

Limnol. Oceanogr., 40(6), 1995, 1159–1165
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Fiber-optic oxygen microsensors, a new tool in aquatic biology

Abstract—A new fiber-optic oxygen microsensor (microoptrode) based on dynamic fluorescence quenching has been developed to measure oxygen gradients in marine sediments and microbial mats. The microoptrodes are fabricated by immobilizing an oxygen-quenchable fluorophore at the tapered tip of an optical fiber. A special optoelectronic system has been designed to measure oxygen with these microoptrodes. It is based on small and cheap optical components and can easily be miniaturized for field applications. In contrast to oxygen microelectrodes, the new oxygen microoptrodes are easy to make, do not consume oxygen, and show no stirring dependence of the signal. In addition, they show excellent long-term stability and storage stability. Hydrogen sulfide, carbon dioxide, and other relevant chemical parameters do not interfere with the measurement. Oxygen profiles in marine sediments obtained from measurements with microoptrodes show good correlation to profiles measured with oxygen microelectrodes.

Sediments, biofilms, and many other compact microbial communities (e.g. marine snow) are characterized by steep gradients of various physical and chemical parameters such as light intensity, pH, and O₂ over distances ranging from <0.5 mm to a few millimeters (Revsbech and Jørgensen 1986; Kühl et al. 1994). Dissolved oxygen is one of the most important parameters in biological systems and knowledge of oxygen concentration gradients is of paramount importance in understanding the function and regulation of most microbial communities. Microsensors are powerful tools for determining oxygen gradients at high spatial resolution and for determining rates of O₂ production and consumption (Revsbech and Jørgensen 1986).

The first amperometric oxygen microsensors were developed for use in physiology (Silver 1965; Whalen et al. 1967) and were based on coated platinum cathodes. Revsbech et al. (1980) adapted these cathode-type O₂ microelectrodes for use in marine sediments and developed Clark-type oxygen microelectrodes with improved measuring characteristics (Revsbech and Ward 1983; Revsbech 1989). Numerous applications of these sensors in microbial ecology have been reported (*see* Revsbech and Jørgensen 1986). Clark-type O₂ microelectrodes equipped with a guard cathode (Revsbech 1989) can be made with tip diameters <5 μm and have excellent measuring properties such as short response times (<1 s), small stirring sensitivity (1–2%), and a low zero current (<5 pA). Thus they are ideal tools for most applications in aquatic environments.

The construction of well-working Clark-type O₂ microelectrodes is, however, time consuming and requires a significant amount of training. The cathode-type sensors are easier to make but have less ideal measuring prop-

erties (Gust et al. 1987). Oxygen microelectrodes are commercially available but they are expensive and do not always have good measuring properties. Thus, there are major limitations on more frequent use of O₂ microelectrodes in aquatic biology. Furthermore, in applications where long-term measurements are required (e.g. gas exchange studies with flux chambers), the signal stability is often inadequate. Our aim was to develop an alternative oxygen microsensor for use in aquatic environments without these disadvantages.

Optical sensors for chemical species, the so-called optrodes (i.e. the optical analog to electrodes) have undergone rapid development (Wolfbeis 1991). In optrodes, the analyte interacts with an indicator and changes its optical properties. Such changes could be a pH-dependent color change of an acid-base indicator or changes in the fluorescence properties of oxygen-quenchable fluorophores. Normally the indicator is immobilized in an analyte-permeable matrix to make it insoluble in water. The immobilized indicator is typically illuminated via an optical fiber. Various parameters of the back-transmitted light (e.g. intensity, polarization, or spectral distribution) can be related to the analyte concentration. Optrodes were developed to determine pH (Zhujun and Seitz 1984), gases like CO₂ (Lübbbers and Opitz 1975), NH₃ (Rhines and Arnold 1990), and O₂ (Bergman 1986; Wolfbeis et al. 1986), and various ionic species (e.g. Schaffer and Wolfbeis 1989).

Oxygen optrodes are based on the ability of oxygen to act as a dynamic fluorescence quencher, thereby decreasing the fluorescence quantum yield of an immobilized fluorophore (Kautsky 1939). Several oxygen-quenchable fluorophores, which are suitable as indicators in optical sensors, are reported in the literature, mostly polycyclic aromatic hydrocarbons or transition metal complexes (Wolfbeis 1991; Klimant et al. 1994). Oxygen optrodes compared to O₂ electrodes do not consume oxygen and the signal does not depend on flow velocity (Lübbbers 1992). Furthermore optrode measurements are not disturbed by electromagnetic fields. Oxygen optrodes have been developed mainly as minisensors for use in blood gas analysis and as macrosensors for environmental monitoring and in biotechnological applications (Peterson et al. 1984; Weigl et al. 1994). We describe here a new fiber-optic oxygen microsensor, based on dynamic fluorescence quenching, for measuring oxygen gradients at high spatial resolution. This O₂ microoptrode has been characterized and tested in marine sediments.

The luminescent ruthenium(II)-tris-4,7-diphenyl-1,10-phenanthroline perchlorate complex was selected as the oxygen-sensitive dye (Gruber et al. 1993). This indicator dye absorbs blue light at 450 nm and emits a strong red luminescence with a wavelength maximum at 610 nm. The dye is insoluble in water but is soluble in hydrophobic

polymers. Thus, the dye can be easily immobilized in a polymer and will not leach out into the sample. We used polystyrene as the polymer matrix because of its mechanical stability. The indicator was dissolved in this polymer at a concentration of 10 mM by using methyl-ethyl ketone as a solvent. The high dye concentration was necessary to obtain a sufficiently high fluorescence signal. The polymer-indicator solution is stable and can be stored in a refrigerator for at least a year without degradation of the components.

With the polystyrene-indicator solution, we could form thin polymer films on the fiber tip with excellent adhesion to the glass surface and good mechanical stability. We obtained an additional improvement of the mechanical stability by adding titanium dioxide particles ($\sim 1\text{-}\mu\text{m}$ grain size) to the polymer-indicator solution. The addition of TiO_2 also enables a more efficient excitation of the indicator, due to multiple scattering of the blue excitation light in the polymer-indicator layer, which consequently leads to a higher signal intensity.

The oxygen microoptrodes are constructed from multimode silica-silica step index fibers with 100- μm core diameter and 125- μm cladding diameter (Ensign Bickford Corp.). The design of the O_2 microoptrode is shown in Fig. 1. The protective buffer at the measuring end of the fiber is removed by heating and a fiber taper is formed while the bare fiber is heated in a small flame of a gas burner. Fiber tips from 15 to 40 μm in diameter are prepared by cutting the taper with a small knife. The tapered fiber is mounted in an injection needle (*see* Lassen et al. 1992; Kühl and Jørgensen 1992) to improve mechanical stability (Fig. 1A).

The oxygen-sensitive layer is immobilized by dipping the fiber tip once in the polymer-indicator solution (Fig. 1B,C). During the dip-coating procedure, the fiber is moved with an xyz-micromanipulator. After evaporation of the methyl-ethyl ketone (~ 1 min), the sensor tip is coated with a black layer of silicone to avoid interferences from ambient light and to make the signal independent of the optical properties of the surroundings (Fig. 1B,C). After 2 d of curing the silicone, the sensor is ready to use. The oxygen microoptrodes have a tip diameter of 30–50 μm . It is possible to make smaller microoptrodes, but this leads to a dramatic decrease in signal intensity. The fabrication of the sensors is simple and guarantees good reproducibility of the calibration curves of different O_2 microoptrodes.

A special optoelectronic system has been designed for use with oxygen microoptrodes (Fig. 2). The measuring system consists of an illumination module with the light source, glass filter, and lenses; an optical fiber coupler to split the light beam; the optical microsensor; and a detection module with a fluorescence-detecting photomultiplier tube (PMT) and a reference photodiode. The system was designed to obtain a high signal intensity while at the same time using relatively cheap and small optical components. The fluorescence of the immobilized indicator is excited with a blue light-emitting diode (LED, L200CW1K, Ledtronics Inc.). This LED shows an ex-

traordinary bright emission of blue light that perfectly overlaps the absorption spectrum of the indicator.

In contrast to lasers and broadband-emitting light sources like halogen or xenon lamps, LEDs are favorable light sources, because they offer the advantages of a low price, small size, low power consumption, and negligible heat production. In addition, the light emitted by LEDs can be easily modulated electronically. Thus, no mechanical parts for light chopping are required to separate ambient light from fluorescence. The relative large Stokes' shift between the excitation (450 nm) and the emission (610 nm) maximum of the indicator allows use of cheap glass filters instead of interference filters to separate back-scattered excitation light from the fluorescence signal.

The blue light from the LED passes a blue glass filter (BG 12, Schott), which cuts the red part of the LED emission spectrum, and is coupled into a multimode fiber coupler (Gould Inc.) with step index fibers (HCS 110/125 Ensign Bickford Corp.). The high numerical aperture of 0.37 and high core-cladding diameter ratio of this type of fiber allows an efficient incoupling of LED light. The fiber coupler is a fiber-optic analog to a beam splitter and is used to separate the fluorescence signal from the excitation light.

One fiber channel is used to guide a part of the blue light directly to a reference silicon photodiode to compensate for fluctuations in the LED emission. The second fiber channel is connected with the microsensor fiber via a standard ST-fiber connector. The fluorescent light from the fiber tip passes a longpass glass filter (OG 570 Schott) to cut away excitation light and is detected with a red sensitive PMT (H5701-02, Hamamatsu). The light of the LED is pulsed electronically with a frequency of 20 Hz, and by using an electronic filter the interference of non-modulated ambient light on the fluorescence signal can thus be removed. Interferences due to AC light from artificial light sources cannot be suppressed by this electronic circuit but can be avoided by modulating the LED with higher frequencies (> 500 Hz). The whole setup can be battery operated and miniaturized for field applications.

In contrast to microelectrodes, the optrodes described here show a nonlinear decrease in fluorescence signal intensity with increasing oxygen concentration (Fig. 3A). The signal can be linearized by means of the Stern-Volmer equation, which describes the dynamic fluorescence quenching by molecular oxygen (Stern and Volmer 1919):

$$\frac{I_0}{I} = 1 + K_{\text{SV}} \cdot [\text{O}_2]. \quad (1)$$

Here I_0 and I are the fluorescence intensities in the absence and presence of oxygen. The overall quenching constant K_{SV} quantifies the quenching efficiency and $[\text{O}_2]$ is the oxygen concentration.

Unfortunately, this simple two-parameter equation is valid only for ideal systems such as diluted solutions of fluorophores in liquid solvents. The calibration curve of most described optrodes, including the microoptrodes described here, therefore exhibit nonlinear Stern-Volmer

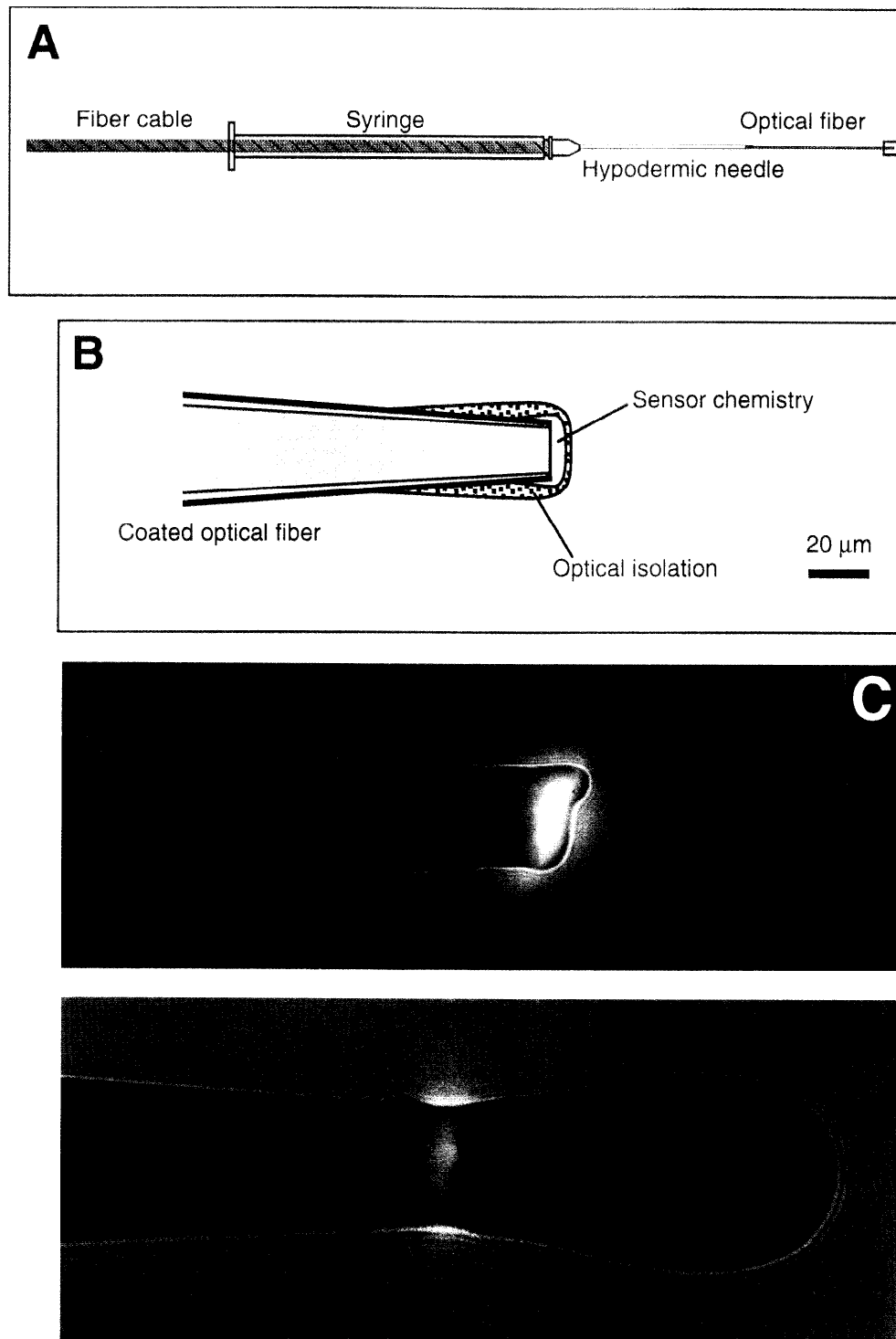


Fig. 1. A, B. Design of oxygen microoptrode. C. Photographs of a sensor tip without (above) and with (below) an optical isolation of black silicone (partly redrawn after Kühl and Jørgensen 1992).

plots. Preliminary studies have shown, however, that the calibration curves of oxygen optrodes based on ruthenium complexes dissolved in polystyrene can be described by a slightly adapted Stern-Volmer equation (Fig. 3B):

$$\frac{I_0}{I} = \left(\frac{0.85}{1 + K_{SV}[\text{O}_2]} + 0.15 \right)^{-1} \quad (2)$$

The basic assumption for use of Eq. 2 is that the flu-

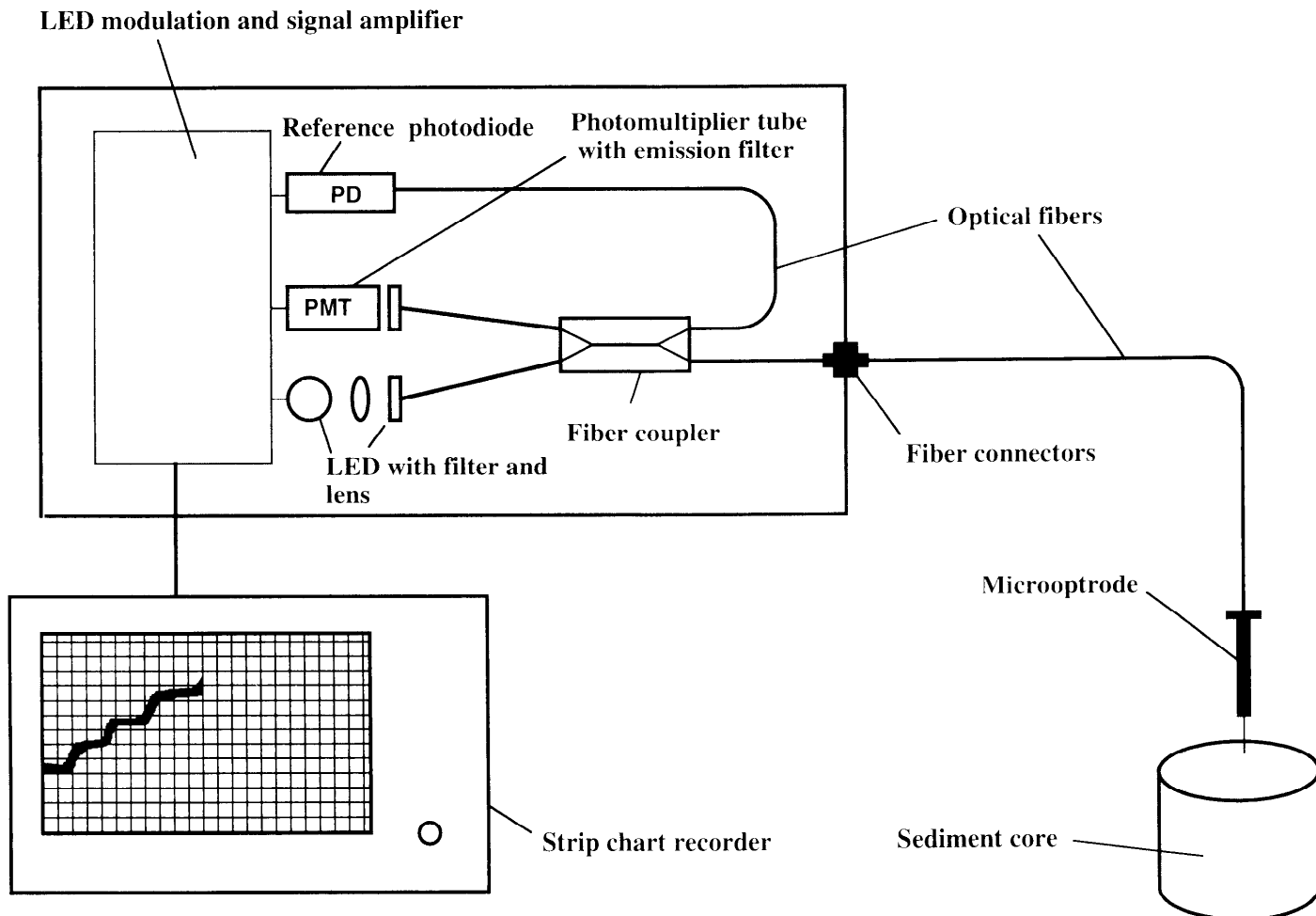


Fig. 2. Schematic diagram of the oxygen microoptrode measuring system.

orescence of a fraction of the indicator (15%) is not quenched significantly by oxygen. Equation 2 looks more complicated than the simple Stern-Volmer equation (Eq. 1) but includes no additional variable parameters. Therefore a two-point calibration is sufficient to describe the calibration curves of the O_2 microoptrodes. The parameter I_0 was calibrated with water, which was made anoxic by adding 1% sodium sulfite. I_0 is a typical intensity parameter and depends on many factors such as sensor preparation, changes in the optical properties of the system (e.g. bending effects), drifting of the detector sensitivity, or degradation of the indicator (photobleaching, leaching). The parameter K_{SV} characterizes the quenching efficiency and therefore the sensitivity of the sensor. K_{SV} depends only on the composition of the sensing layer and was calibrated by measuring the signal in air-saturated water. For a defined composition of the sensing layer and identical conditions during fabrication of the sensor tips, K_{SV} is therefore constant, and a single-point calibration of the sensor is sufficient.

It is important to mention that application of Eq. 1 and 2 is allowed only if all of the back-reflected excitation light is removed by optical filters. In our measuring system, the level of backscattered excitation light is $<2\%$ of the total signal intensity and therefore can be ignored.

Due to the small diameter of the fiber tip, it was necessary to optimize our microoptrodes to obtain a sufficiently high fluorescence signal. By using optical fibers with a high core-cladding diameter ratio and a high indicator concentration in the sensing layer, it was possible to measure with a good signal-to-noise ratio ($\sim 1,000:1$). Depending on the tip diameter of the sensors and the thickness of the sensing layer, we obtained signals up to 80 pW of fluorescent light. Since the noise of the PMT is ~ 1 fW, a resolution of at least $2 \mu M O_2$ in the range up to 100% air saturation was achieved. At higher oxygen concentrations, the resolution decreases due to the hyperbolic form of the calibration curve (Fig. 3A). Sensors without an optical isolation of black silicone have a much higher signal intensity (up to 500 pW) due to an additional excitation of side-bound sensing material by the evanescent light field of the fiber. However, the coating of the fiber tip with black silicone is important for the use of microoptrodes in natural systems.

The silicone layer not only suppresses ambient light and the background fluorescence in sediments and biofilms (e.g. from chlorophyll) but also avoids interferences by changes in the optical properties of the surroundings of the sensor tip (e.g. refractive index, turbidity-reflectivity, or coloration). Especially in natural systems with a

high density of biomass, a dramatic decrease in signal intensity is observed when a noncoated sensor tip penetrates the mat surface. The black silicone coating also acts as a barrier to potentially interfering ionic fluorescence quenchers, such as heavy metals.

The sensors were tested with respect to long-term stability and storage stability. During the first few days after fabrication, the sensitivity of the microoptrodes decreased slightly because of evaporation or trace amounts of the solvent from the polymer. Thereafter, the sensors can be stored for >6 months without any change of the quenching constant K_{SV} . No degradation of the indicator was observed during this period. When the sensors were stored in freshwater for 1 week, we found no significant change of the quenching constant K_{SV} . Thus, only the recalibration of the intensity parameter I_0 was required during long-term measurements. The stability of the whole measuring system was tested by inserting the microoptrode for 50 h in anoxic water. We expected to find considerable photodegradation of the indicator but the signal was stable throughout the 50-h period, although the illumination power was high. This excellent long-term signal stability is one of the most important advantages in comparison to O_2 microelectrodes.

In contrast to Clark-electrodes, the signal of oxygen optrodes is based on a thermodynamical equilibrium and is therefore diffusion-independent (Wolfbeis 1991). We tested the stirring sensitivity of microoptrodes in an artificial sediment of autoclaved glass beads (bead diam, 40–70 μm) covered by 1 cm of air-saturated water. No gradient in oxygen concentration existed in the system. The water was continuously aerated and water circulation was created by directing an air stream toward the water surface. The microoptrode measurements showed the presence of a vertical oxygen profile throughout the water and the sediment as expected (Fig. 4). The same test performed with oxygen microelectrodes showed a high stirring effect ($\sim 10\%$) and a low stirring effect (1–2%), depending on the design of the electrode (*see also* Revsbech 1989).

The oxygen microoptrodes exhibited response times of 5–30 s (t_{90}), depending on the combined thickness of the sensing layer and the black isolating coating. The relatively slow response results mainly from the low oxygen permeability of the rigid polystyrene matrix. The response time of the microoptrodes shown here does not allow their use in photosynthesis measurements based on the light–dark shift method, where response times <0.5 s are required (Revsbech and Jørgensen 1983). First tests have shown, however, that it is possible to design much faster optrodes by careful selection of the polymer matrix. By immobilizing the ruthenium complex in soft polymers like silicone rubber or plasticized PVC, we achieved much shorter response times (<500 ms). Such sensors suffer, however, from poor mechanical stability because the sensing film can be removed from the sensor tip when penetrating coarse sediments or very cohesive biofilms.

Interference tests were performed for chemical parameters that are relevant in sediments (Table 1). In contrast to Clark-type electrodes, high concentrations of CO_2 and H_2S did not affect the signal of our microoptrodes. Changes

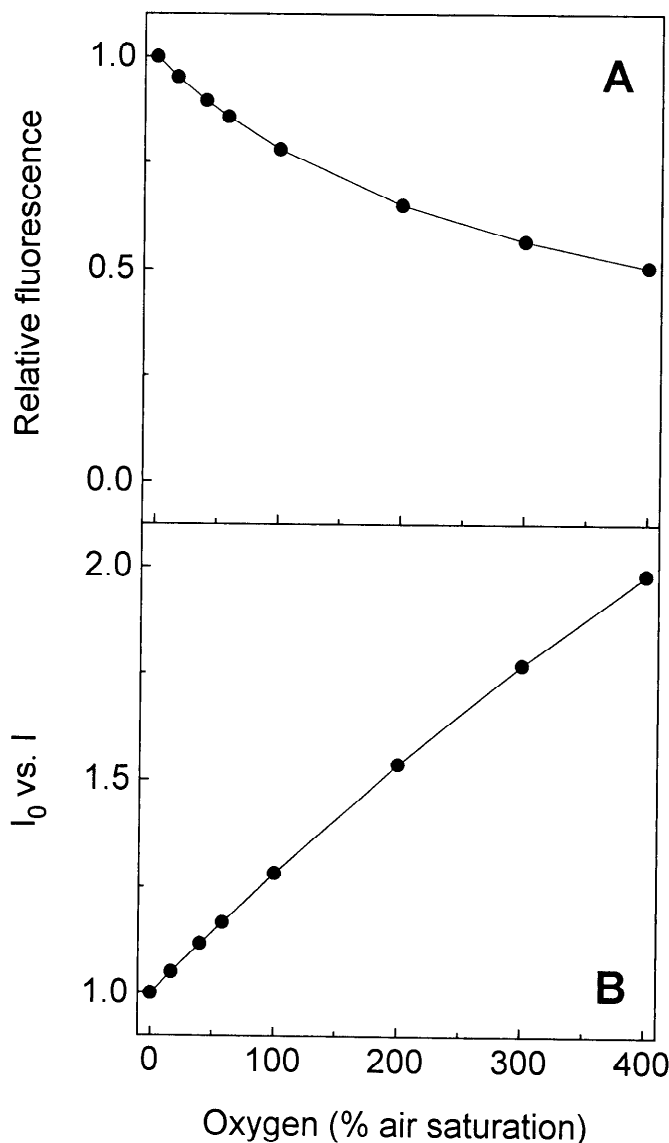


Fig. 3. Calibration curve (A) and Stern-Volmer plot (B) of an oxygen microoptrode by using Eq. 2 (*see text*), temperature 22°C.

in salinity, pH, and high concentrations of ionic fluorescence quenchers did not disturb the measurement.

Steady state oxygen profiles were measured in core samples of sandy coastal sediments by O_2 microoptrodes and with O_2 microelectrodes prepared in our laboratory according to Revsbech (1989). The microoptrodes were calibrated as described above, while a linear calibration of the O_2 microelectrode was done from readings in air-saturated water and in the anoxic part of the sediment. Oxygen profiles were measured both in the dark and during illumination with a 150-W halogen lamp (DC operated to avoid optical interferences). We found that microoptrode measurements with a spatial resolution of 50 μm were possible in the sediments investigated. The oxygen profiles showed a good agreement between the optrode and the electrode oxygen measurements (Fig. 5).

We have tested the microoptrodes in various North

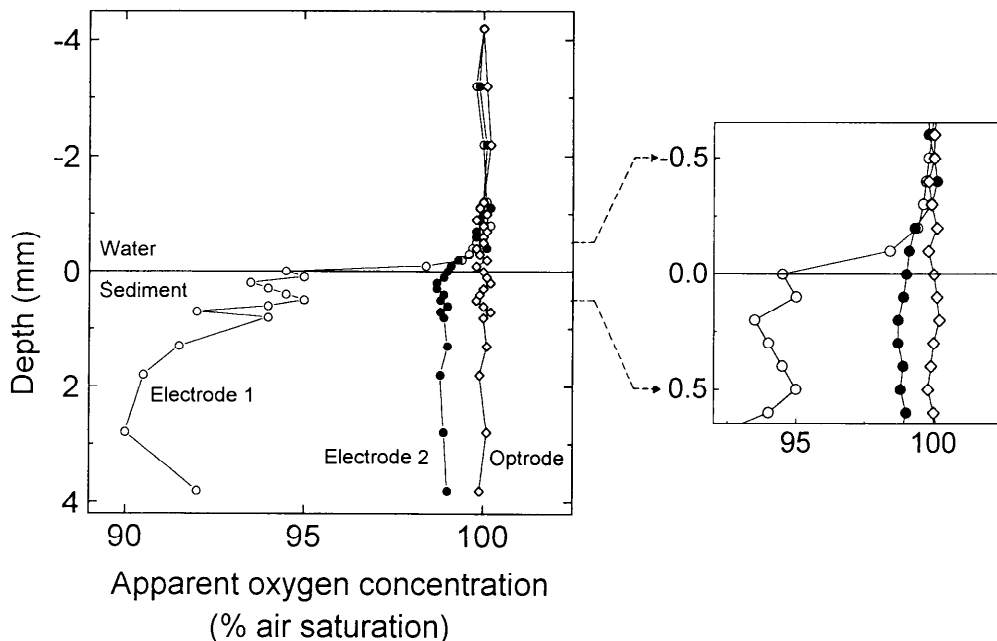


Fig. 4. Apparent oxygen profiles through a stirred-water phase down into an artificial sediment composed of autoclaved glass beads (40–70- μm bead diameter). All layers have the same real oxygen concentration. Measurements were made with microoptrode (\blacklozenge) and with O_2 microelectrodes with a low (\bullet) and high (\circ) stirring sensitivity.

Sea sediments, microbial mats, and in artificial sand with grain sizes ranging from 20 to 200 μm . Because of their silicone overcoat, the microoptrode sensing tips are flexible and do not break easily. Stable oxygen profiles were, however, difficult to measure in very cohesive microbial mats due to bending of the fiber tip and consequent change in the signal intensity. This microbending effect can be avoided by two different strategies. The first is to coimmobilize a second inert fluorophore in the sensor tip, with different excitation and emission wavelengths than the indicator, as an internal reference. This method requires a much more sophisticated optoelectronic system and additional optical components such as a second PMT and interference filter. In addition, photobleaching effects cannot be referenced in this way. A much more efficient method to eliminate the problems is to use the relation between the fluorescence lifetime of the indicator and the oxygen concentration instead of the fluorescence intensity (Lippitsch et al. 1988):

$$\frac{\tau_0}{\tau} = 1 + K_{\text{SV}} \cdot [\text{O}_2]. \quad (3)$$

This relationship is identical to Eq. 1 where the fluorescence intensity values are replaced by the fluorescence lifetime in the absence (τ_0) and presence (τ) of the respective oxygen concentration. Measuring the intensity independent fluorescence lifetime is the preferred way to develop a powerful optical oxygen meter. Such a sensing system is currently under development in our laboratory.

Our first results and experiences with the new fiber-

Table 1. Interferences of relevant chemical parameters in natural marine systems on the oxygen microoptrode signal.

Parameter	Signal change (%)
pH 2 (diluted HCl)	<1
pH 11 (diluted NaOH)	<1
Salinity (5% NaCl)	<1
CO_2 (100% saturation)	<1
H_2S (1 mM)	<1
Heavy metals (1 mM Fe^{2+})	<1

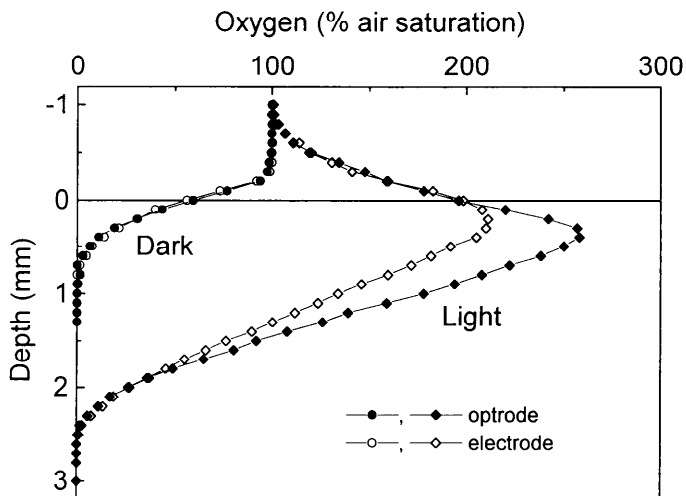


Fig. 5. Oxygen profiles measured in a coastal North Sea sediment with a O_2 microoptrode and a O_2 microelectrode.

optic O₂ microsensors have shown that they are an interesting alternative to oxygen microelectrodes. Concurrent measurements with oxygen microoptrodes and microelectrodes in marine sediments showed no significant difference between the profiles obtained. The spatial resolution of the microoptrode measurements is presently limited to 50 μm. The optoelectrical measuring system for use with the microoptrodes consists of small and inexpensive optical components and can easily be miniaturized for field use. The sensor fabrication is easy and does not require special training. For applications requiring a large number of microsensors, the use of optrodes thus offers advantages to electrodes. The oxygen microoptrodes shown exhibit favorable properties like stable calibration curves allowing long-term applications, no stirring sensitivity, and no interferences from H₂S or CO₂.

The use of optical measurement principles along the lines presented here is an interesting alternative for detecting other relevant compounds, like CO₂ and NH₃, which presently cannot be detected with sufficient spatial resolution and sensitivity by electrodes.

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References

- BERGMAN, I. 1986. Rapid response atmospheric oxygen monitor based on fluorescence quenching. *Nature* **218**: 396.
- GRUBER, W., I. KLIMANT, AND O. S. WOLFBEIS. 1993. Instrumentation for optical measurement of dissolved oxygen based on solid state technology, p. 448-457. *In Ocean Optics 12*, Proc. SPIE 1885.
- GUST, G., K. BOOIJ, W. HELDER, AND B. SUNDBY. 1987. On the velocity sensitivity (stirring effect) of polarographic oxygen microelectrodes. *Neth. J. Sea Res.* **21**: 255-263.
- KAUTSKY, H. 1939. Quenching of luminescence by oxygen. *Trans. Faraday Soc.* **35**: 216-219.
- KLIMANT, I., P. BELSER, AND O. S. WOLFBEIS. 1994. Novel metal-organic ruthenium(II) diimin complexes for use as longwave excitable luminescent oxygen probes. *Talanta* **41**: 985-991.
- KÜHL, M., AND B. B. JØRGENSEN. 1992. Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector. *Limnol. Oceanogr.* **37**: 1813-1823.
- , C. LASSEN, AND B. B. JØRGENSEN. 1994. Optical properties of microbial mats: Light measurements with fiber-optic microprobes, p. 149-166. *In* L. J. Stal and P. Coumme [eds.], *Microbial mats: Structure, function and environmental significance*. NATO ASI Ser. G, V. 35. Springer.
- LASSEN, C., H. PLOUG, AND B. B. JØRGENSEN. 1992. A fibre optic scalar irradiance microsensor: Application for spectral light measurements in sediments. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Ecol.* **86**: 247-254.
- LIPPITSCH, M. E., J. PUSTERHOFER, M. J. P. LEINER, AND O. S. WOLFBEIS. 1988. Fibre optic oxygen sensor with the fluorescence decay time as the information carrier. *Anal. Chim. Acta* **205**: 1-6.
- LÜBBERS, D. W. 1992. Fluorescence based chemical sensors, p. 215-260. *In* *Advances in biosensors*. V. 2. JAI.
- , AND N. OPITZ. 1975. The pCO₂/pO₂ optrode: A new probe for measuring pCO₂ and pO₂ of gases and liquids. *Z. Naturforsch.* **30C**: 532-533.
- PETERSON, J. I., R. V. FITZGERALD, AND D. K. BUCKHOLD. 1984. Fiber-optic probe for in vivo measurement of oxygen partial pressure. *Anal. Chem.* **56**: 62-67.
- REVSBECH, N. P. 1989. An oxygen microelectrode with a guard cathode. *Limnol. Oceanogr.* **34**: 474-478.
- , AND B. B. JØRGENSEN. 1983. Photosynthesis of benthic microflora measured with high spatial resolution by the oxygen microprofile method: Capabilities and limitations of the method. *Limnol. Oceanogr.* **28**: 749-756.
- , AND ———. 1986. Microelectrodes: Their use in microbial ecology. *Adv. Microb. Ecol.* **9**: 293-352.
- , J. SØRENSEN, T. H. BLACKBURN, AND J. P. LOMHOLD. 1980. Distribution of oxygen in marine sediments measured with microelectrodes. *Limnol. Oceanogr.* **25**: 403-411.
- , AND D. M. WARD. 1983. Oxygen microelectrode that is insensitive to medium chemical composition: Use in an acid microbial mat dominated by *Cyandinium caldarium*. *Appl. Environ. Microbiol.* **45**: 755-759.
- RHINES, T. D., AND M. A. ARNOLD. 1990. Determination of ammonia in untreated serum with a fiber-optic ammonia gas sensor. *Anal. Chim. Acta* **231**: 231-235.
- SCHAFFER, B., AND O. S. WOLFBEIS. 1989. A sodium selective optrode. *Microchim. Acta* **3**: 109-116.
- SILVER, I. A. 1965. Some observations on the cerebral cortex with a ultra-micro, membrane-covered, oxygen electrode. *Med. Electron. Biol. Eng.* **3**: 377-387.
- STERN, O., AND M. VOLMER. 1919. Über die Abklingzeit der Fluoreszenz. *Phys. Z.* **20**: 183-188.
- WEIGL, H. B., AND OTHERS. 1994. Optical triple sensor for measuring pH, oxygen and carbon dioxide. *J. Biotechnol.* **32**: 127-138.
- WHALEN, W. J., J. RILEY, AND P. NAIR. 1967. A microelectrode for measuring intracellular pO₂. *J. Appl. Physiol.* **23**: 798-801.
- WOLFBEIS, O. S. 1991. Fiber optic chemical sensors and biosensors. CRC.
- , M. J. P. LEINER, AND H. E. POSCH. 1986. A new sensing material for optical oxygen measurement with the indicator embedded in an aqueous phase. *Microchim. Acta* **3**: 359-366.
- ZHUIJUN, Z., AND W. R. SEITZ. 1984. A fluorescence sensor for quantifying pH in the range from 6.5 to 8.5. *Anal. Chim. Acta* **160**: 47-51.

Submitted: 31 October 1994

Accepted: 11 April 1995

Amended: 23 May 1995