Measurement of microbial oxidation of methane in lake water

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Abstract

A radiotracer method which measures rates of oxidation of methane to cell material, extracellular products, and carbon dioxide has been applied to two lakes and indicates that methane oxidation occurred in a narrow band where methane and oxygen occurred together in the water column. Oxidation rates of 1.0 μM hr^{-1} were recorded in a eutrophic lake; rates in a meromictic lake reached 0.15 μM hr^{-1}. Usually a third of the carbon from oxidized methane was found in cell material and extracellular products and the rest was converted to carbon dioxide. This ratio was observed to change at very low oxygen concentrations.

Methane can be one of the most important end products of the anaerobic decomposition of organic matter. Much decomposition in lakes occurs in or on the sediments. In spite of the fact that such sediments are commonly anaerobic, carbon budgets for lakes frequently note the presence of methane without quantifying it. Although methane is biologically inert in the absence of oxygen, it can be converted to carbon dioxide and cell material in the presence of oxygen; it is thus a potential source of carbon for primary and secondary production.

The culture, physiology, and distribution of methane-oxidizing bacteria have been studied (e.g. Whittenbury et al. 1970; Leadbetter and Foster 1958; Hutton and ZoBell 1949); but only Howard et al. (1971) have studied the rates of methane oxidation in lake water.

Certain lakes in the Experimental Lakes Area (ELA), northwestern Ontario (Johnson and Vallentyne 1971), are either naturally meromictic or have been artificially eutrophied. In these lakes methane is a sizable component of the carbon budget and methods were needed to assist in resolving the role played by methane in lake metabolism. A radiotracer method was developed to measure rates of methane oxidation to cell material and soluble organic products and to carbon dioxide. Its advantages of high sensitivity, short incubation times, and maintenance of in situ gas concentrations produce a minimum of disturbance to the microbial community.

We are indebted to P. Campbell for his advice on Lake 120, R. H. Betts for his advice on gas equilibria, and E. J. Fee for his criticisms of the manuscript. R. J. Flett advised us during development of the gas chromatographic techniques. D. W. Schindler made the Experimental Lakes facility available to us.

Materials and methods

Samples were taken with a Van Dorn sampler. Duplicate subsamples were collected in 120-ml serum bottles following methods for oxygen samples (Strickland and Parsons 1968) except that, to minimize oxygen invasion, at least three times the bottle volume was allowed to overflow. Each bottle was capped immediately with a serum stopper, which was just pierced with a 26-G hypodermic needle (1.3 cm), to ensure that all entrapped air bubbles were displaced as the stopper was seated. The hypodermic needle was then removed and one of each pair of subsamples was killed in the field

by injecting NaOH to pH 11. An extra, unfixed subsample was taken from the sample representing the anticipated depth of maximum activity. All samples were transported to the laboratory (0.75 hr) in light-tight containers at temperatures within 5°C of in situ temperatures.

Oxygen samples were collected in 300-ml BOD bottles from the same Van Dorn cast as the methane samples. They were fixed and analyzed in the laboratory by the azide modification of the Winkler method (Am. Public Health Assoc. 1965).

Analysis

Twenty-five milliliters of each sample fixed at the lake, followed by 25 cc of helium, were drawn into a 50-cc disposable plastic syringe fitted with an 18-G 2.5 cm needle. This needle was replaced with a 26-G needle, the tip of which was inserted partially into a rubber bung to provide a temporary seal. Thirty seconds of vigorous shaking was sufficient to strip >97% of the dissolved CH_4 from the sample. The gas phase was then injected into an inverted 6-ml serum bottle filled with distilled water, displacing the water through a second 26-G needle. The excess gas phase was used to flush the serum bottle after all the distilled water had been expelled. This procedure permitted replicate analyses of a sample as well as sample storage for up to 1 week.

Two-tenths of a milliliter of this sample was injected into a Pye 104 gas chromatograph equipped with a flame ionization detector and a phenyl isocyanate/Porasil C column. Methane concentrations were calculated from peak height and expressed as μmoles CH_4 per liter (μM) of lake water.

Stock ^{14}C-CH_4 gas (1.2×10^7 dpm ml^{-1}; 10 μCi μmole^{-1}, Amersham/Searle Corp.) was contained at atmospheric pressure in a mercury manometer. Before each sampling period a suitable volume was withdrawn into a plastic syringe and an equal volume of mercury was displaced into the manometer to re-establish atmospheric pressure in the manometer and the syringe. The volume of ^{14}C-CH_4 gas was then transferred to a second apparatus and diluted tenfold with nitrogen. A half milliliter of this gas mixture was added by 1-cc plastic syringe to each sample bottle and to the single blank sample. The blank was fixed immediately by adjusting to about pH 11 with NaOH. Again, throughout these manipulations the mercury level in the manometer was adjusted to maintain atmospheric pressure as gases were added or removed.

The sample bottles and blank were shaken for 8 min at maximum speed on a wrist shaker, sufficient to equilibrate the ^{14}C-CH_4 label with the ^{12}C-CH_4 present in the sample. The injection of ^{14}C-CH_4 label and the shaking of serum bottles was carried out in diffuse light.

The samples and blank were incubated within 3°C of in situ temperature for from 1 to 5 hr, depending on the rate of CH_4 oxidation. Incubation was in the dark to prevent photosynthetic oxygen production.

Following incubation the samples were killed as before. The samples and blank bottles were opened and a 25-ml portion of each was analyzed for total CH_4 concentration as described above. Three 10-ml portions of each were also taken and processed as described below.

The first 10-ml portion was stripped of CH_4 by air scrubbing (~90 ml min^{-1}) for 5 min in the apparatus described by Schindler et al. (1972). No significant losses of ^{14}C-CO_2 were encountered, presumably by reason of the high pH imposed. Four milliliters of this sample were then placed in 14 ml of a dioxane fluor (Schindler 1966).

The second 10-ml portion was similarly treated after adjustment to pH 2.5, low enough to ensure that all ^{14}C-CO_2 was lost. Both samples were counted in a scintillation counter and the appropriate blank value subtracted. As both CO_2 and CH_4 were found to be scrubbed from the second portion, the results from this sample were taken to represent the amount of methane carbon converted to particulate and soluble materials. The difference between the activity in the second sample and that in the first was taken to represent the amount of methane carbon converted to carbon dioxide.

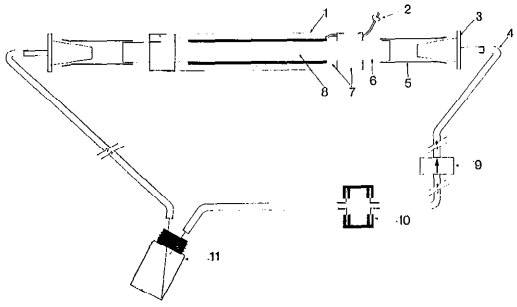

Fig. 1. Apparatus used to oxidize ^{14}C-CH_4 and to trap the resulting ^{14}C-CO_2 in phenethylamine. 1—15-mm-I.D. pyrex tube (16 cm long); 2—22-G Nichrome C winding to power source; 3—plastic reducer; 4—3-mm-I.D. Tygon tubing; 5—6-mm-I.D. silicone tubing; 6—6-mm-I.D. quartz tube (20 cm long); 7—asbestos tape; 8—copper oxide; 9—sealed push-pull air pump; 10—glass injection port with serum stoppers; 11—scintillation vial containing 3 ml of phenethylamine and fitted with a silicone rubber stopper, pierced with 22-G 2.5- and 25-G 7.6-cm needles.

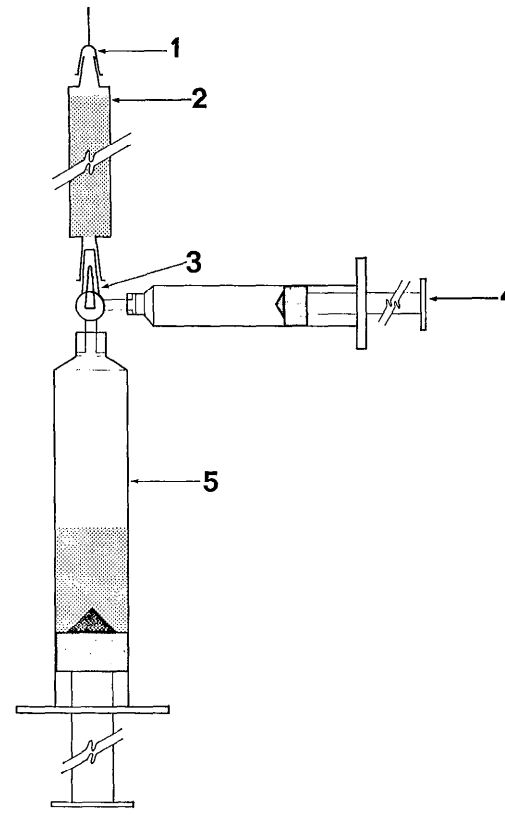

Fig. 2. Apparatus used to inject dry gas sample into the collection apparatus. 1—26-G 1.3-cm needle; 2—magnesium perchlorate drying tube; 3—three-way valve; 4—5-cc plastic syringe holding 4 cc of air; 5—30-cc plastic syringe holding 10 cc of sample and 20 cc of helium.

The third 10-ml portion was used to establish the amount of ^{14}C-CH_4 remaining after incubation. It was necessary to measure dissolved ^{14}C-CH_4 concentrations for each sample since there was significant volumetric error during addition of ^{14}C-CH_4 by the syringes used. The 10-ml sample was drawn from the incubation bottles through a three-way valve into a 30-cc disposable syringe and stripped with 20 ml of helium as described earlier. The stripped ^{14}C-CH_4 was then injected into a collection loop (Fig. 1), a modification of the apparatus described by Thompson and Hamilton (1974). This apparatus converted ^{14}C-CH_4 to ^{14}C-CO_2 which was trapped in phenethylamine.

The injection procedure was as follows. The male opening of the three-way plastic valve on a 30-cc sample syringe (Fig. 2) was inserted into the female connector of a drying tube. A 5-cc plastic syringe (set at 4.0 cc) was fixed to the third outlet of the three-way valve. With the three-way valve in the position shown in Fig. 2 (closed to the atmosphere) the 26-G needle on the end of the drying tube was inserted into the injection port. The valve was then closed to the 5-cc syringe and the vacuum drew the ^{14}C-CH_4 sample into the loop. When all the gas had been withdrawn from the 30-cc syringe the valve was opened to the 5-cc syringe, permitting 4 ml of air to flush the sample through the drying tube and into the loop. Injection of dry gases prevented the formation of a white, phenethylamine-water precipitate in the apparatus which caused clogging and poor replication.

The sample was circulated in the loop for 5 min, during which time 100% of the ^{14}C-CH_4 was converted to ^{14}C-CO_2 and trapped in phenethylamine. Samples were counted in a toluene fluor (7.14 g of PPO, 0.14 g of POPOP and 40 ml of methanol per liter of

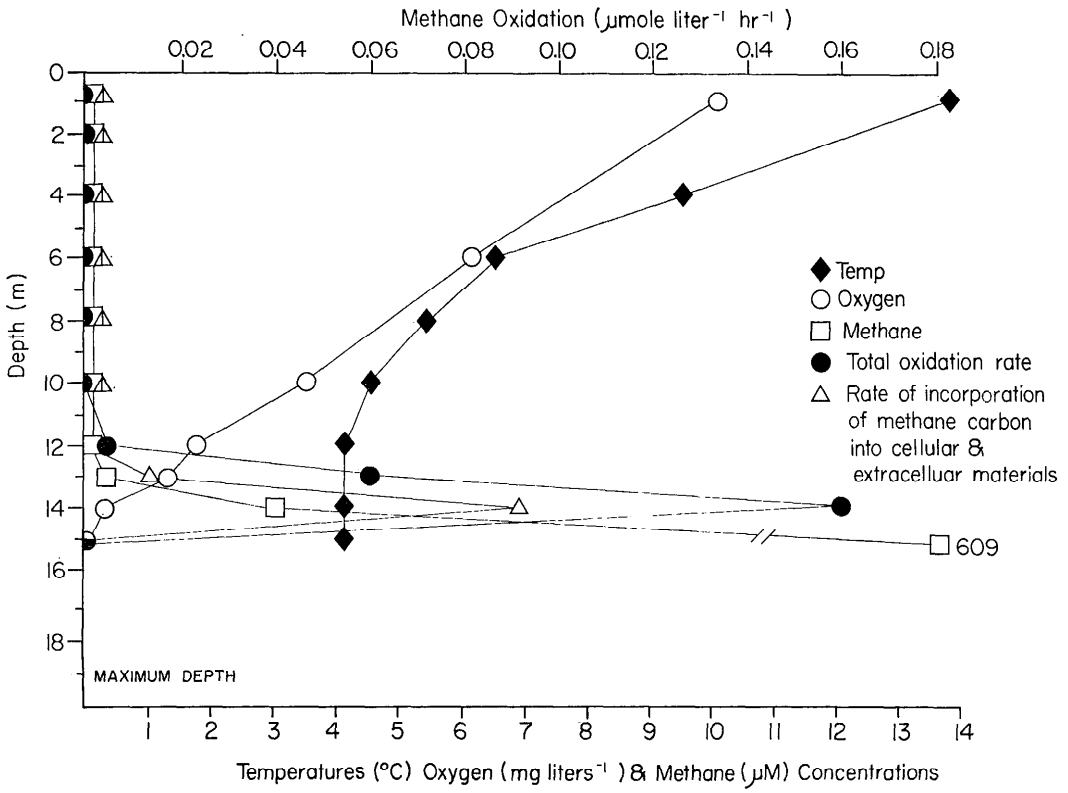

Fig. 3. Profile of methane oxidation in Lake 120 on 24 May 1973.

toluene). By this method replicate samples of a standard ^{14}C-CH_4 concentration had a coefficient of variation of 4%. Sample counts were converted to dpm liter^{-1} of lake water and used to calculate specific activity and rates of methane oxidation.

Results

A profile from Lake 120 of the total rate of CH_4 oxidation (to cell material, extracellular products, and CO_2) is presented in Fig. 3. A lens of methane-oxidizing microbes between 8 and 14 m accounted for all the methane oxidation in this profile; more than 98% of the oxidation occurred in a narrow band between 13 and 14 m. The population was most active where there was >0.3 μM CH_4 and 0.25 mg O_2 liter^{-1}. Between the surface and 8 m oxidation rates were zero and methane oxidation appeared to be CH_4 limited in this region (0.1 μM). Below 14 m the rate falls to zero presumably as a result of anaerobiosis. A lens of methane-oxidizing organisms is thus maintained and regulated by the rate of upward diffusion of CH_4 and the presence of an available electron acceptor.

Also plotted in Fig. 3 is the rate of production of cell material and extracellular products. The difference between this rate and the total rate was the rate of CO_2 production. About two-thirds of the methane oxidized at all depths was converted to CO_2. This agrees closely with the carbon balance data reported by Vary and Johnson (1967) who worked with a mixed culture of methane-oxidizing bacteria, but differs markedly from the 20% conversion of utilized CH_4 to CO_2 reported by Brown et al. (1964).

The results in Table 1 show the high rates of oxidation at 12, 13, and 14 m. These rates

Table 1. Rates of methane oxidation to particulate and soluble carbon and to carbon dioxide in Lake 120, 24 May 1973.

Depth (m)	% of ^{14}C-CH_4 utilized hr^{-1}	dpm ℓ^{-1}* sample pH 11	Total oxidation rate $\mu M\ hr^{-1}$	dpm ℓ^{-1}* sample pH 2.5	Production rate of particulate and soluble C $\mu M\ hr^{-1}$	Production rate of CO_2 $\mu M\ hr^{-1}$
1	0.00	0.00	0.00	0.00	0.00	0.00
4	0.00	0.00	0.00	0.00	0.00	0.00
6	0.00	0.00	0.00	0.00	0.00	0.00
8	0.28%	38,300	0.00037	13,000	0.00013	0.00024
10	0.30%	49,600	0.00037	13,400	0.00010	0.00027
12	1.70%	64,300	0.00176	23,400	0.00064	0.00112
13	5.4%	204,000	0.0202	73,900	0.00732	0.0129
14	7.2%	264,000	0.157	97,300	0.0578	0.0992
15	0.00	0.00	0.00	0.00	0.00	0.00

*Net counts (sample dpm - blank dpm).

necessitated short incubations (1.25 hr) to prevent serious substrate depletion during incubation and to prevent introduction of error into the calculation of specific activity at time zero. The oxidation rate at 14 m indicates a substrate turnover time of about 12.5 hr. This rate is supported by the high CH_4 gradient, illustrated by the contrast between concentrations at 13 m (0.4 μM) and at 15 m (609 μM).

Discussion

The introduction of a gas phase into a water-filled sample bottle changes the gaseous concentrations in the water. This is especially true in this procedure as the gas and liquid phases are shaken to equilibrium at the time of ^{14}C-CH_4 injection. The effect on the concentration of gases in water by the introduction of a gas phase can be calculated as:

$$x = mV_g/1 + \alpha V_l,$$

where V_l is the volume of the liquid phase, V_g the volume of the gas phase, α the volume of the given gas (at STP) dissolved by one volume of liquid at a given temperature and at 1-atm pressure, m the volume of the given gas (at STP) in the liquid and gas phases, and x the volume of given gas (at STP) present in the gas phase at equilibrium.

The volume of dissolved gas (at STP) in the liquid phase (y) at equilibrium is therefore given by:

$$y = m - x.$$

Using the above equation, we have calculated the effect on in situ CH_4 concentration at 4°C of the addition of 0.0006 ml of ^{14}C-CH_4 in a 0.5-ml gas phase. At an in situ CH_4 concentration of 700 μM, the CH_4 concentration would be reduced by 7.8%. At an in situ concentration of 1.0 μM it would be increased by 13.1%. We believe that these concentration changes would not seriously affect CH_4 oxidation rates in the presence of a substantial natural concentration of CH_4. However, at an in situ CH_4 concentration of 0.1 μM the addition of this amount of label would increase the substrate concentration two times: thus even oxidation rates calculated using in situ concentrations could be overestimated. It has been our experience however, that oxidation rates at these low CH_4 concentrations are usually very low or undetectable. Consequently in the consideration of lake budgets this error does not appear important. A similar calculation has been carried out to ascertain the effect on in situ concentration of the injection of a 0.5-ml gas phase containing 2% oxygen. At equilibrium the oxygen concentration in the sample water would be reduced by 8.5% at an in situ oxygen concentration of

7.0 mg liter^{-1}. At 0.5 mg liter^{-1} the oxygen concentration would be increased by 13.0%. Therefore, it is important that as small a gas phase as possible be introduced into the sample bottles and that it be oxygen-free. A small gas phase minimizes the change in in situ gas concentrations and permits a larger percentage of ^{14}C-CH_4 to be dissolved in the water phase at equilibrium, thus reducing the cost of the assay.

The most striking characteristic of the methane-oxidizing population of Lake 120 was its efficiency of utilization of low concentrations of both methane and oxygen; it could oxidize CH_4 at an easily detectable rate at an in situ CH_4 concentration of 0.2 μM and could also carry out oxidation in the presence of less than 0.1 mg liter^{-1} O_2 (3 μM). This observation reinforces the need to ensure that there is no oxygen invasion during either sampling or addition of ^{14}C-CH_4.

These low oxygen concentrations do appear to exert stress on the methane oxidizers and the percent CO_2 production decreases. For example at an in situ oxygen concentration of 0.04 mg liter^{-1} in Lake 227, only 40% of the methane oxidized was converted to CO_2. At low oxygen concentrations methanol may be an end product of methane oxidation instead of CO_2 (Harwood and Pirt 1972). Methanol would be included as a soluble material in this analysis since it is not stripped from water solutions by the conditions of the method (Van Hall et al. 1965).

Hutton and ZoBell (1949) have stated that rates of CH_4 oxidation are very slow at low temperatures (3–5°C); requiring 9 months for conclusive results. In contrast, rates of methane oxidation at 5°C in Lake 227 of the Experimental Lakes Area have been recorded in excess of 1 μM hr^{-1}. The importance of this methane oxidation to the yearly carbon budget of a lake is not well understood. Howard et al. (1971) have reported oxidation rates in Lake Erie of 0.16 μM CH_4 hr^{-1} at 25°C. However, the potential of CH_4 oxidation seems to be much greater than this when the 5°C results from Lake 227 are considered.

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