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A Preliminary Study of the Methane Oxidation in Piburger See (Tyrol, Austria) (A. MARIÑELARENA and W. ELKINS)

Zusammenfassung: Vorläufige Ergebnisse der Methan-Oxidation im Piburger See (Tirol, Österreich).

Es wurde versucht, eine annähernd genaue und anwendbare Methode zur Messung der Methanoxidation im Labor zu entwickeln.

Um die Intensität der Oxidation im Piburger See zu ermitteln, wurden im Abstand von etwa 5 Tagen über der tiefsten Stelle des Piburger Sees Vertikalprofile entnommen, wobei auch die Temperatur sowie der Gehalt an Sauerstoff und Methan erfaßt wurden.

Some authors (e.g. RUDD and HAMILTON 1975, 1978; PATT et al. 1974; HARRITS and HANSON 1980) have shown that during summer stratifications methane oxidation is confined to a narrow zone in the thermocline region of lakes where oxygen concentration is low. By estimating the rate of methan oxidation in the 24 m deep water column of Piburger See during the months of July and August we attempted to establish if their statement also applies to the Piburger See.

Another objective of this study was to determine in laboratory tests the influence of variations in concentrations of oxygen and methane on the oxidation rate.

Materials and methods

Profiles

Weekly profiles were taken of dissolved methane concentrations, methane oxidation, dissolved oxygen concentrations, and temperature.

Methane and methane oxidation: Duplicate samples were taken with a modified SCHINDLER-sampler. Samples were collected in individual 120 ml B.O.D. bottles following methods for oxygen samples (STRICKLAND and PARSONS 1968), allowing the bottle volume to overflow at least three times to minimize oxygen invasion. One of each pair of samples were fixed in the field by injecting NaOH to pH 11. This bottle was used for determining the real methane concentration. An extra, unfixed sample was taken and brought back (in light tight plastic containers at in situ temperature  $\pm 3^{\circ}$  C) to the laboratory for incubation at  $6^{\circ}$  C. Four hours after taking the sample the hereto unfixed sample was also killed. This sample was used for determining the oxidation rate, by comparing the methane concentrations in the bottles fixed at 0 and 4 hours:

$$\text{oxidation rate} = \frac{\text{methane (bottle 4 hrs)} - \text{methane (bottle 0 hrs)}}{4 \text{ hours}}$$

An incubation time of four hours was found to allow sufficient oxidation for an accurate measurement of the oxidation rate and to allow for any lag effect in the activity of the bacterial populations in the water.

The methane concentrations were estimated by gas chromatography, following the method described by RUDD et al. (1974) and ELKINS (in preparation). The apparatus used was a Fractovap GC 2151 (CARLO ERBA), with an automatic gas sample valve, sample volume 12.8  $\mu$ l, Porapak Q column, and flame ionization detector. Signals were recorded on an SP 4100 Computing Integrator (SPECTRA PHYSICS). The values for methane concentrations were calculated from the peak area using the following equations:

$$\text{methane conc. } (\mu\text{M l}^{-1}) = \frac{\text{Area (sample)}}{\text{Area (standard)}} \times 0.57143 \times F \times 78125$$

where Area (sample) and Area (standard) are the area of the peaks for the sample and the standard (methane 99.995 %) integrated, the factor 0.57143 represents the amount of methane in a 12.8  $\mu$ l sample at 100 %, F is a factor for adjusting the determined concentration to 760 mm Hg and a 100 % stripping of the sample, the factor 78125 allows for calculating the concentration in 1 liter.

Oxygen and temperature: Oxygen samples were collected in 70-ml B.O.D. bottles from the same Schindler cast as the methane samples. They were fixed and prepared at the lake and analyzed in the laboratory by the Winkler method (Am. Public Health Assoc. 1975).

Temperature was measured with a blue alcohol thermometer attached to the inside wall of the Schindler sampler.

#### Culture jar

As an integral part of the study of the methane oxidation we tried to cultivate and estimate the number of  $\text{CH}_4$ -oxidizing bacteria present in the depths in which oxidation was found. The method used was the MPN-technique. The culture medium selected was a slightly modified version of the one described by NAGUIB and OVERBECK (1970). The medium used was an inorganic nutrient salt solution without any carbon-source.

The inoculated tubes were incubated under a methane-air (1 : 1) atmosphere, the  $\text{CH}_4$  being the only C-source for bacterial growth.

The reported incubation time required for finding positive results (visible growth) goes from one to several weeks, depending on the author. After a number of inconclusive attempts it was decided to discontinue this aspect of the study, there not being enough time in the allowed two months to solve the different problems encountered with the medium.

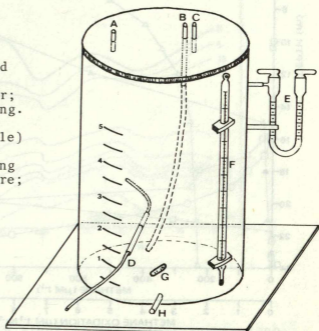
Greater expectations were put into an experiment to test the influence of different concentrations of methane and

oxygen, for which a culture jar was designed and built (Fig.1). A plexiglass cylinder with a tight closing lid was to be used as a closed system, from which gas and culture samples could be taken at intervals without disturbing the equilibrium of the reaction system. The temperature was kept at a constant 21<sup>0</sup> C.

After adding 5 liters of lake water to the jar from the depths in which oxidation had been measured Helium was bubbled through the lake water to decrease the oxygen concentration to  $<0.5 \text{ mg l}^{-1}$ . Then methane (commercial grade) was added through bubbling to reach approximately lake water concentrations. This procedure created a  $\text{CH}_4\text{-He}$  atmosphere in the gas phase above the water. This was kept uniform by attaching B and C to a peristaltic pump and bubbling the mixture through the water. The system was mixed continuously by a magnetic stirrer and during the experiment the temperature, dissolved oxygen and methane concentrations were measured and the oxidation rates were determined. The methane concentrations were measured using 10 ml plastic syringes so as not to upset the system by extracting too much water at one time.

Fig. 1: Culture jar

- A - water inlet, attached to a reservoir with additional lake water;
- B - gas inlet for bubbling.
- C - gas outlet;
- D - plastic tube (turnable) for taking samples;
- E - U-tube for controlling the gas phase pressure;
- F - thermometer;
- G - magnetic stirrer;
- H - water outlet.



Results

Profiles

Fig. 2 shows an example of a typical summer profile of methane oxidation for Piburger See. Profiles were always taken at the deepest point of the lake at 0-5-10-14 m and then in 1 m-intervals to 24 m. Samples were taken from the same Schindler cast for measuring methane concentrations, methane oxidation, dissolved oxygen and temperature. Every other week parallel profiles were made at other locations to compare the results.

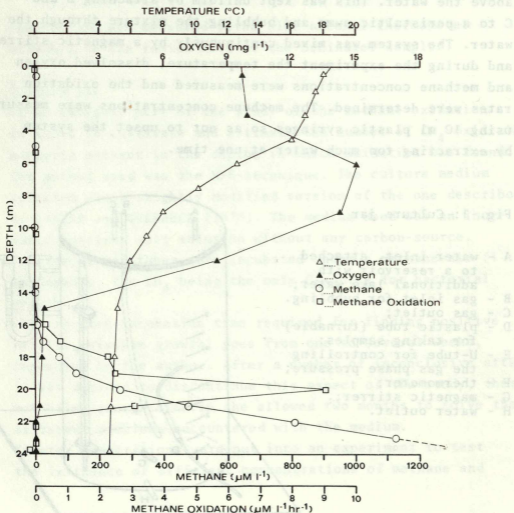


Fig.2: Profile of methane oxidation on 30 July 1980

In this particular profile taken on 30 July the methane concentration shows a steep gradient in the hypolimnion, decreasing rapidly from a maximum of  $1450 \mu\text{M}$  (calculated) at 24 m to  $<0.1 \mu\text{M}$  at 15 m. No methane could be detected in the thermocline and only small traces ( $<3 \mu\text{M}$ ) in the epilimnion. The oxygen curve has a maximum at 6 m ( $15.16 \text{ mg l}^{-1}$ ) and decreases to  $<0.6 \text{ mg}$  at 15 m and 0 mg at 22 m. The thermocline lies between 5 and 15 m. Methane oxidation on this day was limited to a narrow zone of activity between 15.5 and 21.5 m where the oxygen concentration was  $<1.0 \text{ mg}$ . We found no oxidation below 21.5 m because of anoxia.

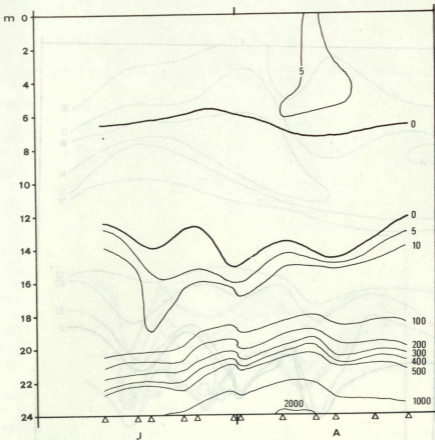


Fig. 3: Methane ( $\mu\text{M l}^{-1}$ ) in Piburger See July - August 1980.

The methane concentrations measured throughout the water column in the two month sampling period are presented in Fig.3. Stratification has already set in. During these two months methane ( $< 6 \mu\text{M l}^{-1}$ ) was to be found in the epilimnion down to 6 m. However, in the bottom 3/4 of the thermocline, which reached down to 14 m at the beginning of July and 11 m towards the end of August, almost no dissolved methane could be found. The methane produced in the sediments of Piburger See and dissolved into the water column appears to accumulate almost totally in the hypolimnion, its concentration showing a steep gradient near the sediment (19 - 24 m). The maximum methane measured in this period was on 7 August at  $2080 \mu\text{M}$  at 24 m.

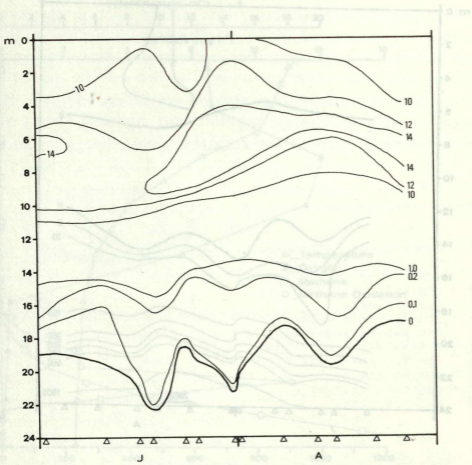


Fig.4: Dissolved oxygen ( $\text{mg l}^{-1}$ ) in Piburger See July - August 1980.



Fig.4 shows the dissolved oxygen concentrations for the sampling period. The oxygen maximum was found in the thermo-cline region, typical for this lake at this time of year. Microaerobic conditions on the other hand were found between 16 and 23 m. However, the 0- oxygen isopleth fluctuated considerably (up to 3 m difference in 8 days) within short periods of time. There would appear to be two possible explanations for this phenomenon: on the one hand, the fluctuations could be caused by differences in methane diffusion rates away from the sediment resulting in fluctuations in the oxidation rates in the lower regions. On the other hand, a series of raising and lowering of the Olszewski-tube was carried out between the middle of July and the end of August. The Olszewski-tube is an artificial outlet for nutrient-rich hypolimnetic water put into operation in 1970.

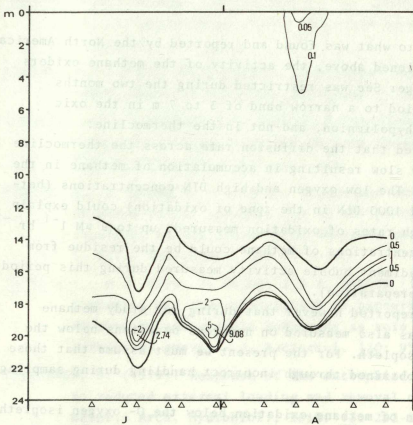


Fig.5: Methane oxidation rates ( $\mu\text{M l}^{-1} \text{hr}^{-1}$ )



The rates of methane oxidation measured in Piburger See are shown in Fig.5. The results establish that the oxidation activity was limited to a narrow band between 15.5 and 21.5 m, with a fluctuation of the 0- oxidation isopleth corresponding to the 0- oxygen isopleth seen in Fig.4. One exception to the limiting of the methane oxidation to the oxic hypolimnion is found in the middle of August, with a rate of  $<0.11 \mu\text{M hr}^{-1}$  between 0 and 5 m. The methane oxidizers were most active in a narrow lens where there were  $>10 \mu\text{M CH}_4$  and  $<0.2 \text{ mg O}_2$ . During two short periods we measured oxidation rates in excess of  $2 \mu\text{M hr}^{-1}$ , with a maximum rate of  $9.08 \mu\text{M hr}^{-1}$  on 30 July at 20 m.

### Discussion

In contrast to what was found and reported by the North American authors mentioned above, the activity of the methane oxidizers in the Piburger See was restricted during the two months sampling period to a narrow band of 3 to 7 m in the oxic zone of the hypolimnion, and not in the thermocline.

It is believed that the diffusion rate across the thermocline must be very slow resulting in accumulation of methane in the hypolimnion. The low oxygen and high DIN concentrations (between 300 and 1000 DIN in the zone of oxidation) could explain the very high rates of oxidation measured, up to  $9 \mu\text{M l}^{-1} \text{ hr}^{-1}$ . The low concentrations of methane could be the residue from the heavy sediment bubble activity measured during this period (ELKINS in preparation).

It must be reported however that during our study methane oxidation was also measured on numerous occasions below the 0- oxygen isopleth. For the present we must assume that those rates were obtained through incorrect handling during sampling.

The question of methane oxidation below the 0- oxygen isopleth will however be the subject for further study in the near future.

No definite conclusions were obtained from the tests with the culture jar, there not remaining enough time during the limited period of this study to overcome the difficulties encountered. It was found especially difficult to maintain low oxygen concentrations once these were reached through bubbling. The results obtained in this experiment, even though few and not always consistent, were nevertheless interesting. More experiments will be made with the culture jar to study the influence of different methane and oxygen concentrations on the methane oxidation in the coming months.

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