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Optical measurement of oxygen and temperature in microscale: strategies and biological applications [1]

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Abstract

Sediments, microbial mats, biofilms and other microbial communities are characterized by steep gradients of physical and chemical parameters. Microsensors are powerful tools to measure these parameters with a sufficient spatial resolution and with a small disturbance of the micro-environment in natural systems. Recently, fiber-optical microsensors have been introduced in the field of aquatic biology as an alternative to existing electrochemical microsensors. Such micro-optodes have already been developed for high-resolution measurement of dissolved oxygen and for temperature measurements. They are easy to fabricate and show an improved long-term and storage stability. An overview is given on the development and characterization of different types of micro-optodes for oxygen and temperature. A luminescence lifetime-based device has been developed which is portable and enables microsensing both in the laboratory and under field conditions. Limitations in practical work with optical microsensors are demonstrated, and strategies to overcome them briefly discussed. A micro-optode array as well as a method for high-resolution oxygen imaging in sediments are presented as two different ways to investigate the two-dimensional oxygen distribution in heterogeneous living systems. Future applications and developments in micro-optode research will be discussed briefly.

Keywords: Oxygen micro-optodes; Temperature micro-optodes; Luminescence lifetime; Sensor arrays; Lifetime imaging

1. Introduction

Marine and freshwater sediments, microbial mats and biofilms are populated by dense communities of microalgae and other bacteria and are therefore characterized by steep gradients of physical and chemical parameters in the upper few millimeters. This includes light intensity, temperature, spectral distribution of light, O_2, pH, hydrogen sulfide, CO_2, and nutrients [1,2]. A knowledge of their microscale distribution is of paramount importance in understanding the function and regulation of such communities [1]. The use of microsensors is the best way to measure gradients of physical and chemical parameters with suffcient spatial resolution ($<50\ \mu m$). Such microsensors have very small tip diameters (5 to 50 μm), which enable an almost non-invasive measurement to be made in intact biological systems.

Microelectrodes for various analytes have been developed over the last 20 years and have been successfully applied to various aquatic systems. Oxygen microelectrodes are applied most frequently, but microsensors for the measurement of pH, sulfide, nitrate and nitrous oxide or physical parameters such as scalar irradiance have been used as well for detailed measurements in sediments and biofilms [3–8].

A disadvantage that limits a more frequent use of microelectrodes is their time-consuming fabrication. In contrast, optodes are simple to design and therefore offer an interesting alternative for chemical sensing with high spatial resolution.

Different optical-sensing schemes exist which are potentially useful for developing optical microsensors for such relevant metabolic products as carbon dioxide and ammonia. The optode technique offers a way for simultaneous determination of various relevant chemical and physical parameters with a single microsensor. Such sensors are essential to calculate the total concentration of inorganic carbon and sulfide in sediments from data for the acidic gases carbon dioxide and hydrogen sulfide and the pH value.

Tan et al. have introduced fiber-optical submicroprobes for intracellular pH and oxygen measurement with an immobilized sensing chemistry at the tip of pulled optical fibers [9,10] having a tip diameter of less than 1 μm. However, for measurement in harsh sediments or other complex biological systems, such probes are not useful, since they are too fragile

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due to their very small size. In addition, the optical fiber guides the excitation light only, whereas fluorescence was detected externally with a photomultiplier lens system mounted at the microscope table. The blue argon ion laser used as the light source makes it difficult to design a portable device for field studies.

We have introduced oxygen micro-optodes in the field of aquatic biology as an alternative to existing Clark-type oxygen micro-electrodes [11,12]. These sensors are simple to fabricate and show an improved long-term stability and storage stability compared to micro-electrodes.

Yet another motivation for our optode work was to combine optical sensing with imaging techniques in order to obtain two-dimensional microscale measurements of chemical parameters by use of planar optodes. The following gives an overview of this new field of optode research and examples of applications to natural systems are given.

2. Oxygen micro-optodes

2.1. Relevance of fine-scale oxygen measurement

Dissolved oxygen is one of the key parameters in biological systems, and is either produced by autotrophic photosynthesis in the presence of light, or consumed via different respiration processes. The oxygen production or uptake of a sediment layer can be calculated from the measured microprofile in the diffusive boundary layer via the first Fick law. The diffusive boundary layer covers the sediment with a typical thickness of about 0.5 mm.

In sediments or biofilms which are frequently populated by dense bacterial communities, the oxygen concentration can vary from hypersaturation to fully anaerobic conditions only in the upper few millimeters. Oxygen micro-optodes with a tip diameter of $\approx$10-30 μm are therefore required, which are characterized by a moderate sensitivity, to enable the measurement of oxygen within the full dynamic range from 0.1 to 100% oxygen saturation with a sufficient spatial resolution.

2.2. Selection of indicators and polymers

Optical oxygen sensors most frequently are based on dynamic quenching of luminescence. The luminescence of the oxygen indicator which is suitable for designing micro-optodes should be excitable by light-emitting diodes (LEDs) and should have luminescence lifetimes >1 μs. This is necessary for a potential realization of a compact lifetime-based measuring system. The indicator should be photostable, since the light density at the tip of the tapered optical fiber can be very high. Non-solubility in water is another essential property in order to minimize dye leaching. A high solubility in hydrophobic polymers is necessary to obtain a sufficiant signal intensity.

In the last few years various oxygen-quenchable luminophores have been presented for use in oxygen optodes [13–18]. From our point of view, highly luminescent ruthenium(II) complexes with diimine ligands and phosphorescent platinum and palladium porphyrins are the most useful oxygen indicators for designing micro-optodes. Table 1 summarizes selected sensing materials which have been found useful for designing micro-optodes for different applications. The ruthenium(II)-tris-4,7-diphenyl-1,10 phenanthroline-perchlorate ($Ru[diph]_3$) fulfils almost all of the desired requirements and therefore has been selected for designing micro-optodes [19]. The absorption spectrum of $Ru[diph]_3$ perfectly overlaps the emission spectrum of bright blue LEDs with their peak emission at 450 nm. $Ru[diph]_3$ dissolves in hydrophobic polymers such as polystyrene in concentrations up to 10 mM, which results in high signal intensities. In addition, dissolved in hydrophobic polymers its displays an extraordinarily high photostability. Therefore $Ru[diph]_3$ is the preferred indicator for intensity-based oxygen measurements.

Phosphorescent palladium and platinum porphyrins have been evaluated as indicators for oxygen micro-optodes as well. They show strong room-temperature phosphorescence with quantum yields up to 60% and have been frequently used in optical oxygen sensing [18,20]. The phosphorescence of porphyrin-based indicators is highly susceptible to quenching by oxygen. It is possible to embed them in most polymeric matrices, yielding oxygen-sensitive materials. This gives a high flexibility in selection of the optimal polymer to design efficient micro-optodes. The platinum and palladium porphyrins can be excited by either blue or blue-green LEDs or even yellow or orange LEDs. As their photostability is inferior to that of $Ru[diph]_3$, they are less suitable for intensity-based micro-optodes. On the other hand, their long luminescence lifetimes makes them ideally suited for lifetime-based oxygen sensing. Platinum(II)–octaethylporphyrin (Pt–OEP) [20] and platinum(II)–octaethylketoporphyrin (Pt–OEKP) [1] are insoluble in water but well soluble in hydrophobic polymers.

When deposited on the tip of micro-optodes, the matrix must be loaded with a high concentration of the indicator, since the sensing spot on the fibre tip is extremely small and the luminescence signal relatively low. Furthermore, the coating should adhere very well to obtain mechanically stable micro-optodes. It is essential because the sensors are applied in systems such as course sandy sediments or cohesive microbial mats, where strong shearing forces act on the sensing tip when penetrating the sample. Coatings made of soft polymers such as silicone or plasticized poly(vinyl-chloride) (PVC) are not stable in such systems. Ethyl cellulose and cellulose acetate in turn exhibit significant water uptake and, consequently swell.

Mechanically stable micro-optodes were obtained if the indicators were dissolved in rigid hydrophobic polymers like polystyrene (PS) or poly(methyl-methacrylate) (PMMA). Organically modified sol-gels (ormosils) also appear to be

Table 1
Composition and properties of selected sensing materials which have been found useful as coatings for oxygen micro-optodes

Indicator/matrix	Signal	Response time (t_{90})	Sensitivity [f]	τ_0 [g]/modulation frequency	Ex/Em (nm) / used LED	Comments
Ru[diph]$_3$/PS [a]	very high	<2 s	22%	5 μs/45 kHz	450/600 blue	excellent photostability moderate sensitivity
Ru[diph]$_3$/plasticized PVC	very high	<200 ms	50%	5 μs/45 kHz	450/600 blue	useful for monitoring fast oxygen dynamics poor mechanical stability limited storage stability due to plasticized leaching
Pt–OEP [c]/PS	high	<2 s	80%	90 μs/5 kHz	400, 535/640 blue	high sensitivity (not useful for high oxygen conc.) moderate photostability
Pt–OEP/PMMA [b]	high	<5 s	35%	90 μs/5 kHz	400, 535/640 blue	optimal sensitivity to cover the whole dynamic range of oxygen
Pt–OEKP [d]/PS	moderate	<2 s	75%	60 μs/8 kHz	400, 592/760 blue, yelow	more photostable than Pt–OEP excitation with yellow LEDs avoids background fluorescence of the optical components
Pd–OEP [e]/PS	moderate	<2 s	98%	900 μs/500 Hz	420, 545/670 blue	poor photostability very high sensitivity allows detailed investigations on nearly an aerobic conditions

[a] Polystyrene.
[b] Poly(methyl-methacrylate).
[c] Platinum(II)–octaethylporphyrin.
[d] Platinum(II)–octaethylketoporphyrin.
[e] Palladium(II)–octaethylporphyrin.
[f] Signal loss if changes from nitrogen to air.
[g] Luminescence lifetime in absence of oxygen.

promising matrices and their use in micro-optode techniques is currently under investigation.

2.3. Micro-optode fabrication

Multimode silica fibers with a core diameter of 100 μm have been found to be most suitable for micro-optode fabrication. Polymer-cladded multimode glass fibers would allow an efficient incoupling of light from LEDs due to their high numerical aperture. On the other hand, they cannot be tapered, since the optical cladding is damaged during the heating step. Single-mode fibers are useful only in the case of laser light sources. The intensity of light from an LED that can be coupled into a monomode fiber is too low.

The micro-optodes were fabricated using a preparation procedure described in detail elsewhere [11]. Fiber tips with a diameter up to 10 μm were prepared by tapering the optical fibers in the flame of a small torch. Alternatively, a fiber puller may be used to prepare the fibre tips [9]. The oxygen-sensitive coating was deposited by dipping the fiber tip into the polymer solution and subsequently evaporating the solvent. Micro-optodes used for intensity-based measurements must be coated with an additional layer of black silicone to suppress any optical effects from the surrounding sample. In a last step, the optical fiber is fixed in a glass capillary or in an injection needle for more convienient handling of the microsensors. This is a general fabrication scheme which may easily be adapted to the preparation of other micro-optodes. A cross section through an oxygen micro-optode (as well as temperature micro-optodes) is shown in Fig. 1.

2.4. Optical setup

The measuring system for working with oxygen micro-optodes is shown in Fig. 2. A fiber coupler was found to be

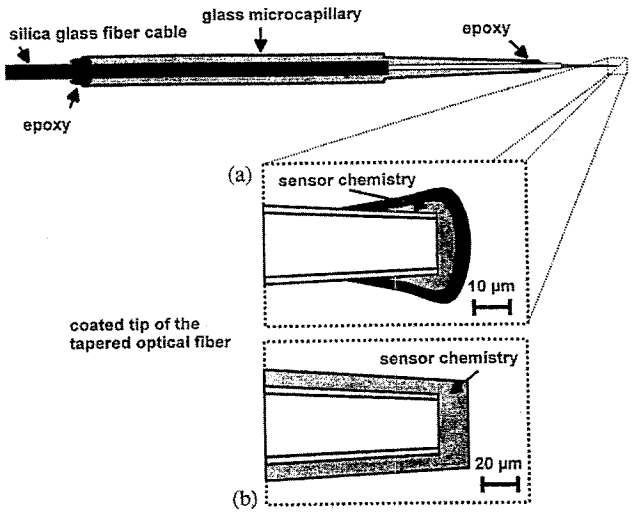

Fig. 1. Designs for micro-optodes: [a] a tapered optical fiber is coated with an oxygen- or temperature-sensitive material (if the luminescence intensity is measured, the sensing layer is covered by an additional layer of black silicone); [b] an optical fiber is inserted in a tapered glass capillary, which is filled with a temperature-sensitive indicator solution.

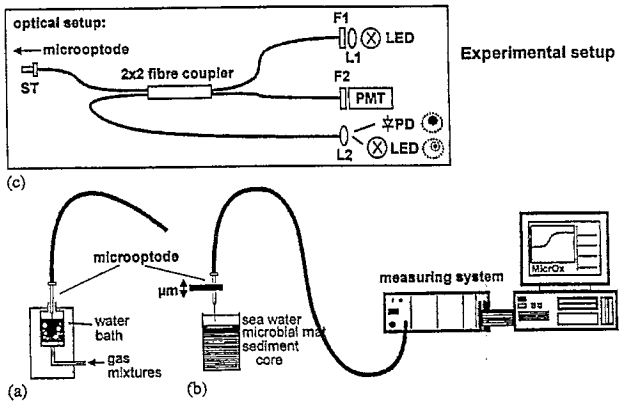

Fig. 2. Schematic drawing of the optical unit and measuring setup used for working with oxygen or temperature micro-optodes.

ideally suited for separating luminescence from backscattered excitation light. Such couplers are commercially available, relative cheap, and well specified due to their use in telecommunications. It is mandatory to select fiber couplers of low intrinsic fluorescence under illumination with blue LEDs.

LEDs are the preferred light sources due to their small size, low power consumption, negligible heat production and the ease of electronic modulation. Bright LEDs with colors ranging from blue to orange are commercially available and an efficient incoupling of light into the core of the optical fiber is not a problem any longer. At the fiber taper light is focused onto the sensing tip and this results in efficient excitation of the immobilized indicator. Depending on the respective oxygen indicator used, bright blue LEDs (for $Ru[diph]_3$, Pt–OEP, Pd–OEP) or yellow LEDs (for Pt–OEKP) were selected as excitation sources. Combinations of gelatin filters or glass filters were found to separate the backreflected excitation light adequately from luminescence.

At this time the luminescence is detected with a compact red-sensitive photomultiplier module, but work is in progress to replace it by a photodiode. As a result, a portable oxygen meter based on measurement of luminescence intensity was developed for use along with micro-optodes. It was successfully tested under laboratory conditions as well as in the field. Since intensity measurements suffer from a number of disadvantages (which include photobleaching of the indicator, effects on the scattering properties and color of the sample, fiber bending and drifts in the electronic system), a luminescence lifetime-based instrument was developed and successfully introduced [21]. The system is based on an optical unit very similar to the intensity-based device, but with a phase-modulation technique being used in order to measure the luminescence lifetime [22]. As expected, the performance of such an instrument is superior to that of the old intensity-based system.

2.5. Fine-scale oxygen measurements

The micro-optodes were successfully tested in various biological systems. For example, typical steady-state oxygen

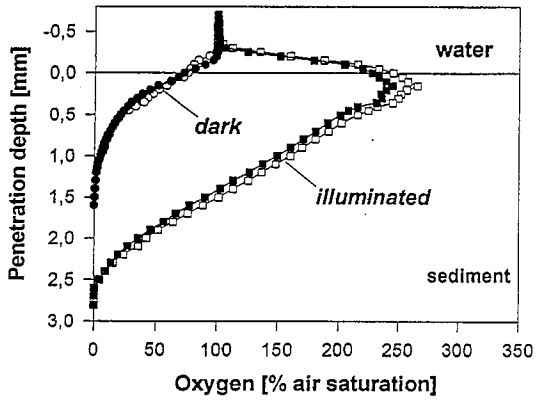

Fig. 3. Typical oxygen profiles in the dark and under strong illumination in a coastal marine sediment. Measurements were performed simultaneously with an opxygen micro-optode (open symbols) and an oxygen micro-electrode (closed symbols); both sensing tips had a separation of 50 μm.

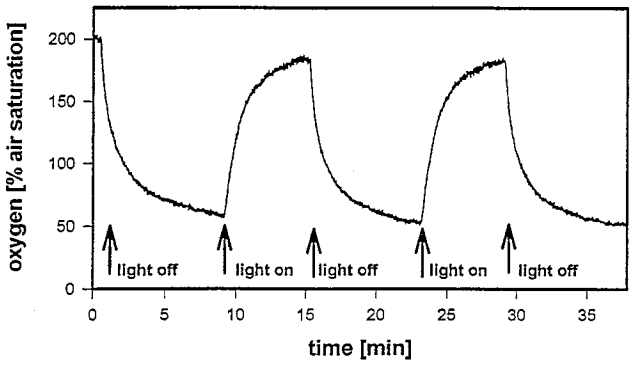

Fig. 4. Study of the oxygen dynamics in a phototrophic layer of cyanobacteria growing on the top of a coastal marine sediment, during alternate light–dark cycles; measurement was performed with a fast-responding micro-optode ($t_{90} \approx 1$ s).

profiles were measured in a core sample of a coastal sandy sediment populated by phototrophic microalgae and are shown in Fig. 3. The profiles were measured with a combined sensor (where an oxygen micro-optode was fixed at the tip of an oxygen micro-electrode). The distance between the two sensors was ≈ 50 μm. The oxygen profiles from the optode and the respective electrode measurement agree quite well. The production of oxygen in the upper 1 mm of the sediment layer in the presence of light (due to photosynthesis), as well as the consumption of oxygen in the dark (due to respiration), is obvious from the measured profiles.

The oxygen dynamics in the photosynthetically active layer of such a sediment during alternating light–dark cycles may also be measured with the micro-optodes, as shown in Fig. 4. Such measurements give information on the rates of photosynthesis and the respiratory activity. The new micro-optodes were also used to study photosynthesis and respiration in lichens populated with cyanobacteria under in situ conditions. Fig. 5 shows typical oxygen gradients in lichens growing on the bottom of a water-filled rockpool. Measurements were performed at different illumination intensity over the day. These field studies were performed with a portable battery-operated and intensity-based oxygen meter. It was

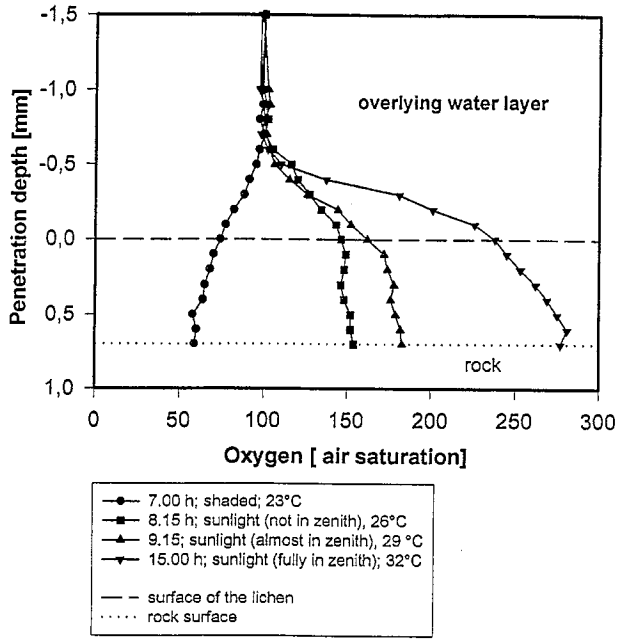

Fig. 5. In situ measurement of oxygen profiles measured at different light conditions, i.e., different times of day, in lichens growing at the bottom of a water-filled rockpool. The micro-optode measurements were performed outdoors under field conditions.

also employed under harsh field conditions. Currently the oxygen micro-optodes are being adapted on free-falling underwater vehicles to measure oxygen gradients in situ in deep-sea sediments. For this application it is first necessary to study the response of oxygen optodes at high hydrostatic pressures.

Oxygen sensors based on measurement of light intensity have certain practical limitations. Bending effects, for example, change the signal. If the sensing tip penetrates a rigid or very cohesive material, the microbending of the fibre tip may invalidate the measurements. Furthermore, the black isolating coating that is necessary to exclude optical interferences from the heterogeneous sample (caused, for example, by chlorophyll fluorescence, scattering effects, etc.) can decrease the signal intensity significantly. Micro-optodes coated with black silicone and with a tip diameter smaller than 20 μm show a too low signal and do not allow measurements to be made with sufficient accuracy. Oxygen measurements with a lifetime-based device will overcome these disadvantages and are the preferred technique for future measurements. However, the lack of an optical isolation can cause another problem. In phototrophic communities the excitation light can stimulate photosynthesis at the sensing tip, causing a change in the oxygen conditions. Strategies to overcome this problem could be new oxygen indicators, excitable with NIR LEDs, or a proper time regime to reduce the photosynthesis effect.

3. Temperature micro-optodes

Temperature may affect metabolic as well as chemical turnover rates in biological systems. The knowledge of the

microdistribution of temperature can give important information about the microbial metabolism in different layers. If chemical parameters are to be measured with microsensors, temperature gradients would cause errors in the measurement due to the lack of temperature compensation of the signal. Currently no microsensors exist which allow the measurement of temperature gradients in complex microbial communities with a sufficient spatial resolution.

Temperature optodes which measure the luminescence lifetime of certain phosphors are known and also commercially available, but are not optimal to design temperature micro-optodes [23]. Demas and DeGraff suggested the use of ruthenium(II)complexes with a strongly temperature-dependent quantum yield as well as of the luminescent lifetime for temperature sensing [24]. The ruthenium(II)-tris-1,10-phenanthroline complex ($Ru[phen]_3$) was selected as a promising temperature indicator, since dissolved in water its luminescence lifetime is strongly affected between 0 and 50°C ($\approx 2.5\%\ °C^{-1}$). Two designs of temperature micro-optodes were presented [25]. The cross section of both microsensors is shown in Fig. 1. In the first a tapered and sealed glass capillary is filled with a deaerated solution of $Ru[phen]_3$ and an excess of sodium dodecylsulfate. A tapered optical fiber is then introduced into the tip of the glass capillary to measure the luminescence. Sodium dodecylsulfate was added to dissolve the indicator in a micellar form, giving a higher quantum yield as well as a longer luminescence lifetime. Sodium sulfite was added to remove oxygen. This micro-optode is characterized by a very high temperature coefficient (Fig. 6) and has the advantage that chemical species do not interfere since they cannot penetrate through the glass. Unfortunately the design limits the miniaturization of the sensors down to a size $\approx 35\ \mu m$.

The design of the second type of temperature micro-optodes is identical to that of the oxygen micro-optodes and based on tapered optical fibers coated with a thin temperature-sensitive film of PVC which contains the dissolved $Ru[phen]_3$ complex. In this matrix the indicator exhibits only a negligible cross sensitivity to oxygen. Micro-optodes based on this concept allow miniaturization down to 10 μm. Unfortunately $Ru[phen]_3$ in PVC has a significantly lower temperature coefficient (Fig. 6). Temperature measurements were performed with the device already developed for oxygen micro-optodes [21].

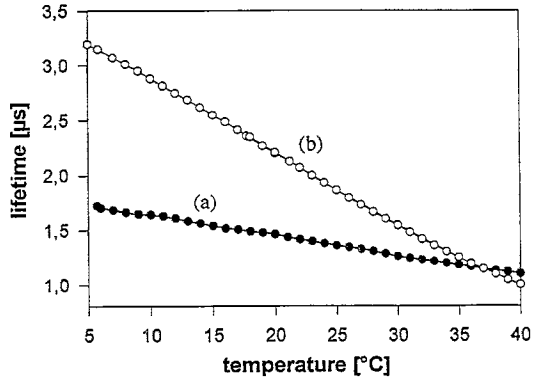

Fig. 6. Calibration curves (temperature vs. luminescence lifetime) of selected temperature micro-optodes: (a) $Ru[phen]_3$ dissolved in PVC; (b) $Ru[phen]_3$ micellar dissolved in an aqueous sodium dodecylsulfate solution (deaerated by adding 1% of sodium sulfite).

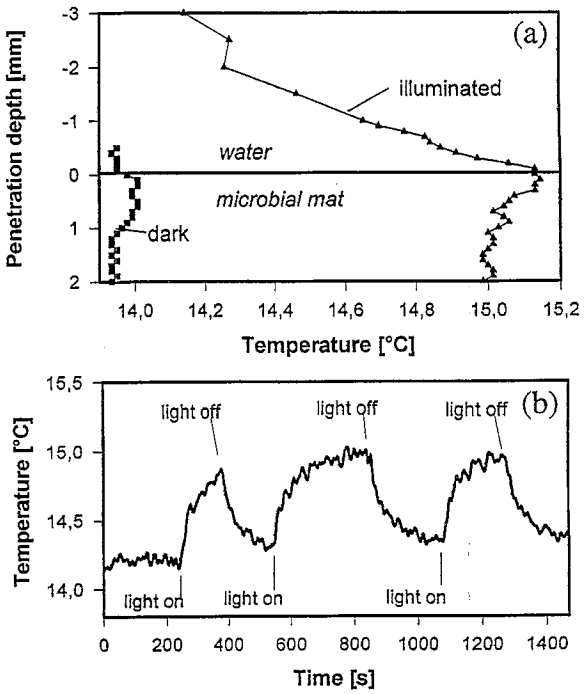

Fig. 7. Temperature gradients in the dark and under strong illumination in a microbial mat (a), and study of the temperature dynamics in the phototrophic layer of a strong light-absorbing cyanobacteria community during alternate light–dark cycles (b). Both data sets were measured with a temperature micro-optode of type B (see Fig. 1[B]).

An example of measurement of temperature gradients in a dense light-absorbing microbial mat is given in Fig. 7(a). A significant increase of temperature in the biological system was observed during illumination with the visible light of a cold light source. Fig. 7(b) shows the temperature dynamics in the same microbial mat during alternating light–dark cycles.

4. Oxygen micro-optode array

The spatial distribution of many chemical and physical parameters in natural systems is characterized by significant vertical as well as horizontal heterogeneity. Therefore, the measurement of a single oxygen profile often gives no representative information on the oxygen conditions in the whole system. In addition, the oxygen uptake or production of a larger sediment can normally not be inferred from a single (or a few) oxygen profiles. Measuring several profiles to describe the temporal and spatial heterogeneity is tedious and does not allow studies of the dynamics of the oxygen distribution over time. Simultaneous measurements of oxygen gradients at several points require a series of microelectrodes

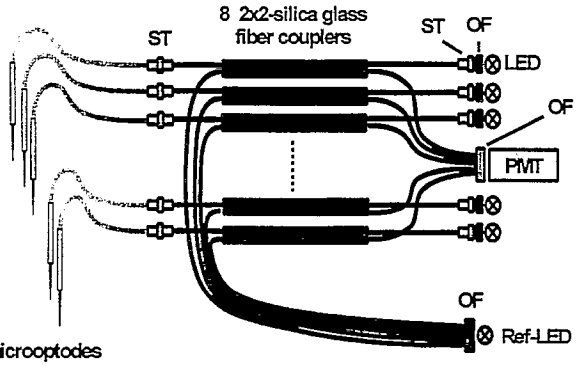

Fig. 8. Optical unit of a device designed for measurements with a micro-optode array; each sensor is illuminated via a separate LED/fiber coupler assembly, whereas a single PMT was used for detection of the luminescence signal of all micro-optodes.

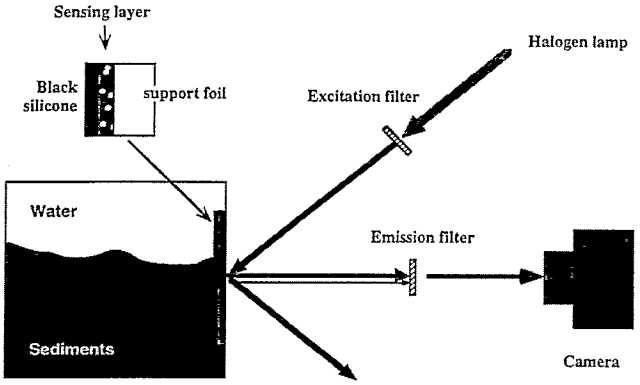

Fig. 9. Illustration of the experimental setup to measure the two-dimensional oxygen distribution in a sediment by an imaging technique (not to scale); a cross section of the sensing foil is shown in the upper left corner (mylar support, sensing layer, black optical isolation).

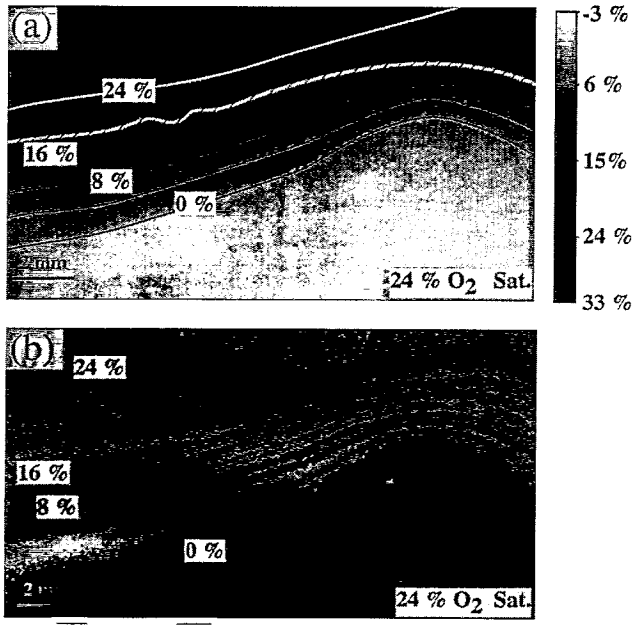

Fig. 10. Example of a high-resolution image of the oxygen distribution at the sediment/water interface of a marine sediment. The dashed line indicates the sediment surface. The respective oxygen concentrations are expressed on a linear grey scale.

with associated recording devices, which is expensive and in most cases impractical due to the considerable effort necessary for the fabrication of microelectrodes that work well.

The presented oxygen micro-optodes are easy to prepare and measurements of oxygen gradients with several sensors simultaneously are relatively easy, provided that the signals of several oxygen microsensors can be recorded simultaneously with a single opto-electronic device.

A multichannel instrument was developed which allows the measurement of oxygen with eight micro-optodes simultaneously, but it may be easily extended for working with even more sensors. The opto-electronic unit of the system is almost identical to the previously described system for lifetime-based oxygen sensing with micro-optodes [21]. The optical system was, however, extended by using a separate LED as light source and a separate fiber coupler for each micro-optode (Fig. 8). A more detailed description of the design and the performance of an oxygen micro-optode array is given elsewhere [26]. It was tested for the simultaneous measurement of oxygen gradients in a microbial mat and for time-series measurements of oxygen in a diffusion chamber [26]. Yet another advantage of the array is the possibility to calibrate and characterize multiple micro-optodes simultaneously.

5. High-resolution oxygen imaging

Luminescence imaging is another possibility to measure the two-dimensional oxygen distribution and dynamics in heterogeneous communities, e.g., around inhabited animal burrows and in heavily bioturbated communities. An imaging method using oxygen-sensing foils and a CCD camera-based setup was recently introduced by Glud et al. [27]. A scheme of the setup is shown in Fig. 9. The oxygen-sensitive film was glued inside the wall of a small aquarium which was filled with a natural sandy sediment, populated with heterotrophic respiring bacteria. The luminescence of the sensing film was excited by the blue light of a halogen lamp equipped with a cut-off filter and luminescence was detected with a computer-controlled CCD camera system equipped with a long-pass filter. The system is luminescence intensity based, but a system that measures the luminescence lifetime instead of the intensity is under development. A three-point calibration procedure was required to calculate the respective oxygen concentrations and was performed before the sediment was put into the aquarium. Fig. 10 shows a typical two-dimensional oxygen distribution at the sediment–water interface within the upper few millimeters of the sediment. More detailed information on the measuring system and its application in sediments is given elsewhere [27].

6. Conclusions

New fiber-optic oxygen microsensors, micro-optodes, offer a good alternative to existing oxygen micro-electrodes.

The fabrication of the sensors is simple and does not require special training. For applications requiring a large number of microsensors, optodes offers advantages over electrodes. Oxygen micro-optodes show stable calibration curves and no interference by hydrogen sulfide. The temperature micro-optodes open a way for fine-scale measurements of the temperature distribution in sediments and biofilms.

Activities to develop micro-optodes for other relevant parameters like pH, carbon dioxide and hydrogen sulfide are still in progress. It is also planned to design sensors that allow the simultaneous measurement of several parameters with a single microsensor.

Luminescence imaging is a powerful method to measure the two-dimensional distribution of chemical parameters with planar foils and was successfully applied for fine-scale two-dimensional oxygen sensing in sediments.

The micro-optodes presented in this overview are the first true optical microsensors allowing the measurement of environmental parameters directly in complex biological systems with high spatial resolution and negligible disturbance of the microenvironment. It is obvious that such micro-optodes will find important future applications in many areas of environmental and biotechnological research.

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Biographies

Ingo Klimant, born in 1964, graduated in analytical chemistry at the Mining Acadamy, Freiberg, Germany in 1990. In 1993 he received a Ph.D. in chemistry from the Karl-Franzens

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Michael Kühl, born in 1964, studied biology at the University of Aarhus, Denmark, where he received an M.Sc. degree in microbial ecology in 1988 with a thesis on the development of fiber-optic microprobes and a measuring system for microscale light measurements in sediments and biofilms. From 1988 to 1992 he completed his Ph.D. at the Department of Microbial Ecology, University of Aarhus, Denmark. His Ph.D. work involved the use of both optical and electrochemical microsensors to study the microenvironment in compact microbial communities. In 1992 he joined the Max-Planck Institute for Marine Microbiology, to build up the microsensor research group. Since 1995 he has been heading the Microsensor Research Group. His scientific interests are in the development and use of fiber-optic and electrochemical microsensors in microbial ecology.

Ronnie N. Glud, born in 1963, studied biology at the University of Aarhus, Denmark, where he received an M.Sc. degree in microbial ecology in 1990 with a thesis on the application of microsensors in photosynthetic biofilms. From 1990 to 1993 he completed his Ph.D. at the Department of Microbial Ecology, University of Aarhus, Denmark. His Ph.D. work focused on in situ studies of oxygen dynamics and early diagenetic processes in marine sediments. In 1993 he joined the Max-Planck Institute for Marine Microbiology as a post-doctoral fellow. His scientific interest are in situ studies of benthic mineralization with special emphasis on oxygen dynamics.

Gerhard Holst, born in 1962, studied electronic engineering at the RWTH University in Aachen, Germany, where he received the diploma in 1990 with a final work about reflectance pulse oximetry with electrooptical and hybrid fiber-optical sensors. From 1991 to 1994 he completed his Ph.D. in the group of Professor Lübbers at the Max-Planck Institute for Molecular Physiology, Dortmund, Germany, about a new optical sensing principle, the flux optode, and its phase-modulation-based measuring system. In 1994 he joined the Microsensor Research Group of the Max-Planck Institute for Marine Microbiology as a post-doctoral fellow, where he is currently working on the development of new fiber-optic microsensors and on the development of measuring systems for laboratory and field applications.