

Chapter III.2 In Situ Measurement of Oxygen Profiles of Sediments by Use of Oxygen Microelectrodes

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1 Introduction

Until recently, the measurement of oxygen was restricted to the water column (Chap. III.1), since no adequate methods were available for its measurement in sediments. The use of microsensors is essential for the resolution of the steep O₂ gradients often found along the sediment core. Measurements of oxygen in extracted pore water were made by several authors [2, 3, 8, 9, 12], but it is difficult to obtain reliable results this way. Teal and Kanwisher [22] attempted to measure the oxygen concentration of sediments in situ by insertion of a polarographic oxygen sensor (POS), but their sensor was large and was sensitive to stirring. Although unable to record an accurate oxygen profile, their measurements indicated that the sediment was anoxic below a few mm depth. In situ polarographic analysis of sediments was made by several authors [4, 11] using large platinum electrodes without membranes [15]. Because of their sensitivity to environmental conditions, e.g., interstitial water flow and the diffusion coefficient of oxygen in the sediment, it is difficult to calibrate these electrodes in terms of oxygen concentration. The readings are interpreted as oxygen availabilities, defined as the amount of oxygen reduced per unit time and per unit area of the active platinum surface. Standardized size and shape of the platinum electrode and a constant polarization voltage are necessary for reproducible results. This method is useful only in sandy sediments, and there are still difficulties in obtaining reliable results [5, 16]. Oxygen availability measurements made in coastal marine sediments generally indicate an oxygen penetration down to several cm depth in contrast to the results reported below.

The use of membrane-covered oxygen microelectrodes largely eliminates the experimental errors described above. Such electrodes, with tip diameters ranging from 2 to 10 μm , constructed according to Baumgärtl and Lübbers ([1]; Chap. I.4) have been successfully used for the measurement of dissolved oxygen in marine sediments [17]. Oxygen microelectrodes offer three advantages: accurate measurement of oxygen tension in water without the need of stirring; excellent spatial resolution due to the very small oxygen-reducing platinum or gold surface [7]; extremely fast response to change in oxygen tension (Chap. I.4). The application of oxygen microelectrodes was previ-

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ously restricted to a physiological context. The results presented in this section were obtained from marine sediments. The microelectrodes could, without doubt, also be used to measure oxygen in other media, such as soils, activated sludge, and bacterial colonies.

2 Microelectrode Design

The electrode design described by Baumgärtl and Lübbers has been modified for oxygen analysis in sediments. Experiments were conducted with electrodes having an active surface consisting of platinum or gold. The glass tubes used for the construction of the electrodes must be fairly thick to prevent water currents from causing vibration of the electrode tip. I used 5 mm (outer diameter) AR-glass tubes with a wall thickness of 0.9 mm (Glaswerk Wertheim, W. Germany). Risk of vibration was further reduced by limiting the length of the thin part of the electrode to 2–4 cm (Fig. 1). Vibration of the electrode tip was difficult to avoid when very long shafts were employed.

The best results are obtained with electrodes with tip diameters from 5 to 10 μm . With a tip diameter of less than 5 μm , the electrodes are generally too fragile and the current output too low. Electrodes with a tip diameter greater than approximately 10 μm must be covered by very thick membranes for accurate measurements in non-stirred media. For application in coarse-grained sediment the glass casing near the electrode tip should have a wall thickness of at least 1 μm . Electrodes with a reasonably thick glass casing can be pushed through sandy sediment, but any electrode will break if it hits a larger particle. The electrodes operate best if the membrane is protected by a recess of a few μm at the tip. The electrode tips were coated with polymer by immersion in polymer solution. Various polymers were tested and the best results were obtained with solutions of D.P.X., which is used for mounting microscope slides. Most problems during the use of oxygen microelectrodes are caused by poor membranes, and the procedure I use for the application of the membrane is therefore described in detail. The viscosity of the D.P.X. solution is adjusted with xylene so that the solution

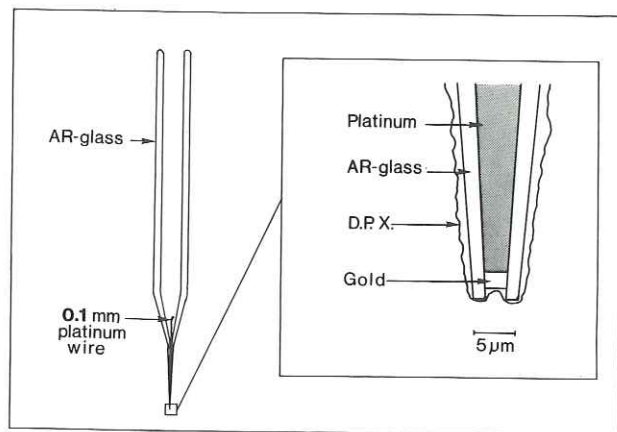


Fig. 1. Oxygen microelectrode used in sediment. The electrode tip is shown in detail on the right. The gold layer at the tip is not essential and also electrodes without gold were used

is just able to float down the wall of a test tube when this is put upside down. One cm^3 of the solution is then poured into a test tube (10 cm high, 1.3 cm inner diameter). Seven to eight mm of the microelectrode tip is immersed into the solution and fixed at this level with a slit stopper. The test tube with the microelectrode is then placed in a desiccator and vacuum is applied for 5 min, followed by 10 min at atmospheric pressure. The test tube is now placed upside down at an angle of 45° . When the solution slowly flows down along the wall of the test tube, the tip of the microelectrode leaves the solution before the shaft and this is very important. If the microelectrode were simply pulled out of the solution, large drops of polymer would aggregate near the tip and this might increase the diameter of the microelectrode considerably. The microelectrodes with the new membranes are baked at 60°C for 1 h after which the tips are immersed into distilled water. The performance of the new electrodes may be tested after hydration for 1 day. Baumgärtl and Lübbers coat the glass insulation of their microcoaxial needle sensors with a Ag/AgCl layer which forms a reference electrode (Chap. I.4). However, in environments where sulfide may occur, another type of reference electrode should be used (Chap. I.6).

3 Equipment for Field Use

The circuit for oxygen measurements with microelectrodes is very similar to those used for ordinary POS. The use of a highly sensitive ammeter and efficient electrical shielding is essential. All coaxial cables should have an additional graphite shielding underlying the copper shielding ("low noise" quality). If several electrodes are glued together (N.P. Revsbech, B.B. Jørgensen, and Y. Cohen, in preparation), cables with several individually shielded conductors contained within a common shield may be used.

The ammeter used by us in the field was a battery-operated Keithley 480 picoammeter. Since picoammeters do not operate satisfactorily at high air humidities, the meter was housed in a watertight Plexiglas box dried with a desiccant. A mercury cell connected to an adjustable voltage divider yielded the polarization voltage.

The reference electrode should have a large capacity. If free sulfide was present in the medium, a double junction reference electrode (Orion, model 90-02-00) was used to prevent contamination. Otherwise a normal calomel electrode (Radiometer, model K401) sufficed, but care should be taken to prevent clogging of the porous electrolyte bridge. Low-capacity reference electrodes caused erratic readings during field experiments when all electrical shielding must "float" at the potential of the reference electrode.

The holder for the p_{O_2} microelectrode is an extremely critical part of the equipment. It should provide electrical contact with the platinum wire of the microelectrode without adding electrical noise and must ensure absolute dryness of the inner surfaces of the electrode. Even if the inner surfaces of the electrode are only slightly humid, short circuits interfere with the readings and cause unstable zero currents. An electrode holder with an internal reservoir of desiccant (Fig. 2) solves all these problems. This electrode holder can be submersed into water and is adjustable to electrodes

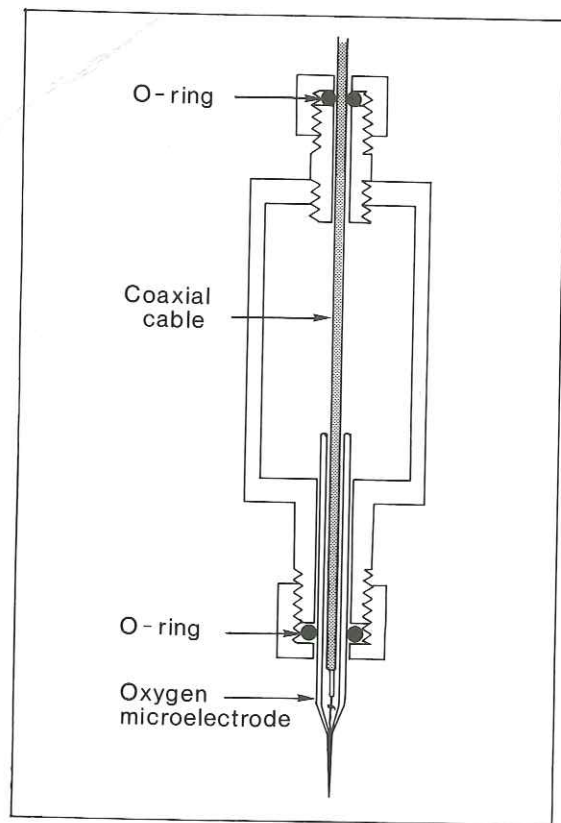


Fig. 2. Adjustable electrode holder made of Plexiglas. The shielding of the coaxial cable is removed from the lowest mm. The uninsulated wire thus protruding from the lower end of the cable is in electrical contact with the platinum wire of the microelectrode. The space inside the electrode holder can be filled with a desiccant, in which case a cotton plug should be used to protect the inner surfaces of the electrode from contamination

of different lengths. In less than 20 cm of water it is more convenient to use long electrodes to avoid submersion.

The microelectrodes were pushed into the sediment by means of a micromanipulator mounted on a sturdy rack, which was placed on a concrete slab during field measurements.

4 Microelectrode Operation

The electrodes were checked in a 2% NaCl solution for satisfactory current output at N_2 saturation and air saturation prior to the application of the membranes. Before each experiment the membrane-covered electrode should be tested in water from the sampling locality. The signal drift should be small and the stirring effect in water should be less than 5%. In a pure NaCl solution linear calibration curves are usually obtained, but in seawater there may be significant deviation from linearity. However, electrodes with thick membranes showed good linearity even in a hypersaline brine (tested up to 80‰ salinity). Measurements with such electrodes gave current ratios for air-saturated seater/oxygen saturated seawater (both currents corrected for zero cur-

rent) of 4.6 to 4.8. The theoretical ratio is 4.77. Some electrodes show a transient increase in sensitivity to oxygen ("anoxic overshoot") after exposure to low oxygen tension [14]. Electrodes used in illuminated sediments often signalled oxygen saturations changing from 0% to 100% within a few seconds and the anoxic overshoot therefore represents a potential source of error. Large anoxic overshoots indicate poor membranes and only electrodes with negligible overshoots should be used. Electrodes with the characteristics described above normally had a current output (at 20°C) of about 100 pA for air saturation and 3 to 12 pA for N_2 saturation (zero current). Electrodes with a current output as low as 20 pA for air saturation and 1 pA for N_2 saturation have also been successfully used.

Two different techniques were used for the measurement of oxygen in sediments. In the "fixed point" technique, a pO_2 -electrode was fixed in a certain depth of the sediment and the oxygen tension was continuously recorded. The sensitivity of the electrode must be constant during these experiments. Electrodes which show a drift in the sensitivity to oxygen can only be used with the "dip" technique, in which the electrode is pushed stepwise into the sediment and the oxygen tension recorded at 0.5 mm or 1.0 mm intervals. Only a few seconds are needed to record the oxygen tension at each depth and the electrode can be recalibrated between "dips", thus minimizing the error of drift. The reading in the deeper, anoxic part of the sediment was used as zero calibration. Winkler analysis of the water above the sediment ([20], App. D) yielded the second calibration point for a linear calibration curve.

The electrodes could be pushed through a sheet of latex rubber without damage so that a diffusion barrier of this kind can be introduced experimentally between the sediment and the overlying water [17]. The fact that the electrodes can be pushed through soft polymers may be advantageous in several other contexts.

5 Environmental Factors Interfering with the Readings

Mg^{2+} and Ca^{2+} , which are very abundant in seawater, may both poison the electrodes (Chap. I.4). These ions and dissolved organic matter account for the observation that oxygen microelectrodes are often much less stable in seawater than in a pure solution of NaCl and that electrodes with thin membranes show non-linear calibration curves in seawater.

Hydrogen sulfide interferes with the measurement of oxygen in sediments [17]. A new electrode with an active surface consisting of platinum often showed a decline in oxygen sensitivity of up to 50% after the first exposure to sulfide, but once poisoned its sensitivity to sulfide was low. The current output of a poisoned electrode declined by only 1% to 2% when, to continuously aerated 0.2 mol dm^{-3} HEPES buffer (pH = 7.0), sulfide was added up to a concentration of 10^{-4} mol dm^{-3} . When sulfide was added up to 10^{-3} mol dm^{-3} , the current output was lowered by 15% to 20%. In addition to the immediate decrease in the sensitivity to oxygen caused by the addition of sulfide, an increased drift in the electrode current was observed. Since sulfide rarely reaches a concentration of 10^{-4} mol dm^{-3} in normal O_2 -containing marine sediments, corrections for interference by sulfide are unnecessary. Only microelectrodes with an

active surface of platinum have been checked for the quantitative interference of H_2S . Other authors have, however, found gold cathodes to be less affected by sulfide than platinum cathodes ([10], Chap. I.6). The results I have obtained with gold-microelectrodes indicate that the interference by sulfide was negligible, and the gold microelectrodes also showed less current drift than the platinum microelectrodes. All the oxygen microelectrodes made at our laboratory are now gold-plated at the tip.

Changes in pH below pH = 7 may exert an influence on the O_2 readings (Chap. I.4). This is irrelevant in the surface layers of marine sediments where pH values range from 7.2 to 10.2 ([6], N.P. Revsbech, unpublished results).

Like all POS, oxygen microelectrodes are sensitive to changes in temperature with temperature coefficients of 2.1% to 2.7% per $^{\circ}C$ when calculated as oxygen tension (Chap. I.4), corresponding to about 4.8% per $^{\circ}C$ when calculated as oxygen concentration. Simultaneous temperature measurements may therefore be necessary if there is a temperature gradient in the profile.

6 Examples of Data Obtained Using Oxygen Microelectrodes

The oxygen microelectrodes described above were used to measure the oxygen profiles in undisturbed, dark incubated sediment [18, 21]. In addition, oxygen profiles under varying conditions of illumination and water flow above the sediment surface were recorded [13, 17]. It is possible to calculate from the oxygen profiles the rates of oxygen consumption and the diffusion coefficient of oxygen in the sediment [17]. Furthermore, a new method for the determination of benthic primary production by oxygenic photosynthesis was created [19]. The new method is based on microelectrode measurements of the change in oxygen concentration within the photic layer of the sediment when the sediment is darkened for a few seconds. In contrast to previous methods, this new method also shows the vertical distribution of photosynthetic activity.

The effect of stirring of the overlying water on the oxygen profile in dark-incubated sediment is shown in Fig. 3. During "vigorous stirring" some sediment material went into suspension, a new steady state oxygen profile was quickly attained, and oxygen penetration increased by only 2 mm. After 12 min of reduced stirring, the oxygen profile differed slightly from that at the beginning probably due, to some extent, to changes of the sediment surface during the experiment. The boundary between sediment and water is a continuum, rendering an exact definition of "sediment surface" difficult. The zero depths at Figs. 3 and 4 were defined as the depth at which the oxygen tension, in the dark and during gentle stirring, was about 20% less than that of the overlying water. These zero depths coincided reasonably well with the surface observed directly through a microscope. Since the zero depth is more or less influenced by the degree of stirring (Fig. 3), a standardized gentle stirring should be used to obtain reproducible zero depths.

The dip technique was also used to record the oxygen profiles during a light-dark cycle as shown in Fig. 4. The light intensity used in the experiment ($700 \mu\text{Einst m}^{-2} \text{s}^{-1}$) was much higher than the in situ intensity at a water depth of 4 m. During the ex-

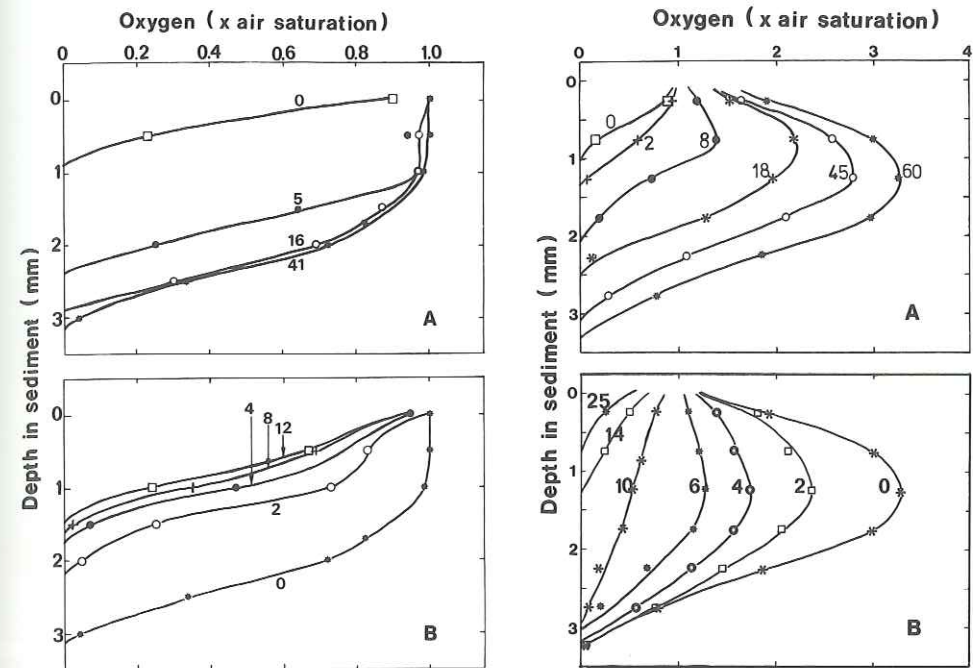


Fig. 3

Fig. 4

Fig. 3A,B. The effect on the oxygen profiles of a dark incubated, sandy sediment produced by stirring of the overlying water. The time indicated for each curve refers to the start of the dip. The deepest part of the profile was recorded ca. 1 min later. Temperature, $10^{\circ}C$. A Oxygen profiles during vigorous stirring. The numbers indicate the duration in minutes of vigorous stirring. B Oxygen profiles during gentle stirring after vigorous stirring for 41 min. Numbers indicate the time in minutes after cessation of vigorous stirring

Fig. 4A,B. Oxygen profiles in a sandy sediment during a light-dark cycle. The light intensity was $700 \mu\text{Einst m}^{-2} \text{s}^{-1}$ and the temperature was $15^{\circ}C$. The time [min] indicated for each curve refers to the start of the dip. The deepest part of the profile was recorded ca. 1 min later. A Oxygen profiles in the light. Numbers indicate the time in minutes after the light was turned on. B Oxygen profiles in the dark following illumination for 60 min. Numbers indicate the time in minutes after the light was turned off

periment a gentle water current was maintained above the sediment. Illumination resulted in increased oxygen tensions in the surface layers of the sediment and the oxygen penetration increased from 1 to 3 mm. The steady-state dark profile was reestablished within 25 min of turning off the light. The profiles shown in Fig. 4 were recorded in the laboratory, but similar results have been obtained in the field [13].

The oxygen tension as a function of time can be continuously recorded by the "fixed point" technique. The results from such an experiment are shown in Fig. 5. The water depth at the sampling locality was only a few cm, and the experimental light intensity ($1000 \mu\text{Einst m}^{-2} \text{s}^{-1}$) was comparable to the maximum in situ intensity. In the uppermost millimeter, the oxygen tension rose immediately after commencement of illumination, and very high values were obtained within a few minutes. The resulting steep oxygen gradients in the surface layer led to an increased loss of oxygen to the

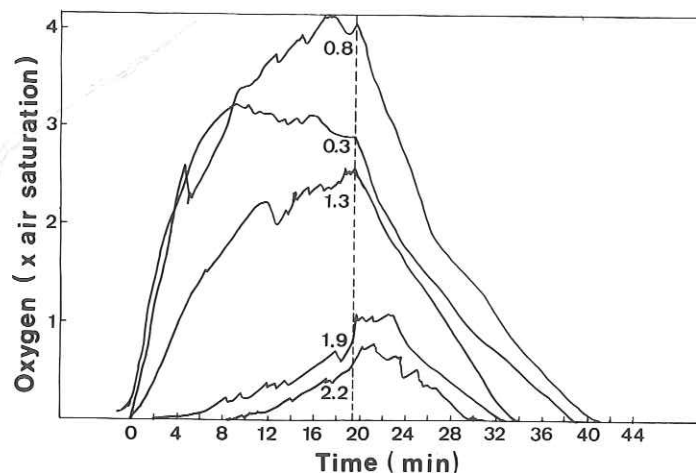


Fig. 5. Oxygen tension at various depths in the sediment during a light-dark cycle. Numbers indicate the depth in the sediment in mm. Light ($1000 \mu\text{Einst m}^{-2} \text{ s}^{-1}$) was turned on at time 0 and turned off at 19.5 min. Temperature, 15°C

water and to the deeper sediment layers. The curves therefore only approximate straight lines during the first minute, after which a diminishing rate of increase was observed. At depths of 1.9 and 2.2 mm there was no initial rise in oxygen tension after illumination, and consequently no net oxygen production. After 2 and 8 min, respectively, the oxygen tension began to rise in these two layers, indicating that oxygen was supplied by diffusion from photosynthetically more active layers. The reverse sequence of events occurred during the initial dark period.

The application of oxygen microelectrodes in sediments reveals otherwise unobtainable data on the distribution and dynamics of oxygen in sediments. It would be valuable to follow, with similar spatial resolution, the changes of other chemical parameters. It is now possible to construct pH and sulfide microelectrodes. Their application in sediments (N.P. Revsbech, B.B. Jørgensen, and Y. Cohen, in preparation) will give us further information about sediment metabolism.

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