive to BES than CO$_2$ reduction (50% inhibition of CO$_2$ reduction required a 20-fold higher BES concentration than 50% inhibition of methane oxidation). Since nonspecific inhibition of fermenting organisms would likely be manifest in the CO$_2$ reduction rates (due to altered substrate supply), the apparent insensitivity of CO$_2$ reduction to 2-30 mM BES indicates fermenters were relatively unaffected.

BES also partially inhibited net methane oxidation in sulfate-containing sediments (Figure 5b). The degree of inhibition was somewhat less than for the methanogenic sediments (~50% at 60 mM BES). Sulfate reduction rates dropped only slightly with increasing BES concentration, again indicating that nonspecific inhibition probably did not occur.

Sulfate/temperature manipulation experiment. During the initial three weeks, methane oxidation rates in the treatments were similar and increased with time (Figure 4a). Similarly, CO$_2$ reduction rates were approximately equal, with a slight decrease through time (Figure 4b). The MOR/CRR ratio during this period ranged from 0.05 to 0.1 (Figure 4c) and pore water H$_2$ concentrations remained roughly constant at ~10.0 ± 0.4 nM (Figure 4d).

Cooling the sediment from 26°C to 10°C had a large effect on rates and concentrations. Both methane oxidation and CO$_2$ reduction rates decreased substantially (Figures 4a and 4b) while the MOR/CRR ratio increased from -0.08 to -0.15 (Figure 4c). The H$_2$ concentration dropped to 1.82 ± 0.04 nM (Figure 4d), consistent with the work of Conrad et al. [1987] and Westermann [1994], who observed that steady state H$_2$ concentrations in methane producing sediments decrease at lower temperatures.

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Discussion

Methane Oxidation by Methanogens

In sulfate-depleted Cape Lookout Bight sediments, methane oxidation occurred throughout the year at a fraction of the concurrent methane production rate. Evidence from the field and laboratory studies suggests that this methane oxidation is mediated by methanogenic bacteria.

During the summer and in the sediment incubation experiment, methane oxidation did not occur in the presence of sulfate, despite the availability of methane as a substrate (Figures 1 and 3). The exclusion of methane-oxidizing bacteria from sulfate-containing sediments could be due to either inhibition or competition for common substrates. Since it is unlikely that marine bacteria are inhibited by sulfate at pore water concentrations (0-29 mM), competition is the most likely mechanism of exclusion. If competition is responsible for exclusion of methane oxidizers from sulfate-containing sediments, it follows that the organism responsible for methane oxidation requires a substrate used by sulfate reducers (i.e., H$_2$ or acetate). Since the only substrates necessary for methane oxidation are (presumably) methane and an electron acceptor, it seems likely that the organism responsible for methane oxidation also carries out a reaction that requires hydrogen or acetate.
On the basis of these criteria, it seems likely that methanogenic bacteria are involved in methane oxidation. Methanogens use H₂ and acetate and are excluded from sulfate-containing sediments on the basis of competition [Lovley et al., 1982]. Further, methanogens have demonstrated ability to oxidize methane anaerobically [Zehnder and Brock, 1979]. This evidence, along with the finding that methane oxidation is partially inhibited by BES, suggests that methanogenic bacteria are responsible for methane oxidation in sulfate-depleted Cape Lookout sediments. In the simplest case the reaction might be thought of as the reverse of CO₂ reduction (see (2)).

Net Methane Oxidation by a Methanogen-Sulfate Reducer Consortium

In sulfate-containing Cape Lookout Bight sediments, net consumption of methane occurred during the winter in an interval at the base of the sulfate-reducing zone. The boundaries of this interval indicate that a consortium of methanogenic and sulfate-reducing bacteria may be responsible for the net oxidation. This hypothesis is supported by the results of the laboratory studies.

The zone of net methane oxidation is bounded below by the sulfate-depletion depth (Figures 2a-2c). Net consumption of methane did not occur in the absence of sulfate in any of the field or laboratory studies, suggesting that sulfate is necessary for net oxidation. This hypothesis is supported by the sulfate/temperature manipulation experiment, which showed that net methane oxidation can be brought about by adding sulfate to methanogenic sediments (Figure 4c). The sulfate dependence suggests that sulfate-reducing bacteria are involved in net methane oxidation.

The zone of net oxidation also appears to be limited to depths below ~10 cm (Figures 2a-2c). Given methane concentrations of 0.1 to 1.0 mM in this portion of the sediment column, the lack of methane oxidation is probably not due to methane limitation. The 10-cm upper boundary may be the result of seasonal changes in the minimum depth of methanogenesis. The sulfate depletion depth in Cape Lookout sediments oscillates from ~8 cm in the summer to >25 cm in the winter. Since methanogenesis is generally limited to below the sulfate depletion depth, the sediment column can be divided into three zones; above 8 cm there is never an active population of methanogens; from 8-25 cm a large population is active during the summer; below 25 cm, conditions always favor an active methanogen population.

Applying this scheme to the depth profiles of methane oxidation, it is apparent that above 8 cm, where there is no established methanogen population, methane oxidation does not occur; in the 8-25 cm zone an active population of methanogens is periodically flooded with sulfate and net methane oxidation occurs; below 25 cm, where the sediment is always sulfate depleted, methane oxidation occurs at a fraction of the methane production rate. The coincidence in zonation of net methane oxidation and methanogen population suggests that methanogenic bacteria are involved in net methane consumption. The partial inhibition of net methane oxidation in sulfate-containing sediments by BES supports this hypothesis.

The field and laboratory studies suggest that net methane oxidation occurs by a mechanism involving both methanogenic and sulfate-reducing bacteria. We have proposed that methane oxidation in sulfate-depleted sediments is mediated by methanogens and occurs via a reversal of CO₂ reduction (see (2)). In sulfate-containing sediments the hydrogen produced by methanogens during methane oxidation could be used by sulfate reducers in a syntrophic association:

\[
\text{HSO}_4^- + 4\text{H}_2 \rightarrow \text{H}^- + 4\text{H}_2\text{O}\ .
\]

A precedent exists for the type of "reversible metabolism" methanogens would require for this mechanism to be feasible. Lee and Zinder [1988] isolated an acetogenic bacterium that could convert H₂ and CO₂ to acetate when grown in pure culture. When cocultured with a methanogen (which acted as a hydrogen sink), the organism catalyzed the reverse reaction, converting acetate to H₂ and CO₂. The acetogen-methanogen consortium is directly analogous to the methanogen-sulfate reducer consortium we propose to account for anaerobic methane oxidation.

Thermodynamic Considerations

Application of thermodynamics to the methane oxidation system provides constraints on the feasibility of the consortium hypothesis. We assume that oxidation of methane with water (see (2)) must yield sufficient free energy for growth of the methanogen. The Gibbs free energy (ΔG) of the methane oxidation reaction can be calculated using the following equation:

\[
\Delta G = \Delta G^\circ + RT \ln \left( \frac{P_{\text{H}_2}P_{\text{CO}_2}}{P_{\text{CH}_4}} \right),
\]

where ΔG^\circ (kJ mol⁻¹) is free energy at standard concentrations corrected for temperature (using the van't Hoff equation [Atkins, 1990]), T is Kelvin temperature, R is the universal gas constant, and P_{\text{H}_2}, P_{\text{CO}_2}, and P_{\text{CH}_4} are partial pressures. Because fugacities differ from partial pressures by <0.5% over the range of temperatures and pressures encountered in this study [Atkins, 1990], fugacity coefficients can be neglected. It is clear from (4) that ΔG of methane oxidation is strongly dependent on the hydrogen partial pressure; the lower P_{\text{H}_2}, the more favorable the methane oxidation reaction. In Cape Lookout sediments, ΔG = 0 when P_{\text{H}_2} = 1.12 ± 0.009 atm ([H₂] = 0.84 ± 0.007 mM), given typical conditions at the 20-cm horizon (P_{\text{CH}_4} = 2 atm, P_{\text{CO}_2} = 0.07 atm, and ΔG^\circ = +136.8 ± 0.1 kJ mol⁻¹). This calculation indicates that methane oxidation via (2) is thermodynamically favored at H₂ concentrations below 0.84 mM.

It is unlikely, however, that methane oxidation would occur at ΔG values close to zero. Bacteria must couple the energy obtained from metabolic reactions to the formation of the energy carrier adenosine triphosphate (ATP) [Thauer and Morris, 1984]. The predominant mode of energy coupling in chemotrophic anaerobes is thought to be chemiosmosis [Vogels et al., 1988], in which the energy from metabolic reactions is stored as an electrical potential and pH gradient across a membrane [Mitchell, 1961]. The energy released when protons are transported back across the membrane (with the gradient) is coupled to ATP formation. The total energy released by transporting n protons must be at least -44 kJ mol⁻¹ to produce ATP under typical intracellular conditions [Alberty, 1969; Hill, 1977]. Since the energy released from reaction of one substrate molecule must be sufficient to transport one proton across the membrane (against the pH gradient and electrical potential), reactions which yield less than -44/n kJ mol⁻¹ will not allow cell growth. The number of protons required to make one ATP is of the order of 3 [Ferguson and Sorgato, 1982], but changes in intracellular conditions may allow the H⁺/ATP ratio to increase [Komens and Booth, 1981]. If n is roughly 3-5, then an organism would require an energy
yield of at least 9-15 kJ mol\(^{-1}\) substrate for growth. This calculation assumes 100% efficiency in energy conservation so the actual minimum energy requirement is probably somewhat higher.

The implication of this "biological energy quantum" on the proposed reversal of CO\(_2\) reduction is schematized in Figure 6. Assuming an energy quantum of 10 kJ mol\(^{-1}\), three situations can occur: for \(\Delta G\) (methane oxidation) < -10 kJ mol\(^{-1}\), methane oxidation predominates; for \(\Delta G > +10\) kJ mol\(^{-1}\), methane production predominates; between -10 and +10 kJ mol\(^{-1}\), no reaction occurs. The same relationship exists between predominant reaction and \(H_2\) concentration.

For typical Cape Lookout conditions \((P_{CH_4} = 2\) atm, \(P_{CO_2} = 0.07\) atm, and \(\Delta G_{10^\circ C} = +136.8 \pm 0.1\) kJ mol\(^{-1}\) and assuming an energy requirement of 10 kJ mol\(^{-1}\)), CO\(_2\) reduction will only occur at \(H_2\) concentrations above 2.4 nM; methane oxidation, at concentrations below 0.29 nM. Between these concentrations, neither reaction provides sufficient energy for cell growth. This may explain why sulfate stimulates methane oxidation in the winter but not in the summer. While sulfate reducers might maintain \(H_2\) concentrations too low to allow CO\(_2\) reduction in sulfate-containing sediments throughout the year, \(H_2\) concentrations low enough to support methane oxidation might only occur during the winter.

The results of the sulfate/temperature manipulation experiment indicate that the methanogen-sulfate reducer mechanism is consistent with the constraints of thermodynamics and the biological energy quantum. The onset of net methane oxidation (caused by cooling and sulfate addition) was concurrent with a decrease in \(H_2\) concentration to 0.16 ± 0.017 nM (Figure 4d). The control treatment (no added sulfate) shows that the \(H_2\) concentration of 0.16 nil/(Pro = 0.21 + 0.023 g/atm) is consistent with the constraints of thermodynamics and the biological energy quantum. The onset of net methane oxidation was greater than that of CO\(_2\) reduction (-9.6 ± 0.8 kJ mol\(^{-1}\), with \([H_2] = 1.82\) nM, and the same experimental conditions), indicating the energy yield for methane oxidation probably exceeds the biological quantum requirement of CO\(_2\) reducers.

It is interesting to note in this context that CO\(_2\) reducers in freshwater sediments derived an energy yield as low as about -8 kJ mol\(^{-1}\) from methane production [Westermann, 1994]. Further, the 7 to 15-\(\mu\)M acetate concentrations typical of sulfate-depleted Cape Lookout sediments in the winter (D. B. Albert et al., manuscript in preparation, 1994) provide a similar energy yield of -9.1 to -10.9 kJ mol\(^{-1}\) for acetoclastic methanogenesis in Cape Lookout sediments (using \(\Delta G_{10^\circ C} = -45.3 \pm 0.1\) kJ mol\(^{-1}\), \(P_{CH_4} = 2\) atm, \(P_{CO_2} = 0.07\) atm, \(PH = 7.2 \pm 0.1\), and \(\gamma_{Acetate} = 1.15 \pm 0.05\), estimated from Garrels and Thompson [1962]). Methane oxidation and both modes of methanogenesis appear to proceed with minimum energy yields in a range consistent with chemiosmotic energy conservation.

**Other Bacteria in a Methane-Oxidizing Consortium**

Field and laboratory studies suggest that a consortium of CO\(_2\) reducers and sulfate reducers is responsible for net methane oxidation in Cape Lookout Bight sediments. However, since the requirements of the mechanism are general (one bacterium to oxidize methane and a second to maintain the end product at low concentrations), it is possible that other bacteria could fill these roles.

While the \(H_2\) concentration data are consistent with mediation of net methane oxidation by CO\(_2\) reducers, acetoclastic methanogens could perform the same function. Zehnder and Brock [1979] showed that pure cultures of acetate fermenters can convert methane to acetate (though, as in the case of CO\(_2\) reducers, the amount of acetate produced was always much smaller than the amount of methane simultaneously formed).

![Figure 6. "Biological energy quantum" concept for CO\(_2\) reduction in Cape Lookout Bight sediments. Diagonal line plots the relation between \(\Delta G\) and \(H_2\) concentration under typical Cape Lookout Bight conditions. Shaded region indicates the range of \(\Delta G\) and \(H_2\) concentrations over which neither methane production nor oxidation will occur, if a minimum energy requirement of -10 kJ mol\(^{-1}\) is assumed.](image-url)
This mechanism, with subsequent use of acetate by sulfate reducers, could equally well result in net methane oxidation. Equation (6) shows that for aceticlastic methanogenesis, $\Delta G = 0$ when $[\Sigma CH_3COO^-] = 0.15 \pm 0.018 \mu M$, and $\Delta G = -10 \text{ kJ mol}^{-1}$ (in the case of a biological energy quantum requirement) when $[\Sigma CH_3COO^-] = 0.0021 \pm 0.00026 \mu M$:

$$\Delta G = \Delta G^0 + RT \ln \frac{\gamma_{Acetate}[CH_3COO^-]}{\gamma_{CO2}[P_{CH_4}]}$$

(6)

with $\Delta G^0$ = 45.3 $\pm$ 0.1 kJ mol$^{-1}$, $P_{CH_4} = 2.0$ atm, $P_{CO2} = 0.07$ atm, $pH = 7.2 \pm 0.1$, and $\gamma_{Acetate} = 1.15 \pm 0.05$. In sulfate-containing Cape Lookout Bight sediments, typical acetate concentrations of 1-3 $\mu M$ (D. B. Albert, et al., manuscript in preparation, 1994) are an order of magnitude too high to yield any free energy (and 3 orders of magnitude too high to satisfy the biological energy quantum requirement). These calculations indicate that net methane oxidation via reversal of aceticlastic methanogenesis would not be a thermodynamically favored process in Cape Lookout Bight.

It is also possible that the role of "hydrogen sink" in the microbial consortium might not be limited to sulfate reducers. In theory, any terminal bacteria capable of maintaining low enough hydrogen concentrations (e.g., $NO_3^-$, $Fe^{3+}$, or $Mn^{4+}$ reducers [Lovley and Goodwin, 1988]) might enable methanogens to bring about net methane oxidation. This raises the possibility that net methane oxidation might occur in freshwater sediments with higher concentrations of such electron acceptors. However, if methane was present throughout the sediment column, as in many freshwater systems, then the oxidation might be expected to occur near the sediment-water interface, making it difficult to distinguish from aerobic oxidation.

Attempts to grow a coculture of methanogens and hydrogenotrophs on methane alone would constitute a test of the consortium hypothesis. With careful monitoring of $H_2$ concentrations (to ensure that energy requirements for "reverse methanogenesis" are met) such a coculture could establish whether the consortium hypothesis is feasible and which bacteria could participate in such an arrangement.

### Synthesis of Previous Geochemical and Microbiological Studies

The methanogen-sulfate reducer consortium hypothesis is consistent not only with data presented here but with much of the current microbiological and geochemical evidence as well. The involvement of methanogens accounts for anaerobic methane oxidation in both freshwater [Zehnder and Brock, 1980] and marine [Reeburgh, 1980; Iversen and Blackburn, 1981] sediments with a single mechanism. The necessity for a hydrogen scavenger (with high concentrations of terminal electron acceptor) may explain why net methane oxidation has thus far only been observed in marine systems, and it provides a basis for the apparent coupling of methane oxidation and sulfate reduction [Devol and Ahmed, 1981; Alperin and Reeburgh, 1985; Iversen and Jørgensen, 1985].

This hypothesis does not require that sulfate reducers have the ability to grow on methane (an ability which has not been demonstrated), as would be required if sulfate reducers alone accounted for methane oxidation in some environments [Iversen and Jørgensen, 1985]. Additionally, the requirement of a consortium explains why net methane oxidation could not be induced in pure cultures of methanogens [Zehnder and Brock, 1979]. The proposed influence of $H_2$ concentration on $CO_2$ reduction might also explain the discrepancy between MOR/CRR ratios in pure culture and the environment. Pure cultures, which are grown under an $H_2$-rich atmosphere, generally have an MOR/CRR ratio $>0.005$ [Zehnder and Brock, 1979]; in freshwater sediments, where $H_2$ concentrations are considerably lower than in pure culture [Lovley and Goodwin, 1988], the MOR/CRR ratio is higher ($<0.08$) [Zehnder and Brock, 1980]. This is in keeping with thermodynamic predictions that lower hydrogen concentrations favor methane oxidation and result in a higher MOR/CRR ratio.

The inconsistent results obtained from inhibition studies of methane oxidation are not as easily explained. Inhibition of methane oxidation in Cape Lookout sediments required BES concentrations substantially higher than those typically used in laboratory studies. It is possible that previous studies treated sediments with BES concentrations too low for an effect to have been evident [Alperin and Reeburgh, 1985; Sandbeck, 1987].

With the proposed consortium mechanism, attempts to inhibit methane oxidation with sulfate reduction inhibitors such as molybdate and tungstate would not provide unambiguous results. In Cape Lookout, inhibition of sulfate reduction would allow $CO_2$ reducers to become active (with the availability of $H_2$ at higher concentrations). Methane oxidation would then occur at a fraction of the $CO_2$ reduction rate. In the sulfate/temperature manipulation experiment, rates of methane oxidation in sulfate-containing and sulfate-depleted sediments (Figure 4a) were not sufficiently different that any inhibitory effects of molybdate on methane oxidation would be apparent. A similar mechanism might be responsible for the lack of inhibition of methane oxidation in previous studies [Alperin and Reeburgh, 1985; Sandbeck, 1987; Iversen et al., 1987].

### Summary

Anaerobic methane oxidation in Cape Lookout Bight occurs in two distinct modes. In sulfate-depleted sediments, methane oxidation occurs throughout the year at a fraction of the concurrent methane production rate. We hypothesize that methanogenic bacteria are responsible for this mode of oxidation. In sulfate-containing sediments, net methane oxidation occurs during the winter in an interval at the base of the sulfate reducing-zone. We propose that this latter mode of oxidation is carried out by a consortium of methanogenic and sulfate reducing bacteria. In this mechanism, methane is oxidized by $CO_2$ reducers, using water as an electron acceptor (see (2)). The hydrogen produced in the reaction is utilized by sulfate reducers in a syntrophic association. When $H_2$ is maintained at sufficiently low concentrations, net methane oxidation occurs.

Anaerobic methane oxidation by methanogens appears to be both biochemically and thermodynamically feasible. Pure culture studies indicate that enzymatic pathways for the conversion of methane to carbon dioxide exist in methanogens [Zehnder and Brock, 1979]. Further, $H_2$ concentrations measured in the sulfate/temperature manipulation experiment show that methane oxidation with water would produce $-13.5 \text{ kJ mol}^{-1}$, a greater energy yield than for $CO_2$ reduction at the same temperature. These data indicate that net methane oxidation via a reversal of $CO_2$ reduction is possible.
Evidence from the field and laboratory studies indicates the involvement of methanogens in methane oxidation is likely in Cape Lookout Bight. In the sediment incubation experiment and summer field studies, methane oxidation is predicated on the activity of methanogens and occurs at a fraction of concurrent methane production rates. Net methane oxidation in the winter does not occur in sediments which have not supported an active population of methanogens (above ~8 cm). Methane oxidation in both sulfate-depleted and sulfate-containing sediments was partially inhibited by BES. These data suggest that methanogens bring about the actual chemical transformation of methane to CO₂.

The sulfate/temperature manipulation experiment and winter field studies showed, however, that sulfate reducers are required to bring about net consumption of methane. In Cape Lookout Bight, net methane oxidation occurred only in sulfate-containing sediments and could be brought about in the laboratory by adding sulfate to methanogenic sediments. The presence of sulfate allows sulfate reducers to maintain H₂ concentrations approximately tenfold lower than those in sulfate-depleted sediments. The maintenance of low hydrogen concentration provides the means of coupling methanogens and sulfate reducers to bring about net methane oxidation.

The methanogen-sulfate reducer consortium appears not only to be a biochemically and thermodynamically feasible mechanism of net methane oxidation but also to be a likely explanation for the seasonal variations in methane oxidation observed in Cape Lookout Bight. Such a mechanism might also account for methane oxidation observed in other anoxic marine sediment and water column environments.

Acknowledgments. This work was supported by Environmental Protection Agency technical service contract OD4067TEX (M.J.A.) and National Science Foundation grant OCE-9017979 (C.S.M., D.B.A., and M.J.A.). T.M.H. received support from the NSF Research Experience for Undergraduates program, the Barry Goldwater Excellence in Education Sciences provided lab and boating facilities for field research. We are thankful to Larry Benninger and Neal Blair for thoughtful reviews of an early draft of the manuscript.

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(Received May 10, 1994; revised June 29, 1994; accepted July 7, 1994.)