M.Kühl & N.P. Revsbech (2001). Biogeochemical Microsensors for Boundary Layer Studies, pp. 180-210. In: B.P. Boudreau & B.B. Jørgensen (eds.), The Benthic Boundary Layer, Oxford University Press, New York, 2001.

# 8 BIOGEOCHEMICAL MICROSENSORS FOR BOUNDARY LAYER STUDIES

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Interfacial processes in sediments, biofilms, and other benthic aquatic systems can be strongly affected by the presence of hydrodynamic boundary layers. The boundary layer (BL) can limit mass transfer between the overlying water and the solid matrix, thus affecting biological processes. The BL region adjacent to reactive surfaces that contains significant chemical gradients is known as the diffusive boundary layer (DBL). The DBL thickness is dependent on the flow velocity, surface topography and porosity of the solid matrix, and it ranges from <0.1 mm to several millimeters in thickness (see also chapters 5, 9, 14 and 15). As the interface is approached, mass transfer of solutes in the DBL becomes less dependent on advective motions (turbulence) and increasingly dependent on diffusion. Any net solute consumption or production within the matrix relative to the surrounding water, therefore, leads to the formation of concentration gradients over the DBL as well as within the matrix. The sediment-water interface thus can be the site of steep gradients of physicochemical variables over distances of <0.1 mm, and methods for studying these variables near the interface must resolve variations over very small distances without significant disturbance to these gradients. Noninvasive optical analysis, by direct microscopic observation, analysis of water current with laser-Doppler anemometry, nuclear magnetic resonance imaging (Wieland, 2000) or positron emission tomography (Khalili et al., 1998) fulfills these requirements, but most methods with high spatial resolution are based on microsensors.

Different microsensors for environmental use, based on a wide variety of principles, have been developed during the last couple of decades. This development seems to be accelerating as microscale construction makes it possible to apply principles that would not work in macroscale analogs. The latter observation is directly connected to the fact that diffusion is a fast transport process in microscale. As most electrochemical sensor principles rely on diffusion-dependent electrode reactions, scaling down the active surface area and diffusion distances leads to better signal stability, faster response, and practical independence of the microsensor signal on stirring of the external medium. Such measuring characteristics can normally not be realized with macrosensors. For electrochemical microsensors these advantageous measuring characteristics occur when the characteristic sensor tip dimension (i.e., the diameter of the sensor membrane or the diameter of the reactive surface) becomes so small that the diffusion to and from the sensor changes from a planar to a hemispherical geometry. It is now generally agreed on, that this happens with tip dimensions of <20 µm. An excellent review on the principles and advantages of electrochemical microsensors was given by Heintze (1993).

Confusion exists about the term *microsensor* in both analytical chemistry and aquatic sciences, where terms such as ultramicroelectrode and nanode are also used. Here, we use a rather broad definition of microsensors as being any sensor that allows for measurements with a spatial resolution of ≤0.1 mm. In most cases, the actual spatial resolution is equal to the tip diameter of the sensor, if the sensor does not consume so much of the analyte that it disturbs the analyte gradient to be measured. Sensors allowing measurements at a millimeter scale are termed *minisensors*.

Below we present a review of microsensors and microsensor-based methods for analysis of transport and reaction processes near the sediment-water interface. Details regarding construction and data acquisition are beyond the scope of this chapter, which focuses on a description of the microsensors now available, as well as a few examples of the type and quality of data that can be obtained. An excellent introduction to the practical aspects of microsensor construction is given by Thomas (1978) and Amman (1986). Earlier reviews offer detailed information about microsensor construction and application to aquatic systems (Revsbech and Jørgensen, 1986; Van den Heuvel et al., 1992; Kühl et al., 1994b; Revsbech, 1994; Klimant et al., 1997b; Buffle and Horvai, 2000; Varney, 2000). A recent review by Amann and Kühl (1998) addresses the combined use of microsensors and molecular biological techniques. Examples of microsensor data can be found in chapters 10, 14, and 15. Tables 8.1–8.3 summarize currently available microsensors, their measuring characteristics, and key references for construction.

## 8.1 Electrochemical Microsensors

All the sensors described in this review are based on either electrochemical or optical signal detection. For some sensors a biological reaction precedes the electrochemical reaction, and these sensors are labeled as *microbiosensors*. A new microsensor for flow and diffusivity uses an electrochemical principle, but it provides information about a physical parameter and so will be described separately. The remaining electrochemical microsensors can be divided into (1) simple $Ag/Ag^+$ half cells; (2) ion-exchanger based electrodes; (3) simple cathodes or anodes with continuous, varying, or no polarization; and (4) Clark-type gas sensors where the chemical species must pass an ion-impermeable membrane before oxidation or reduction or, alternatively, induction of pH changes in the internal electrolyte (i.e., the Severinghaus principle).

### 8.1.1 Reference Electrodes

For many applications a reference electrode must be in direct electrical contact with the environment being analyzed. Such reference electrodes are usually commercial Ag/AgCl or Calomel electrodes, sometimes equipped with a double junction to minimize risk of sulfide precipitation in the inner liquid junction. There are many misunderstandings and misuses surrounding such reference electrodes. First of all, many investigators have tried to keep them as near the sensing electrode as possible and therefore pushed them into sediments. In this case the potential at the liquid junction may be affected by colloidal particles in the sediment, and the potential of the overall reference is changed (Brezinski, 1983; Yang et al., 1989). The exact mechanism behind the suspension effect is still under debate and such aneffect is not always found in sediment studies (e.g., Cai and Reimers, 1993). Furthermore, contact with sulfide leads to formation of metal sulfide precipitates that may clog the reference. Thus, in practice the most reliable results are obtained by keeping the reference in the water phase. For high-accuracy measurements it may be necessary to shield the reference from water currents, as exposure to flow may lead to changes in potential. A simple way to lower this effect is to place folded paper towel around the tip of the reference.

### 8.1.2 $Ag/Ag^+$ Half-Cell Microelectrodes

The simplest possible microsensor for environmental use consists of a small, exposed silver surface that is coated with the silver salt of the ion to be analyzed. The sensors are usually made of a tapered platinum wire coated with an insulating glass. The glass is removed from the very tip, and silver is subsequently electroplated onto the plati-

**Table 8.1a.** Part I. Electrochemical microsensors used in biogeochemical systems (status in 1998). Gas-sensor types.

| Microsensor Type Gas Sensors | Detection Principle | Tip Diameter | Life-Time |
|---|---|---|---|
| $O_2$, cathode type | Reduction of $O_2$ on Au-cathode. | 1–100 µm | Drift during use; shelf life >1 year |
| $O_2$, Clark-type | Reduction of $O_2$ on Au-cathode behind silicone membrane. | 1–100 µm | Months up to >1 year |
| $H_2$, anode type | Oxidation of $H_2$ at Pt- or Pd- anode. | 1–100 µm | Days |
| $H_2$, Clark-type | Oxidation of $H_2$ at Pt- or Pd- anode behind silicone membrane. | 1–100 µm | Days |
| $N_2O$ | Reduction of $N_2O$ on Ag-cathode behind silicone membrane. | 1–100 µm | 1 month |
| Combined $N_2O/O_2$ | Reduction of $O_2$ and $N_2O$ on Au- and Ag-cathodes behind a silicone membrane. | 10–100 µm | 1 week |
| $H_2S$ | Oxidation at a Pt-anode of ferrocyanide formed by $H_2S$ oxidation with ferricyanide behind a silicone membrane. | 10–100 µm | Weeks |
| $CO_2$ | $CO_2$-induced pH change of bicarbonate solution behind a silicone membrane. | 10–100 µm | Few days to 1 week |

**Table 8.1a** Part II. (continued)

| Gas Sensors | Detection Limit | Interference | Key References |
|---|---|---|---|
| $O_2$, cathode type | 0.1 µM $O_2$ | $H_2S$, $Mg^{2+}$, $Ca^{2+}$ | Whalen et al. (1967)<br>Baumgärtl and Lübbers (1973) |
| $O_2$, Clark-type | 0.1 µM $O_2$ | $H_2S$ | Revsbech and Ward (1983)<br>Revsbech (1989) |
| $H_2$, anode type | 1 µM $H_2$ | $CO_2$, CO, $CH_4$ | Baumgärtl and Lübbers (1973) |
| $H_2$, Clark-type | 1 µM $H_2$ | CO, $CH_4$ | Witty (1991)<br>Ebert and Brune (1997) |
| $N_2O$ | 0.1 µM $N_2O$ | $H_2S$, acetylene | Revsbech et al. (1988) |
| Combined $N_2O/O_2$ | 0.1 µM $O_2$ / $N_2O$ | $H_2S$, acetylene | Revsbech et al. (1988) |
| $H_2S$ | 1 µM $H_2S$ | $SO_2$, intense ambient light | Jerochewski et al. (1996)<br>Kühl et al. (1998) |
| $CO_2$ | <5 µM $CO_2$ | acid gases, $H_2S$ | De Beer et al. (1997a)<br>Zhao and Cai (1997) |

**Table 8.1b** Part I. Other types of sensors.

| Microsensor Type | Detection Principle | Tip Diameter | Life-Time |
|---|---|---|---|
| **Ion Sensors** | Build-up of potential across solid or liquid ion-exchanger membrane | | |
| pH, glass | | 20–200 µm | Months to 1 year |
| pH, solid state | | 3–15 µm | Weeks |
| pH, LIX | | 1–20 µm | <1 to a few days |
| $NO_3^-$, LIX | | 1–20 µm | <1 to a few days |
| $NO_2^-$, LIX | | 1–20 µm | <1 to a few days |
| $NH_4^+$, LIX | | 1–20 µm | <1 to a few days |
| $Ca^{2+}$, LIX | | 1–20 µm | <1 to a few days |
| $S^{2-}$, solid state | $S^{2-}$ activity–dependent potential of $Ag^+$ over $Ag/Ag_2S$ poly-crystalline membrane | 10–100 µm | A few days |
| **Voltammetric Sensors** | | | |
| $O_2$, Total S, $Fe^{2+}$, $Mn^{2+}$, $H_2O_2$, $I^-$ | Fast scan voltammetry with a mercury-gold amalgam electrode | >100–300 µm | |
| **Physical Parameters** | | | |
| Temperature | Microthermocouple wire | 10-100 µm | Months |
| Diffusivity | Amperometric detection of a tracer gas diffusing out of a capillary | 20-300 µm | Months |
| Flow | Same as the diffusivity sensor | 20-50 µm | Months |

**Table 8.1b** Part II. (continued)

| Microsensor Type | Detection Limit | Interference | Key References |
|---|---|---|---|
| **Ion Sensors** | | | Reviewed by Amman (1986) |
| pH, glass | pH 1 to 14 | | Thomas (1978), Amman (1986) |
| pH, solid state | pH 1 to 12 | $H_2S$ | VanHoudt et al. (1992) |
| pH, LIX | pH 3 to 11 | | De Beer et al. (1997a) |
| $NO_3^-$, LIX | 5 µM $NO_3^-$ in freshwater | $Cl^-$, $HCO_3^-$, $H_2S$ | De Beer and Sweerts (1989) |
| $NO_2^-$, LIX | 0.1 µM $NO_2^-$ in freshwater; 10 µM $NO_2^-$ in seawater. | $H_2S$ | De Beer et al. (1997b) |
| $NH_4^+$, LIX | 1 µM $NH_4^+$ in freshwater | $K^+$, $Na^+$ | De Beer and Van den Heuvel (1988) |
| $Ca^{2+}$, LIX | <0.1 µM $Ca^{2+}$ in freshwater; 10 µM $Ca^{2+}$ in seawater | | Amman (1986) Fluka (1997) |
| $S^{2-}$, solid state | pH dependent: 1-10 µM $S_i$ in neutral to alkalinewaters | $Hg^+$, $Ag^+$, $O_2$, thiourea | Revsbech and Jørgensen (1986) Kühl and Jørgensen (1992a) Visscher et al. (1991) |
| **Voltammetric Sensors** | | | Measuring principles reviewed |
| $O_2$, Total S, $Fe^{2+}$, $Mn^{2+}$, $H_2O_2$, $I^-$ | | salinity, pH | by Heintze (1993) Brendel and Luther (1995) Luther et al. (1998) |
| **Physical Parameters** | | | |
| Temperature | 0.1–0.01 °C | | Omega Engineering Inc. |
| Diffusivity | Full relevant scale | | Revsbech et al. (1998) |
| Flow | 5 µm $s^{-1}$– 5 cm $s^{-1}$ | | J. Gundersen (unpublished) |

**Table 8.2a** Fiber-optic microprobes and microsensors used in biogeochemical systems (status in 1998). Part I. Fiber-optic microsensors.

| Type | Detection Principle | Tip Diameter | Life Time |
|---|---|---|---|
| **Fiberoptic** | Monitoring the light field at the tip of an optical fiber | | |
| Field radiance | Directional light collection with tapered optical fiber | 5–140 µm | >1 year |
| Irradiance | Small cosine collector at tapered end of optical fiber | 40–140 µm | >1 year |
| Scalar irradiance | Isotropic light collection via small diffusion sphere at tapered end of an optical fiber | 70–200 µm | >1 year |
| Relative pigment distribution via induced Chl *a* fluorescence | Combined excitation/ emission via a tapered optical fiber | 10–140 µm | >1 year |
| Position of water-solid interface | Change in reflection of NIR light emitted and collected via a tapered optical fiber | 20–50 µm | >1 year |
| Diffusivity and flow | Detection of time-dependent change of fluorescence injected a known distance from fiber tip | 10 µm micro-injection capillary and 20 µm field radiance probe 100–250 µm from injection spot | months |

**Table 8.2a** Part II. Fiber-optic microsensors. (continued)

| Type | Detection Limit | Interference | Key References |
|---|---|---|---|
| **Fiberoptic** | | Changes in refractive index at tip | Reviewed in Kühl et al. (1994b) |
| Field radiance | UVB–NIR <1µmol photons $m^{-2}s^{-1}$ | | Jørgensen and Des Marais (1986), Kühl and Jørgensen (1992b, 1994) |
| Irradiance | VIS–NIR <1µmol photons $m^{-2}s^{-1}$ | | Lassen and Jørgensen (1994), Kühl et al. (1994a) |
| Scalar irradiance | UVB–NIR <1µmol photons $m^{-2}s^{-1}$ | | Lassen et al. (1992), Kühl et al. (1994b, 1998), Garcia-Pichel (1995) |
| Relative pigment distribution via induced Chl *a* fluorescence | VIS–NIR <1µmol photons $m^{-2}s^{-1}$ | | Schreiber et al. (1996) |
| Position of water-solid interface | NIR 50 µm or better spatial resolution | | Klimant at al. (1997a) |
| Diffusivity and flow | flow velocity: 3–500 µm $s^{-1}$ ; diffusivity: time dependent | | De Beer and Stoodley (1996), De Beer (1997) |

**Table 8.2b** Part I. Micro-opt(r)odes.

| Type | Detection Principle | Tip Diameter | Life Time |
|---|---|---|---|
| **Micro-opt(r)odes** | Change in optical properties of indicator dye immobilized at the tip of a tapered optical fiber | | |
| Temperature | Temperature–dependent change in luminescence lifetime | 20–100 µm | Months |
| $O_2$ | $O_2$–dependent change of luminescence intensity or lifetime | 20–100 µm | Weeks to months |
| pH | pH–dependent change in absorption/reflection | 20–100 µm | Weeks to months |
| $CO_2$ | Optical detection of a $CO_2$–dependent pH change behind a gas–permeable membrane | 20–100 µm | A few days to 1 week |

**Table 8.2b** Part II. Micro-opt(r)odes. (continued)

| Type | Detection Limit | Interference | Key References |
|---|---|---|---|
| **Micro-opt(r)odes** | | | Reviewed in Klimant et al. (1997b) |
| Temperature | 0.1–0.5 °C | $O_2$ | Holst et al. (1997a) |
| $O_2$ | Dependent on sensor chemistry, down to <0.1 µM | $SO_2$, high background fluorescence | Klimant et al. (1995, 1997b) |
| pH | pH 7–9.5, can be modified by choice of pH indicator chemistry | | Kohls et al. (1997a) |
| $CO_2$ | 5 µM $CO_2$ | Acid gases, $H_2S$ | Kohls et al. (1997b) |

**Table 8.3** Part I. Micro-biosensors for biogeochemical systems (status in 1998).

| Microsensor Type | Detection Principle | Tip Diameter | Life Time |
|---|---|---|---|
| **Enzyme Based Biosensors** | | | |
| Glucose | $O_2$ microsensor with immobilized glucose oxidase | 10 µm | A few days |
| **Whole Cell Based Biosensors** | | | |
| $CH_4$ | $O_2$ microsensor with immobilized $CH_4$–oxidizing bacteria | 25 µm | Months |
| $NO_3^-$ and $NO_2^-$ | $N_2O$ microsensor with immobilized denitrifying bacteria | 25 µm | Weeks |
| Dissolved organic carbon (DOC) | $O_2$ microsensor with immobilized yeast cells | 50 µm | Not determined |

**Table 8.3** Part II. Micro-biosensors for biogeochemical systems (status in 1998) (continued).

| Microsensor Type | Detection Limit | Interference | Key References |
|---|---|---|---|
| **Enzyme Based Biosensors** | | | |
| Glucose | 1 μM | $H_2O_2$, variables affecting the enzyme activity | Cronenberg et al. (1991), Van Den Heuvel et al. (1992) |
| **Whole Cell Based Biosensors** | | | |
| $CH_4$ | 2 μM | $H_2S$, $H_2$ | Damgaard and Revsbech (1997), Damgaard et al. (1998) |
| $NO_3^-$ and $NO_2^-$ | 0.1 μM | $N_2O$ | Larsen et al. (1997) |
| Dissolved organic carbon, DOC | 50 μM glucose | $O_2$ | Neudörfer and Meyer-Reil (1997) |

num. Coating with the silver salt in question is done electrochemically. The resulting electrode is basically a $Ag/Ag^+$ half cell, where the $Ag^+$ activity, and thus the potential, is governed by the solubility of the silver salt in the analyzed medium. Variations in the anion activity will affect the $Ag^+$ activity, as the solubility product must be constant; the potential of the electrode is thus a function of the anion activity. This type of sensor only works for a specific ion if other anions giving a less soluble silver salt are absent or almost absent. Chloride can thus be measured when sulfide is absent and bromide is very low, whereas sulfide under anaerobic conditions can be measured without interference, due to the extremely low solubility product of silver sulfide.

In an environmental context, sulfide is often considered an important variable, and sulfide microsensors of the $Ag/Ag_2S$ type have therefore been used quite extensively (Revsbech and Jørgensen, 1986; Visscher et al., 1991). Calibration must be performed under strictly anaerobic conditions. Besides leading to oxidation of sulfide in the calibration solution, oxygen can be reduced on the negatively charged electrode surface, which thus depolarizes the electrode. Log-linear calibration curves down to micromolar concentrations, with a slope near the theoretical 29.5 mV per concentration decade at room temperature, can be obtained by use of such simple sulfide microsensors, but calibration must start with high concentrations (>100 μM) and then move toward lower concentrations. A meaningful signal usually will not be obtained if a sensor is immersed directly in a solution containing, for example, 10 μM total dissolved sulfide. Another complication in use of sulfide microsensors is the need for parallel pH measurements to compensate for the change in $S^{2-}$ activity with changes in pH.

The construction and use of functioning $Ag/Ag_2S$ electrodes can be problematic (Kühl and Steuckart, 2000). These problems arise from nonideal response (Revsbech and Jørgensen, 1986), signal drift and very long response times at low sulfide levels (Kühl and Jørgensen, 1992a), susceptibility of the polycrystaline $Ag_2S$ membrane to physical damage, and mixed potentials due to coprecipitates in the membrane (see also Jeroschewski et al., 1996; Kühl et al., 1998, and references therein).

### 8.1.3 Ion-Exchange-Based Microsensors

The oldest ion-exchange-based sensor (and also the best) is the glass pH electrode. The glass in the pH-sensitive part of the electrode is selectively permeable to hydrogen ions, and based on the specific external activity of hydrogen ions, a potential will

build up across the pH glass. This potential is then read by a high-impedance voltmeter. Miniaturized versions of the glass pH electrode have been known for many years (Hinke, 1969; Thomas, 1978; Revsbech and Jørgensen, 1986), and except for increased fragility, they are identical to macroscopic analogs in terms of accuracy of determination and lack of interferences.

Ion exchangers can also be liquid, and in constructing microsensors, such a liquid ion exchanger (LIX) is positioned in a capillary tip. The solvents used for construction of LIX membranes are hydrophobic, and to prevent displacement of the LIX by water, the inner surfaces of the microsensor are rendered hydrophobic by silanization (Amman, 1986; Thomas, 1978). A generalized LIX-type sensor is shown in figure 8.1, which also shows how a liquid coaxial shielding can be made around the sensor. With such a coaxial shielding, noise pickup is negligible and the signal can be read at 0.1 mV accuracy, whereas extreme variations in the signal without the shielding makes it virtually impossible to use such sensors in a normal laboratory environment. The entire setup can be enclosed in a large Faraday cage, but even by doing so, reproduction of the same signal stability as with the simple coaxial shielding shown in figure 8.1 is difficult.

Liquid ion exchangers of relevance for the study of the sediment-water interface that have been tested and are commercially available (see e.g., Fluka, 1997) include those for $H^+$, $NH_4^+$, $Ca^{2+}$, $NO_3^-$, and $NO_2^-$. LIX-type microsensors for $H^+$ have been used extensively for physiological purposes and are characterized by a very high selectivity. Glass pH microsensors can have lifetimes of about one year, which cannot be matched by LIX sensors. LIX-type sensors can, however, be made extremely small, with tip diameters from <1–10 μm, allowing a similar spatial resolution during measurements (Van Den Heuvel et al., 1992). Glass pH electrodes can also be made quite small (Thomas, 1978), but such small sensors are difficult to make, and they are also characterized by much longer response times than LIX-based pH sensors. Thus, the usual fast responding glass pH sensors used for environmental applications (Revsbech and Jørgensen, 1986) have a minimum pH sensing tip of about 20 μm diameter and a length of 100 μm.

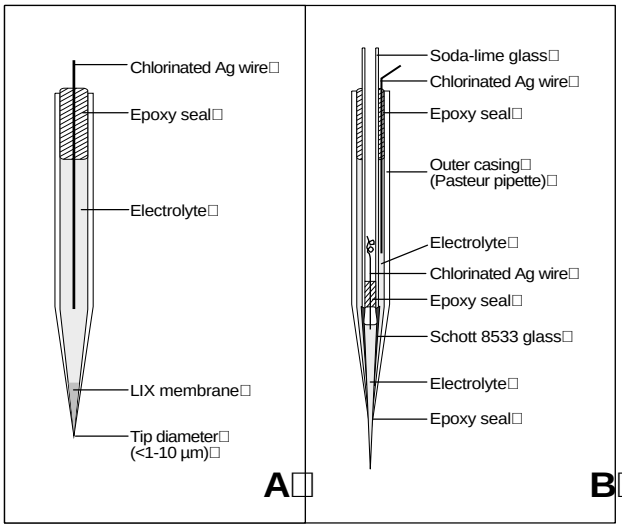


**Figure 8.1** A, Schematic representation of an LIX-type microsensor. B, Efficient coaxial shielding of microelectrod for LIX microsensors. (Redrawn after Jensen et al. [1993]).

The ammonia microsensors suffer from interference from $K^+$ ions, but they can be used in freshwater environments with low $K^+$ activity (De Beer and Van den Heuvel, 1988). The detection limit in an ordinary freshwater environment is usually a few micromolar and about 1 μM in demineralized water. Nitrate can be measured by LIX microsensors in freshwater (De Beer and Sweerts, 1989), but due to a pronounced interference from $HCO_3^-$ and $Cl^-$, use in saline waters is not possible. Even in freshwater under optimal conditions of low $HCO_3^-$ (1 mM) and virtual absence of $Cl^-$, calibrations are curved below 100 μM nitrate. Furthermore, the accuracy of depth profiles in sediments can be adversely affected by concurrently increasing $HCO_3^-$ concentrations with depth (Jensen et al., 1993). An example of nitrate profiles obtained in a sediment with a LIX microsensor is shown in figure 8.2, together with profiles of oxygen. A diffusion-reaction model was used to calculate rates of $NO_3^-$ production (nitrification), consumption (denitrification) from the nitrate profiles, and a depth profile of oxygen consumption. Note that the values for nitrate do not reach zero in the deepest layers, due to $HCO_3^-$ interference, but this zero-concentration condition was imposed on the model. The performance of nitrate electrode and any other LIX electrode does however rely heavily on the LIX properties. In a recent comparison of different LIX materials for nitrate microsensors, Verschuren et al. (1999) found several LIX compounds with better selectivity and overall performance as compared to that used by Jensen et al. (1993).

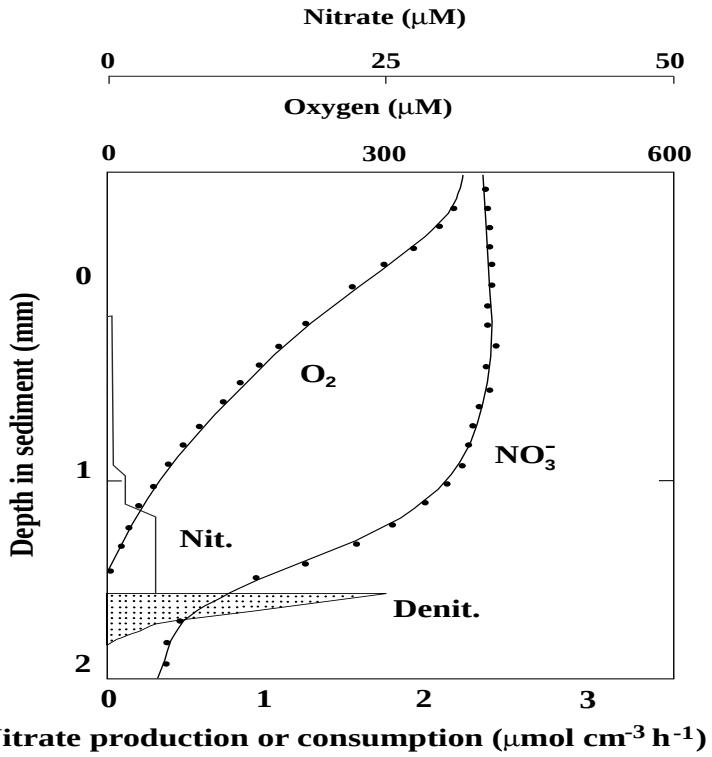


**Figure 8.2** Profiles of oxygen and nitrate in a freshwater sediment and modeled distributions of oxygen consumption and nitrification.

Calcium ions are frequently measured with LIX microelectrodes in physiology and other biomedical applications. However, due to the excellent performance of the $Ca^{2+}$ LIX available, it is possible to analyze $Ca^{2+}$ in both seawater and freshwater, and $Ca^{2+}$ microelectrodes have been used to study $Ca^{2+}$ dynamics in macroalgae (McConnaughey and Falk, 1991) and biofilms (Hartley et al., 1996). Furthermore, $Ca^{2+}$ LIX microelectrodes were used to measure $Ca^{2+}$ dynamics in calcifying corals (de Beer et al., 2000) and planktonic foraminifera, and some first measurements in sediments indicate that calcium microelectrodes also may be useful in studies of calcite dissolution (Wenzhöfer, 1999). Finally, Müller et al. (2000) demonstrate various mini-LIX electrodes for fresh porewaters, including $Ca^{2+}$ and $CO_3^{2-}$ sensors.

Recently, an LIX microelectrode for nitrite measurement in biofilms has been developed (de Beer et al. 1997b) with good measuring characteristics in both freshwater and seawater. The $NO_2^-$ microelectrode exhibits a log-linear response towards nitrite down to 1 µM $NO_2^-$ in freshwater and 10 µM $NO_2^-$ in seawater. This most recently developed LIX microelectrode is also shielded against mechanical and hydrophobic interactions with the biofilm matrix by coating the microsensor tip with a thin hydrophilic proteinaceous matrix (see de Beer et al., 1997, for more details). This hydrophilic protective coating, which can also be used with other LIX microelectrodes, minimizes problems described above, such as shifting calibration curves, drift, and loss of signal by the use of LIX microelectrodes in cohesive biofilms and sediments.

Solid-state iridium/iridium oxide electrodes can be used for pH in biofilms (VanHoudt et al., 1992), but we still do not know if sediments can be analyzed by such potentiometric equilibrium-based electrodes, as dissolved sulfide may present a significant interference.

### 8.1.4 Simple Microsensors with Continuous or No Polarization

The first oxygen microsensors were simple cathodes, often coated with collodium or a similar polymer to provide some chemical and physical shielding from the environment. Microsensors constructed in this way can have sensing tips down to about 1 µm diameter (Whalen et al., 1967; Baumgärtl and Lübbers, 1973), and they have proven very useful for physiological applications. The first analyses of the oxygen distribution near the sediment-water interface were also conducted using such electrodes (Reimers, 1987). To increase sturdiness and ease of signal amplification, minielectrodes with diameters of 0.1–1 mm have also been made this way (Visscher et al., 1991). However, such simple oxygen electrodes cannot be manufactured with a perfectly linear response and are also characterized by continuous current drift. The reason for this non-ideal behavior is poisoning of the electrode surface by organic matter and precipitation of carbonate minerals due to the alkaline microenvironment created by the reduction of oxygen. Furthermore, such cathode-type $O_2$ microelectrodes, especially needle electrodes, can exhibit a significant consumption of oxygen, which leads to disturbances of the gradients and to a high dependency of the microsensor signal on changes in the diffusive supply of $O_2$ to the sensor tip with changes in flow or diffusivity, that is, a high stirring sensitivity. This can lead to significant artifacts in measured $O_2$ profiles when measurements are taken in a velocity gradient, for example, at the sediment-water interface (Gust et al., 1987; Revsbech, 1989; see also section 8.2.4).

Redox electrodes are basically just bare platinum or gold surfaces exposed directly to the environment. They interact with redox couples of the environment, and the resulting potential, $E_h$, is read against a standard reference. There is apparently no investigation available about how small a noble metal surface can be made and still result in reasonable redox values, but microscale redox sensors have been used for the determination of redox potentials in the gut of termites (Ebert and Brune, 1997). $E_h$ is not emphasized in this chapter because such values cannot be used for quantitative calculations, and even with large redox electrodes, reproducible and meaningful readings are difficult to obtain. Furthermore, electron exchange of most redox couples is

slow, and environmental systems are controlled by mixed potential (fixed by several couples acting together).

Fast-scan voltammetric methods are frequently used in electrochemistry but have had limited application to environmental analysis (e.g., Davison and Whitfield, 1977; Davison et al., 1988). Simple cathode/anode-type microelectrodes or Clark-type microsensors (section 8.1.5) for single chemical species measure a current at a fixed electrode potential. Fast scan voltammetry, with a simple glass-coated metal microelectrode allows in principle the simultaneous detection of all redox species reacting at the metal surface within the range of a potential scan. Brendel and Luther (1995) developed such a microelectrode system and used it for simultaneous measurements of $O_2$, $H_2O_2$, sulfide, Fe(II), and Mn(II) in the porewaters (see also Luther et al., 1998). Their method is based on the use of a rather large mercury/gold amalgam electrode with a diameter of >100 μm. Reactions at such large electrodes depend on planar rather than hemispherical diffusion (Heintze, 1993; see our introductory remarks), and the electrodes exhibit currents in the nanoampere range, due to their high consumption of analyte, which may disturb local gradients and pH (see also Buffle, 1988). The latter will also interfere with the electrode reactions (Davison et al., 1988). Voltammetric techniques require frequent conditioning of the microelectrode surface, and the resulting measurements are therefore rather slow as compared to other microsensors, that is, in the range of minutes per measuring scan. Thus, both spatial and temporal resolutions of present direct-voltammetric techniques are limited and would benefit from further optimization.

There is much literature on voltammetric techniques and their applicability for environmental analysis, which we cannot address here (a review appears in Tercier and Buffle, 1993). Other electrode materials like mercury-plated iridium microelectrodes seem better suited for environmental analysis (Tercier et al., 1995) as compared to gold electrodes, which are notorious for exhibiting adsorption and memory effects, especially in the presence of sulfide (J. Buffle and W. Davison personal communication). Iridium microelectrodes coated with a protective gel layer have been used for in situ measurements in natural waters and may also prove useful in sediments (Tercier and Buffle, 1996). The potential for porewater analysis in sediments by direct fast-scan voltammetry with microelectrodes needs further investigation, and such data should be critically evaluated by simultaneous analyses with other microanalytical techniques. Such a comparison of techniques should preferentially be performed both in defined solutions as well as in systems with defined gradients of analytes. Nevertheless, in situ voltammetry represents an interesting new approach for porewater analysis, and presently, it is the only available method,in addition to high resolution gel-sampling techniques (Davison et al., 1997), for fine-scale measurements of iron and manganese species in sediments (e.g., Huettel et al., 1998; Luther et al., 1998) and natural waters (Tercier-Waeber et al., 1998).

### 8.1.5 Gas Microsensors with Ion-Impermeable Membranes

Gas microsensors based on ion-impermeable membranes measure the partial pressure of a gas. To convert to molar concentrations, the temperature- and salinity-dependent solubility of the gas must be known. In practice, empirically determined relations for the concentration of dissolved gases in gas-equilibrated water of a known salinity and temperature (e.g., Garcia and Gordon, 1992) can be used for this purpose, if independent concentration measurements of the calibration solutions, for example, by Winkler titration for oxygen, do not exist.

The best known gas microsensor is the Clark-type (Clark et al., 1953) oxygen microsensor (Revsbech, 1989) that measures $pO_2$. The tip of such a sensor is shown in figure 8.3. An Ag/AgCl reference is also immersed into the internal mildly alkaline electrolyte, and the electrochemistry in the sensor is thus electrically insulated from the environment by the glass casing and the gas-permeable silicone membrane. The

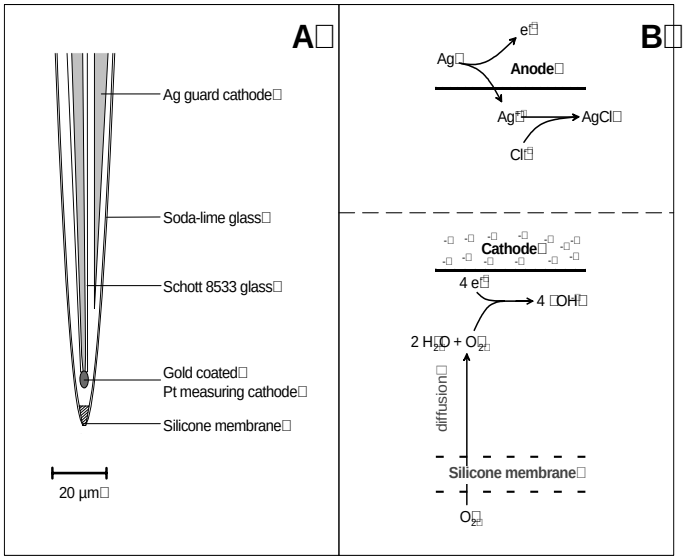


**Figure 8.3** A Clark-type $O_2$ microsensor with a 90% response time of about 0.2 s. A, Detailed view of the sensor tip. B, Schematic of the measuring principle. The top shows the reaction at the counter electrode in the bulk electrolyte, and the bottom illustrates the reaction at the polarized (–0.8V) measuring electrode in the microsensor tip. The alkaline electrolyte (pH 10) contains 0.5 M KCl in 0.5 M carbonate buffer.

guard cathode prevents diffusion of oxygen from the bulk electrolyte toward the sensor tip. These microsensors are characterized by absence of interferences, perfectly linear calibration curves, response times ($t_{90}$) as fast as 0.1 s, and low effects on the signal (<1–2%) from changes in stirring or diffusivity of the external medium (see also sections 8.1.4 and 8.2.4). In general, a current that is measured in an electrochemical system that is totally electrically shielded from the environment is more reliable than measurements with an electrode that is in direct physical and electrical contact with the environment.

The Clark-type $pO_2$ microsensor is probably the most frequently and widely used microsensor for environmental applications, and for a wide variety of purposes, including studies of photosynthesis and respiration in biofilms, sediments, and symbiotic consortia (Revsbech and Jørgensen, 1986; Revsbech, 1994; Kühl et al., 1995, 1996), $O_2$ distribution in soils and rhizospheres (Christensen et al., 1994; Højberg et al., 1994), $O_2$ microdistribution in a termite gut (Brune et al., 1995), and *in situ* $O_2$ profiles in deep-sea sediments (Gundersen and Jørgensen, 1990). An example of an oxygen microprofile in a sediment is shown in figure 8.2. An important result of the use of $pO_2$ microsensors has been the detailed characterization of the diffusive boundary layer properties via the measurement of oxygen profiles across the sediment-water interface (Jørgensen and Revsbech, 1985; Jørgensen and Des Marais, 1990; also chapters 10, 14, and 15). A significant part of our knowledge of the DBL thus comes from the use of Clark-type microsensors. Their functioning is well understood and has been optimized for environmental applications over the last decade to a stage where it is now possible to predict its measuring characteristics from the tip geometry dimensions, temperature, salinity, and $O_2$ concentration (Gundersen et al., 1998).

A similar sensor as the one shown in figure 8.3 can be used for analysis of nitrous oxide, if the gold tip is substituted with a silver tip and the electrolyte is made very

alkaline (pH > 13). The best catalytic activity with respect to $N_2O$ reduction occurs when the electrolyte is a pure NaOH solution. When using an electrolyte without chloride, a small exposed metal surface of the guard is needed to prevent an excessive current intensity in the guard electrode circuit. The $pN_2O$ microsensors can be equipped with a gold cathode in front of the silver cathode so that oxygen interference is avoided. The procedure for constructing such combined sensors was described by Revsbech et al. (1988). Simple $pN_2O$ sensors constructed with only a guard and a silver cathode for $N_2O$ detection are now used as sensing elements in nitrate biosensors (see section 8.3.3).

By substitution of the gold tip with a platinized (i.e., electrolytically coated with colloidal platinum) tip and acidification of the electrolyte, the sensor shown in figure 8.3 can be employed as a hydrogen sensor when a positive potential is applied to the platinum tip. The hydrogen sensors are usually operated without a guard. While the $pH_2$ microsensor has proven useful for studies in special environments like root nodules (Witty, 1991) and the digestive tract of termites (Ebert and Brune, 1997), the $H_2$ concentrations in nature are generally too low to be detected by the sensor, which has a detection limit around 1 µM. Hydrogen can, however, be used as a tracer in a sensor for flow and diffusivity, and such a sensor can then use a $H_2$ microsensor as an internal sensing element (see section 8.4).

A Clark-type microsensor with platinum electrodes can be employed to analyze dissolved hydrogen sulfide if a ferri-cyanide solution at alkaline pH is used as the electrolyte (Jerochewski et al., 1996; Kühl et al., 1998; fig. 8.4A). Hydrogen sulfide diffuses through the silicone membrane and is deprotonated to $HS^-$, which is oxidized by ferricyanide in the alkaline electrolyte. The ferrocyanide formed by this reaction is subsequently oxidized back to ferricyanide at a platinum measuring electrode (fig.

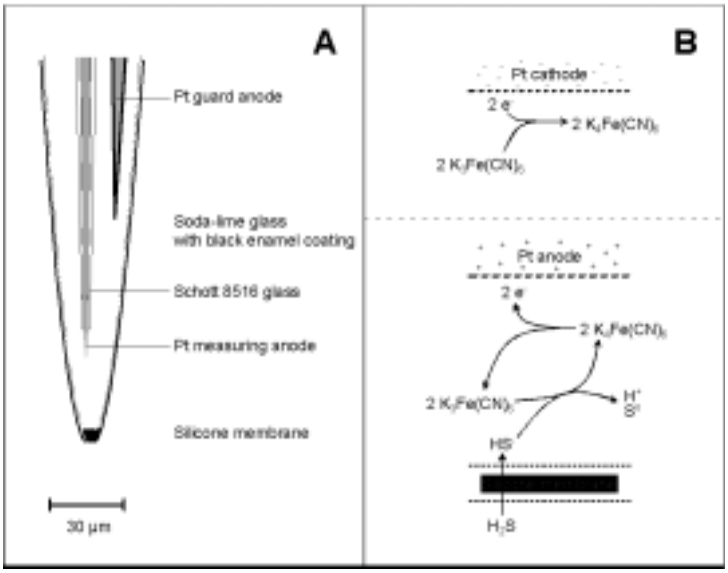


**Figure 8.4** A Clark-type microsensor for $H_2S$. A, Detailed view of the sensor tip. B, Schematic drawing of the measuring principle. The top shows the reaction at the counter electrode in the bulk electrolyte, and the bottom shows the reaction at the polarized (+85 mV) measuring electrode in the microsensor tip. The alkaline electrolyte (pH 10) contains 0.05 M $K_3Fe(CN)_6$ in 0.5 M carbonate buffer (from Kühl et al., 1998).

8.4B). The $pH_2S$ microsensor can be used for analysis of low sulfide concentrations without the need of a recent exposure to high concentrations, as needed with the simple $Ag/Ag_2S$ electrodes described in section 8.1.2, and they can also be made very thin (< 10–20 μm). The $pH_2S$ microsensor exhibits a linear response to dissolved $H_2S$, and as its basic design is similar to the Clark-type $pO_2$ microsensor, it also has a fast response time and low stirring sensitivity. Used in combination with pH microsensors, the $pH_2S$ microsensor has been successfully measured microprofiles of sulfide and anoxygenic photosynthesis in neutral to moderately alkaline sediments and biofilms up to pH 8.5 (Pringault et al., 1999; Wieland and Külh, 2000). Furthermore, fine-scale sulfide measurements in acidic environments now become possible with the new $pH_2S$ microsensor (Kühl et al., 1998). This new sensor may therefore replace the $Ag/Ag_2S$ microelectrode for most environmental applications.

Sensors based on pH changes in an electrolyte behind a membrane that allows acid-base active gases to pass have been known for several decades (Severinghaus and Bradley, 1958). Miniature $pCO_2$ sensors based on this principle were made for physiological applications at an early stage (e.g., Pul et al., 1978), but a high detection limit for $CO_2$ and long response times made them unsuitable for environmental applications. Microscale $pCO_2$ sensors with sufficient sensitivity for analysis at the sediment-water interface were constructed and used by Cai and Reimers (1993). Their sensors suffered, however, from very long response time, which is caused by the slow hydration of $CO_2$ and therefore a slow pH change in the buffered electrolyte of the sensor tip.

A more sensitive and considerably faster responding $pCO_2$ microsensor has been developed by De Beer et al. (1997a), who used a fast-responding pH-LIX microelectrode as the internal pH-sensing transducer in combination with the addition of the enzyme carbonic anhydrase to the electrolyte. Carbonic anhydrase catalyzes the hydration of $CO_2$ significantly and leads therefore to a faster response of the sensor. The 90% response time at concentrations around 10 μM $CO_2$ is, however, still in the range of 20–60s. The detection limit is around 5 μM. Zhao and Cai (1997) employed a similar design concept to construct a slightly larger $pCO_2$ microsensor, which possesses a longer life-expectancy (i.e., about 1 month), due to the use of a different glass type and reference electrode. When using this type of $pCO_2$ microsensor, care should be taken in any analysis of environments containing hydrogen sulfide, as $H_2S$ will also diffuse into the sensor tip and affect the pH behind the gas-permeable membrane.

### 8.1.6 Instrumentation for Electrochemical Microsensors

The basic circuitry requirements for electrochemical microsensors were described by Revsbech and Jørgensen (1986). Suppliers of sensitive pA meters and high impedance mV meters are numerous, but these systems often need special modifications for use with microsensors. Sources of dedicated microsensor instrumentation are listed in table 8.4. A general warning for the recording of microsensor signals is the risk of electrical short circuits caused by humidity. All electrochemical microsensors used for environmental purposes are constructed of glass, and glass surfaces will conduct electrical currents at high air humidity. A threshold air humidity that results in problems cannot be estimated a priori as this depends on the type and corrosion (i.e., hydration) of the glass surface. To avoid any problems, the parts likely to create problems should be enclosed in an electrode holder containing a desiccant (Revsbech and Jørgensen, 1986). The total current leakage in a measuring circuit should not exceed $10^{-13}$ Å.

Alternatively, the measuring circuits can be miniaturized and mounted directly on the microsensors via a waterproof plug (V. Meyer personal communication, Germany, MasCom GmbH). Such measuring systems exhibit a low signal noise and a further reduction of the response time of the measuring circuit. Microsensors equipped in this way can easily be interchanged even under harsh field conditions (e.g., on board

**Table 8.4** Some commercial sources for micro (<0.1 mm) and mini (<1 mm) sensors[a]

| Company | Products |
|---|---|
| Diamond Electrodes Inc. | $O_2$, pH, $S^{2-}$, LIX, and other microsensors |
| Fluka Chemie AG | LIX materials and minisensors |
| Lazar Research Lab. Inc. | $O_2$, pH, $CO_2$,and LIX minisensors |
| MasCom GmbH | $O_2$, pH, $S^{2-}$, redox, and $H_2S$ microsensors |
| Microelectrodes Inc. | pH, $CO_2$ , LIX minisensors |
| Precision Measurement Engineering (PME) | $O_2$, temperature, conductivity minisensors |
| Presens GmbH | $O_2$, pH, temperature, and other fiber-optic microsensors |
| UniSense Aps | $O_2$, pH, $N_2O$, $H_2S$ and other electrochemical microsensors; $CH_4$ and $NO_3^-$ microbiosensors; flow and diffusivity microsensors |
| World Precision Instruments (WPI) | $O_2$, pH, and LIX minisensors, NO microelectrodes |

a Most companies also supply necessary meters and accessory equipment.

ship. Furthermore, the waterproof design allows for robust in situ shallow-water measurements with the system mounted on platforms or operated by a SCUBA diver. For in situ microsensor analysis of boundary layers and sediments in deeper waters, however, sophisticated autonomous instruments, that is, benthic landers and remotely operated vehicles that can be deployed down to >5 km depth, have been designed and are reviewed by Tengberg et al. (1995) and in chapter 10.

In the literature, a Faraday cage is sometimes recommended for microsensor work to minimize electrical noise. One should remember, however, that a Faraday cage does only work properly if it also encloses the person operating the microsensor setup. In practice, it is our experience that an effective liquid coaxial shielding of microsensors (see section 8.1.3 and fig. 8.1) in combination with efficient grounding of the experimental setup, that is, avoiding formation of ground loops, leads to almost noise-free sensor signals. These relatively simple precautions are much more cost-effective than using an expensive Faraday cage and allow for simple experimental setups with an equal or even better performance with respect to electric noise.

## 8.2 Optical Microsensors

Electrochemical detection principles have a long tradition in environmental monitoring and are implemented in various macro- and microsensor designs (see sections 8.1, 8.2, and 8.4). However, as an alternative, optical sensing principles where analyte-dependent changes in an optical indicator are monitored have become more popular in recent years (e.g., Wolfbeis, 1991). While most developments and applications have come from analytical chemistry and biomedical research, such as blood gas analysis, the development of environmental sensors has only begun. Optical microsensors are based on the use of single-strand fiberoptics that collect and direct light signals between the sensor fiber tip and the opto-electronic measuring system (Holst et al., 2000). Below, we discriminate between fiber-optic microprobes that collect an inherent light field variable (e.g., light intensity, spectral composition, or fluorescence) from the tip surroundings, and fiber-optic microsensors, called *micro-opt(r)odes*, that measure physicochemical variables via optical changes, for example, changes in fluorescence or absorption of an indicator dye that is immobilized at a sensor tip (fig. 8.5).

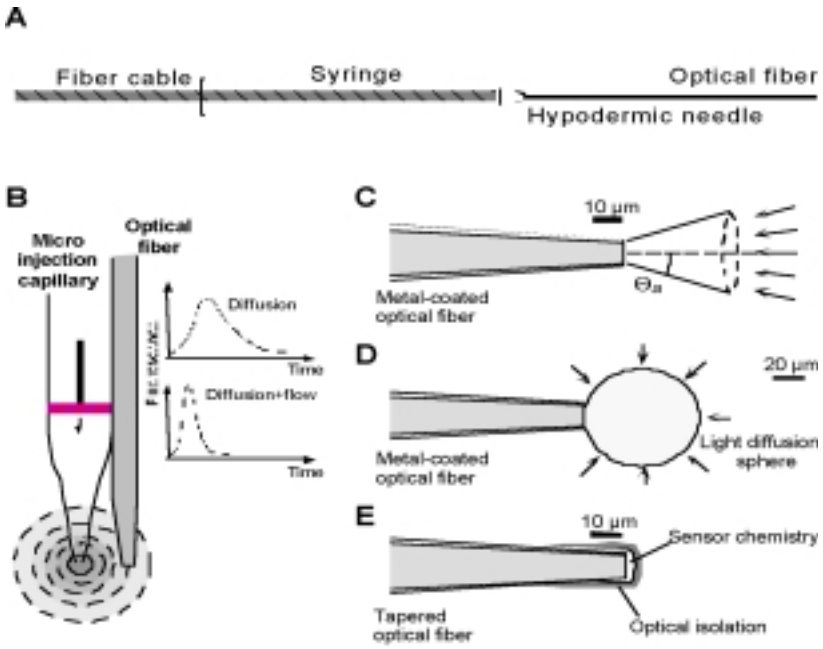


**Figure 8.5** Representative examples of fiber-optic microprobes and microsensors. A, Basic design of a fiber-optic microprobe/microsensor. B, A fiber-optic device for fine-scale diffusivity and flow measurements (see text for details). C, A simple tapered and coated optical fiber for measuring directional radiant flux (i.e., the field radiance). D, A scalar irradiance microprobe, which collects the available light from all directions isotropically via a small diffusing sphere cast at the end of a tapered fiber. Diagram E: A fiber-optic microsensor, micro-opt(r)ode, for O with a fluorescent indicator dye fixed at the fiber tip. (Partly redrawn after Kühl and Jørgensen [1992b], Klimant et al. [1995], and De Beer [1997])

### 8.2.1 Field Radiance Microprobes

The first fiber-optic microprobes for aquatic systems were developed to measure microscale spectral light distributions in sediments and biofilms. By using a simple tapered or untapered optical fiber, which has a well-defined directional response due to the inherent narrow acceptance angle of such fibers, the radiant flux incident from a small solid angle in a defined direction can be measured; this is the field radiance, which is the most fundamental variable of benthic light fields, as illustrated in fig. 8.5A (Jørgensen and Des Marais, 1986; Kühl and Jørgensen, 1992b, 1994). While field radiance microprobes are ideal for studies of zonations and migrations of photosynthetic microorganisms (Bebout and Garcia-Pichel, 1995; Kühl et al., 1994b; Pringault et al., 1998), their practical use for measuring light intensity available for photosynthesis is limited. Due to the diffuse nature of light fields in benthic environments (Kühl and Jørgensen, 1994; Kühl et al., 1994b), microorganisms experience light incident from all directions. Thus, estimation of the total light available for their photosynthesis would require field radiance measurements over all directions and subsequent spherical integration of the radiant fluxes in order to calculate the scalar irradiance; this is the total radiant flux from all directions about a point, which is the most relevant measure of light available for photosynthesis in the strongly scattering and absorbing sediment and biofilm matrix of cells, mineral grains, and polymers. Such measurements are not practical in general, and have only been done in a few detailed studies of sediment and biofilm optics (Jørgensen and Des Marais, 1988; Kühl and

Jørgensen, 1994; Kühl et al., 1994b). Furthermore, the procedure involves a relatively complicated mathematical treatment of the radiance data (Fukshansky-Kazarinova et al., 1996).

### 8.2.2 Irradiance and Scalar Irradiance Microprobes

Microprobes for measuring light intensity in benthic environments have been developed for measuring irradiance and scalar irradiance. Irradiance microprobes measure the incident quantum flux from the upper or lower hemisphere on a horizontal surface area element. This is realized by fixing a small light scattering disk on the end of an optical fiber (Kühl et al., 1994a; Lassen and Jørgensen, 1994) which has the properties of a microcosine collector. Scalar irradiance microprobes integrate the quantum flux from all directions; this is done by fixing a small light-scattering sphere at the end of an optical fiber (Lassen et al., 1992) that collects the incident light isotropically (fig. 8.5B). By use of these microprobes in connection with oxygen microsensors, detailed studies of photosynthesis regulation by light can be performed in benthic systems (Ploug et al., 1993; Kühl et al., 1997).

### 8.2.3 Microprobes for Fluorescence and Surface Detection

In addition to measuring the directional radiation with field radiance microprobes, probes in this class can also be used to monitor induced fluorescence in the surroundings of the tapered optical fiber or to detect the penetration of the fiber tip into a solid matrix (e.g., the sediment-water interface). Klimant et al. (1997a) have recently developed a simple method for detecting the sediment-water interface with a field radiance microprobe. By directing near-infrared (NIR) light from the tapered fiber to the sediment surface and collecting the backscattered light through the same fiber, the penetration of the fiber tip into the sediment or any other optical boundary can be detected. When the fiber tip approaches the sediment surface, an increased backscattering is recorded, and the highest relative signal change will occur at the water-sediment interface. By combining such a simple fiber-probe with a microsensor, autonomous surface detection becomes possible. This is particularly important for in situ applications of microsensors that are mounted on benthic landers (see chapter 10).

By directing blue excitation light out of the fiber tip, pigments such as chlorophyll (Chl a) can be excited at the fiber tip and the induced fluorescence can be collected via the same fiber. This allows for high-resolution measurements of pigment distributions in sediments and biofilms. Furthermore, such a probe also allows the use of a more sophisticated microscale fluorescence analysis, where the redox status of photosystem II in oxygenic phototrophs can be monitored and photochemical as well as nonphotochemical quenching can be quantified (Schreiber et al., 1996).

Additionally, by gluing a field radiance microprobe together with a microinjection capillary, a simple device for finescale flow and diffusivity measurements can be realized (fig. 8.5D; De Beer, 1997). After injection of a nanoliter-sized plume of a fluorescent dye, the dye will move away from the injection point due to diffusion and flow. By monitoring the time-dependent change in dye fluorescence measured at some distance from the injection spot with the field radiance microprobe, diffusivity or flow velocity can be calculated (De Beer, 1997; De Beer and Stoodley, 1996; see also chapter 15).

### 8.2.4 Fiber-Optic Microsensors: Micro-opt(r)odes

Optical detection of various physicochemical variables can also be accomplished by immobilization of an optical indicator in a sensor fiber tip. Such indicators change

their absorption or fluorescence characteristics as a function of the variable measured (fig. 8.5C). While such detection principles are well known and implemented as larger sensors (i.e., opt(r)odes; Wolfbeis, 1991), the first fiber-optic microsensors (micro-opt(r)odes) for environmental application have only recently appeared (Klimant et al., 1995; Holst et al., 1997a, 1997b; Kohls et al., 1997). Most optical sensors are either based on analyte concentration dependent quenching of the indicator fluorescence (e.g., oxygen and temperature sensors) or on an analyte concentration-dependent change in the absorption characteristics of the indicator chemistry (e.g., pH and $CO_2$ sensors). A common requirement for such microscale sensors is a suitable photostable sensor chemistry immobilized in a polymer matrix that adheres well to the fiber tip and exhibits a high signal intensity. Once the sensor chemistry and matrix materials have been properly chosen, micro-opt(r)odes are easy to manufacture, as the process involves only tapering of the fiber and subsequent dip-coating in a cocktail of the sensor chemistry and the dissolved matrix material, followed, in some cases, by a final coating of the sensor tip with an optical insulation. Furthermore, a sensitive opto-electronic measuring system is required that directs light to and from the fiber tip via the same sensor fiber without significant losses (see section 8.2.5).

Oxygen micro-opt(r)odes are based on the oxygen-dependent dynamic fluorescence quenching of various ruthenium complexes, or platinum- and palladium-porphyrins (see Klimant et al., 1997b) that are excited by blue and yellow light emiting diodes (LEDs), respectively, and emit red light. The indicators are immobilized in sol-gel or polystyrene matrices that are applied as a thin layer of sensor chemistry on the fiber tip (fig. 8.5C). The calibration curves of micro-opt(r)odes based on fluorescence quenching exhibit a hyperbolic shape; that is, the largest relative signal changes occur at low oxygen concentrations (Klimant et al., 1995). Either the fluorescence intensity or the fluorescence lifetime can be used as the measured variable, but the latter allows for more robust measurements independent of ambient light and photo-bleaching effects (Holst et al., 1997b). Various sensor chemistries are available, dependent on the dynamic range that is required (Klimant et al., 1997b). While ruthenium-based micro-opt(r)odes exhibit sensitivities equal to electrochemical $O_2$ microsensors in the range of 0–100% $O_2$, the porphyrin-based microsensors are best in the 0–100 % air saturation range. It is also possible to make porphyrin-based sensors that exhibit a very high sensitivity at trace levels of oxygen, that is, <0.1% air saturation, where electrochemical $O_2$ microsensors are less useful.

In addition to simple construction and a good storage and measuring signal stability, one of the most important characteristics of $O_2$ micro-opt(r)odes is the fact that these microsensors do not consume $O_2$ and therefore exhibit no stirring sensitivity. An illustration of the effect of stirring sensitivity on measured microprofiles is shown in figure 8.6, where a comparison of electrochemical and fiber-optic $O_2$ microsensors was done in an artificial sediment of glass beads without any oxygen gradient present between the glass beads and the overlying water. While the $O_2$ micro-opt(r)ode measured a constant concentration, as expected, both $O_2$ microelectrodes showed a decrease in signal as they approached the sediment surface (see also section 8.1.4). It is, however, possible to construct Clark-type oxygen microsensors with less stirring sensitivities than the ones used for recording the data in figure 8.6. Due to their high stability and easy construction, $O_2$ micro-opt(r)odes are ideal for deployment on benthic landers or other measuring platforms. The first adaptation and test of $O_2$ micro-opt(r)odes on benthic landers were, thus, recently completed (Glud et al., 1998a,b). During this study, the pressure dependency of the microsensors was tested, and the results showed no need for a pressure compensation even when used in the deep sea.

A fiber-optic temperature microsensor has been developed around the principle developed for $O_2$ micro-opt(r)odes (Holst et al., 1997a). While the measuring system and the basic sensor-chemistry remain the same, the immobilization procedure and the design of the microsensor tip were adapted to exclude $O_2$. The fluorescence thus depends only on the ambient temperature at the very fiber tip. This sensor may find application as an internal temperature sensing element for temperature compensation

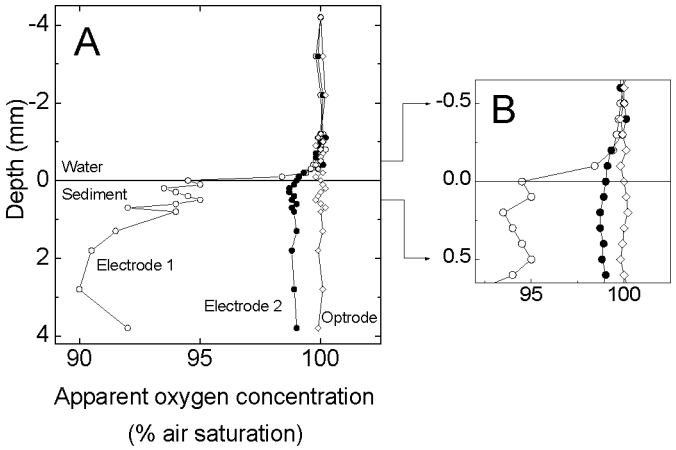


**Figure 8.6** Microprofiles of apparent $O_2$ concentration in an artificial sediment with a stirred water phase above, as measured by a $O_2$ micro-opt(r)ode and Clark-type $O_2$ microelectrodes with a high (electrode 1) and a low (electrode 2) stirring sensitivity, respectively. There was no real gradient of $O_2$ present in the system. (From Klimant et al. [1995]).

of other microsensors. Furthermore, the high spatial resolution of the temperature micro-opt(r)ode is ideal for resolving thermal gradients in benthic environments.

A pH micro-opt(r)ode has been developed by Kohls et al. (1997a). This sensor consists of a pH-indicator matrix, fixed at the fiber tip. The pH is evaluated by the pH-dependent change of spectral reflectance, due to a change in the absorption properties of the indicator, guided back from the sensor tip. The sensor has been calibrated successfully for marine systems in the pH range of 7–9.5. Based on fiber-optic pH sensing, the first fiber-optic $CO_2$ microsensors were recently developed (Kohls et al., 1997b; Holst et al., 2000) and employed in the deep-sea (Wenzhöfer, 1999).

### 8.2.5 Instrumentation for Fiber-Optic Microsensors

While most fiber-optic microsensors are relatively easy to construct, they require more sophisticated opto-electronic measuring systems and high-quality optical components. The small light-collection areas pose a need for efficient excitation and light detection with high sensitivity. For many years, this limited measuring systems to relatively complicated, often microscope-based, laboratory setups with laser light sources and large expensive light detection components (photomultiplier tubes or cooled diode array and charge-coupled devise [CCD] cameras). The rapid development of opto-electronics within telecommunications and information storage technology, however, has resulted in the availability of small, robust, and relatively inexpensive components that are ideal for optical sensor measuring systems. Simple sensitive spectrometers and photomultipliers for light detection, bright LEDs for fluorescence excitation, as well as robust optical components like fiber-optic couplers, that is, a fiber-optic analog to beam-splitters, are now commercially available. Consequently, relatively simple and robust measuring systems have recently been developed for use in laboratory and field applications or on autonomous measuring platforms sush as benthic landers. A fundamental design of a measuring system for fiber-optic microsensors is shown in figure 8.7. More detailed information on measuring systems for use with fiber-optic microsensors can be found elsewhere (Kühl and Jørgensen, 1992b; Holst et al., 1995, 1997b; Klimant et al., 1995, 1997b; Kühl et al., 1997; Glud et al., 1998a,b).

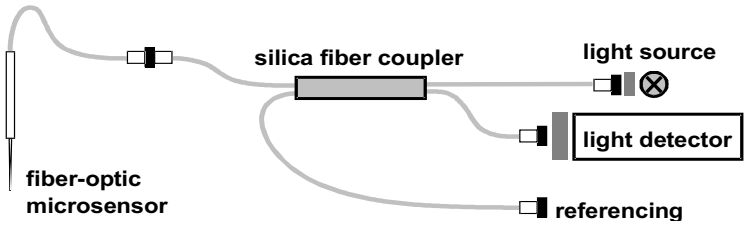


**Figure 8.7** General scheme of a measuring system for fiber-optic microsensors. In the case of fluorescence measurements, a low-pass filter must be inserted in front of the excitation light source and a high-pass filter in front of the detector. The light source can be an LED or a broad-band miniature xenon or halogen lamp, while the detector can be a small photomultiplier tube, a single photodiode, an intensified diode array, or a CCD chip. The fiber-optic coupler divides the light input from each fiber strand equally between the opposite two fibers and thus acts as a fiber-optic beam-splitter. In the case of fluorescence measurements, either the intensity or the fluorescence lifetime can be monitored by using special electronic modulation schemes of the light source and detector.

## 8.3 Microbiosensors

The simplest possible biosensors are the biological oxygen demand (BOD) sensors, made by immobilizing a layer of heterotrophic microorganisms in front of an oxygen sensor. Dissolved organic matter in the medium being analyzed stimulates microbial respiration by the immobilized microorganisms, and the oxygen signal decreases. A microsensor based on this principle has been made by Neudörfer and Meyer-Reil (1997) and used for a semiquantitative determination of dissolved organic matter in sediment porewater. Of the problems with such sensors, we should mention correction for ambient oxygen concentration, induction of enzymes for uptake and metabolism of the various dissolved electron donors, and the need for oxic conditions. Revsbech et al. (2000) offer a detailed review of microscale biosensors.

The first selective microscale biosensor to be described was a glucose microsensor based on detection of hydrogen peroxide, produced by enzymatic oxidation of glucose with oxygen (Cronenberg et al., 1991). However, glucose is not a species that builds up near the benthic boundary layer. Recently biosensors for the two more relevant species, nitrate and methane, have been described (Larsen et al., 1996, 1997; Damgaard and Revsbech, 1997; Damgaard et al., 1998). Both of these sensors are based on the same general principle that bacteria, which transform the chemical species to be sensed, are grown in the tip of a capillary behind a membrane permeable to that chemical species. As growth depends on the chemical species entering through the membrane, growth of the bacteria is limited to an extremely small volume, typically contained within a cylinder volume of 20 μm in diameter and 100 μm in length. Bacterial growth is limited by the flux through the membrane. All other substrates for growth are supplied in excess by diffusion from a large (1 mL) medium present in the shaft of the sensor. Due to the $10^5$ times larger volume of this reservoir as compared to the volume with growth, the supply from the reservoir never becomes limiting.

### 8.3.1 Methane Microbiosensor

Methane is the end product of anaerobic degradation in environments with limited availability of sulfate, and a method for accurate in situ measurement of $CH_4$ concentration profiles would therefore be of great value. A procedure based on gas sampling through a small "membrane inlet" probe has been developed (Rothfuss and Conrad,

1994; Lloyd et al., 1996), but due to the relatively large membrane area needed, these devices are sensitive to the transport characteristics (water current or diffusivity) of the external medium, and they have a limited spatial resolution of approximately 1 mm. A better spatial resolution can be realized (e.g., Baumgardner et al., 1995), but this leads to a significant drop in sensitivity.

A microscale biosensor for $CH_4$ can be constructed by growing methane oxidizing bacteria in a gradient of $CH_4$ that diffuses from the medium and a gradient of $O_2$ that diffuses from an internal gas reservoir (fig. 8.8A). The $O_2$ consumption within the sensor is dependent on the $CH_4$ concentration in front of the silicone membrane at the sensor tip, and this is reflected in changes in the $O_2$ profile within the sensor (Damgaard and Revsbech, 1997). These changes in oxygen profile are monitored by the internal $O_2$ microsensor, which continuously senses the $O_2$ concentration in front of the silicone membrane that seals the internal gas reservoir. The calibration curves obtained by monitoring the signal from the internal $O_2$ microsensor as a function of methane concentration are linear at low concentrations, but some sensors exhibit nonlinear curves at higher concentrations.

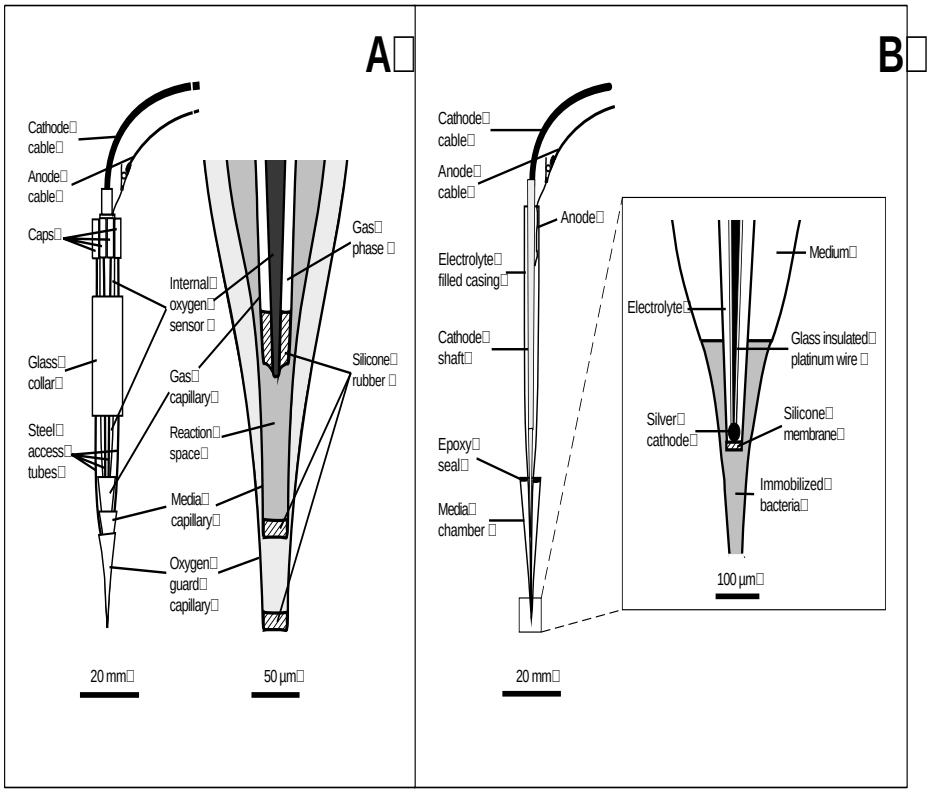


**Figure 8.8** Two new microbiosensors. A, Methane microbiosensor based on methane oxidation by methanotrophic bacteria growing in opposing gradients of methane diffusing in from the exterior and oxygen diffusing from an internal reservoir. Interference from external oxygen is avoided by use of an oxygen guard capillary filled with heterotrophic bacteria. (Redrawn after Damgaard et al. [1998]) B, Nitrate microbiosensor, based on a special mutant strain of bacteria that denitrify nitrate (and nitrite), diffusing in from the exterior, to nitrous oxide, which is measured by an internal nitrous oxide microsensor. (Redrawn after Larsen et al. [1997])

Oxygen and $H_2S$ are the only environmentally relevant interfering chemical species, but $O_2$ interference may be avoided by an $O_2$ scavenging system in front of the sensor (Damgaard et al., 1998). In the same publication, a strategy for removing the $H_2S$ interference was also described, but yet has to be realized (Damgaard et al., 1998). There can also be a small but significant interference from $H_2$ (L. R. Damgaard, personal communication); however, the $H_2$ concentrations in most sediments are so low (<<1 μM) that the interference is irrelevant. The difference between stagnant and vigorously stirred water usually corresponds to about 5 μM $CH_4$ for the microsensor without an $O_2$ guard, independent of $CH_4$ concentration. Sensors with an $O_2$ guard do not exhibit any stirring sensitivity. The lifetime of $CH_4$ biosensors is several months.

### 8.3.2 Nitrate Microbiosensor

The transformation rates and concentrations of inorganic nitrogen compounds are key variables in the biogeochemistry of aquatic environments, and for freshwater environments LIX-based microsensors can be used to analyze of $NO_3^-$, $NO_2^-$, and $NH_4^+$ (section 8.1.3). In marine environments, the high concentrations of interfering ions prevent the use of LIX-based sensors. Alternatively, $NO_3^-$ can be reduced to $N_2O$ by use of a culture of denitrifying bacteria, and the $N_2O$ can subsequently be quantified by an electrochemical $N_2O$ microsensor (see section 8.1.4).

A biosensor based on this principle was originally made with a very simple design (Larsen et al., 1996), which was not suited for environmental analysis. This design has been improved to allow determination of microprofiles in sediments and biofilms (fig. 8.8B; Larsen et al., 1997). The detection limit is better than 0.1 μM. High oxygen concentrations and low temperature both decrease the dynamic range of the sensor, but they do not interfere with the linearity of the signal. As an example of the temperature effect, a sensor with a 20 μm tip exhibited a linear calibration in the 0–300 μM range at 20°C, but only 0–50 μM at 4°C. At the same time the 90% response time to changes in $NO_3^-$ concentration increased from 20 to 35 s. The lifetime of $NO_3^-$ biosensors is at present only a few days, but work is being carried out to improve long-term stability. Except for $H_2S$ and nitrogen oxides (i.e., $NO_2^-$ and $N_2O$), there are no interfering agents. The nitrate micro-biosensors can thus be used in a wide variety of media, oxic or anoxic, and in a temperature range of 3–40°C.

Nitrate biosensors have been used for analysis of nitrate profiles in marine sediments, lake sediments, and waste water biofilms (Schramm et al., 1996). Continuous on-line monitoring of water-phase $NO_3^-$ in natural environments and in waste water treatment plants has been performed for periods of up to 10 days, and with the latest improvements in $N_2O$ detection and tip membrane application (T. Kjær, unpublished data) ,considerably longer lifetimes should be possible. The latest improvements have also removed the sulfide interference.

## 8.4 Microsensors for Diffusivity and Water Velocities

### 8.4.1 Diffusivity Microsensor

Measurements of microscale concentration gradients near the sediment-water interface can be of limited value unless diffusion-reaction models can be used to extract reaction rates and fluxes. Consequently, detailed information about the diffusive properties of the investigated media is essential. Resistivity probes (Andrews and Bennett 1981) have been employed by many investigators for this purpose. However, resistivity probes are not ideal for analysis of the uppermost few millimeters of the sediment, as the abrupt change in diffusive properties at the interface cannot be resolved. A microsensor is now available for this purpose (Revsbech et al., 1998). The microsensor consists of a glass capillary with a silicone membrane in the tip. The membrane is

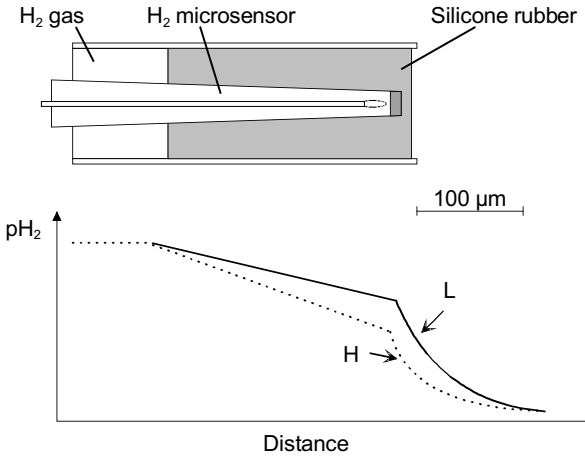


**Figure 8.9** Microsensor for diffusivity based on hydrogen as the diffusing tracer, which is monitored by a hydrogen microsensor (see text for more details). The lower part of the figure indicates the different internal gradients of H in the case of a low (L) and a high (H) diffusivity of the external medium surrounding the sensor tip. A similar but smaller sensor can be used to quantify water flow over a very high dynamic range. (Redrawn from Revsbech et al. [1998])

two to four times thicker than the inner diameter of the capillary. The lumen behind the membrane is filled with a gas that can be sensed by some type of microsensor, and the appropriate microsensor is contained within the capillary, with its tip inserted into the membrane (fig. 8.9).

The bulk volume of the sensor is a source of gas that diffuses from the gas reservoir, through the membrane, and out into the environment. The concentration sensed by the internal gas microsensor is governed by the relative diffusional resistance within the membrane in comparison to the diffusional resistance in a sphere around the tip of the sensor. Revsbech et al. (1998) have shown that the signal, S, from the gas sensor is described by the equation

$$S = a/(\phi D_s + b) \tag{8.1}$$

where $\phi$ is the porosity, $D_s$ is the apparent diffusion coefficient, and a and b are characteristic constants for a specific sensor. The sensors to date have been based on $O_2$ or $H_2$ as the diffusing gases. The practical applicability of $O_2$ is limited, but $H_2$ is acceptable as a tracer in most contexts. However, some environments may consume hydrogen at rates that interfere with the assay. Nitrous oxide could also be used a tracer gas, but oxygen-insensitive $N_2O$ sensors are tedious and difficult to fabricate.

The spatial resolution of $\phi D_s$ measured by the new diffusivity microsensor as shown in figure 8.9 is dependent on the tip diameter. Sensors can easily be made with tip diameters down to 25 µm, and such a sensor would have a spatial resolution of about 100 µm; however, most of the effect on the signal is caused by the environment within one electrode diameter from the sensor. Such high spatial resolutions may seem advantageous, but most environments are too heterogeneous for high-resolution analysis. The use of 100-µm-thick sensors can only be recommended in fine-grained sediments, and the sensor should always be at least twice as thick as the mean diameter of the grains within the sediment. The response time is about 4 min for a 100-µm-thick sensor, and as a threefold larger diameter would result in approximately a response time that is nine times longer, the practical applicability is limited to sensors <300 µm. The latter fact also limits practical investigations to sediments with grains sizes smaller or equal to fine sands.

### 8.4.2 Flow Microsensor

The sensor shown in figure 8.9 can also act to measure water flow (J. Gundersen, personal communication). For this purpose the tip diameter should be made much smaller, and the membrane in the tip should be as thin as possible (i.e., 10–20 μm). Such sensors have been calibrated and employed to analyze flow fields above solid surfaces. Sensors with a tip diameter of about 25 μm can usually be used to quantify flows ranging from 5 μm $s^{-1}$ up to 5 cm $s^{-1}$. The 90% response time to changes in flow regime may be as short as 0.2 s. Detailed investigations of flow at a microscale using the sensor principle illustrated in fig. 8.9 have not been reported to date; alternatively, see section 8.2.3 and Chapter 15.

## 8.5 Physical Disturbance Caused by Sensor Insertion

Microscale analyses of chemical species have many advantages over conventional methodologies, including high spatial resolution, rapid response, and minimal disturbance of the analyzed substrate. The minimal disturbance of the analyzed substrate does not mean, however, that no disturbance occurs. Although microsensor tips may be very thin, introduction of the sensor to deeper layers may lead to considerable disturbance from the microsensor shaft, and in such a case the recording of multiple concentration profiles should not be performed at the same location. Furthermore, even very slim electrodes with tip diameters of a few micrometers cause changes in the thickness of the DBL, corresponding to an apparently higher flow rate when inserted from above the sediment (Glud et al., 1994; see also chapter 14). This last effect can be overcome by introduction of the sensor from below, so that only the tip of the sensor enters the diffusive boundary layer, but such a procedure can only be accomplished in some laboratory setups. Compression of the DBL by the insertion of microsensors from above was found to be less important in sediments and biofilms with a heterogeneous surface topography (Lorenzen et al., 1995). The effect on the overall concentration profiles is most pronounced when a major part of the concentration change occurs in the DBL and when the microsensor is thick. For example, the analysis of a highly active biofilm from a waste water treatment plant was corrupted by the use of a 25-μm-thick nitrate biosensor (Schramm et al., 1996), whereas the effect will be minor by analysis of ordinary sediments with 10-μm-thick oxygen microsensors.

## 8.6 Summary and Some Directions for Future Work

This chapter has reviewed the possibilities for microsensor analysis of the benthic boundary layer and interfacial biogeochemical processes as they stood in mid-1998. However, development of new microsensor-based techniques has accelerated during the last few years, and this is likely to continue. We do not expect that many new chemical species will be added to the list of those we can detect at present, but the existing technologies will be refined or improved by the introduction of more sensitive and more reliable ones. Fiber-optic techniques may become more prominent due to a generally higher signal stability with optical detection, while new optical detection principles need to be devised for the analysis of ions, where optical sensing still lags behind electrochemistry. Furthermore, microbiosensors, based on immobilized enzymes and whole cells, along the lines of sensing principles developed for nitrate and methane, will become more common.

New microsensors for flow and diffusivity allow for much more detailed studies of mass transfer phenomena in benthic environments, and we envision many interesting applications in boundary layer research. A better understanding of the mass transfer phenomena in sediment and biofilms is also a prerequisite for developing better and

more accurate diffusion-reaction models to interpret measured microprofiles and to calculate conversion rates.

When applying microsensors in natural systems and interpreting data, it is important to keep in mind that such measurements are point measurements in a complex heterogeneous matrix, which can exhibit a pronounced spatial and temporal heterogeneity due to animal activity, for example. While microsensor data often allow for detailed studies of fundamental regulatory mechanisms and interactions of various biogeochemical processes, an extrapolation of process rates to larger sediment areas can be problematic and requires a thorough consideration of the system's heterogeneity. Often microsensor measurements are interpreted assuming a 1D diffusion geometry for sediments, while the system is, in reality, 3D. A good example of the importance of such considerations can be found in chapter 15, where microsensor measurements in biofilms are discussed in terms of diffusion geometry, sample heterogeneity, and mass transfer phenomena.

Ideally, numerous microsensor measurements should be combined with techniques that integrate over larger areas, e.g., flux chambers, and with studies on structural heterogeneity when quantitative questions about biogeochemical processes must be resolved. New microscopic and imaging techniques have become available, that allow 2D and 3D studies of biofilms and sediments, and the 2D distribution of chemical parameters in sediments can now be studied with planar optrodes (Glud et al., 1996, 1998b, 1999; Klimant et al., 1997b), combined with sophisticated imaging systems (Holst et al., 1998). In situ gel sampling devices are now available for measuring chemical porewater composition at high spatial resolution (Harper et al., 1997; Davison and Zhang, 2000), and these techniques can probably be further extended to allow for 2D mapping of porewater composition (Davison et al., 1994, 1997). By use of dissolved $O_2$ sensitive dyes or coated microparticles in connection with laser scanning and imaging techniques, it should also be possible to realize noninvasive measurements of the 3D oxygen distribution near the sediment-water interface and around other interfaces. In connection with modern particle velocimetry techniques, this would ultimately allow the simultaneous visualization of hydrodynamic and diffusive boundary layers. Microsensor studies in combination with these new techniques appear to us as an important new direction, and we foresee numerous applications in boundary layer research and biogeochemistry over the next years.

A long wish list for new microsensors continues to exist. In biogeochemistry, probably the largest gap in our ability to directly measure relevant variables is in the analysis of sulfate, phosphate, metal ions, and simple organic substrates such as acetate; fine-scale detection of some of these compounds is already possible with gel-sampling techniques. The present arsenal of microsensors permits detailed studies of the most important biogeochemical processes, and the widespread use of microsensors throughout the scientific community is not limited by the number of attractive techniques, but rather by the practical availability of microsensors. Commercial production of various types of microsensors is now becoming more common, and several companies sell micro- and minisensors and necessary measuring equipment (see table 8.4).

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## References


Amann, R., and Kühl, M. (1998) In situ methods for assessment of microorganisms and their activities. Curr. Opin. Microbiol. 1, 352–358.

Amman, D. (1986) Ion-Selective Microelectrodes: Principles, Design and Applications. Springer Verlag, Heidelberg.

Andrews, D., and Bennett, A. (1981) Measurements of diffusivity near the sediment-water interface with a fine-scale resistivity probe. Geochim. Cosmochim. Acta 45, 2169–2175.

Baumgardner, J. E., Quin, J. A., and Neufeld, G. R. (1995) Micropore membrane inlet mass spectrometer probes suitable for measurements of tissue surface gas tensions. Jour. Mass Spectr. 30, 563–571.

Baumgärtl, H., and Lübbers, D. W. (1973) Platinum needle electrodes for polarographic measurement of oxygen and hydrogen, *in* Oxygen Supply (Kessler, M., ed.), p. 130–136. Urban and Schwarzenberg, München.

Bebout, B. M., and Garcia-Pichel, F. (1995) UVB-induced vertical migration of cyanobacteria in a microbial mat. Appl. Environ. Microbiol. 61, 4215–4222.

Brendel, P. J., and Luther, G. W. III (1995) Development of a gold amalgam voltammetric microelectrode for the determination of dissolved Fe, Mn, $O_2$, and S(II-) in porewaters of marine and freshwater sediments. Environ. Sci. Technol. 29, 751–761.

Brezinski, D. P. (1983) Influence of colloidal charge on response of pH and reference electrodes: the suspension effect. Talanta 30, 347–354.

Brune, A., Emerson, D., and Breznak, J. A. (1995) The termite gut microflora as an oxygen sink: microelectrode determination of oxygen and pH gradients in guts of lower and higher termites. Appl. Environ. Microbiol. 61, 2681–2687.

Buffle, J. (1988) Complexation reactions in aquatic systems. An analytical approach. Ellis Horwood, Chichester, U.K.

Buffle, J., Horvai, G. (2000) In situ Monitoring of Aquatic Systems: Chemical Analysis and Speciation. John Wiley, New York.

Cai, W.-J., and Reimers, C. E. (1993) The development of pH and $pCO_2$ microelectrodes for studying the carbonate chemistry of pore waters near the sediment-water interface. Limnol. Oceanogr. 38, 1762–1773.

Christensen, P. B., Revsbech, N. P., and Sand-Jensen, K. (1994) Microsensor analysis of oxygen in the rhizosphere of the aquatic macrophyte *Littorella uniflora* (L.) Ascherson. Plant Physiol. 105, 894–852.

Clark, L. C, Wolf, R., Granger, D., and Taylor, A. (1953) Continuous recording of blood oxygen tension by polarography. Jour. Appl. Physiol. 6, 189–193.

Cronenberg, C. C. H., Van Groen, H., De Beer, D., and Van den Heuvel, J.C. (1991) Oxygen-independent glucose microsensor based on glucose oxidase. Anal. Chim. Acta 242, 275–278.

Damgaard, L. R., and Revsbech, N. P. (1997) A microscale biosensor for methane. Anal. Chem. 69, 2262–2267.

Damgaard, L. R., Revsbech, N. P., and Reichardt, W. (1998) Use of an oxygen-insensitive microscale biosensor for methane concentration profiles in a rice paddy. Appl. Environ. Microbiol. 64, 864–870.

Davison, W., Buffle, J., and DeVitre, R. (1988) Direct polarographic determination of $O_2$, Fe(II), Mn(II), S(-II) and related species in anoxic waters. Pure Appl. Chem. 60, 1535–1548.

Davison, W., Fones, G. R., and Grime, G. W. (1997) Dissolved metals in surface sediment and a microbial mat at 100 μm resulution. Nature 387, 885–888.

Davison, W., and Whitfield, M. (1977). Modulated polarographic and voltammetric techniques in the study of natural water chemistry. Jour. Electroanal. Chem. 75, 763–789.

Davison, W., and Zhang, H. (2000) In situ measurements of labile species in water and sediments using DGT, *in* Chemical Sensors in Oceanography (Varney, M., ed.).

Gordon and Breach, Newark, N.J.
Davison, W., Zhang, H., and Grime, G.W. (1994) Performance characteristics of gel probes used for measuring the chemistry of pore waters. Environ. Sci. Technol. 28, 1623–1632.
de Beer, D. (1997) Microenvironments and mass transfer phenomena in biofilms and activated sludge studied with microsensors, *in* Proceedings of the International Symposium on Environmental Biotechnology (Verachtert, H., and Verstraete, W., eds.), p. 217–224. Koninklijke Vlaamse Ingenieurs Verininging, Antwerpe.
de Beer, D., Glud, A., Epping, E., and Kühl, M. (1997a) A fast responding $CO_2$ microelectrode for profiling in sediments, microbial mats and biofilms. Limnol. Oceanogr. 42, 1590–1600.
de Beer, D., Kühl, M., Stambler, N., and Vaki, L. (2000) A microsensor study of light enhanced $Ca^{2+}$ uptake and photosynthesis in the reef-building coral *Favia sp*. Mar. ecol. Prog. ser. 194, 75-85.
de Beer, D., Schramm, A., Santegoeds, C. M., and Kühl, M. (1997b) A nitrite microsensor for profiling environmental biofilms. Appl. Environ. Microbiol. 63, 973–977.
de Beer, D., and Stoodley, P. (1996) Relation between the structure of an aerobic biofilm and transport phenomena. Water Sci. Technol. 32, 11–18.
de Beer, D., and Sweerts, J. P. R. A. (1989) Measurement of nitrate gradients with an ion-selective microelectrode. Anal. Chim. Acta 219, 351–356.
de Beer, D., and Van den Heuvel, J. C. (1988) Response of ammonium-selective microelectrodes based on the neutral carrier nonactin. Talanta 35, 728–730.
Ebert, A., and Brune, A. (1997). Hydrogen concentration profiles at the oxic-anoxic interface: A microsensor study of the hindgut of the wood-feeding lower termite *Reticulitermes flavipes* (Kollar). Appl. Environ. Microbiol. 63, 4039–4046.
Fluka (1997) Selectophore, Ionophores for Ion-Selective Electrodes and Optrodes. Fluka Chemie, Buchs, Switzerland.
Fukshansky-Kazarinova, N., Fukshansky, L., Kühl, M., and Jørgensen, B. B. (1996) Theory of equidistant three-dimensional radiance measurements with optical microprobes. Appl. Optics 35, 65–73.
Garcia, H. E., and Gordon, L. I. (1992) Oxygen solubility in sea-water: better fitting equations. Limnol. Oceanogr. 37, 1307–1313.
Garcia-Pichel, F. (1995) A scalar irradiance fiber-optic microprobe for the measurement of ultraviolet radiation at high spatial resolution. Photochem. Photobiol. 61, 248-254.
Glud, R. N., Gundersen, J. K., Revsbech, N. P., and Jørgensen, B. B. (1994) Effects on the benthic diffusive boundary layer imposed by microelectrodes. Limnol. Oceanogr. 39, 462–467.
Glud, R. N., Kühl, M., Kohls, O., and Ramsing, N. B. (1999) Heterogeneity of oxygen production and consumption in a photosynthetic microbial mat as studied by planar optodes. Jour. Phycol. 35, 270-279.
Glud, R. N., Ramsing, N. B., Gundersen, J. K. and Klimant, I. (1996) Planar optrodes: a new tool for fine scale measurements of two-dimensional $O_2$ distribution in benthic communities. Mar. Ecol. Progr. Ser. 140, 217–226.
Glud, R. N., Klimant, I., Holst, G., Kohls, O., Meyer, V., Kühl, M., and Gundersen, J.K. (1998) Adaptation, test and in situ measurements with $O_2$ microoptodes on benthic landers. Deep-Sea Res. I 46, 171–183.
Glud, R. N., Santegoeds, C. M., de Beer, D., Kohls, O., and Ramsing, N. B. (1998) Oxygen dynamics at the base of a biofilm studied with planar optodes. Aquat. Microbial Ecol. 14, 223–233.
Gundersen, J. K. and Jørgensen, B. B. (1990) Microstructure of diffusive boundary layers and the oxygen uptake of the sea floor. Nature 345, 604–607.
Gundersen, J. K., Ramsing, N. B., and Glud, R. N. (1998) Predicting the signal of $O_2$ microsensors from physical dimensions, temperature, salinity, and $O_2$ concentration. Limnol. Oceanogr. 43, 1932–1937.
Gust, G., Booij, K., Helder, W,. and Sundby, B. (1987) On the velocity sensitivity

(stirring effect) of polarographic oxygen microelectrodes. Netherlands Jour. Sea Res. 21, 255–263.

Harper, M. P., Davison, W., and Tych, W. (1997) Temporal, spatial, and resolution constraints for *in situ* sampling devices using diffusional equilibration: Dialysis and DET. Environ. Sci. Technol. 31, 3110–3119.

Hartley, A. M., House, W. A., Leadbetter, B. S. C., and Callow, M. E. (1996) The use of microelectrodes to study the precipitation of calcite upon algal biofilms. Jour. Colloid Interface Sci. 183, 498–505.

Heinze, J. (1993) Elektrochemie mit Ultramikroelektroden. Angew. Chemie 105, 1327–1349.

Hinke, J. A. (1969) Glass microelectrodes in the study of binding and compartmentalization of intracellular ions, *in* Glass Microelectrodes (Lavallee, M., Schanne, O. F., and Herbert, N. C., eds.), p. 349–375. John Wiley, New York.

Højberg, O., Revsbech, N. P., and Tiedje, J. M. (1994) Denitrification and nitrous oxide accumulation in soil aggregates as measured with a combined microsensor for oxygen and nitrous oxide. Soil Sci. Soc. Amer. Jour. 58, 1691–1698.

Holst, G., Kühl, M., and Klimant, I. (1995) A novel measuring system for oxygen microoptodes based on phase modulation technique. SPIE Proc. 2508, 387–398.

Holst, G., Kühl, M., Klimant, I., Liebsch, G., and Kohls, O. (1997a) Characterization and application of temperature microoptodes for use in aquatic biology. SPIE Proc. 2980, 164–171.

Holst, G., Glud, R. N., Kühl, M., and Klimant, I. (1997b) A microoptode array for fine-scale measurement of oxygen distribution. Sensors Actuators B 38–39, 122–129.

Holst, G., Klimat, I., Kohls, O., and Kühl, M. (2000) Optical microsensors and microprobes, *in* Chemical Sensors in Oceanography (Varney, M., ed.), p. 143-188. Gordon and Breach, Newark, N.J.

Holst, G., Kohls, O., Klimant, I., König, B., Richter, T., and Kühl, M. (1998) A modular luminescence lifetime imaging system for mapping oxygen distribution in biological samples. Sensors Actuators B 51, 163–170.

Huettel, M., Ziebis, W., Forster, S., and Luther, G. W. III (1998) Advective transport affecting metal and nutrient distributions and interfacial fluxes in permeable sediments. Geochim. Cosmochim. Acta 62, 613–631.

Jensen, K, Revsbech, N. P., and Nielsen, L. P. (1993) Microscale distribution of nitrification activity in sediment determined with a shielded microsensor for nitrate. Appl. Environ.l Microbiol. 59, 3287–3296.

Jeroschewski, P., Steuckart, C., and Kühl, M. (1996) An amperometric microsensor for the determination of $H_2S$ in aquatic enviroments. Anal. Chem. 68, 4351–4357.

Jørgensen, B. B., and Des Marais, D. J. (1986) A simple fiber-optic microprobe for high resolution light measurements: application in marine sediment. Limnol. Oceanogr. 31, 1376–1383.

Jørgensen, B. B., and Des Marais, D. J. (1988) Optical properties of benthic photosynthetic communities: fiber-optic studies of cyanobacterial mats. Limnol. Oceanogr. 33, 99–113.

Jørgensen, B. B., and Des Marais, D. J. (1990) The diffusive boundary layer of sediments: oxygen microgradients over a microbial mat. Limnol. Oceanogr. 35, 1343–1355.

Jørgensen, B. B., and Revsbech, N. P. (1985) Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnol. Oceanogr. 30, 11–21.

Khalili, A. J., Basu, A. J., and Pietrzyk, U. (1998). Flow visualization in porous media via positron emission tomography. Phys. Fluids 10, 1031–1033.

Klimant, I., Meyer, V., and Kühl, M. (1995) Fiber-optic oxygen microsensors, a new tool in aquatic biology. Limnol. Oceanogr. 40, 1159–1165.

Klimant, I., Holst, G., and Kühl, M. (1997a) A simple fiber-optic sensor to detect the penetration of microsensors into sediments and other biogeochemical systems. Limnol. Oceanogr. 42, 1638–1643.

Klimant, I., Kühl, M., Glud, R. N., and Holst, G. (1997b) Optical measurement of oxygen and temperature in microscale: strategies and biological applications. Sensors Actuators B 38–39, 29–37.

Kohls, O., Klimant, I., Holst, G., and Kühl, M. (1997a) Development and comparison of pH microoptodes for use in marine systems. SPIE Proc. 2978, 82–94.

Kohls, O., Epping, E., Holst, G., and Kühl, M. (1997b) Microoptodes for $CO_2$ measurements in marine systems. Paper presented at the SPIE Conference on Chemical, Biochemical and Environmental Fiber Sensors VII, June 16–18, München.

Kühl, M., Cohen, Y., Dalsgaard, T., Jørgensen, B. B., and Revsbech, N. P. (1995) Microenvironment and photosynthesis of zooxanthellae in scleractinian corals studied with microsensors for $O_2$, pH, and light. Mar. Ecol. Progr. Ser. 117, 159–172.

Kühl, M., Glud, R. N., Ploug, H., and Ramsing, N. B. (1996) Microenvironmental control of photosynthesis and photosynthesis-coupled respiration in an epilithic cyanobacterial biofilm. Jour. Phycol. 32, 799–812.

Kühl, M., and Jørgensen, B. B. (1992b) Spectral light measurements in microbenthic phototrophic communities with a fiber-optic microprobe coupled to a sensitive diode array detector. Limnol. Oceanogr. 37, 1813–1823.

Kühl, M., and Jørgensen, B. B. (1992a) Microsensor measurements of sulfate reduction and sulfide oxidation in compact microbial communities of aerobic biofilms. Appl. Environ. Microbiol. 58, 1164–1174.

Kühl, M., and Jørgensen, B. B. (1994). The light field of microbenthic communities: radiance distribution and microscale optics of sandy coastal sediments. Limnol. Oceanogr. 39, 1368–1398.

Kühl, M., Lassen, C., and Jørgensen, B. B. (1994a) Light penetration and light intensity in sandy marine sediments measured with irradiance and scalar irradiance fiber-optic microprobes. Mar. Ecol. Progr. Ser. 105, 139–148.

Kühl, M., Lassen, C., and Jørgensen, B. B. (1994b) Optical properties of microial mats: light measurements with fiber-optic microprobes, *in* Microbial Mats: Structure, Development, and Environmental Significance (Stal, L. J., and Caumette, P., eds.), p 149–167. Springer Verlag, Heidelberg.

Kühl, M., Lassen, C., and Revsbech, N. P. (1997) A simple light meter for measurements of PAR (400 to 700 nm) with fiber-optic microprobes: application for P vs. $E_0$ (PAR) measurements in a microbial mat. Aquat. Microbial Ecol. 13, 197–207.

Kühl, M., and Steuckart, C. (2000) Sensors for in-situ analysis of sulfide in aquatic systems, *in* InSitu Monitoring of Aquatic Systems: Chemical Analysis and Speciation (Buffle, J., and Horvai, G., eds.), p. 121-159. John Wiley, New York.

Kühl, M., Steuckart, C., Eickert, G., and Jeroschewski, P. (1998) A $H_2S$ microsensor for profiling biofilms and sediments: application in an acidic lake sediment. Aquat. Microbial Ecol. 15, 201–209.

Larsen, L. H., Revsbech, N. P., and Binnerup, S. J. (1996). A microsensor for nitrate based on immobilized denitrifying bacteria. Appl. Environ. Microbiol. 62, 1248–1251.

Larsen, L. H., Kjær, T., and Revsbech, N. P. (1997) A microscale $NO_3^-$ biosensor for environmental applications. Anal. Chem. 69, 3527–3531.

Lassen, C., and Jørgensen, B .B. (1994) A fiber-optic irradiance microsensor (cosine collector): application for in situ measurements of absorption coefficients in sediments and microbial mats. FEMS Microbiol. Ecol. 15, 321–336.

Lassen, C., Ploug, H., and Jørgensen, B. B. (1992) A fibre-optic scalar irradiance microsensor: application for spectral light mesurements in sediments. FEMS Microbiol. Ecol. 86, 247–254.

Lloyd, D., Thomas, K., Price, D., O'Neil, B., Oliver, K., and Williams, T. N. (1996) A membrane-inlet mass spectrometer miniprobe for the direct simultaneous measurement of multiple gas species with spatial resolution of 1 mm. Jour. Microbiol. Meth. 25, 145–151.

Lorenzen, J., Glud, R. N., and Revsbech, N. P. (1995) Impact of microsensor-caused changes in diffusive boundary layer thickness on $O_2$ profiles and photosynthetic

rates in benthic communities of microorganisms. Mar. Ecol. Progr. Ser. 119, 237–241.

Luther, G. W., Brendel, P. J., Lewis, B. L., Sundby, B., Lefrancois, L, Silverberg, N., and Nuzzio, D. B. (1998) Simultaneous measurement of $O_2$, Mn, Fe, $I^-$ and S(II-) in marine porewaters with a solid-state voltammetric microelectrode. Limnol. Oceanogr. 43, 325–333.

McConnaughey, T. A., and Falk, R. H. (1991) Calcium-proton exchange during algal calcification. Biol. Bull. 180, 185–195.

Müller, B., Buis, K., Stierli, R., and Wehrli, B. (2000) High spatial resolution measurements in lake sediments with PVC based liquid membrane ion-selective electrodes. Limnol. Oceanogr. 43, 1728-1733.

Neudörfer, F., and Meyer-Reil, L.-A. (1997) A microbial biosensor for the microscale measurement of bioavailable organic carbon in oxic sediments. Mar. Ecol. Progr. Ser. 147, 295–300.

Ploug, H., Lassen, C., and Jørgensen, B. B. (1993) Action spectra of microalgal photosynthesis and depth distribution of spectral scalar irradiance in a coastal marine sediment of Limfjorden, Denmark. FEMS Microbiol. Ecol. 102, 261–270.

Prigault, O., Epping, E., Guyoneaud, R., Khalili, A., and Kühl, M. (1999) Dynamics of anoxygenic photosynthesis in an experimental green sulfur bactial film. Environ. Microbiol. 1, 295-305.

Pringault, O., Kühl, M., de Wit, R., and Caumette, P. (1998) Growth of green sulphur bacteria in experimental benthic oxygen, sulphide, pH and light gradients. Microbiol. 144, 1051–1061.

Pul, C. P., Rechnitz, G. A., and Miller R. F. (1978) Micro-size potentiometric probes for gas and substrate sensing. Anal. Chem. 50, 330–333.

Reimers, C.E. (1987) An in situ microprofiling instrument for measuring interfacial pore water gradients: methods and oxygen profiles from the North Pacific Ocean. Deep-Sea Res. 34, 2017–2035.

Revsbech, N. P. (1989) An oxygen microelectrode with a guard cathode. Limnol. Oceanogr. 34, 474–478.

Revsbech, N. P. (1994) Analysis of microbial mats by use of electrochemical microsensors: recent advances, *in* Microbial Mats: Structure, Development, and Environmental Significance (Stal, L.J., and Caumette, P., eds.), p. 135–147. Springer Verlag, Heidelberg.

Revsbech, N. P., Jørgensen, B. B., and Blackburn, T. H. (1980) Oxygen in the sea bottom measured with a microelectrode. Science 207, 1355–1356.

Revsbech, N. P. and Jørgensen, B. B. (1986) Microelectrodes: their use in microbial ecology. Adv. Microbial Ecol. 9, 293–352.

Revsbech, N. P., Kjaer T., Damgaard, L.R., Lorenzen, J., and Larsen, L. H. (2000) Biosensors for analysis of water, sludge, and sediments with emphasis on microscale biosensors, *in* In Situ Monitoring of Aquatic Systems: Chemical Analysis and Speciation (Buffle, J., and Horvai, G., eds.), p. 195-222. John Wiley, New York.

Revsbech, N. P., Nielsen, L. P., Christensen, P. B., and Sørensen, J. (1988) Combined oxygen and nitrous oxide microsensor for denitrification studies. Appl. Environ. Microbiol. 54, 2245–2249.

Revsbech, N. P., Nielsen, L. P., and Ramsing, N. B. (1998) A novel microsensor for determination of diffusivity in sediment. Limnol. Oceanogr. 43, 986–992.

Revsbech, N.P., and Ward, D. M. (1983) Oxygen microelectrode that is sensitive to medium chemical composition: use in an acid microbial mat dominated by *Cyanidium caldarium*. Appl. Environ. Microbiol. 45, 755-759.

Rothfuss, F., and Conrad, R. (1994) Development of a gas diffusion probe for the determination of methane concentrations and diffusion characteristics in flooded paddy soil. FEMS Microbiol. Ecol. 14, 307–318.

Schramm, A., Larsen, L. H., Revsbech, N. P., Ramsing, N. B., Amann, R., and Schleifer, K.-H. (1996) Structure and function of a nitrifying biofilm as determined by in situ hybridization and microelectrodes. Appl. Environ. Microbiol. 62, 4641–4647.

Schreiber, U., Kühl, M., Klimant, I., and Reising, H. (1996) Measurement of chlorophyll fluorescence within leaves using a modified PAM fluorometer with a fiber-optic microprobe. Photosynth. Res. 47, 103–109.

Severinghaus, J. W., and Bradley, A. F. (1958) Electrodes for blood $pO_2$ and $pCO_2$ determination. Jour. Appl. Physiolo. 13, 515–520.

Tengberg A., et al. (1995) Benthic chamber and profiling landers in oceanography — a review of design, technical solutions and functioning. Progr. Oceanogr. 35, 253–294.

Tercier, M.-L., and Buffle, J. (1993) In situ voltammetric measurements in natural waters. Future prospects and challenges. Electroanal. 5, 187–200.

Tercier, M.-L., and Buffle, J. (1996) Antifouling membrane-covered voltammetric microsensor for in situ measurements in natural waters. Anal. Chem. 68, 3670–3678.

Tercier, M.-L., Parthasarathy, N., and Buffle, J. (1995) Reproducible, reliable and rugged Hg-plated Ir-based microelectrode for in situ measurements in natural waters. Electroanal. 7, 55–63.

Tercier-Waeber, M.-L., Belmont-Hebert, C., and Buffle, J. (1998). Real-time continous Mn(II) monitoring in lakes using a novel voltammetric in situ profiling system. Environ. Sci. Technol. 32, 1515–1521.

Thomas, R.C. (1978). Ion-Sensitive Intracellular Microelectrodes: How to Make and Use Them. Academic Press, New York.

Van Den Heuvel, J. C., de Beer, D., and Cronenberg, C. C. H. (1992). Microelectrodes: a versatile tool in Biofilm research, *in* Biofilms — Science and Technology , NATO ASI Ser. E 223 (Melo, L. F., Bott, T. R., Fletcher, M., and Capdeville, B., eds.), p. 631-644. Kluwer, Amsterdam.

VanHoudt, P., Lewandowski, Z., and Little, B. (1992) Iridium oxide pH microelectrode. Biotechnol. Bioeng. 40, 601–608.

Varney, M. (2000) Chemical Sensors in Oceanography. Gordon and Breach, Newark, N.J.

Verschuren, P. G., van der Baan, J. L., Blaauw, R., de Beer, D., and van den Heuvel, J. C. (1999) A nitrate-selective microelectrode based on a lipophilic derivative of iodocobalt(III)-salen. Fresenius Jou. Anal. Cham. 364, 595-598.

Visscher, P. T., Beukema, J., and van Gemerden, H. (1991) In situ measurements of oxygen and sulfide profiles with a novel combined needle-electrode. Limnol. Oceanogr. 36, 1476–1480.

Wenzhöfer, F. (1999) Biogeochmical processes at the sediment water interface and quantification of metabolically driven calcite dissolution in deep sea sediments. Ph.D. thesis, University of Bremen.

Whalen, W. J., Riley, J., and Nair, P. (1967) A microelectrode for measuring intracellular $pO_2$. Jour. Appl. Physiol. 23, 798–801.

Wieland, A. (2000) Microsensor analysis of oxygen and sulfide turnover in hypersaline microbial mats. Ph.D. thesis, University of Bremen.

Wieland, A., and Külh, M. (2000) Short term effects on oxygen and sulfide cycling in a hyper-saline cyanobacterial mat (Solar Lake, Egypt). Mar. Ecol. Prog. ser. 197, 87-102

Witty, J.F. (1991) Microelectrode measurements of hydrogen concentrations and gradients in legume nodules. Jour. Exper. Bot. v. 42, 20-36.

Wolfbeiss, O. S., ed. (1991) Fiber Optic Sensors and Biosensors. CRC Press, New York.

Yang, S. X., Cheng, K. L., Kurtz, L. T., and Peck, T. R. (1989) Suspension effect in potientiometry. Particle Sci. Technol. 7, 139–152.

Zhao, P. and Cai, W.-J. (1997). An improved potentiometric $pCO_2$ microelectrode. Anal. Chem. 69, 5052–5058.