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PART I

Instruments and methods

Adaptation, test and in situ measurements with O₂ microopt(r)odes on benthic landers

Ronnie Nøhr Glud^{a,*}, Ingo Klimant^b, Gerhard Holst^c,
Oliver Kohls^c, Volker Meyer^c, Michael Kühl^{c,*},
Jens Kristian Gundersen^a

^a*Institute of Biological Sciences, Department of Microbial Ecology, University of Aarhus, Ny Munkegade, DK- 8000 Aarhus C, Denmark*

^b*Institute of Analytical Chemistry, Chemo- and biosensors, University of Regensburg, D-93040 Regensburg, Germany*

^c*Max Planck Institute for Marine Microbiology, Microsensor research group, Celsiusstr. 1D-28359 Bremen, Germany*

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Abstract

Oxygen microopt(r)odes have recently been introduced as an alternative to microelectrodes in the field of aquatic biology. We here describe adaptation, test results and first in situ measurements made with O₂ microopt(r)odes on deep-sea benthic landers. This includes a detailed description of the sensors, the mechanical mounting, and the necessary measuring system. Hydrostatic pressure effects on the sensors and the optical penetrators are evaluated and discussed. Further, in situ microopt(r)ode data obtained by a profiling lander (Profilur) and a benthic chamber lander (Elinor) are presented, discussed and compared to measurements obtained simultaneously by Clark type O₂ microelectrodes. The obtained data demonstrated that opt(r)odes are a realistic and good alternative to electrodes for landers and other measuring platforms during deep-sea deployments. © 1999 Elsevier Science Ltd. All rights reserved.

1. Introduction

Since O₂ microelectrodes were introduced to the field of microbial ecology in the early eighties (Revsbech et al., 1980), they have significantly increased our

* Corresponding author. Present address: Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000, Helsingør, Denmark. Fax: 0045 49 26 11 95; e-mail: mblrg@mail.cen-trum.dk.

understanding of O₂ dynamics in a wide range of aquatic environments (e.g. Revsbech and Jørgensen, 1986). Microelectrode measurements have been performed in various communities from photosynthetic microbial mats to deep-sea sediments (Revsbech et al., 1983; Reimers et al., 1986). Their small size, fast response time, low stirring sensitivity and relatively good long-term stability have made microelectrodes an excellent tool for studying O₂ concentrations at a high spatial and temporal resolution (Revsbech, 1989). However, despite the many applications of O₂ microelectrodes, the relatively complicated manufacturing procedure has limited the number of people producing and applying these sensors. This is especially true for in situ applications in marine sediments, where rough handling and autonomous profiling can result in a high rate of demolished microelectrodes. Further, total O₂ uptake measurements with benthic chamber landers in deep-sea environments require a stability that is not always fulfilled by available electrodes (Tengberg et al., 1995).

Recently, a new type of fiber optic O₂ microsensor, the microopt(r)ode, was introduced to aquatic biology (Klimant et al., 1995). The measuring principle is based on dynamic quenching of an immobilised luminophore by O₂, which decreases the luminescence quantum yield (Kautsky, 1939). The immobilised luminophore is fixed to the tip of a tapered glass fiber, which directs the excitation light to the sensor tip. This fiber also transmits the O₂ quenchable luminescent light back to the measuring circuit (Klimant et al., 1995). As a result of the quenching process, the intensity of the luminescent light decreases as the O₂ concentration experienced by the immobilised luminophore at the fiber tip increases. The main advantages of these new microsensors are a far simpler manufacturing procedure, no stirring sensitivity, and a good long-term measurement and storage stability of the sensors (Klimant et al., 1995, 1997).

We describe here the first application of O₂ microopt(r)odes on benthic lander systems. This includes a description of the sensor construction, sensor chemistry, calibration procedures, the required electronics and the pressure compensated mounting on the landers. Potential hydrostatic pressure effects on O₂ microopt(r)odes are evaluated and discussed. Based on in situ tests, microopt(r)ode measurements are discussed and their potential as an alternative to microelectrodes in deep-sea applications is evaluated.

2. Technical description

2.1. Preparation and calibration of O₂-microopt(r)odes

Various designs for O₂ opt(r)odes are described in the literature, but most of these were developed for blood-gas analysis (e.g. Bacon and Demas, 1987; Papkovsky, 1993). Because of poor mechanical- and photostability these sensors were not optimal for microopt(r)odes or for deep sea application. We have used ruthenium(II)-tris-4,7-diphenyl-1,10-phenanthroline perchlorate (Rudpp) immobilised in a polystyrene matrix (Aldrich, Germany) as the optical indicator material. This ensured a low compressibility and a high mechanical stability of the indicator matrix. The

polystyrene was dissolved in chloroform and Rudpp was added to a final concentration of 5 mM. The dye–matrix solution was stable and could be stored for several months in a refrigerator.

For construction of the opt(r)odes we used a 100/140 μm silica/silica step index optical fiber (Radiall, Germany) with a standard ST-plug added to one end. The other end of the fiber was tapered by heating to a diameter of approximately 10 μm . Afterwards the taper was cut to a diameter of approximately 30 μm . The fiber tip was dipped in the dye–matrix solution, and after complete evaporation of the solvent (three days at room temperature) the polymer matrix showed a good adhesion to the glass surface. The matrix was hydrophobic and insoluble in water and tests showed that no dye leached from the sensor tip on a time scale relevant for the measurements. The immobilised dye had a light emitting diode (LED)—compatible excitation maximum at 450 nm and exhibited a large Stokes-shift for the emission maximum to 610 nm. These spectral characteristics allowed us to use simple glass filters for the separation of emission and excitation light (see opto-electronic sections below). To reduce the influence of stray light and reflections from the sediment during measurement, the dye matrix was dip-coated with black silicone (thickness < 5 μm). The tip diameter of an O_2 microopt(r)ode thus depends on the dimensions of the tapered fiber, sensor chemistry and optical isolation, and is typically within the range 40–50 μm (Fig. 1). Construction of O_2 microopt(r)odes with a tip diameter < 40 μm is possible, but in strongly tapered fibers the loss of excitation light increases to an unacceptable level. The coated glass fiber was fixed for support and easier handling in a 8 mm glass capillary as shown in Fig. 1.

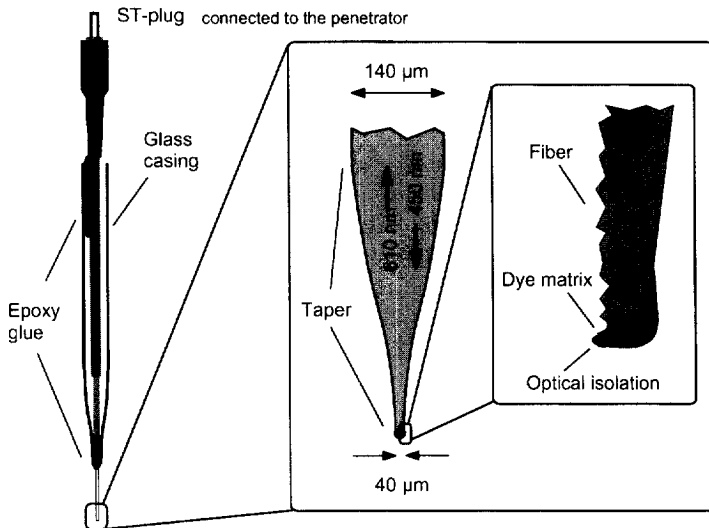


Fig. 1. A schematic drawing of a microopt(r)ode and the sensing tip. Arrows indicate outgoing light (towards the sensor tip) and incoming light (emitted from the sensing tip).

The luminescence from an ideal O₂-quenchable dye is described by the Stern–Volmer equation (Stern and Volmer, 1919):

$$\frac{I_0}{I_C} = (K_{sv}C) + 1 \quad (1)$$

where I_0 and I_C are the luminescent light intensities in the absence and presence of O₂ at a concentration C , respectively. The quenching coefficient (K_{sv}) expresses the efficiency of the quenching process (Stern and Volmer, 1919). However, in non-ideal cases, as for immobilised luminophores, a fraction model has been suggested (Carraways et al., 1991a and b). Based on this model, we used an empirical relation proposed by Klimant et al. (1995), with a fraction index of 0.15 (i.e. the non-quenchable part of the luminescent light signal)

$$\frac{I_0}{I_C} = \left[\frac{0.85}{(K_{sv}C) + 1} + 0.15 \right]^{-1} \quad (2)$$

Eq. (2) looks more complicated than Eq. (1) but contains no additional parameters; thus a simple two point calibration is sufficient. Laboratory tests verified that the calibration curves of lander mounted opt(r)odes were completely described by this equation (data not shown). Typically, I_0 and K_{sv} can be obtained by measuring the luminescent intensities in a small calibration jar with 0 and 100% air saturated water (in reality dissolved O₂ equilibrates with the polymer matrix and undergoes phase separation, so that sensors truly sense the partial pressure and not the concentration of O₂). The probe to probe reproducibility of microopt(r)odes is not better than any other microsensor, and sensors have to be individually calibrated. However, since the intensity of the luminescent light is strongly temperature dependent, it is important to perform the calibration of the microopt(r)odes at the application temperature (Demas and DeGraff, 1992; Klimant et al., 1997). The applied microopt(r)odes had a response time of 3–5 s, precision > 1% of the signal, detection limit < 1 μM and tip diameter < 40 μm. Previous investigation has proven that Ru(diph)/polystyrene sensors can be applied continuously for 50 h without any change in signal (Klimant et al., 1995). Continuous illumination of the sensor tip may, however, result in photobleaching. By illuminating the sensor tip only during actual measurements, the long-term stability can be correspondingly increased. The microopt(r)odes could be stored for several months before use without a signal decrease. Previous investigations have also shown that no solutes present in marine sediments cause significant interferences with O₂ measurements by ruthenium based microopt(r)odes (Klimant et al., 1995).

2.2. Opto-electronic measuring system

The two electronic boards carrying the circuits necessary for the opt(r)ode measurements have a size of 3 × 7 × 7 cm and fit into the electronic casing originally designed for our benthic landers. The organisation of the electronics is schematically presented in Fig. 2 and basically consists of six modules: an optical switch, a 2 × 2 multimode

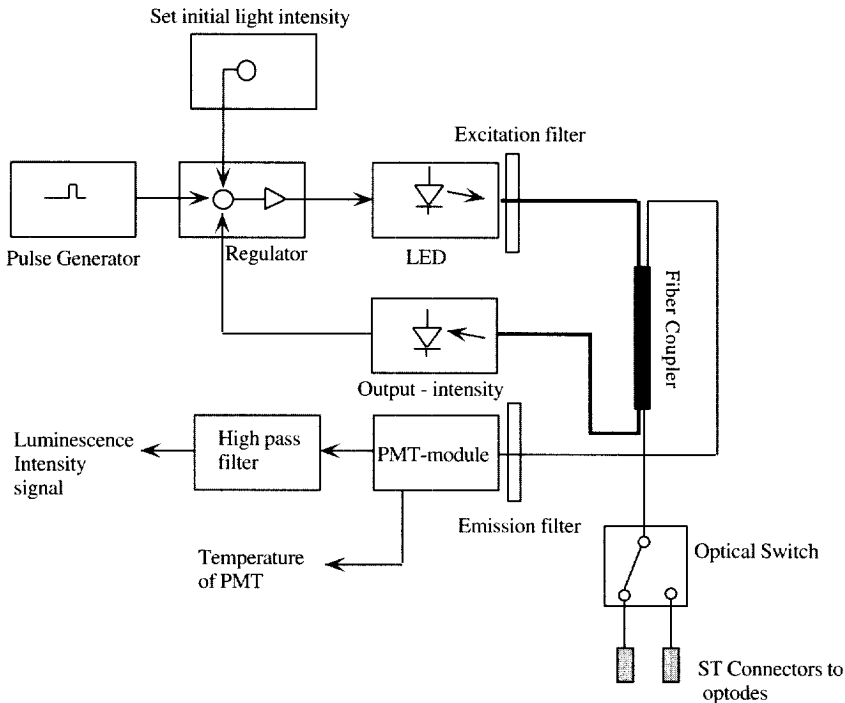


Fig. 2. Schematic drawing of the opto-electronic circuit for the opt(r)ode measurements. See text for more details. LED = light emitting diode, PMT = photomultiplier.

fiber coupler, a regulated excitation light source (LED), an electronic filtering system, an optical filtering system, and a photomultiplier (PMT).

The light from the LED (NSPB 500S Nichia Chemical Europe) is regulated in order to keep the excitation light intensity constant. This is achieved by measuring the LED output through one arm of the fiber coupler (50/50–100/140, Gould Inc.); any deviation in the LED signal is then compensated for by regulating the power supply to the LED. Since the absolute signal level varies among opt(r)odes, the excitation intensity can initially be manually adjusted in order to prevent the opt(r)ode signal from going off-scale during measurements.

The excitation filter (LEE, HT 141) is placed in front of the LED, and the light beam is guided through the fiber coupler to the optical switch. The position of the optical switch is controlled by the software, and after switching it takes approximately 2 s before a steady excitation intensity becomes established. For the present prototype we have chosen to work with an optical switch with only two channels. The number of channels can, however, be increased by applying a multichannel optical switch (Holst et al., 1997). The luminescence signal from the microopt(r)ode tip is carried through the fiber coupler to the PMT detector (H5701-02, Hamamatsu), which is equipped with a long pass filter (OG 570 Schott, Germany).

The power supply to the LED is modulated with a frequency of 5–6 kHz. This allows the removal of interfering signals from ambient light after the signal amplification by the PMT. This is often convenient during tests and calibrations in the laboratory but is not necessary during a deep-sea deployment. Unfortunately, the gain of the PMT is temperature sensitive, and the signal decreases by approximately 1% as the ambient temperature is increased by 1°C. This effect can be corrected for if the temperature of the PMT is measured, but opt(r)ode measurements should preferably be done at steady-state in situ temperatures. Due to heat production by the electronics, steady-state is typically reached after 1 h at the application temperature.

The power consumption of the opt(r)ode electronics is approximately 60 mA during measurements and 10 mA in the “stand by” mode, which, compared to the electronic units equipped solely with electrodes, corresponds to an increase in power consumption of approximately 20 and 3%, respectively. The estimated total cost of the necessary hardware for the measuring system is 2500 US\$.

2.3. Mechanical adaptation to the landers

In Profilur, the profiling lander, microsensors were mounted directly on a movable electronic casing, which after landing was moved stepwise towards the sediment (Gundersen and Jørgensen, 1990). Specially manufactured, pressure-stable optical penetrators (D.G. O'Brian, UK partnumber 1535016-115) were adapted to the electronic casings of both landers. The opt(r)odes were mounted in oil filled transparent adaptors constructed of cast Plexiglas (PMMA) (Fig. 3). The adaptors were equipped with a small rubber bladder, which allowed for pressure compensation. The profiling lander was equipped with both microopt(r)odes and microelectrodes in parallel. We used Clark-type O₂ microelectrodes with internal reference and a guard cathode (response time for 90%-signal < 1 s, precision > 1% of the signal, detection limit app. 1 μM, tip diameters < 10 μm and stirring effects < 1%) (Revsbech, 1989).

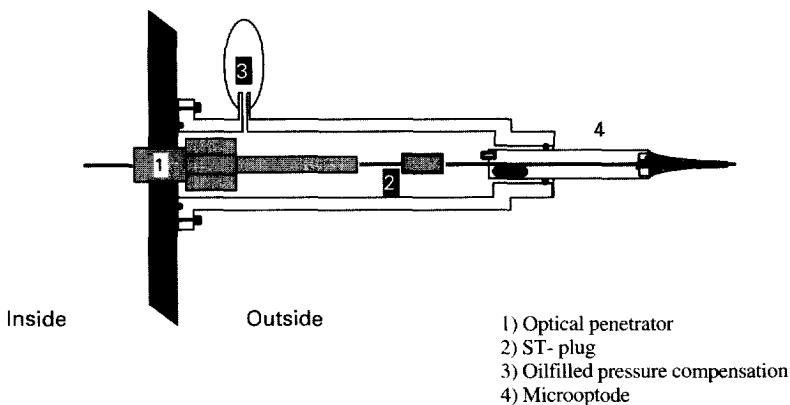


Fig. 3. Schematic drawing of the pressure compensated mounting of microopt(r)odes on submersible platforms (not to scale).

The benthic chamber lander, Elinor, was equipped with two microopt(r)odes and with two minielectrodes (Glud et al., 1995). The sensors were placed in the lid, which closed the incubation chamber 7.5 h after the deployment (Glud et al., 1995). The minielectrodes had an outside tip diameter of approximately 2 mm, but because of a small internal sensor opening the minisensors had the same sensor characteristics as the microelectrodes. In Elinor, the distance of 1.60 m from the electronic casing to the benthic chamber required an oil-filled flexible silicone tubing to carry the optical fiber and the coaxial cables to the chamber lid (Glud et al., 1994). The sensors were mounted in adaptors comparable to the one shown in Fig. 2.

3. Results and discussion

3.1. Hydrostatic pressure effects

In order to investigate hydrostatic pressure effects on the optical penetrators, they were mounted in the lid of a pressure tank in which hydrostatic pressure could be increased in steps of 5–10 atm up to 600 atm. Initially, no sensor chemistry was used, and immobilised titanium oxide was applied to the fiber tip. In this way the excitation light was partly reflected back to the non-filtered measuring system. Several pressure cycles between 1 and 600 atm were performed, and the signal change due to mechanical stress induced by the hydrostatic pressure on well functioning penetrators and fiber cables never exceeded 0.3% (data not shown). Most benthic landers obtain calibration values for the O₂ signal at the seafloor, and therefore such a slight change in signal during descending or ascending will not cause problems for the sensor application.

After these initial tests, the pressure effects on the sensing chemistry were investigated. A circular glass window of 5 mm in diameter was placed in the lid of the same pressure tank as used above. The luminophore-polymer matrix was applied on the inside glass surface, and excitation light was supplied from outside the pressure tank. As seen in Fig. 4A the luminescence intensity measured from the outside gradually increased at higher pressures (for safety reasons it was only possible to apply a pressure of up to 200 atm in this set-up). Without O₂ in the surrounding water (no quenching) there was only a slight, approximately linear signal increase of 0.75‰ atm⁻¹ (Fig. 4A), which probably was caused by small pressure induced changes in the light path. However, as O₂ was added to the water the pressure effect gradually increased and reached a maximum of 0.02% atm⁻¹ at 301 μM O₂ (Fig. 4A). The effect of the decreased quenching efficiency at higher pressures caused the O₂ sensitivity at 200 atm to be 8% lower than at 1 atm (i.e. the distance between the upper and lower line in Fig. 4A). The decreased sensitivity was also reflected in the change of the calibration curves obtained at different pressures (Fig. 4B).

Subsequently, O₂ microopt(r)odes as described above were mounted on the optical penetrators, and the luminescence intensity was measured during pressure cycles between 1 and 600 atm with 0, 61, 104, 218 and 278 μM O₂ in the surrounding water. At 600 atm the O₂-sensitivity of O₂ microopt(r)odes at 280 μM O₂ had decreased by

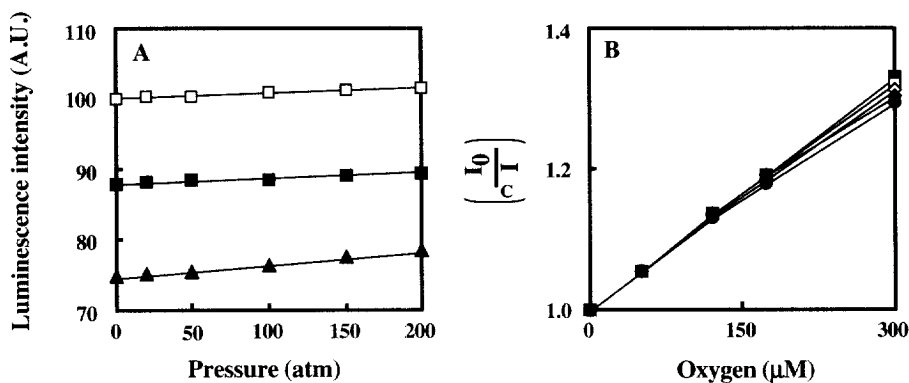


Fig. 4. Panel A shows the luminescence intensity at three different O₂ concentrations in the surrounding water (□–□ = 0 μM; ■–■ = 120 μM; ▲–▲ = 301 μM) as a function of the applied hydrostatic pressure. The intensity at 1 atm hydrostatic pressure and 0 μM O₂ was set as 100 A.U. Panel B shows the calibration curve of the immobilised luminophore at six different hydrostatic pressures from 1 (■–■) to 200 atm (●–●) (see panel A).

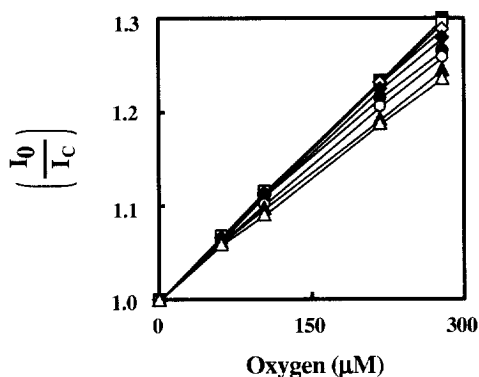


Fig. 5. Calibration curves for an O₂ microopt(r)ode at eight different hydrostatic pressures: 1 atm. (■–■); 50 atm. (□–□); 100 atm. (◇–◇); 200 atm. (◆–◆); 300 atm. (●–●); 400 atm. (○–○); 500 atm. (▲–▲); 600 atm. (△–△).

approximately 20%. The effect of hydrostatic pressure on the microopt(r)ode signal (Fig. 5) was similar to the effect observed solely with immobilised luminophore (Fig. 4B). This indicated that the sensor signal remained unaffected by potential pressure effects on penetrators, cables and mechanical parts of the sensor.

We ascribe the pressure sensitivity to physical changes in the polymer matrix or a decrease in the quenching efficiency at increased hydrostatic pressure. Despite the decrease in sensitivity the sensors were still fully operational at a pressure that corresponded to full ocean depth. Since most landers obtain at least two water samples for sensor calibration or a zero reading is inherent in the measurements (microprofiles) at the seafloor (constant pressure) (Tengberg et al., 1995), a relatively simple two point calibration of in situ data can be performed by applying Eq. (2).

However, for water column studies the pressure effect has to be accounted for and must be included (together with the temperature) in the calibration equation, as is the case with O₂ microelectrodes (Gundersen et al., in press). The pressure sensitivity did not appear to be significantly different between opt(r)odes.

3.2. *In situ microprofiles*

The profiling lander equipped with 2 microopt(r)odes and 5 microelectrodes was deployed three times at 10 m water depth in Aarhus Bight, Kattegat (Denmark). The lander was allowed to settle to the seafloor, and after a 1 h delay the electronic casing was moved down towards the sediment surface in steps of 100 µm. The waiting time allowed the lander to settle firmly at the seafloor and also ensured that the temperature of the PMT reached a steady state of 19°C (in situ temperature 4°C). The micro-sensors were positioned at each depth for 10 s before 10 values from each microopt(r)ode were recorded simultaneously with 20 values from each microelectrode.

The applied microopt(r)odes had a response time of approximately 3–5 s, which was due to the time it took to reach thermodynamic equilibrium between the O₂ in the sensor matrix and the surrounding water. Therefore the opt(r)odes were positioned at a given depth 10 s prior to the actual measurements. Subsequently, the signal from each of the microopt(r)odes was recorded 10 times. The first two measuring points were, however, affected by the changed position of the optical switch (see above) and were discarded. The eight non-affected values were averaged, calibrated and used as a measuring point at a given depth. The signal of the microelectrodes was recorded simultaneously with the microopt(r)odes. This measuring procedure required 30 s at each measuring depth, but this could have been further optimized (10 s at each depth would actually have been sufficient to obtain the required measurements). The Ru(diph)/polystyrene opt(r)odes applied here have a relatively long response time; the sensors can be made with a response time of approximately 2 s. In cases where a faster response time is required O₂ microelectrodes are still the only alternative.

Measured O₂ microprofiles contain two inherent calibration points; the constant reading in the anoxic sediment and the constant reading in the bottom water with a known O₂ concentration (determined by Winkler titration of a bottom water sample). The calibrated data obtained at the very first in situ deployment are presented in Fig. 6. Unfortunately, two microelectrodes broke during handling of the lander, and consequently only three microelectrode and two microopt(r)ode profiles are shown. As observed by simultaneous video recordings, the sediment surface was heterogeneous with an extended microtopography, and for comparison the profiles here aligned to the depth where the first significant decrease was observed. The profiles were similar, and the observed scatter was probably caused by heterogeneity in the sediment. The difference in tip size between the two types of microsensors did not affect the measured profiles.

3.3. *In situ chamber data*

The benthic chamber lander, Elinor, was deployed at another station in Aarhus Bight a few days later (water depth 16 m). As before, 10 values from each microopt(r)ode

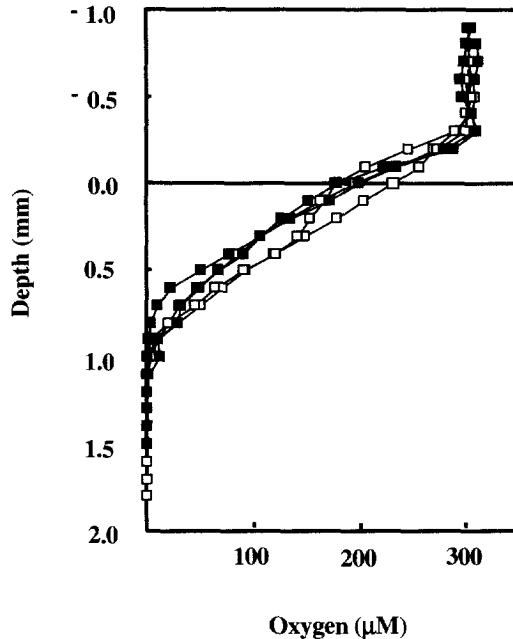


Fig. 6. Benthic O_2 microprofiles measured in situ with microelectrodes (\square - \square) and microopt(r)odes (\blacksquare - \blacksquare) at 10 m water depth. The profiles were aligned to the depth where the first significant decrease in O_2 concentration was registered. Fat horizontal line indicates the estimated position of the sediment surface.

were recorded simultaneously with the values from each microelectrode, and this measuring procedure was initiated every 2 min. The sensors were calibrated using a zero reading measured at in situ temperature *on board* and the constant reading in the bottom water before lid closure (determined by Winkler titration of a bottom water sample). The complete, calibrated readout from one microopt(r)ode and one minielectrode during the deployment is shown in Fig. 7. Initially, the signals differed, but after the PMT temperature reached a steady-state it stayed more or less constant until lid closure at approximately 450 min. The O_2 concentration thereafter decreased linearly in the enclosed water volume. During the incubation, stirring ensured an efficient mixing of the water phase without any resuspension (Glud et al., 1995). The data from the two sensor types resulted in identical total benthic O_2 consumption rates (Fig. 7). However, the noise level of the opt(r)odes was higher than that of the electrodes; this was related to microbending of the fiber cables and the ST-connectors by the free flowing water at the seafloor. Laboratory tests showed that this could be avoided by better attachment of the fiber cables and the ST-connectors to the lander frame before deployment.

3.4. Opt(r)odes as an alternative to electrodes

As demonstrated in Figs. 6 and 7, data obtained by microopt(r)odes have the same quality as data measured by mini- or microelectrodes. Although the practical

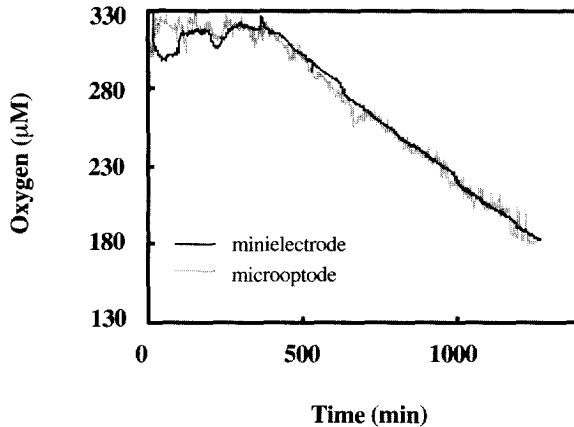


Fig. 7. Calibrated readout obtained simultaneously with a microopt(r)ode and a minielectrode during deployment of a benthic chamber at 16 m water depth. Lid was closed after approximately 450 minutes of deployment.

experience with opt(r)odes in oceanography is still limited, the better long-term stability, higher storage potential and the simpler manufacturing procedure make microopt(r)odes a good alternative to microelectrodes. Furthermore, microopt(r)odes do not consume O_2 , and the signal is therefore insensitive to stirring.

If opt(r)odes are applied for measurements in the water column, or in benthic chambers, miniaturisation and optical isolation of the sensor tip is not needed. Application of such macroopt(r)odes would thus increase the signal output and thereby simplify the opto-electronics (e.g. the PMT could be omitted). This would reduce the costs of the electronics and further simplify the use of the sensors. However, for practical reasons we have preferred to work with identical electronic units for our lander systems. Since opt(r)odes do not consume the quenching solute, a correction for sensor consumption during chamber incubations as for macroelectrodes is not needed. We have applied Ru(diph)/polystyrene microopt(r)odes, but many alternative luminophores and matrix materials could potentially be applied if other features of the sensors are required (e.g. faster response time). The fields of development and application of opt(r)odes are expanding, and more sensitive and stable material combinations may appear within the next few years. A promising development is the use of luminescence lifetime, instead of the luminescence intensity, as the signal carrier (Lippitsch, 1988; Holst et al., 1995). This would further improve the long-term stability, eliminate the noise related to microbending and simplify the calibration procedure, but lifetime based reading would also require relatively sophisticated electronics (Holst et al., 1995).

In conclusion, we have successfully adapted fiber-optic O_2 microsensors for use on benthic landers and have shown their potential for in situ application in the marine environment. Although the operational experience with microopt(r)odes on submersible platforms is still limited and future optimized microsensor designs and opto-electronic measuring systems may be developed (e.g. luminescence lifetime based

O₂ measurements in situ), the present sensor and measuring systems allow O₂ micro-opt(r)odes to be applied in marine environments, including the deep sea as an alternative to microelectrodes.

Acknowledgements

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