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Microsensors as a tool to determine chemical microgradients and bacterial activity in wastewater biofilms and flocs

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Abstract

Microsensors used in microbial ecology are reviewed with emphasis on new sensor developments (NO_3^-, NO_2^-, NH_4^+, CO_2, H_2, H_2S and CH_4 microsensors as well as fiberoptical microsensors for O_2, temperature and pH). Examples of microsensor applications in biofilms and activated sludge flocs are presented, in which sulfate reduction and denitrification were studied.

Introduction

Isolation and cultivation are classical microbiological techniques to characterize microorganisms and their activities. These methods have, however, two major drawbacks: first, many microorganisms cannot be cultivated, second, microorganisms in pure or defined cultures do not necessarily reflect their behavior in natural environments. Especially growth conditions in complex ecosystems, like biofilms or aggregates, are difficult to simulate in defined cultures. Therefore, our knowledge about bacterial activity, community structure and composition in such systems is limited.

Molecular methods were developed during the last years to identify the bacteria present in environmental samples and to reveal their spatial distribution, i.e. by genetic fingerprinting and *in situ* hybridization techniques (Amann et al. 1995; Muyzer & Smalla 1998). Techniques based on nucleic acid analysis do not include cultivation and have therefore less biases. However, most of these molecular tools do not provide information about the *in situ* activity of the bacteria. Both the *in situ* bacterial activity and the microenvironmental conditions can be determined with microsensors.

Microsensors can measure chemical and physical variables with high spatial resolution (< 50 μm) due to their small tip-diameter (1–20 μm). Especially in microbial mats, biofilms and aggregates, where high metabolic rates of the dense microbial population and molecular diffusion cause steep chemical gradients and narrow zones of microbial activity, microsensors are powerful tools. Due to the small dimensions and analyte consumption of microsensors, microgradients can be measured without significant disturbance of the sample and the gradients themselves. Furthermore, such measurements allow studies of internal cycling processes, e.g. coupled sulfate reduction and sulfide oxidation measurements in biofilms and sediments with an internal sulfur cycle (Kühl & Jørgensen 1992; Kühl et al. 1998).

Microsensors have been developed during the last years for measuring important metabolites like O_2 (Klimant et al. 1995; Revsbech & Jørgensen 1986), H_2S (Jeroschewski et al. 1996), S^{2-}(Revsbech & Jørgensen 1986), pH (Hinke 1969; Kohls et al. 1997), H_2 (Ebert & Brune 1997), NO_3^- (De Beer & Sweerts 1989; Larsen et al. 1997), NO_2^- (De Beer et al. 1997b), N_2O (Revsbech et al. 1988), NH_4^+ (De Beer & Van den Heuvel 1988), CO_2 (De Beer et al. 1997a), and CH_4 (Damgaard & Revsbech 1997). The microsensors used in environmental studies can be divided in two groups: electrochemical microsensors and fiber-optical microsensors. Here, we give a brief review of these microsensors together with some examples of electrochemical microsensor applications

in biofilms and activated sludge flocs. More detailed reviews of microsensors and their application in environmental analysis can be found elsewhere (Kühl & Revsbech 1998; Revsbech & Jørgensen 1986). A recent review of Amann and Kühl (1998) addresses the combined use of molecular techniques and microsensors in microbiology.

Electrochemical microsensors

Three types of electrochemical microsensors are most often used in environmental applications: potentiometric microsensors, amperometric microsensors and micro-biosensors, which are actually amperometric microsensors including a biological reaction in the sensor tip. The principles and some examples of each type will be described below. Environmental application of voltametric microelectrode methods have also been reported recently (Brendel & Luther III 1995), but will not be discussed here.

Potentiometric microsensors

Potentiometric microsensors are based on charge separation of ions across a membrane. An electrical potential difference is hereby generated according to the Nernst equation:

$$\Delta E = \frac{RT}{zF} ln(\frac{a_i}{a_e}) = E_o + k \cdot log(a_i) \qquad (1)$$

with R is the gas constant, T the absolute temperature, z the charge of the ion, F the Faraday constant, a_i and a_e the ion activity, in the sample and in the electrolyte solution, respectively. As the activity in the electrolyte solution (a_e) may be considered constant, the electrical potential difference across the membrane becomes proportional to the logarithm of the ion activity in the sample. The formula can then be simplified as shown above introducing a constant, k, and an offset potential, E_0. The potential is measured with a high-impedance (10^{15} Ω) voltmeter.

There are three major types of potentiometric microsensors: (a) full glass, (b) Ag/Ag^+ half cell and (c) liquid ion-exchange (LIX) based microsensors.

(a) Full glass microsensor

The only full glass microsensor relevant for microbial ecology is the pH glass electrode described by Hinke (1969) and Thomas (1978). This microelectrode is a small version of the commercially available pH electrodes, in which the H^+ selective membrane is made of a special glass. The sensors have a long lifetime, but have a sensing length of approximately 20 μm, which might be a disadvantage if high spatial resolution is demanded. In that case LIX pH microsensors are recommended (see below).

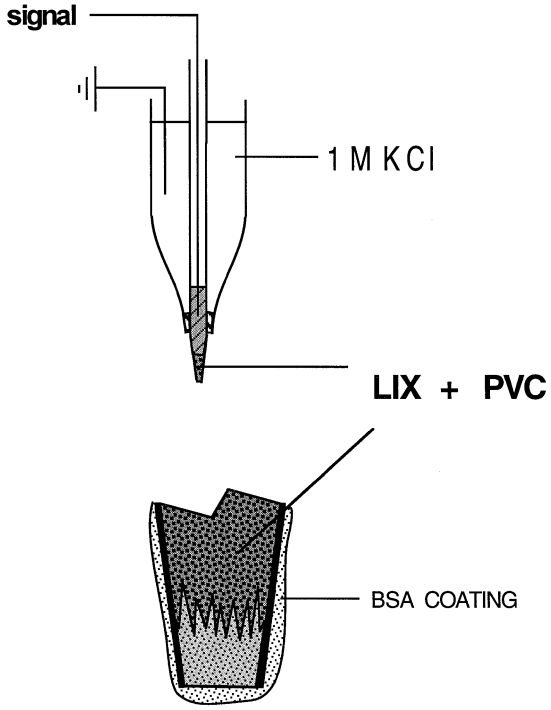

Figure 1. Schematic drawing of a LIX microsensor.

(b) Ag/Ag^+ half cell microsensor

The sulfide microsensor described by Revsbech et al. (1983) and Kühl & Jørgensen (1992) works as an Ag/Ag^+ half cell. A platinum wire coated at the tip with silver and a silver salt of the ion to be analyzed (in this case Ag_2S) functions as a sensor in combination with an external reference electrode. The Ag^+ activity determines the potential of the sensor and is depending on the solubility of the silver salt and therefore on the S^{2-} activity in the analyzed medium. Dissolved S^{2-} is in equilibrium with H_2S, HS^- and H^+, according to the following equation:

$$H_2S \overset{K_1}{\longleftrightarrow} H^+ + HS^- \overset{K_2}{\longleftrightarrow} 2H^+ + S^{2-} \qquad (2)$$

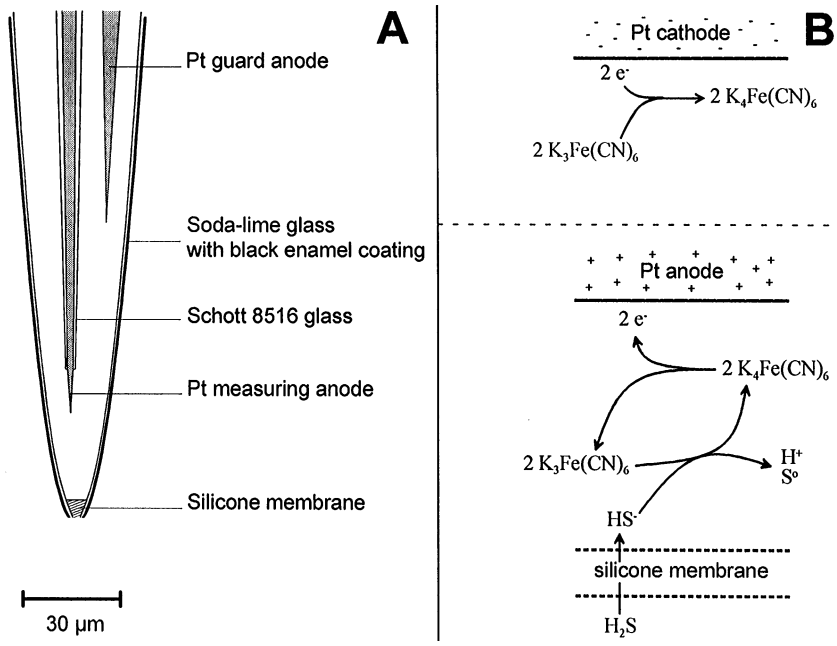

Figure 2. Scheme of a H_2S microsensor from Kühl et al. (1998), with kind permission from Inter-Research Science Publisher. (A) View of the sensor tip. (B) Chemical reactions at the counter electrode (upper part) and at the working electrode (lower part).

K_1 and K_2 are dissociation constants. Since the sensor measures only S^{2-}, also the pH has to be measured for determination of the total dissolved sulfide concentration. A disadvantage of the microsensor is the slow response time, especially at low concentrations. Furthermore, it can only be used under anoxic conditions, since the high potential of the microsensor can reduce oxygen.

(c) Liquid ion-exchange (LIX) based microsensor

The principle of the LIX microsensor is the same as of the other potentiometric microsensors. The ion-selective membrane is in this case a liquid ion exchanger (Figure 1). Liquid ion exchangers are commercially available (e.g. from Fluka). To seal the capillary tip of the microsensor with the hydrophobic membrane the glass has to be silanized (Ammann 1986; Thomas 1978). The LIX membrane can be solidified in the tip with PVC. A protein coating (BSA) of the tip prevents interaction of hydrophobic substances from the sample with the LIX (De Beer et al. 1997b). The microsensor is shielded with an outer casing containing 1 M KCl to reduce electrical noise (Kühl & Revsbech 1998). The LIX microsensors currently used in environmental applications are H^+ (Ammann et al. 1981; Schulthess et al. 1981), NO_3^- (De Beer & Sweerts 1989), NO_2^- (De Beer et al. 1997b), NH_4^+ (De Beer & Van den Heuvel 1988), and Ca^{2+} (Ammann et al. 1987). The CO_2 microsensor contains a pH-LIX sensor (De Beer et al. 1997a). The LIX microsensors can be made very small (approximately 1 μm tip diameter) and allow, therefore, an extremely high spatial resolution (~ 5 μm). However, the short lifetime of LIX microsensors (usually a few days) is a disadvantage, as well as the low selectivity of the NH_4^+ sensor for Na^+ and K^+, and the NO_3^- and NO_2^- sensors for Cl^-, which prohibits their use in seawater. For measurements in marine environments a recently developed NO_3^- biosensor (Larsen et al., 1997) can be recommended.

Amperometric microsensors

Amperometric microsensors for O_2 (Revsbech 1989; Revsbech & Jørgensen 1986), H_2S (Jeroschewski et al. 1996), H_2 (Ebert & Brune 1997), N_2O (Revsbech et al. 1988) and HClO (De Beer et al. 1994a), measure the current caused by electrochemical reactions of the analyte at the tip of the microsensor. The current, measured by a sensitive pico-ampere meter, is proportional to the concentration of the substrate. The electrochemical reaction is driven by a potential difference between the working electrode and the reference.

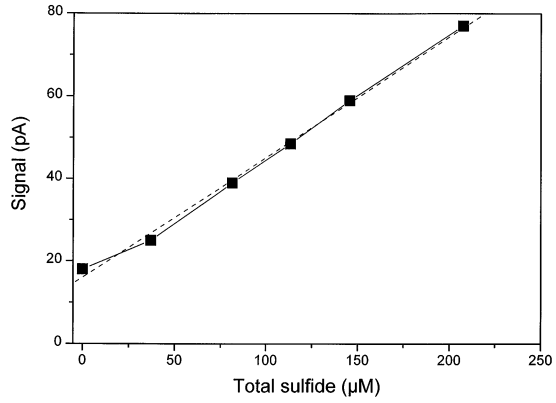

Figure 3. A calibration curve of the H_2S microsensor obtained in buffered, anaerobic artificial wastewater at pH 7.5. The dashed line indicates the linear regression of the data points.

The oxygen microelectrode is a well known and often described sensor, which has improved drastically over the years. From a simple cathode-type oxygen microelectrode (Baumgärtl & Lübbers 1983; Revsbech et al. 1983), it developed to a Clark-type microsensor (Revsbech & Ward 1983) with a cathode situated behind a silicone membrane and immersed in an electrolyte solution. This sensor has a more stable signal, is insensitive for calcium and magnesium ions and is pH independent. Later a guard cathode was added to prevent diffusion of oxygen to the cathode from behind and thus reduce the background signal (Revsbech 1989).

The use of the combined O_2/N_2O microsensor for denitrification studies (Revsbech et al. 1988) is limited by its complex construction. Other microsensors may be preferred (e.g. the NO_3^- biosensor or the LIX sensors for NH_4^+, NO_3^- and NO_2^-).

The H_2S microelectrode is a new Clark-type sensor (Jeroschewski et al. 1996; Kühl et al. 1998). H_2S penetrates through a silicone membrane and is oxidized to sulfur by ferricyanide in the electrolyte solution behind the membrane (Figure 2.). The formed ferrocyanide is then re-oxidized to ferricyanide at the platinum working electrode. The sensor detects only H_2S, therefore, the local pH must be known to determine the total dissolved sulfide concentration. In acidic and moderate alkaline environments (pH < 9) the following formula can then be used to calculate the total dissolved sulfide concentration (Jeroschewski et al. 1996):

$$\left[S_{tot}^{2-}\right] = \left[H_2S\right]\left(1 + \frac{K_1}{[H_3O^+]}\right) \qquad (3)$$

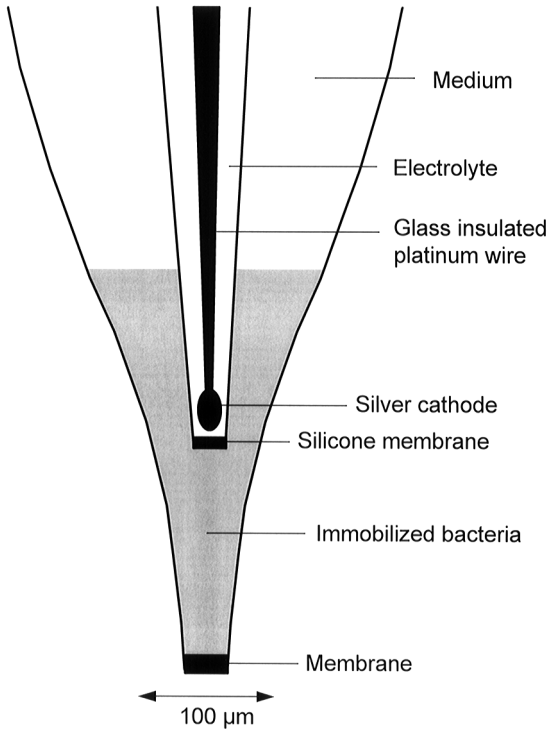

Figure 4. Scheme of a nitrate micro-biosensor. Redrawn from Larsen et al. (1997).

K_1 is the dissociation constant from equation 2 (K_2 can be neglected at this pH range). The microsensor can be calibrated in dilution series of sulfide. The dilution series consist of the same medium as used for the microsensor measurements, at constant pH (phosphate buffered), flushed with nitrogen, to avoid chemical oxidation of the sulfide. The signal in the dilution series is proportional to the total dissolved sulfide, determined by chemical analysis (Cline 1969). An example of a calibration curve can be seen in Figure 3. The detection limit of the sensor is 1–2 μM H_2S. Contrary to the (old) Ag/Ag_2S sensor the H_2S microsensor is insensitive to oxygen, which is highly advantageous for studies at oxic-anoxic interfaces, which are often encountered in environmental applications. This, together with its fast response time (< 0.5 s) and low stirring sensitivity, makes it a much more preferable tool than the Ag/Ag_2S microsensor (Kühl et al. 1998).

Micro-biosensors

A glucose microsensor, based on a glucose oxidase reaction, was the first micro-biosensor developed (Cronenberg et al. 1991). Two new types of microbiosensors, that are more relevant for environmental

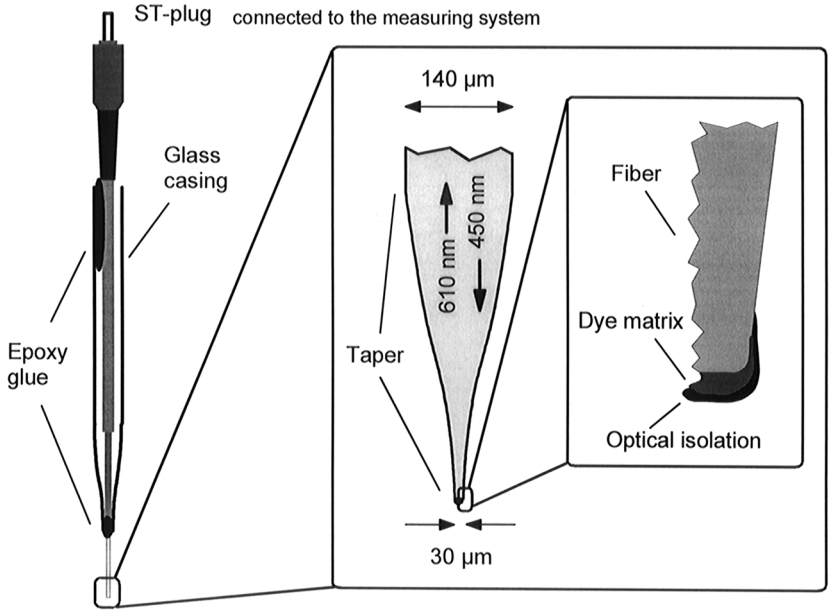

Figure 5. Schematic drawing of an optical microsensor, a micro-optode (O. Kohls, unpublished).

studies, are based on the specific activity of bacteria in the tip of the sensor (Figure 4.); a nitrate (Larsen et al. 1997; Larsen et al. 1996) and a methane biosensor (Damgaard et al. 1995; Damgaard & Revsbech 1997). The nitrate biosensor converts nitrate to N_2O by incomplete denitrification, which is measured by an internal N_2O microsensor. The methane sensor contains methane oxidizing bacteria, that oxidize the methane diffusing into the tip with O_2 from a reservoir in the microsensor. The O_2 consumption is proportional to the methane concentration and is measured by an internal oxygen microelectrode. The biosensors have a tip diameter of 20–50 μm, a detection limit of ca. 0.1 μM for nitrate and ca. 2–10 μM for methane with lifetimes of a few days for the nitrate biosensor and several months for the methane biosensor.

Micro-optodes have recently been developed as an alternative to existing electrochemical microsensors. They have the advantage of easy manufacturing, long term stability and mechanical strength. The optical fiber microsensors are based on analyte dependent changes in luminescence or absorption of an indicator chemistry fixed at the micro-fiber tip. A general scheme of a micro-optode can be found in Figure 5. Three types of optical microsensors have been developed for environmental studies, the oxygen (Klimant et al. 1995), the temperature (Klimant et al. 1997) and the pH micro-optode (Kohls et al. 1997). The fiber optical sensors have tips of 20–30 μm diameter and have response times of a few seconds. As there is no analyte consumption the micro-optodes are insensitive to stirring.

Optical microsensors

Fiber-optical microsensors (micro-optodes) are made of tapered fibers that collect and direct light from the sensor tip to the opto-electronic measuring equipment. The measuring system is rather sophisticated, containing bright light emitting diodes (LED) for fluorescence excitation, excitation – and emission – filters, a fiber coupler (beam splitter) and a photomultiplier as detector (Klimant et al. 1997; Klimant et al. 1995; Kühl & Revsbech 1998).

Applications

Microsensors can be applied in many different environmental studies. They have been used for instance in microbial mats for photosynthesis studies (Jørgensen & Des Marais 1988; Kühl et al. 1994), in sediments (marine and freshwater) to study the boundary layer and microbial conversions (Jørgensen & Des Marais 1990; Jørgensen & Revsbech 1985), in biofilms and marine snow aggregates for structure and function analysis (De Beer et al. 1997b; De Beer et al. 1994b;

Kühl & Jørgensen 1992; Ploug et al. 1997), but also in more exotic environments like the termite gut (Ebert & Brune 1997; Brune et al. 1995). The use of the O_2, H_2S, NO_3^-, NO_2^- and pH microsensors will be illustrated here in two examples: microsensor measurements in biofilms and in activated sludge flocs.

Microsensor measurements in biofilms

The development of a biofilm in a wastewater treatment plant in Bremen, Germany, was followed over time by combining microsensor measurements with molecular analysis (Santegoeds et al. 1998). The biofilm grew on plastic foil in the activated sludge basin. The biofilm was sampled weekly and analyzed in a flow cell with circulating artificial wastewater. The ambient oxygen concentration and temperature were similar to the conditions in the activated sludge basin, i.e. ca. 70 μM O_2 and 20 °C. Oxygen and hydrogen sulfide were measured with amperometric O_2 and H_2S microsensors, and pH was measured with potentiometric glass microelectrodes. Within one week, a patchy, 400 μm thick biofilm developed on the plastic foil. During the following weeks, the thickness increased to a final thickness of 1000-1200 μm from the 8th week on. The oxygen measurements showed a depletion of oxygen in the top 200-400 μm in all stages of biofilm development. The pH in the biofilm was constant (pH 7.5 $\pm$ 0.1). Sulfide was not detected in the first 6 weeks, although anaerobic zones were present from the first week on. It was concluded, therefore, that sulfate reduction did not take place, which is surprising considering the favorable conditions for sulfate reduction and the presence of sulfate reducing bacteria (detected by molecular tools) from the first week on. The H_2S measurements showed that sulfate reduction took place only after the 6th week. Molecular analyses of the biofilm samples showed that additional sulfate reducing bacteria appeared after the 6th week, which might be responsible for the sulfate reduction in the biofilm from that time on. The question what the activity (if any) of the sulfate reducing bacteria is during the first 6 weeks remains unanswered and requires further investigation.

The oxygen and sulfide profiles from the 11 week old biofilm are shown in Figure 6. It can be seen that no sulfide is diffusing out of the biofilm, since all sulfide produced by sulfate reduction is oxidized in the oxic part of the biofilm. From the profiles, the oxygen and sulfide fluxes can be calculated. Comparing the

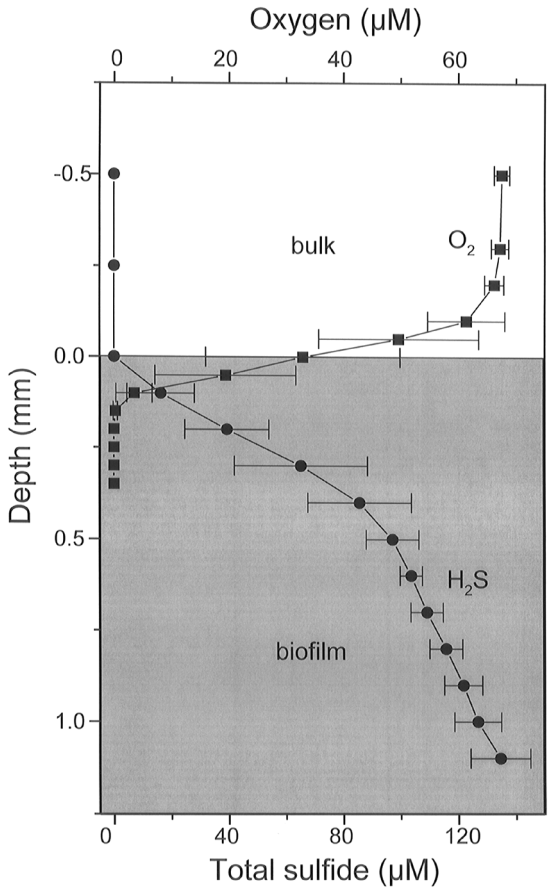

Figure 6. Microsensor measurements of O_2 (■) and total sulfide (●) in an 11 week old biofilm from an activated sludge basin. The biofilm surface is at depth = 0, and the substratum of the biofilm at depth = 1.25 mm.

fluxes, we find for the oxygen flux towards the biofilm a value of ca. 0.6 μmol $m^{-2}s^{-1}$ and for the sulfide flux in the biofilm ca. 0.3 μmol $m^{-2}s^{-1}$. Thus, if sulfide is completely oxidized to sulfate, all oxygen would be needed for re-oxidation. This indicates that sulfate reduction in this biofilm can be more important than the aerobic mineralization in the biofilm. In another study of a trickling-filter biofilm Kühl & Jørgensen (1992) found that sulfate reduction accounted for 50% of the total mineralization of organic carbon.

Microsensor measurements in activated sludge flocs

To measure the chemical gradients in flocs or aggregates a vertical net-jet flow system was developed by Ploug et al. (1998; 1997). The flow cell is made from two Plexiglas tubes with a nylon stocking in between. An upward flow is applied, that keeps the flocs or

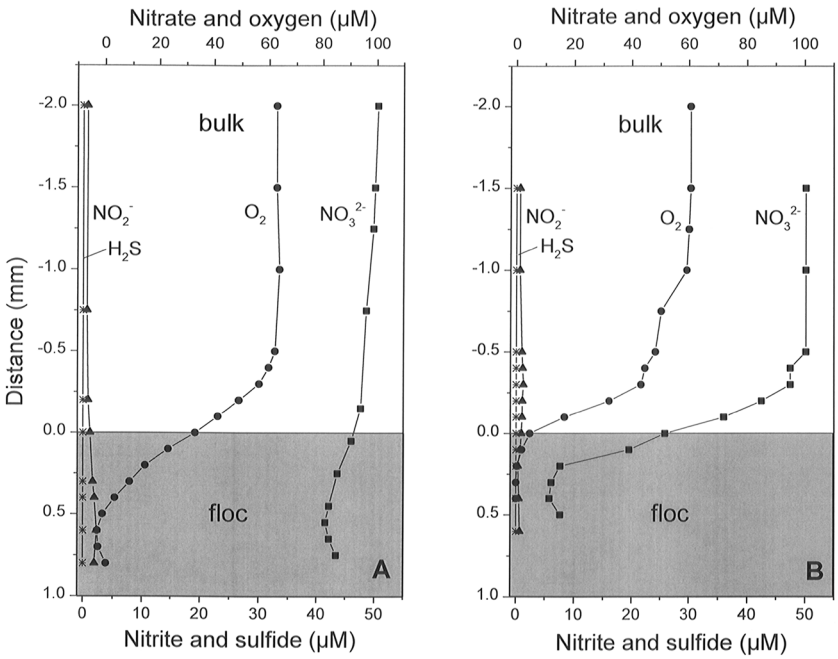

Figure 7. Microsensor profiles of nitrate (■), nitrite (▲), oxygen (●) and sulfide (x) in an activated sludge floc from a wastewater treatment plant in Bremen (A) and from a pilot plant in Prague (B). The floc surface is at distance = 0. The diameter of the flocs is 1–1.5 mm.

aggregates floating just above the netting, also if a microsensor is penetrating the particle. Here we show data of an activated sludge floc from a wastewater treatment plant in Bremen (Germany) and an oxic-anoxic pilot plant in Prague (Czechia). The flocs had a diameter of 1–1.5 mm. Artificial wastewater was used with 100 μM nitrate. The oxygen concentration was kept at 70 μM O_2, the same as in the activated sludge basin. Oxygen and sulfide were measured with amperometric microsensors, pH with a glass microelectrode, and nitrate and nitrite with LIX microsensors. Figure 7 shows the measured profiles. The pH remained constant (pH 7.5), and is not plotted in the graph. Nitrite was present in negligible amounts (ca. 2 μM). Oxygen was completely consumed in the floc from the pilot plant in Prague and only partially in the floc from the Bremen plant (5 μM O_2). The floc from Bremen had only low denitrification activity as the concentration of nitrate decreased from 100 μM to 83 μM in the floc center. The Prague floc had a higher denitrification rate (a decrease from 100 μM to 11 μM nitrate). In air-saturated medium, oxygen penetrated the whole floc and nitrate consumption was not detected (data not shown). Thus, the higher denitrification rate in the Prague floc can be explained by the lower oxygen concentration in the floc. Differences in oxygen penetration can be caused by differences in community composition, structure and density of the flocs. No sulfide could be detected, even at anaerobic conditions (data not shown). Molecular analysis showed that sulfate reducers were present, in higher numbers for flocs from Bremen than for the flocs from Prague. Further investigations will be made to find an explanation for the absence of sulfate reduction.

Perspectives

The combination of microsensor and molecular techniques opens new possibilities in microbial ecology. It is now possible to study the microorganisms in their natural habitat, to localize the organisms, to quantify them, determine their activity and study their *in situ* microenvironment (Amann & Kühl 1998). The strength of this approach was shown by pioneering studies from Ramsing et al. (1993) and Santegoeds et al. (1998) on the sulfate reducing population in biofilms and by Schramm et al. (1996) describing the nitrifying population in a trickling-filter biofilm. Also in other microbial systems the combined use of microsensors and molecular techniques will improve our understanding of the functioning of the microbial communities.

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References

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

Amann R, Ludwig W & Schleifer K-H (1995) Phylogenetic identification and in situ detection of individual microbial cells without cultivation. Microbiol. Rev. 59: 143–169

Ammann D (1986) Ion-selective microelectrodes: principles, design and applications. Springer, Berlin

Ammann D, Bührer T, Schefer U, Müller M & Simon W (1987) Intracellular neutral carrier based Ca^{2+} microelectrode with subnanomolar detection limit. Pflügers Arch. 409: 223–228

Ammann D, Lanter F, Steiner RA, Schulthess P, Shio Y & Simon W (1981) Neutral carrier based hydrogen-ion selective microsensor for extra- and intracellular studies. Anal. Chem. 53: 2267–2269

Baumgärtl H & Lübbers DW (1983) Platinum needle electrodes for polarographic measurement of local O_2 pressure in cellular range of living tissue. Its construction and properties. In: Gnaiger E & Forstner H (Eds) Polarographic oxygen sensors: Aquatic and physiological applications, (pp 37–65). Springer-Verlag, Heidelberg

Brendel PJ & Luther III GW (1995) Development of a gold amalgam voltametric microelectrode for determination of dissolved Fe, Mn, O_2, and S(II-) in porewaters of marine and freshwater sediments. Environ. Sci. Technol. 29: 751–761

Brune A, Emerson D & Breznak JA (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

Cline JD (1969) Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol. Oceanogr. 14: 454–458

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

Damgaard LR, Larsen LH & Revsbech NP (1995) Microscale biosensors for environmental monitoring. Trends Anal. Chem. 14: 300–303

Damgaard LR & Revsbech NP (1997) A microscale biosensor for methane containing methanotrophic bacteria and an internal oxygen reservoir. Anal. Chem. 69: 2262–2267

De Beer D, Glud A, Epping E & Kühl M (1997a) A fast responding CO_2 micro-electrode for profiling sediments, microbial mats and biofilms. Limnol. Oceanogr. 42: 1590–1600

De Beer D, Schramm A, Santegoeds CM & Kühl M (1997b) A nitrite microsensor for profiling environmental biofilms. Appl. Environ. Microbiol. 63: 973–977

De Beer D, Srinivasan R & Stewart PS (1994a) Direct measurements of chlorine penetration into biofilms during disinfection. Appl. Environ. Microbiol. 60: 4339–4344

De Beer D, Stoodley P, Roe F & Lewandowski Z (1994b) Effect of biofilm structures on oxygen distribution and mass transfer. Biotechnol. Bioeng. 43: 1131–1138

De Beer D & Sweerts JPRA (1989) Measurements of nitrate gradients with an ion-selective microelectrode. Anal. Chim. Acta 219: 351–356

De Beer D & Van den Heuvel JC (1988) Response of ammonium-selective microelectrodes based on the neutral carrier nonactin. Talanta 35: 728–730

Ebert A & 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*. Appl. Environ. Microbiol. 63: 4039–4046

Hinke J (1969) Glass microelectrodes for the study of binding and compartmentalisation of intracellular ions. In: Lavallee M, Schanne OF & Herbert NC (Eds) Glass microelectrodes, (pp 349–375). Wiley

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

Jørgensen BB & Des Marais DJ (1988) Optical properties of benthic photosynthetic communities: Fiber-optic studies of cyanobacterial mats. Limnol. Oceanogr. 33: 99–113

Jørgensen BB & Des Marais DJ (1990) The diffusive boundary layer of sediments: Oxygen microgradients over a microbial mat. Limnol. Oceanogr. 35: 1343–1355

Jørgensen BB & Revsbech NP (1985) Diffusive boundary layers and the oxygen uptake of sediments and detritus. Limnol. Oceanogr. 30: 11–21

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

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

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

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

Kühl M, Lassen C & Jørgensen BB (1994) Optical properties of microbial mats: Light measurements with fiber optic microprobes. In: Stal LJ & Caumette P (Eds) Microbial mats: Structure, development, and environmental significance, (pp 149–167). Springer-Verlag, Berlin

Kühl M & Revsbech NP (1998) Microsensors for the study of interfacial biogeochemical processes. In: Boudreau BP & Jørgensen BB (Eds) The bentic boundary layer, in press. Oxford University Press, Oxford

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

Larsen LH, Kjaer T & Revsbech NP (1997) A microscale NO^{3-} biosensor for environmental applications. Anal. Chem. 69: 3527–3531

Larsen LH, Revsbech NP & Binnerup SJ (1996) A microsensor for nitrate based on immobilized denitrifying bacteria. Appl. Environ. Microbiol. 62: 1248–1251

Muyzer G & Smalla K (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie van Leeuwenhoek, 73: 127–141

Ploug H & Jørgensen BB (1998) A net-jet flow system for mass transfer studies in sinking aggregates. Aquat. Microb. Ecol., in press

Ploug H, Kühl M, Buchholz-Cleven B & Jørgensen BB (1997) Anoxic aggregates – an ephemeral phenomenon in the pelagic environment? Aquat. Microb. Ecol. 13: 285–294

Ramsing NB, Kühl M & Jørgensen BB (1993) Distribution of sulfate-reducing bacteria, O_2, and H_2S in photosynthetic biofilms determined by oligonucleotide probes and microelectrodes. Appl. Environ. Microbiol. 59: 3840–3849

Revsbech NP (1989) An oxygen microelectrode with a guard cathode. Limnol. Oceanogr. 55: 1907–1910

Revsbech NP & Jørgensen BB (1986) Microelectrodes: their use in microbial ecology. Adv. Microb. Ecol. 9: 293–352

Revsbech NP, Jørgensen BB, Blackburn TH & Cohen Y (1983) Microelectrode studies of the photosynthesis and O_2, H_2S and pH profiles of a microbial mat. Limnol. Oceanogr. 28: 1062–1074

Revsbech NP, Nielsen LP, Christensen PB & Sørensen J (1988) A combined oxygen and nitrous oxide microsensor for denitrification studies. Appl. Environ. Microbiol. 45: 2245–2249

Revsbech NP & Ward DM (1983) Oxygen microelectrode that is insensitive to medium chemical composition: Use in an acid microbial mat dominated by *Cyanidium caldarum*. Appl. Environ. Microbiol. 45: 755–759

Santegoeds CM, Ferdelman TG, Muyzer G & De Beer D (1998) Structural and functional dynamics of sulfate reducing bacterial populations in biofilms. Appl. Environ. Microbiol. 64: in press

Schramm A, Larsen LH, Revsbech NP, Ramsing NB, Amann R & Schleifer K-H (1996) Structure and function of a nitrifying biofilm as determined by in situ hybridization and the use of microelectrodes. Appl. Environ. Microbiol. 62: 4641–4647

Schulthess P, Shijo Y, Pham HV, Pretsch E, Ammann D & Simon W (1981) A hydrogen ion-selective liquid-membrane electrode based on tri-n-dodecylamine as neutral carrier. Anal. Chim. Acta 131: 111–116

Thomas RC (1978) Ion-sensitive intracellular microelectrodes, how to make and use them. Academic Press, London