Big Soda Lake (Nevada). 3. Pelagic methanogenesis and anaerobic methane oxidation[1]

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Abstract

In situ rates of methanogenesis and methane oxidation were measured in meromictic Big Soda Lake. Methane production was measured by the accumulation of methane in the headspaces of anaerobically sealed water samples; radiotracer was used to follow methane oxidation. Nearly all the methane oxidation occurred in the anoxic zones of the lake. Rates of anaerobic oxidation exceeded production at all depths studied in both the mixolimnion (2–6 vs. 0.1–1 nmol liter-' d^{-1}) and monimolimnion (49–85 vs. 1.6–12 nmol liter-' d^{-1}) of the lake. Thus, a net consumption of methane equivalent to 1.36 mmol m^{-2} d^{-1} occurred in the anoxic water column. Anaerobic methane oxidation had a first-order rate constant of $8.1 \pm 0.5 \times 10^{-4}$ d^{-1}, and activity was eliminated by filter sterilization. However, in situ methane oxidation was of insufficient magnitude to cause a noticeable decrease of ambient dissolved methane levels over an incubation period of 97 h.

The significance of and the organisms responsible for methane oxidation within anoxic waters and sediments are current subjects of debate. Both supportive and nonsupportive data exist. Concentration profiles of methane within anoxic environments frequently exhibit "concave-up" shapes, which suggest removal of the gas by an oxidative process (Barnes and Goldberg 1976; Reeburgh 1976; Reeburgh and Heggie 1977; Martens and Berner 1977; Devol et al. 1984). Evidence indicating that removal is biological in nature is supported by the production of $^{14}CO_2$ from $^{14}CH_4$ during incubation of anoxic water (Panganiban et al. 1979) and sediments (Reeburgh 1980; Iversen and Blackburn 1981; Devol 1983; Iversen and Jørgensen 1985). To our knowledge, Lidstrom (1983) was the first to report biological consumption of unlabeled methane in anoxic water samples. In addition, because aerobic, methane-oxidizing bacteria are capable of sizable isotope fractionation (Silverman and Oyama 1968; Barker

and Fritz 1981; Coleman et al. 1981), reports of ^{13}C-enriched methane (Bernard 1978; Oremland and Des Marais 1983) and ^{13}C-depleted carbon dioxide (Claypool and Kaplan 1974) within the sulfate-reduction zone of anoxic sediments provide circumstantial evidence for an anaerobic counterpart to aerobic bacterial oxidation.

The zone in which anaerobic methane consumption occurs in sediments coincides with one of sulfate depletion, which strongly suggests the involvement of sulfate-reducing bacteria in the oxidation (Iversen and Jørgensen 1985). Oxidation of $^{14}CH_4$ has been reported in enrichment cultures (Panganiban et al. 1979) and pure cultures of sulfate reducers (Davis and Yarbrough 1966: Iversen in prep.). In all of these reports methane could not serve as the sole carbon source for growth of the organisms. The latter is not surprising because of the poor energy yield involved in the oxidation (Wake et al. 1977). On the other hand, Zehnder and Brock (1979) suggested that methanogenic bacteria were responsible for methane oxidation. Pure cultures of methanogens were shown to produce $^{14}CO_2$ from $^{14}CH_4$. However, the amount of methane oxidized

was minor (0.001-0.3%) compared with the quantity of methane formed. In subsequent studies with anaerobic sludges and sediments, net consumption of methane was never demonstrated and the systems always achieved a net production of methane (Zehnder and Brock 1980). These results raised the possibility that reports of $^{14}CO_2$ production from $^{14}CH_4$ in anaerobic microcosms may be only minor "back reactions" mediated by rnethanogens and would not result in net consumption of methane. Because methanogens can be active within zones of sulfate reduction (Oremland and Polcin 1982; Oremland et al. 1982), $^{14}CO_2$ production from $^{14}CH_4$ in these regions could be an artifact of methanogenic bacterial activity rather than an oxidation carried out by sulfate reducers. It is therefore necessary to measure rates of methanogenesis in conjunction with measurements of anaerobic methane oxidation ($^{14}CH_4$ technique) to determine whether a true net consumption of methane occurs.

Big Soda Lake is a particularly good environment in which to examine anaerobic methane oxidation. Sulfate reduction takcs place in the anoxic water (Smith and Oremland 1987) where methanogenic bacteria are also present (Oremland and Dcs Marais 1983). In addition, $\delta^{13}CH_4$ values in the anoxic mixilimnion (-48 to -20‰) and monimolimnion (-60 to -55‰) were considerably heavier than those in the bottom sediments (-74 to -70‰), implying that anaerobic methane oxidation occurs in the water column (Oremland and Des Marais 1983). We performed concurrent incubations designed to measure in situ rates of inethanogenesis and anaerobic methane oxidation to determine whether a net consumption of methane occurs in the lake's anoxic waters. We here report that rates of anaerobic methane oxidation exceed by several-fold those of methanogenesis. However, rates of net consumption were too low to cause a noticeable decrease in ambient levels of methane over the course of a 97-h incubation.

Materials and methods

Sampling site -- In situ experiments were conducted on water samples collected from Big Soda Lake in October 1982 and 1983 and in July 1984. The salient features of the lake have been summarized elsewhere (Zehr et al. 1987).

Methanogenic activity -- Methane production in the water column was studied in field experiments. Water samples were collected from depth in 7-liter Niskin bottles and transferred to glass bottles (2 or 4 liters) by gently filling them to overflowing. followed by sealing with ground-glass stoppers. Bottles were stored at 6°C for about 3 h before processing. The high initial background of methane was reduced by sparging the samples with N_2 emanating from gas dispersion tubes (flow, 300 ml min^{-1} for 20 min). Because this procedure may have also removed volatile methanogenic substrates like trimethylamine, amendment experiments were also conducted (see below). After each bottle was stripped free of methane, subsamples (145 ml) were dispensed into serum bottles (total vol., 165 ml). All transfers and manipulations were made under a flow of N_2. Bottles were sealed under N_2 with butyl rubber stoppers (Bellco Biological Glassware, Inc.) which were crimped in place. Final traces of methane were flushed from the headspace with a vented N_2 gassing syringe (flow, 150 nil min^{-1} for 5 min). Samples were incubated overnight (about 10 h) at 6°C to achieve equilibration between the gas phase (vol., 20 ml) and liquid phase before initial methane headspace determinations by gas chromatography (Oremland and Des Marais 1983). Methane remaining in the liquid phase was calculated by applying solubility coefficients at various salinities (Weisenburg and Guinasso 1979) to the equations of Flett et al. (1976). Dissolved methane was 26% of headspace content for the mixolimnion (S, 26‰) and 17% for the monimolimnion (S, 88‰). A headspace was purposely chosen as a strategy to minimize anaerobic methane oxidation because most of the gas would be partitioned into the gaseous rather than liquid phase.

In situ experiments were conducted during October 1983 and July 1984. Water

samples were incubated in triplicate sets of serum bottles (165 ml) at their respective depths by using nylon bags attached to a float-tethered wire cable. In addition a fourth bottle from each depth contained 2-bromoethanesulfonic acid (BES), an inhibitor of methanogenic bacteria (Gunsalus et al. 1978). Concentrations were 10 mM (October 1983) and 37 mM (July 1984). Amendment experiments were done with additional triplicate sets of bottles incubated at 40 m with supplements of methanol (50 μM) or trimethylamine (50 μM). During July 1984 only methanol (1 mM) was added. Sample bottles were removed from the water column every 2–3 d (as indicated) for analyses of headspace methane. Samples were held at 6°C during this interval (4 h) before being returned to the water column. Incubation periods were 10 (October 1983) or 8 d (July 1984). Rates of in situ methanogenesis were calculated from regression lines fitted through the linear portions of the production curves.

Methane-oxzdatron activity—In situ experiments were conducted during October 1982 and October 1983. Water samples were collected as described above. Subsamples (10 ml) were taken with sterile 10-ml Glaspak syringes (Becton-Dickenson, Inc.). The syringes were flushed several times with water from the Niskin bottle before samples were taken. Syringes were closed with female Luer hubs fitted with butyl rubber septa inserts. Care was taken to exclude gas bubbles from the syringes. The syringes were stored in an ice bath until the experiments began (3–5 h). The in situ rate of methane oxidation was determined in duplicate or quadruplicate sets of syringes (as indicated) by the $^{14}CH_4$ tracer technique of Iversen and Blackbui-n (1981). An aqueous solution of $^{14}CH_4$ was made by equilibrating $^{14}CH_4$ (88 mCi mmol^{-1} CH$_,$; Arnersham, Inc.) with 8.5 ml of anoxic water. The labeled methane was previously cleansed of any contaminant $^{14}CO_2$ by exposure to NaOH solution. Subsequent analysis by gas radio-chromatographic techniques (Culbertson et al. 1981) revealed only the presence of $^{14}CH_4$ in the tracer (detection limit, 150 dpm 250 μl^{-1}). In addition, the NaOH absorption solution was free of $^{14}CO_2$ when analyzed by liquid scintillation counting after being exposed to $^{14}CH_4$. The $^{14}CH_4$ was also found to be free of any other ^{14}C-labeled hydrocarbons (methods of Iversen and Jørgensen 1985). The $^{14}CH_4$ solution was injected through the septa into the sample syringes (50 μl, containing 0.25 μCi syringe') which were then incubated in the lake's water column in nylon mesh bags at their respective depths for periods of 18–120 h. The incubation was ended by injecting the contents of each syringe into evacuated 48-ml serum vials containing 10 ml of 0.63 N NaOH (to fix $H^{14}CO_3^-$ and stop bacterial activity). The vials were then shaken and left until analyses for $^{14}CO_2$ were performed as described elsewhere (Iversen and Blackburn 1981). Filter-sterilized (0.2-pm Nuclepore) water samples served as a control. Parallel syringes containing either BES or Na_2WO_4 (each 40 mM) were used to inhibit methanogenesis and sulfate reduction respectively.

The rate of methane oxidation was calculated from the amount of $^{14}CO_2$ formed and the specific activity of the total methane pool. Recovery of added label was >70% at all depths sampled. In addition to the radioisotope experiments described above, we attempted to determine if the activity of methane oxidation was high enough to cause a decrease in the methane content of water taken from 40-m depth. Triplicate sets of syringes were filled as described above and returned to depth. At various intervals (up to 97 h), sets were withdrawn and injected into evacuated serum bottles (48 ml). Bottles were shaken vigorously, and headspace methane levels were determined by gas chromatography (see below).

The kinetics of anaerobic methane oxidation were followed during 97-h time-course experiments with water from 40 m. The rate constant (k ^{1}) for methane oxidation was determined from plots of the natural logarithm of $A/(A - B)$ vs. time, where A is the initial activity of $^{14}CH_4$ and B is the amount of $^{14}CO_2$ produced at each time. We also measured rates of methane oxidation at different methane concentrations, using water from 40 m. To achieve concentrations of methane below ambient levels (about 50 μM), we first "stripped"

water samples by bubbling them with N_2 as described above. Various amounts of methane were added by syringe to the headspaces of 165-ml serum vials. For methane concentrations greater than ambient, bottles were filled directly from the Niskin sampler and were not bubbled with N_2. A headspace was then generated in these vials by replacing water with methane gas. Bottles were then shaken and left to equilibrate. After equilibration, Glaspak syringes (8) were filled with water from each serum vial (10 ml). The water removed was displaced with N2. Rates of methane oxidation in these samples were determined by the $^{14}CH_4$ method outlined above. The samples were incubated for 48 and 72 h, and rates were calculated from the difference in production between these two times.

Analytical methods—Dissolved methane in lake waters was extracted by either syringe equilibration (Oremland and Des Marais 1983) or by injection of water samples (10 ml) into evacuated vials (vol., 48 ml) followed by headspace analyses. Methane dissolved in monimolimnion sediments was extracted by extruding sections (8–9 cm) of a 2-m core (diam, 5 cm) into syringes (i.d., 2.4 cm; sed. vol., 35 ml). The sediment-filled syringes were emptied into Erlenmeyer flasks (125 ml) containing 50 ml of a 25% NaCl solution. Flasks were sealed under air and shaken vigorously on a reciprocal shaker (250 rpm) for 30 min, after which methane in the headspace was measured. All methane analyses were performed on a flame ionization gas chromatograph (Oremland and Des Marais 1983) with an 80% Porapak N + 20% Porapak Q column (25 × 0.32 cm) to separate hydrocarbons. Dissolved oxygen and temperature were measured by electrode (Cloern et al. 1983*a*).

Flux of methane from the surface water of the lake to the atmosphere was estimated by measuring the increase of methane with time in the headspace of a floating chamber. The chamber was constructed of Plexiglas (wall thickness, 0.64 cm; length, 50 ctn; i.d., 14.5 cm) closed at its top with a piece of flat Plexiglas fitted with a hole in its center (for a serum stopper). The stopper allowed for syringe sample removal (4 ml) from the 1-liter helium headspace. The headspace was generated by vertically submerging the chamber, capping the sampling hole, and then puncturing the serum cap with a gassing needle attached to a helium tank. Because helium was added from the top of the chamber, bubble stripping of methane from solution was avoided. The chamber was deployed on two conseculive calm days during October 1984 for incubation periods of 3–4 h. Samples were withdrawn initially and at 1-h intervals thereafter and stored in small, sealed test tubes. Analyses for methane were made within 8 h of collection.

Results

The concentration of dissolved oxygen was constant from the surface down to the oxycline. The surface concentration of oxygen in October 1983 (250 μM) was similar to that in October 1982 (235 μM), and the depth at which the oxycline was located was the same. The oxycline began at 13 m and oxygen was depleted at 18 m (Fig. 1).

Methane concentrations—Concentrations of methane at the surface of the lake were about 0.1 μM or 50-fold higher than that required for equilibration with the atmosphere (Lamontagne et al. 1973). Methane concentrations increased below the oxycline, though the concentrations were relatively low (<4 μM) in the anoxic part of the mixolimnion. Concentrations of methane increased rapidly in the 35-m chemocline and stabilized at around 53 μM in the monimolimnion. There was no difference in methane concentrations in the monimolimnion measured in October 1982 and in October 1983 (Fig. 1), and these values were equivalent to previous measurements (Oremland and Des Marais 1983).

Methane production rules—Production of methane was evident at all depths during the in situ experiments. In that of October 1983, rates of methane production were linear for the first 10 d (Fig. 2). Production rates were 5–10 times higher in the monimolimnion than in the anoxic mixolimnion (Fig. 3). Comparable results were obtained when the experiment was repeated in July 1984 (data not shown). Addition of 10 mM BES to samples during October 1983 failed to block methanogenesis. Use of higher levels of BES (37 mM) in July 1984 caused
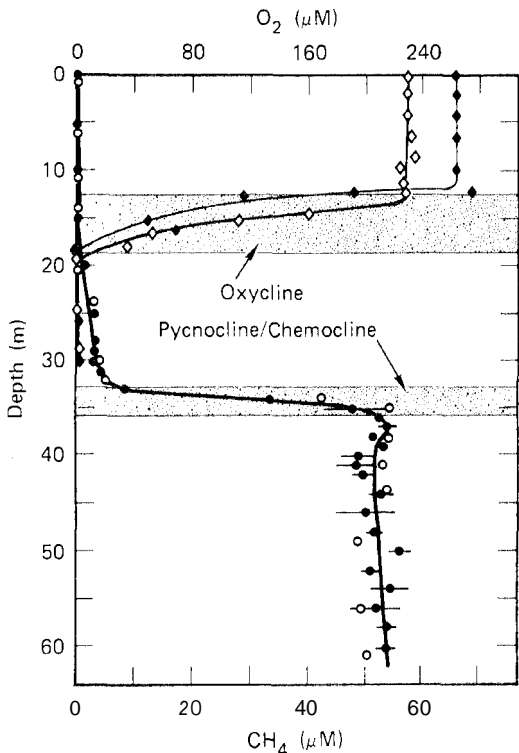
Fig. 1. Vertical profiles of dissolved oxygen ($\Diamond$ — October 1982; +— October 1983) and dissolved methane (0— October 1982; ●—October 1983) in Big Soda Lake. Bars represent the standard errors of duplicate determinations.
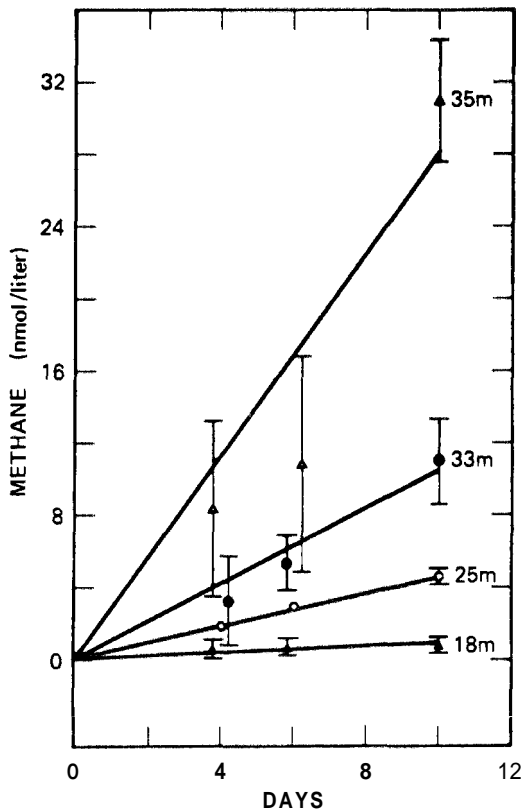

Fig. 2. Production of methane during incubation of anoxic water samples from the mixolimnion (above 35 m) and monimolimnion of Big Soda Lake. Experiments were conducted in October 1983. Results represent the means of three samples, with bars indicating ±1 SD.

a 50–75% inhibition of methanogenesis in mixolimnion waters, but had no effect on samples from the monimolimnion (data not shown). No enhancement of methanogenic activity was observed when 40-m samples were supplemented with either methanol or trimethylamine (data not shown).

-Wethane-oxidation activity — Very low rates of methane oxidation (1.3 nmol liter^{-1} d^{-1}) were detected in the oxic waters of the mixolimnion (above 18 m) where dissolved methane concentrations were about 0.1 μM (Fig. 3). Rates of methane oxidation increased with depth and in general paralleled the concentration profile for methane (Fig. 1). Rates in the monimolimnion were 10-fold higher than those in the anoxic mixolimnion (Fig. 3). Because of the abundance of free sulfide in both the anoxic mixolimnion and monimolimnion waters (e.g. mixolimnion, 0.2–1.2 mM; monimolimnion, 4–

7 mM: Smith and Oremland 1987) as well as the absence of dissolved oxygen at these depths (Fig. 1), it is clear that essentially all of the observed activity occurred under anaerobic conditions. The rate of anaerobic methane oxidation (60.4 nmol liter^{-1} d^{-1}) was highest in the vicinity of the 35-m chemocline. In situ rates of anaerobic methane oxidation exceeded estimates of methanogenesis by a factor of between 5 and 10 for the concurrent experiments of October 1983 (Fig. 3).

In the experiments conducted with 40-m water, the rate of methane oxidation was linear over a 97-h time-course. Mean counts (dpm) above background were 29, 85, 126, and 156 for the initial, 25-, 48-, and 97-h time periods, respectively, equivalent to a
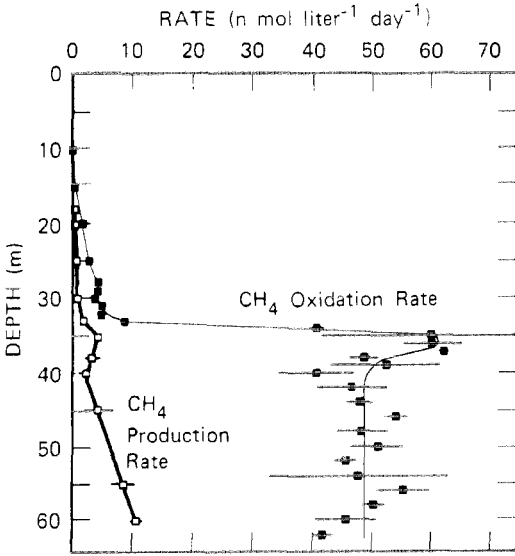
Fig. 3. Activity profiles of in situ methane production and methane oxidation in October 1983. Bars represent the standard errors of duplicate determinations for the methane oxidation experiments. Results are expressed as the means of three samples, with bars indicating ±1 SD for methane production.
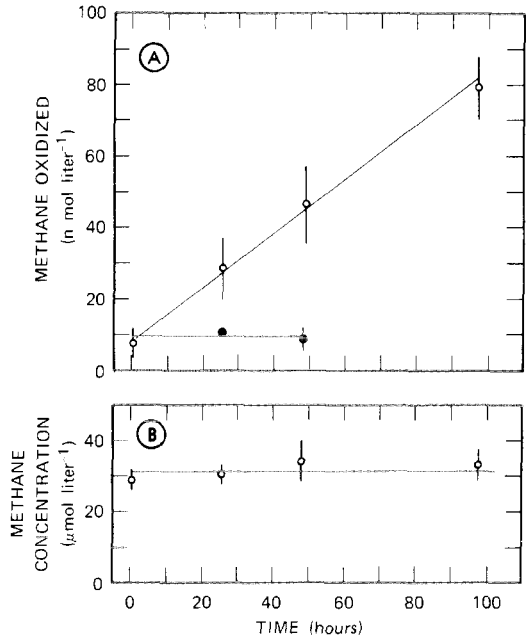

Fig. 4. A. Time-course of methane oxidation at 40-m depth in Big Soda Lake. Untreated samples—○; filter-sterilized controls—●. Bars represent standard errors of triplicate determinations. B. Methane concentration of the syringes used for the time-course. Bars represent standard errors of triplicate determinations.

rate of 25 nmol liter^{-1} d^{-1} (Fig. 4A). Water samples incubated with either BES or Na$_2$WO$_4$ had rates equivalent to the uninhibited samples (data not shown). The quantity of methane oxidized during the 97-h time-course represented only 0.26% of the total methane pool (32 μm), and therefore no discernible decrease in methane concentration could be observed over the incubation period (Fig. 4B).

The rate of anaerobic methane oxidation was proportional to the concentration of methane. A first-order rate constant of $5.6 \pm 1.3 \times 10^{-4}$ d^{-1} was calculated from the regression line of the time-course experiment (Fig. 4A). However, a rate constant that was 25% higher ($8.1 \pm 0.5 \times 10^{-4}$ d^{-1}) was calculated from the methane addition results (Fig. 5). Rates of methane oxidation did not level off at the highest concentrations examined.

Methane flux estimates—A linear increase in the methane content of the flux chambers was observed for both sampling days. Rates of methane loss to the atmosphere in these experiments were 21.6 and 28.3 nmol CH$_4$ chamber^{-1} h^{-1}, which ex-

trapolate to 31.4 and 41.2 μmol CH$_4$ m^{-2} d^{-1}. These values are valid only when the mixolimnion is thermally stratified (May through November).

Assuming that there is a linear gradient of methane in the sediment, we estimated the methane flux from the sediment by applying Fick's first law of diffusion:

$$J(CH_4) = -Ds(dC/dx)$$

where *Ds* is the sediment diffusion coefficient of methane, *C* the methane concentration, and *x* the vertical distance. This equation is valid because of the absence of either bioturbation or bubble transport in the bottom sediment of the lake (Oremland and Des Marais 1983).

Interstitial concentrations of methane measured in July 1984 were 1.71, 1.98, 2.61, and 2.75 mM at respective depth intervals of 0–8.5 cm, 8.5–17 cm, 19.2–27.5 cm, and 27.5–36 cm. Dissolved methane in the water above the sediment was about 60 μM. We estimate the porosity of these sediments to
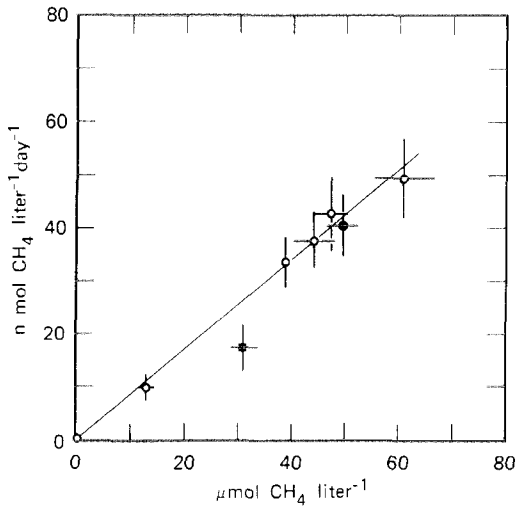
Fig. 5. Methane oxidation rate vs. methane concentration. Horizontal bars represent standard errors of methane concentration; vertical bars represent standard errors of the methane oxidation rate calculated from four determinations. Methane oxidation rate determined from activity profile—●; methane oxidation rate determined from the time-course—■; methane oxidation rates determined at different methane concentrations—○.

be about 0.9 and use a value for Ds of 0.87×10^{-5} cm^2 s^{-1} at 13°C (Iversen and Jørgensen 1985). Thus,

$$
\begin{aligned}
J(CH_4) &= -(0.87 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}) \\
&\quad \times (1.7 \ \mu\text{mol cm}^{-3} - 0.06 \\
&\quad \mu\text{mol cm}^{-3})/4.2 \text{ cm} \\
&= -0.34 \times 10^{-5} \ \mu\text{mol cm}^{-2} \text{ s}^{-1} \\
&\quad \text{or } -2.9 \text{ mmol CH}_4 \text{ m}^{-2} \text{ d}^{-1}.
\end{aligned}
$$

This value is meant to be a crude estimate of the bottom flux of methane for comparative purposes and not a precise determination.

Discussion

The stable anaerobic environment of the monimolimnion was reflected in the methane concentration. The methane concentrations in October 1982 and October 1983 were the same (Fig. 1) and were similar to those reported during 1981 (Oremland and Des Marais 1983). Oxygen was present down to 18 m. The waters below the depth of oxygen depletion contained high concentrations of free sulfide (Smith and Oremland 1987).

Water-column methane production — Methane production occurred in the anoxic part of the water column. The rates increased with depth (Figs. 2, 3) and were of comparable magnitude for the two time periods studied. Thus, mean rates in the anoxic mixolimnion were between 0.08 and 1.9 nmol liter^{-1} d^{-1} in October 1983 and 0.10 and 1.45 nmol liter^{-1} d^{-1} in July 1984. Similarly, mean rates in the monimolimnion were between 2.09 and 10.5 nmol liter^{-1} d^{-1} in October 1983 and between 3.22 and 6.55 nmol liter^{-1} d^{-1} in July 1984. The higher rates in the monimolimnion were probably caused by several factors, including a higher ambient temperature (12° vs. 6°C), greater abundance of dissolved organic carbon (60 vs. 20 mg liter^{-1}: Kharaka et al. 1984), and a lower redox potential (Priscu et al. 1982).

To our knowledge, only one other study has made direct measurements of methanogenic bacterial activity within an anaerobic water column. Winfrey and Zeikus (1979) reported methane production rates ranging from 720 to 1,440 nmol liter^{-1} d^{-1} for the anoxic water column of meromictic Knaack Lake. Their rates are 70–140 times greater than the highest rates we observed in Big Soda Lake. A major difference in the water chemistry of the two lakes is the abundance of sulfate in Big Soda Lake (58–68 mM), compared with undetectable concentrations in Knaack Lake. Sulfate reduction rates in the monimolimnion of Big Soda Lake are at least 200-fold greater than those of methane production (Smith and Oremland 1987). The importance of methane production in anaerobic mineralization in the water column is therefore minor when compared with sulfate reduction (Table 1).

Methanogens in the water column of Big Soda Lake could be outcompeted by sulfate-reducers for common substrates, as has been shown to regulate methane production in other sulfate-containing environments (Oremland and Taylor 1978; Lovley and Klug 1983; Schönheit et al. 1982). Methane production proceeds from noncompetitive substrates, e.g. trimethylamine rather than acetate or hydrogen (Oremland et al. 1983; Oremland and Polcin 1982). Since it was necessary initially to remove background

methane from the samples, volatile precursors could have been removed as well. However, adding methanol or trimethylamine to water samples from 40 m did not stimulate methanogenesis, indicating that the latter was not a significant problem in this study and suggesting that the substrate did not limit methanogenesis in the water column.

The ineffectiveness of BES at blocking methanogenesis in the lake water was unexpected. No inhibition was observed with 10 mM BES, and 37 mM BES was only partially effective against mixolimnion samples (and not at all effective against monimolimnion samples). These mixed results were probably the result of differential susceptibility by the various components of the methanogenic flora to chemical inhibitors. Eor example, Zinder et al. (1984) demonstrated that aceticlastic methanogenesis in digestors was blocked by 1 mM BES, although that formed via H_2 reduction of CO, required 50 mM. Because we used BES in an attempt to discern whether methanogenic bacteria were involved in anaerobic methane oxidation (see below), verifying its efficacy is important in interpreting our results.

Water-column methane *oxidation*—Rates of aerobic methane oxidation in the waters above 18 m varied between 0.2 and 1.3 nmol liter^{-1} d^{-1} and accounted for only 0.13% of the total methane oxidation. Because the quantity of $^{14}CH_4$ added increased the methane pool by a factor of -2, these rates are probably overestimates. Similar low rates of aerobic oxidation (0-1.13 nmol liter^{-1} d^{-1}) were reported in holomictic Lake Mendota before stratification, when dissolved CH, levels were 0.15–1.9 μM (Harrits and Hanson 1980). Oxidation rates increased greatly (up to 28,800 nmol liter^{-1} d^{-1}), however, as methane became more abundant in the Lake Mendota water column (up to 40 mM). Maximal rates of aerobic methane oxidation are usually found within strong methane gradients. For example, Rudd et al. (1974) reported rates of 3,800 nmol liter^{-1} d^{-1} within the oxycline of a stratified lake where dissolved CH, concentrations went from 609 to 3 μM over a vertical distance of only 1 m. The gradient of methane across the oxycline of Big Soda

Table 1. Range of in situ rates (nmol liter^{-1} d^{-1}) of methanogenesis, anaerobic methane oxidation, and sulfate reduction measured in Big Soda Lake. Experiments were conducted in October 1983. unless noted otherwise. Values for sulfate reduction are from Smith and Oremland (1987).

Process	Mixolimnion	Monimolimnion
Methanogenesis	0.1–1.0	1.6–12
Methane oxidation	2.0–6.0	49–85
Sulfate reduction	25–600	900*–3,000

* Value for May 1983

Lake was much less pronounced. For example, during October 1983, dissolved methane values at 10-, 15-, and 20-m depths were 0.11, 0.53, and 1.49 μM, suggesting that aerobic methane oxidation was limited by the availability of dissolved methane.

Anaerobic methane oxidation, in contrast, was clearly the dominant process of methane removal in the water column of Big Soda Iake (Table 2). In situ incubation of the samples ruled out potential oxygen contamination during incubation. No methane oxidation was observed in the filter-sterilized controls (Fig. 4A), thereby indicating the biological nature of the process. The rate of methane oxidation was linear with time and proceeded without any lag phase (Fig. 4A), indicating that the rate measurements, based on a single time point, were valid. However. oxidation rates were still too low to cause a noticeable decrease in dissolved methane over the time-course of the experiment (Fig. 4B). The rate of methane oxidation displayed first-order kinetics with respect to methane concentrations (Fig. 5). The fact that the reaction was not saturated at the highest concentration of methane examined suggests that the bacteria responsible for anaerobic methane oxidation had a low affinity for methane. The first-order rate constant (k^{-1}) for methane oxidation varied between 5.6×10^{-4} d^{-1} and 8.1×10^{-4} d^{-1}. The measured rates of anaerobic methane oxidation in the monimolinrnion agreed well with the values that were calculated with the rate constant of 8.1×10^{-4} d^{-1} (Fig. 5). However, the maximal rates of methane oxidation measured in the 35-m chemocline were higher than the rates predicted from the rate constant. Turbidity (Cloern et al. 1983b) and cell

Table 2. Integrated inputs and losses of methane in Big Soda Lake. The total methane pool of the water column (0–62 m) was 1,633 mmol m^{-2}.

Process	mmol m^{-2} d^{-1}
Methane oxidation (anaerobic water column)	1.510
Methane oxidation (aerobic water column)	0.002
Methane production (water column)	0.150
Methane flux from sediments	2.900
Methane flux to atmosphere	0.036

numbers (Zehr et al. 1987) are always high in the chemocline. Thus, a larger population density of the bacteria responsible for anaerobic methane oxidation probably resides in the chemocline relative to the rest of the water column.

The depth-integrated rates of methane production and oxidation for the entire water column were 0.15 and 1.51 mmol m^{-2} d respectively (Table 2); thus our data demonstrate a net consumption of methane in the anoxic water column of Big Soda Lake. The net oxidation of methane was also supported by simple flux calculations. The flux of methane from the bottom sediments of the monimolimnion (depth, 62 m) could be estimated either by using data from the water-column biological experiments or by applying simple diffusion models to the interstitial methane concentration of the bottom sediments. In the case of the water-column biological experiments, sedimentary methane flux should equal the difference between consumption and production of methane, plus the quantity of methane lost to the atmosphere from the surface of the lake:

$$J(CH_4) = -[(CH, oxid. - CH, prod.) + J \text{ atmos}].$$

Inserting the measured rate of methane loss of 0.036 mmol m^{-2} d^{-1} to the atmosphere and the depth-integrated rate of oxidation (1.51 mmol m^{-2} d^{-1}) and production (0.15 mmol m^{-2} d^{-1}) into this equation yields a flux of 1.4 mmol m^{-2} d^{-1}. This value is valid only when the mixolimnion is thermally stratified (May through November). The bottom flux calculated from the interstitial methane concentration gradient was 2.9

mmol m^{-2} d^{-1} (Table 2) which is about two times higher than the flux estimate made from the biological experiments. Thus, there is a reasonable agreement between the two determinations. The calculation helps to serve as a check on the order of magnitude of the rate results obtained with the biological experiments conducted in the water column.

The magnitude of the various methane sources and removal processes to the water column of Big Soda Lake are summarized in Table 2. Because the methane content of the water column has not changed with time, an estimate of methane turnover time can be made by dividing the total methane pool (1,633 mmol m^{-2}) by either the total inputs (sediment flux + water column production = 3.05 mmol m^{-2} d^{-1}) or losses (oxidation + atmospheric flux = 1.55 mmol m^{-2} d^{-1}). These calculations yield methane turnover estimates of about 1.5–2.9 yr.

The rates of anaerobic methane oxidation at all depths were higher than the rates of methane production (Fig. 3). Methanogenic activity has been reported to be a prerequisite for anaerobic methane oxidation in freshwater sediments and digested sludge, and net production was always observed (Zehnder and Brock 1980). Although methanogens can oxidize methane while producing methane, the oxidation rate is much lower than the production rate (Zehnder and Brock 1979). However, our results clearly demonstrate net oxidation rather than production of methane. The rates of methane production in Big Soda Lake water varied from 5 to 25% of the methane oxidation in the water column, and therefore it seems unlikely that methanogens could be responsible for the measured rates of methane oxidation. The lack of inhibition of anaerobic methane oxidation by BES would also indicate that methanogens are not involved. Alperin and Reeburgh (1985) reported that BES did not influence anaerobic methane oxidation in Skan Bay sediment. Nonetheless, our observations of a net consumption of methane in the anoxic water column of Big Soda Lake argue against an artifactual involvement of methanogens and support diagenic models of zones of anaerobic methane oxidation.

The involvement of sulfate reduction in anaerobic methane oxidation has been discussed previously. Thermodynamic calculations indicate that oxidation of methane by sulfate-reducing bacteria is slightly favorable (Martens and Berner 1977; Wake et al. 1977), and a similar assessment can be made for conditions in Big Soda Lake. Addition of 40 mM tungstate to monimolimnion waters caused a 64% reduction in the rate of sulfate reduction (Smith and Oremland 1987), but had no effect on methane oxidation. A similar lack of inhibition was reported upon use of molybdate in Skan Bay (Alperin and Reeburgh 1985). In addition, neither removal nor addition of methane from or to 40-m water from Big Soda Lake caused any noticeable change in the endogenous rate of sulfate reduction (Smith and Oremland 1987). Even if they were coupled, a comparison of the rates of oxidation and sulfate reduction indicates that only 1.6–2.3% of the reduction could be a consequence of methane oxidation (Table 1). Similarly, Iversen and Blackburn (1981) reported that anaerobic methane oxidation in marine sediments could account for only 0.01–0.06% of sulfate reduction. although methane may fuel sulfate reduction occurring at the base of the zone of sulfate depletion in sediments (Devol 1983; Iversen and Jørgensen 1985). Our results from Big Soda Lake indicate little or no coupling between the process of sulfate reduction and that of anaerobic methane oxidation. Nonetheless, we were able to demonstrate the occurrence of a biologically mediated, net oxidation of methane which proceeded at rates consistent with rough estimates of methane flux in the lake. The mechanism(s) by which this oxidation occurs and the microorganisms responsible for it remain unknown. One possibility is that several types of microbes may be involved in the reaction — a situation which is analogous to anaerobic acetylene oxidation (Culbertson et al. 1981). In this case, fermentative metabolism of acetylene via an acetaldehyde intermediate results in production of equimolar ethanol and acetate. Sulfate-reducing bacteria subsequently oxidize these compounds to carbon dioxide (Culbertson and Oremland in prep). It is therefore possible that an analogous situation occurs in anaerobic methane oxidation. whereby methane initially is metabolized to an intermediate (e.g. methane thiol, methanol, etc.) which is subsequently oxidized by sulfate reducers.

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