A Novel Measuring System for Oxygen Microoptodes based on a Phase Modulation Technique

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ABSTRACT

New fibre optic oxygen microsensors (microoptodes) for use in aquatic environments have recently been developed as an alternative to commonly used Clark-type oxygen microelectrodes. The microoptodes have the advantage of no oxygen consumption and no stirring sensitivity combined with a simple manufacturing process of the sensors. To avoid problems inherent to luminescence intensity measurements like photobleaching, signal dependency on the optical properties of the surrounding medium and system drifts, a novel measuring system was developed. This system uses a phase modulation method to evaluate a signal phase shift that is caused by the oxygen dependent luminescence lifetime. The measuring system is based on simple solid state technology. High reliability and low costs of the system can therefore be combined with the ability of miniaturisation and low power consumption. The system consists of three units: 1) the microoptode with the optical setup (glass fibre couplers, optical filters, lenses, light source (light emitting diode) and light detection (photo multiplier tube)), 2) the analogue signal processing unit, including a special phase detection module and, 3) the digital signal processing unit, a personal computer or a microcontroller for control of the measuring system, display and data storage. First measurements of oxygen depth profiles in sediments and biofilms at high levels of ambient light demonstrated the advantages of phase shift based O_2 measurements as compared to intensity based measurements with microoptodes.

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1. INTRODUCTION

To understand the metabolic processes and transport phenomena in biofilms and sediments it is necessary to measure physical and chemical parameters near to and within these microbial communities. As these systems exhibit steep gradients of physical and chemical parameters over distances ranging from a few hundred µm to a few mm the sensors must be very small to ensure a maximum spatial resolution and a minimum disturbance of the investigated system. One established method is the use of microelectrodes[9] that enable measurements of chemical parameters at a spatial resolution of <50 µm. Microelectrodes are applied e. g. for the measurement of oxygen but they can be difficult to use due to their oxygen consumption, their stirring sensitivity and their complex manufacturing process[10]. To overcome these problems new fibre optic oxygen microsensors were recently developed for use in aquatic environments[4]. The oxygen microoptode uses the well known effect of dynamic quenching of luminescence by oxygen to measure the concentration of oxygen[1,2,3,5,6,8]. As the oxygen quenching changes both the luminescence intensity and the luminescence lifetime, there are two parameters that can be used for evaluation[1,2,3,5,6,8]. First results obtained with oxygen microoptodes connected to a luminescence intensity measuring system have demonstrated some disadvantages inherent to this type of measurements[4,7]. Especially if the microoptode is not optically insulated, the signal strongly depends on the optical properties of the surrounding medium, e.g. ambient light, refraction index and turbidity. Therefore a new measuring system was developed to obtain the luminescence lifetime information by a phase modulation technique[3,5,6].

2. SENSOR

The microoptode consists of a multimode step index quartz glass fibre (100/140 µm) which is tapered at the measuring end to a diameter of 10 - 30 µm. The fibre tip is dipcoated with a thin sensor layer consisting of a luminescent indicator (ruthenium(II)-tris-4,7-diphenyl-1,10-phenantroline perchlorate) immobilised in polystyrene. The fibre optic microsensor is connected via a standard ST-fibre connector to the optical setup of the measuring system (Fig. 1c). More details about construction and performance of the oxygen microoptodes are given elsewhere[4,7].

3. MEASURING SYSTEM

3.1. Measuring principle

The dynamic quenching of luminescence is used to measure the oxygen at the tip of the microoptode. In the case of collisional quenching of molecular oxygen the quencher has two influences on the luminophore: the emitted light intensity is decreased and the lifetime is shortened. For the immobilised luminophore the relation between luminescence intensity and lifetime towards oxygen concentration is best described by a modified SternVolmer equation[4]. If a luminophore is excited with sinusoidally modulated light it will emit sinusoidally modulated light but with a certain delay. This delay, called the phase angle, Φ, between excitation and emission, is determined by the lifetime, τ, with the following relation[3,5,6]:

$$\tan(\Phi) = 2\pi \cdot f_{mod} \cdot \tau \qquad (1)$$

(Φ - phase angle, f_{mod} - modulation frequency, τ - lifetime of luminophore)

The detection of the phase angle instead of a direct lifetime measurement is favourable because it is easier to reduce the bandwidth of the measuring system and thereby improve the signal to noise ratio. For the design of such a phase modulation measuring system the choice of a proper modulation frequency is necessary because the non-linear nature of the tangens function strongly limits the useful range of phase angles. If the upper and lower lifetime of an indicator for the corresponding lower and upper concentration of the analyte is known, the following equation for the optimum modulation frequency can be derived:

$$f_{opt} = \frac{1}{2\pi \cdot \sqrt{\tau_1 \cdot \tau_2}} \qquad (2)$$

(f_{opt} - optimum modulation frequency, τ_1, τ_2 - upper and lower lifetime of the luminophore at the corresponding concentrations of the analyte)

However, this calculated f_{opt} is only a first estimate like the also used arithmetic average value of τ_1 and τ_2[5]. Due to strong interactions of the luminophore and the surrounding matrix, the indicator changes its properties when immobilised. The decay curve of an immobilised indicator is often best described with

two or more lifetimes, whereas the same indicator in a pure solution may show ideal behaviou[r8]. Consequently the modulation frequency must be experimentally optimised to obtain the optimum combination of sensor and measuring system.

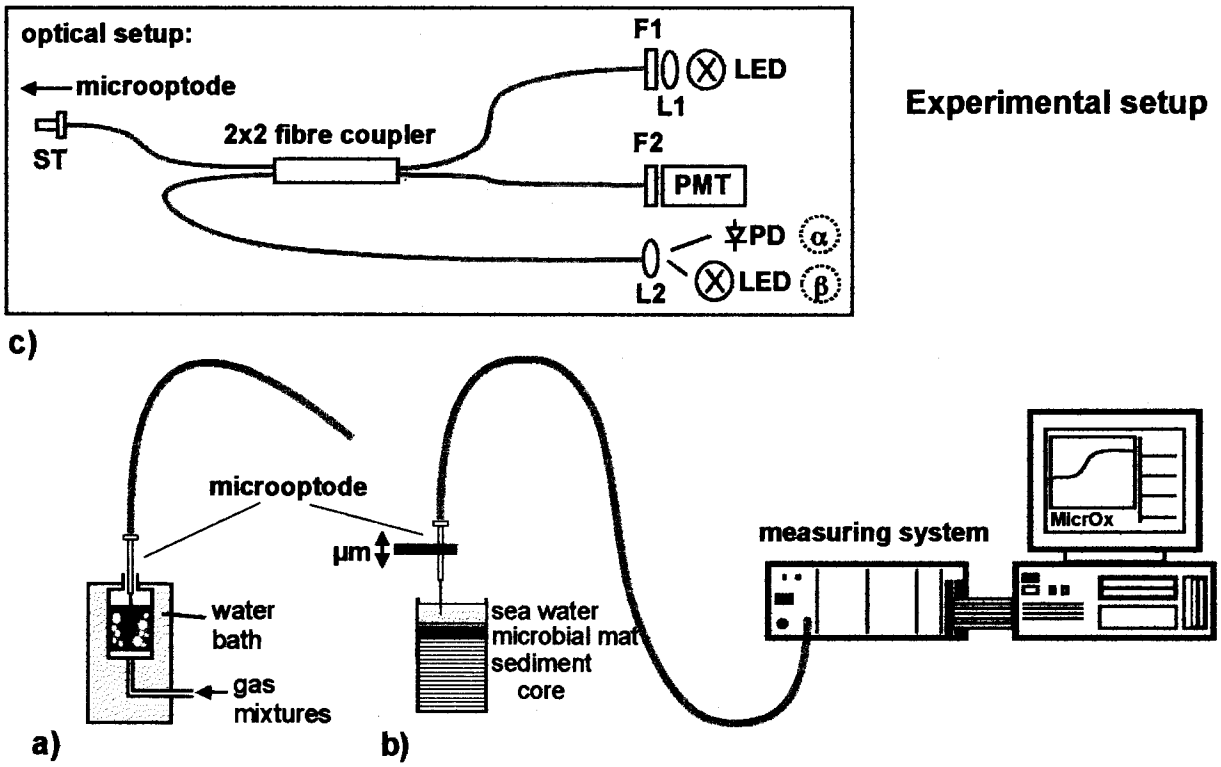

Fig. 1: Experimental setup (schematic drawing).
a) The microoptode is fixed in a water filled calibration vessel that is externally thermostated water bath. Through the bottom controlled gas mixtures of nitrogen and oxygen perfuse the water column in the vessel.
b) The microoptode is fixed to a micro manipulator to measure oxygen profiles in a sediment core that is covered with a microbial mat / biofilm. The water is flushed with air and the whole sediment surface can be illuminated.
c) The optical setup: LED - light emitting diode as excitation or referencing light source, L1, L2 - optical lenses, F1, F2 - optical filters, ST - glass fibre connector, PMT - photo multiplier tube (measuring channel), PD - photo diode (α and β denote the different ways of referencing, for explanation see text)

3.2. Measuring system

The measuring system consists of three main units:

The optical setup (Fig. 1c) - As light source a blue light emitting diode (LED, λ_{peak} = 450 nm,, HPLB 500, Nichia Chemical Europe, Germany) is used. The light is coupled via a blue glass filter (BG12, Schott, Wiesbaden, Germany) into one branch of a 2 x 2 multimode glass fibre coupler (AMS Optotech, München, Germany). One of the outcoming fibres is used for referencing purposes. In version α) (Fig.

1c) the fibre is coupled to a PIN photodiode (S1190-1, Hamamatsu Photonics, Herrsching, Germany) that gives the reference signal for the phase angle measurement. In version β) (Fig. 1c) the red light of a second blue LED (as mentioned above) is coupled through a red foil filter into the fibre to reference the whole signal path in a time multiplex mode. The other outcoming fibre is connected to the micro optode via a ST-connector and guides blue excitation light to the fibre tip. The emitted luminescence at the fibre tip is guided via the same fibre back to the coupler and is distributed between the two incoupling branches. Fifty percent of the emission passes the second branch that is connected to a photomultiplier assembly (PMT, H5783-01, Hamamatsu Photonics, Herrsching, Germany) via a longpass filter (KV550, Schott, Wiesbaden, Germany).

The analogue signal processing (Fig. 2) - the phase angle is measured as the time difference between two sinusoidal current signals that enter identical signal paths at the beginning. The currents are converted into voltage signals (Fig. 2, I/U), high pass filtered (Fig. 2, HP), bandpass filtered (Fig. 2, BP) and amplified. Now the sinusoidal signals are converted into rectangular signals to be logically combined with a "NOT AND". The pulsewidth of the outcoming signal is directly proportional to the phase angle between the reference and the measuring signal. This rectangular signal is converted into a DC signal via a lowpass filter (Fig. 2, LP). Thus the DC signal is linear to the phase angle or the phase shift between measuring signal and reference signal. Additionally the DC and AC parts of reference and measuring signal are measured separately (not shown in Fig. 2). If the referencing with the red LED light is used (Fig. 1c (β)) the signal processing differs. The reference signal is directly generated by the modulation signal (instead of PD in Fig. 2) and in a time multiplex mode alternately the excitation LED and the reference LED are switched on. So the measuring signal is the difference between the measured phase angles for either luminescence signal or reference signal. By this method the whole optoelectronical path is referenced (this idea is based on personal communications with P. O'Leary and C. Kolle, Joanneum Research, Graz, Austria).

The digital signal processing - the sinusoidal modulation signal is generated by a digital frequency synthesiser that is controlled by a personal computer. The five analogue signals are processed either by an I/O board that is plugged into a personal computer (PC, Fig. 1, MicrOx) or by an A/D converter that is connected to a microcontroller board. In the PC based system the signals are continuously displayed, stored and then further evaluated. The microcontroller version will be a smaller laboratory device that displays the data digitally and calculates the oxygen value based on two previously measured calibration values.

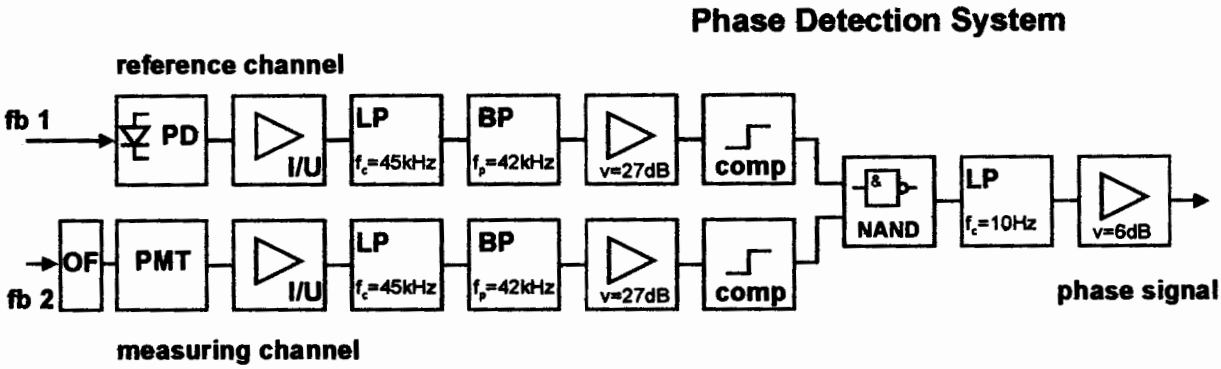

Fig. 1: Phase detection system (schematic drawing of analogue signal processing):
PD - photo diode, I/U - current to voltage conversion, LP - electrical low pass filter, BP - electrical bandpass filter, v - amplifier, comp - comparator, NAND - logical "NOT-AND" combination, OF - optical filter, PMT - photo multiplier tube, f_c - cutoff frequency, f_p - peak frequency, fb1, fb2 - fibre 1 and 2.
The amplitude and direct current evaluation is not drawn.

4. EXPERIMENTAL SETUP

4.1. Characterisation

The microoptode, connected to the PC based measuring system, was positioned in a calibration chamber consisting of two vessels (Fig. 1a). The inner vessel was filled with water and could be perfused by controlled gas mixtures through the gas permeable ceramic bottom of the vessel. The glass gas tube connected to the bottom has a few windings in the outer vessel and it is connected via flexible gas tubes to a oxygen/nitrogen gas mixing system. Circulation of thermally controlled water enabled measurements at constant temperature conditions. With this setup the temperature dependence and the influence of strong ambient light on the measuring signals were investigated. The setup was illuminated with a stepwise controllable fibre optic halogen lamp equipped with a collimating lense (KL 1500, Schott, Wiesbaden, Germany).

4.2. Oxygen profiles

The oxygen microoptode was fixed to a micro manipulator that enables one dimensional movements with µm resolution (Fig. 1, b)). Measurements were done in a North Sea coastal sediment covered with a dense biofilm of photosynthetic micro-organisms. The sediment core was incubated at room temperature

in constantly aerated water. For "light" measurements the setup was illuminated with a fibre optic halogen lamp.

Oxygen profiles were measured simultaneously with an oxygen (Clark-type) microelectrode[10] and an oxygen microoptode, which were glued together. The overall diameter of the sensing tip of the combined sensor was around 50 µm. The combined sensor was positioned in the water 0.5-1.0 mm above the sediment and the sensor was moved down into the sediment in steps of 50-100 µm. This procedure was repeated for light and dark conditions. The microelectrode signal was converted into a voltage signal via a picoampere meter and recorded on a strip chart recorder. The oxygen microelectrode was linearly calibrated from readings in the overlaying air saturated water and in the anoxic deeper parts of the sediment[9]. The same calibration points were used for the microoptode signals. Here the apparent lifetimes were calculated from the measured phase angles according to Eq. 1. These apparent lifetimes were then converted into oxygen values by using a modified Stern-Volmer-relation[4].

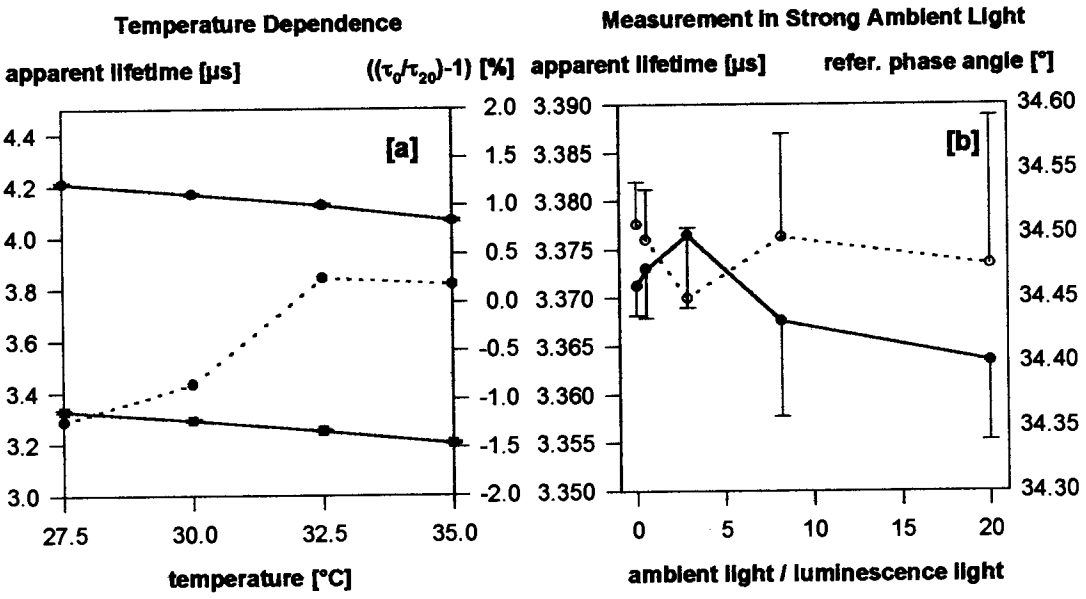

Fig. 3: Temperature dependence and effect of strong ambient light:
a) Apparent lifetimes τ_0 at 0% O_2 and τ_{20} at 20% O_2 measured at increasing temperatures 27.5, 30.0, 32.5 and 35 °C (constant gas mixture 20% O_2 and 80% N_2).
Dashed line: the ratio of $((\tau_0/\tau_{20})-1)$ that corresponds to the quenching constant of the immobilised luminophore.
b) Apparent lifetime at different levels of ambient light expressed as quantity of luminescence light signal (at 27.5 °C and 20% O_2).
Dashed line: the corresponding reference phase angle values obtained by incoupling of red LED light into the signal path.

5. RESULTS

5.1. Temperature dependence and effect of ambient light

The temperature dependence of the oxygen microoptode is shown in Fig. 3a. The apparent lifetime at 0% oxygen, τ_0, decreased from 4.30 to 4.07 µs and the apparent lifetime at 20% oxygen, τ_{20}, decreased from 3.39 to 3.2 µs. While the quenching constant represented by the term $((\tau_0/\tau_{20})-1)$ (Fig. 3, a) does not show a corresponding significant change (between -1.2 to +0.25 %).

When the micro optode without optical insulation penetrates the surface of a sediment the level of background light that enters the fibre changes dramatically. This was simulated under constant temperature and gas conditions in the calibration vessel by increasing the light output of the halogen lamp. Figure 3b shows no significant change of the measured apparent lifetime upon increasing the ambient light level from 0 to 20 times the luminescence signal. For higher ratios of ambient to luminescent light intensity the phase angle that could be measured started to differ from the real value.

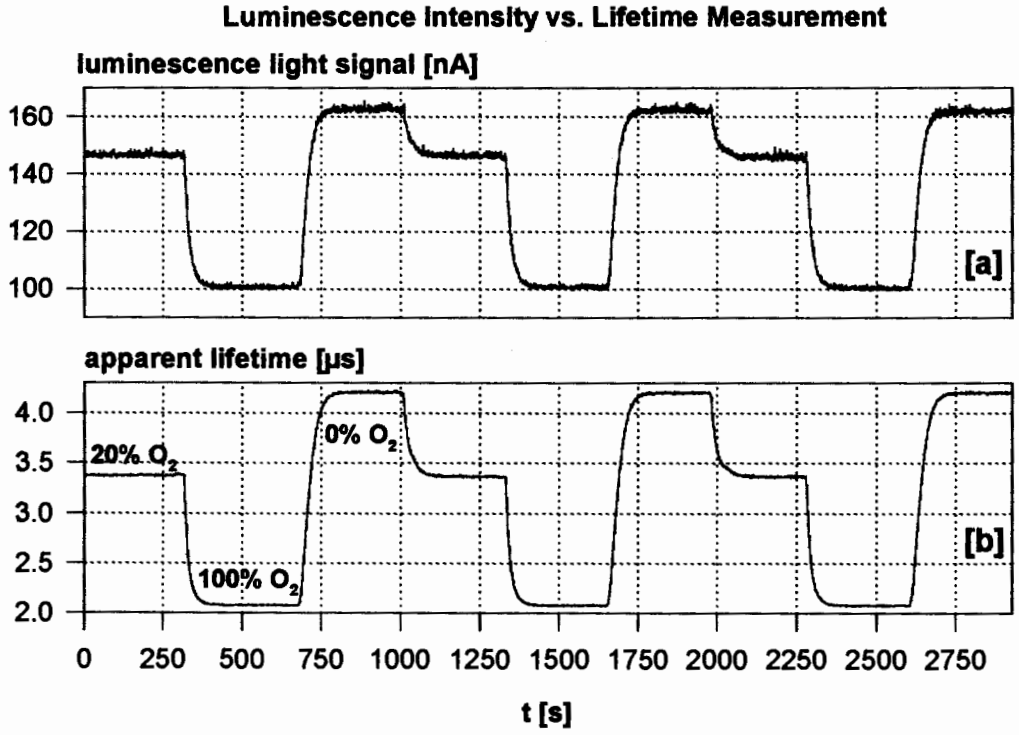

Fig. 4: Comparison of luminescence intensity to apparent lifetime evaluation:
a): luminescence intensity reading versus time during three steps of gas mixtures: 20%-100%-0% O_2
b): the corresponding evaluation of the apparent lifetime versus time.

This is due to non-linear influences from the peak noise of the photomultiplier tube that is amplified by strong ambient light. The measured reference phase angle (Fig. 1c, (β)) also showed no significant change if the ambient/luminescent light ratio stayed in the range from 0 to 20.

Another characteristic of lifetime based measurements can be seen in Fig. 4. Three cycles of 20%-100%-0% oxygen were measured and the measured intensity signal was compared to the apparent lifetime. Because of rising gas bubbles in the calibration vessel the changing light reflecting background produced extra noise in the intensity signal. This noise does not appear in the apparent lifetime measurement. Furthermore a slight shift to lower signal intensities over time could be observed in the intensity curve (Fig. 4a) due to a weak photobleaching. Again this effect does not occur in the lifetime measurement (Fig. 4b).

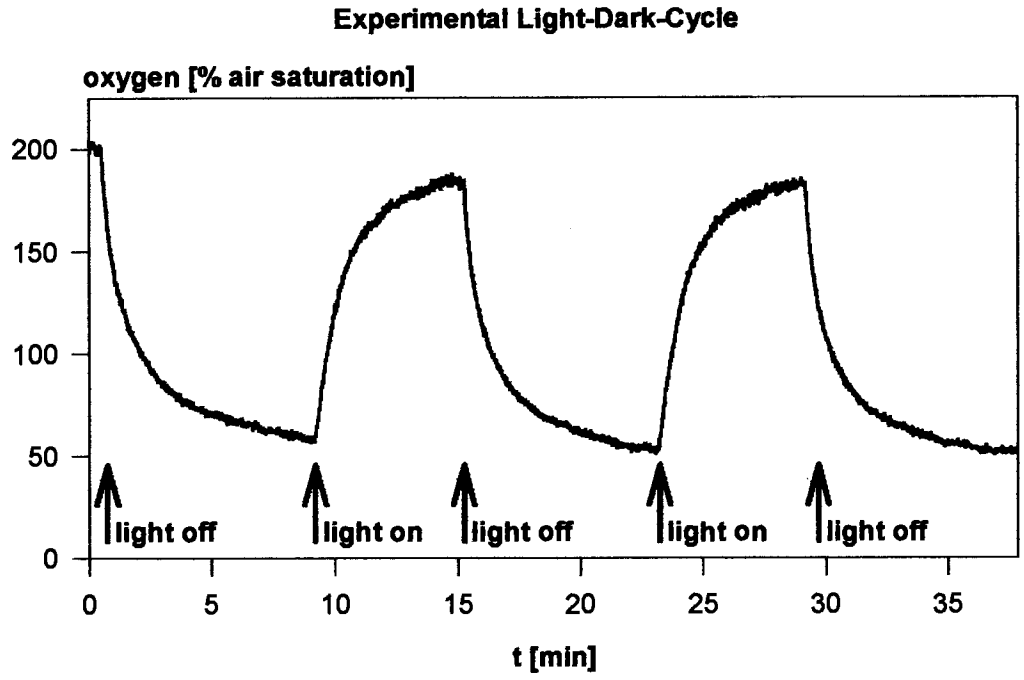

Fig. 5: Measurement of oxygen with a microoptode (not optically insulated) in a photosynthetically active layer of cyanobacteria in a North Sea coastal sediment during experimentally light-dark-cycles.

5.2. Application in sediments

Measurements were done in a North Sea Coastal sediment that was covered with a dense biofilm of photosynthetic micro-organisms. Oxygen was measured in a photosynthetic active layer under alternate

light-dark cycles (Fig. 5). Here the microoptode was positioned at a depth of around 100µm below the sediment surface. In the light oxygen productivity photosynthesis resulted in oxygen supersaturation of the upper sediment layer (Fig. 5, 200 % air saturation). When the light was switched off, the sediment consumed oxygen. This was seen as a decrease of oxygen in the cyanobacterial layer to 52 % within 9 minutes after darkening (Fig. 5).

We also measured steady-state concentration depth profiles of oxygen in the dark and in the light. Figure 6 shows oxygen profiles measured simultaneously by the microelectrode and the microoptode. Each sensor is represented with a dark and a light profile in the graph. For the dark profile the dashed line gives the oxygen reading that is derived from the luminescence intensity measurement. As the micro optode was not optically insulated this curve shows a strong deviation from the other curves due to local changes of optical properties in the surroundings of the sensor tip upon entering the sediment.

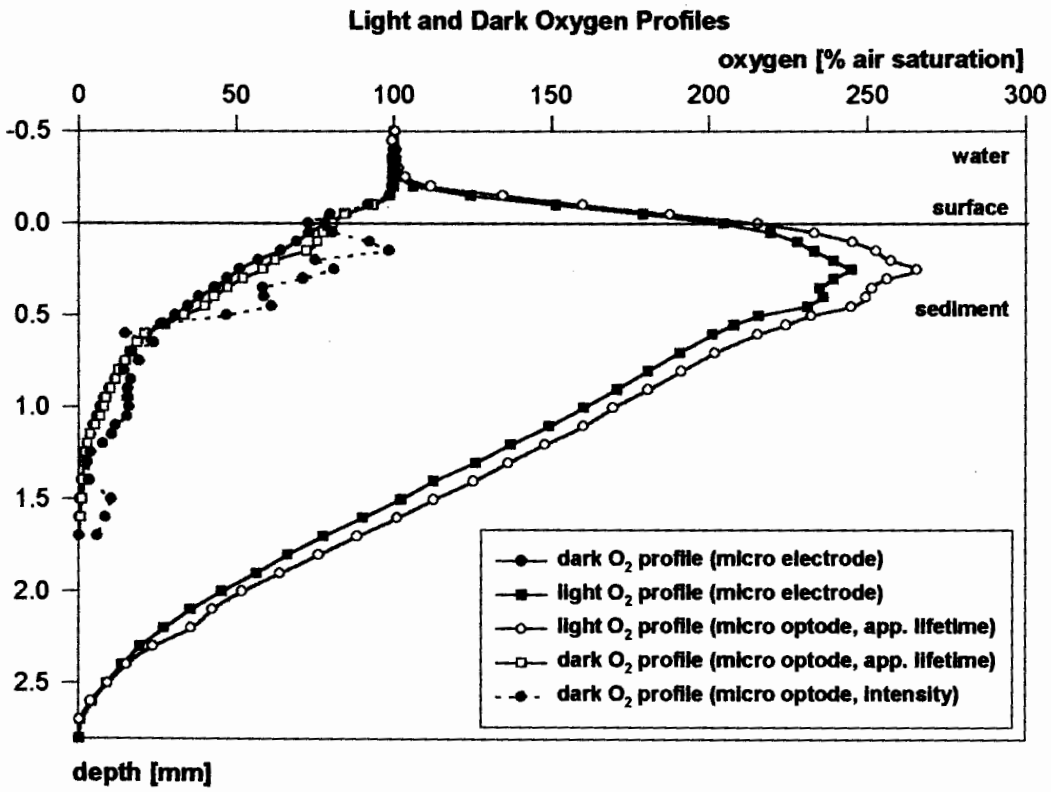

Fig. 6: Oxygen profiles measured with a combined oxygen microoptode (not optically insulated) and an oxygen microelectrode in a North Sea coastal sediment with a dense population of cyanobacteria.

After penetration of the sediment surface a rise in oxygen was observed in both dark and light profiles. Without the optical insulation at the fibre tip excitation and emission light may leave the fibre. This light

seems to be strong enough to induce local photosynthesis leading to a rise in oxygen (Fig. 6). However, oxygen measurements in shallow water sediments without a dense population of phototrophs as well as measurements in continental shelf and deep-sea sediments are not affected by this phenomena.

6. DISCUSSION

The first results with the new phase modulation based measuring system for oxygen micro optodes proved the advantages of this type of oxygen measurements. There is no significant influence of ambient light intensity up to 20 times higher than the luminescence signal, and it is possible to measure oxygen with microoptodes that are not optically insulated. The simultaneous measurements of oxygen profiles with a combined microelectrode and microoptode showed the good performance of the fibre optic oxygen sensors together with the new measuring system. However, the first measurements revealed the problem of induced photosynthesis when measuring in systems with a high density of phototrophs. This will be further investigated and may be solved by use of an oxygen indicator with different spectral characteristics. Additionally, the best compromise has to be found between excitation light intensity of the LED, quantum efficiency of the immobilised indicator and the sensitivity of the detector for luminescent light. For most applications of oxygen measurements in non-photosynthetic aquatic environments the presented combination of oxygen microoptodes with a luminescence lifetime based measuring system already offers advantageous performance with respect to signal stability as compared to both luminescence intensity based microoptode and electrochemical oxygen measurements.

7. ACKNOWLEDGEMENTS

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