CHIMIA 51 (1997) Nr. 12 (Dezember)

Chimia 51 (1997) 878–883

ISSN 0009–4293

Structure and Activity of Microbial Communities in Sediments

Beat Müller[a]*, **Bernhard Wehrli**[a]), **Mary Power**[b]), and **Jan Roelof van der Meer**[b])

Abstract. Mineralization of organic matter in sediments are driven by a dense microbial population. Large chemical concentration gradients perpendicular to the interface are developed. Concentration profiles at the sediment-water interface can be measured *in situ* with miniaturized ion-selective electrodes and fluxes, and reaction rates can be calculated with this information. Direct flux measurements in benthic chambers are performed with isotope tracers to investigate the cycling of nutrients such as nitrogen and sulfur. With the development of molecular genetics new tools have become available to obtain information on the location and activity of microbial communities in the sediment. This opens a fascinating field for collaboration between geochemists and microbiologists. Flux measurements based on microsensors and *in situ* incubation techniques can now be related to microbiological information. In this contribution we give an overview of the current state of research in sensor development, incubation techniques, and molecular genetics at EAWAG.

Why Study Microbial Processes in Sediments?

The sediment-water interface is the biologically most active zone in lakes and coastal regions. Organic material which settles through the water column is mineralized within an active microbial mat at the sediment surface. The intense microbial respiration depletes dissolved electron acceptors such as O_2 and NO_3^- within millimeters. Sulfate reduction and reductive dissolution of iron and manganese oxides occur in the anaerobic zone below the sediment-water interface. In freshwater systems methanogenesis finally decomposes labile organic carbon. In oceans sulfate reduction is often the terminal step. The intensity of these microbial processes directly affects the recycling of nutrients such as nitrogen and phosphorus back into the water column, which is of major interest for the evaluation of nutrient management strategies. On the other hand, the benthic microorganisms also determine the final storage of organic carbon – an important factor in the global carbon balance. Simple methods of porewater analysis became popular 20 years ago to quantify diffusive fluxes in sediments [1][2]. With some mathematical modelling [3] such porewater profiles could be analyzed in terms of microbial reactions. However, the steep and coupled concentration gradients in many microbial biomats require analysis by microelectrodes in order to obtain sufficient vertical resolution [4]. Development of submersible *in situ* profiling instruments made precise measurements of benthic respiration rates possible [5]. The use of radioactive or stable isotopes represents a second approach to measure microbial activity. Methods based on $^{35}SO_4^{2-}$ to trace sulfate reduction rates or $^{15}NO_3^-$ to measure denitrification [6] are now well-established. These 'chemical' methods, however, remain black-box approaches, since only information about rates can be obtained. It is the development of gene-probe methods and the application of the polymerase chain reaction (PCR) to environmental samples that allows to probe the structure of the microbial communities and to detect their activity [7][8]. The possibility to gain insight into the structure of the microbial population and to establish the relevant physiological rates directly in the environment is a fascinating field for cooperation between microbiologists and chemists.

*Correspondence: Dr. B. Müller
[a]) Swiss Federal Institute of Environmental Science and Technology (EAWAG) Department of Biogeochemistry Limnological Research Center CH-6047 Kastanienbaum
[b]) Swiss Federal Institute of Environmental Science and Technology (EAWAG) Department of Microbiology CH-8600 Dübendorf

In situ High-Resolution Chemical Analysis of Sediment Porewater by Ion-Selective Sensors

Introduction to Chemical Sensors

A variety of chemical sensors have recently been constructed to investigate microbial mats and biofilms, and applications in environmental systems are increasing (for an overview see [9]). Chemical sensors exhibit some unique features that make them ideally suited for the *in situ* application in microbial mats. The zone of recently sedimented matter is very active and microbially driven redox reactions occur with high intensities within a layer of a few millimeters to centimeters and are often interdependent. Microbial processes and material fluxes can be interpreted from the course of the concentrations through the interface and quantified from their gradients applying *Fick*'s laws [10] (*Fig. 1*). With adequately miniaturized ion-selective electrodes (ISEs) concentration profiles across thin, highly structured interfaces can be resolved.

The measuring principle of ISEs allows relatively fast, continuous, and nondestructive measurement. No filtration and no discrete samples are needed. Miniaturization of the sensor geometry to micrometer size and negligible sample consumption provide the possibility of high temporal and spatial resolution measurements. Essential parameters for the characterization of performance of ISEs are detection limits, selectivities, sensitivities, and response times. Detection limits for most potentiometric membrane electrodes reach to the lower µmol/l range. Sensitivities should correspond to theory and are quality indicators for the sensors. Selectivity of sensors is important to consider, since interference of disturbing ions always occur when the concentration of the measuring ion falls short of a critical concentration. This is the reason that many potentiometric ISEs can not be used under seawater conditions but are readily applicable in freshwater.

Critical properties of potentiometric electrodes (different from amperometric sensors) are the logarithmic relation between signal response and activity. Concentration changes in environmental systems are often relatively small and subsequently the precision of the measurement is limiting. Moreover, membranes of potentio-

metric sensors are sensitive to microscopic changes on their surfaces and develop a long-term drift.

While the use of the amperometric oxygen microelectrode is more than ten years old, only very few ion-selective liquid membrane electrodes were applied in environmental research. The selectivities of the available specific carriers could not cope with the high electrolyte concentrations in the sea. These potentiometric ISEs can be applied in freshwater, however.

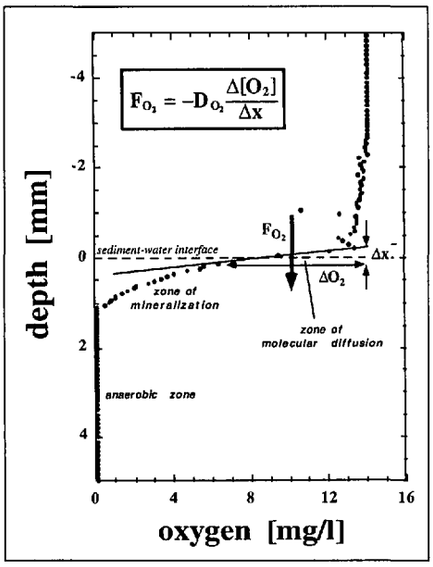

Fig. 1. *Early diagenetic mineralization processes use up O_2 in the sediment: Data show an oxygen profile measured* in situ *with the LISA in Alpnachersee 29.7.97.* The zone of linear decrease of O_2 at the sediment-water interface is caused by molecular diffusion through the diffusive boundary layer. The flux of O_2 can be calculated with *Fick*'s first law of diffusion (in box). The curved part of the profile indicates a zone of intense oxygen demand. The location of the change between the two zones is interpreted as the sediment-water borderline.

On-Site Measurements in Sediment Cores

We determine concentration gradients at the sediment-water interface with liquid membrane (PVC) ion-selective electrodes. For this purpose miniaturized membrane electrodes for pH, NH_4^+, NO_3^-, NO_2^-, Ca^{2+}, CO_3^{2-}, and S^{2-} were developed, and commercial amperometric O_2 microelectrodes are used [9][11]. An optode for CO_2 is under development [12][13]. Electrode bodies have outer tip diameters of 0.6 mm and 8 cm length.

The sediment core including overlaying water sampled with a gravity corer [14] is cut to a length of about 40 cm and placed under a micromanipulator which moves the set of sensors vertically in steps of 0.5 to 0.1 mm. Due to the high impedance of the PVC membrane sensors the setup is placed in a light wire-netting *Faraday* cage to avoid disturbations by electrostatic fields. Calibrations are performed with three solutions immediately before and after the sediment profile is recorded. Calibration curves for solid-state sulfide electrodes are recorded by a pH-titration experiment and are very reproducible. Data recording is performed with an 8-channel *MacLab* system and laptop computer (for details see [11]). In order to minimize alterations of the physical structure of the sediment-water interface, freshly sampled cores were measured on board of a float or at the shore. In this way, measurements can be performed immediately after sampling with a minimum of shaking, temperature changes, or other potentially distressing manipulations involved. *Fig. 2* shows concentration profiles of pH, NH_4^+, Ca^{2+}, and S(–II) across the sediment-water interface of Rotsee.

The experimental technique and setup is versatile and used for specific studies on sediment early diagenetic processes such as nitrogen cycling [15]. All relevant parameters, NO_3^-, NH_4^+, O_2, and pH, are analytically available and concentration profiles can be measured under given conditions of light intensity, O_2 and NO_3^- concentrations.

In situ *Measurements with a Submersible Microprofiling Instrument*

It can not be avoided that the situation at the sediment-water interface of the natural situation is altered due to core handling during sampling, degassing, warming, diffusion, interruption of the flow of bottom water, *etc.* Ideally, core sampling should be avoided at all and chemical gradients should be measured in the lake directly. Classic attempts to measure reaction rates and fluxes *in situ* or to sample porewater are the use of flux chambers and diffusion plates [16]. It was not until 1987 when *Reimers* [17] presented a submersible profiling instrument that allowed to measure gradients at the seafloor with a chemical sensor: First *in situ* measurements with an O_2-sensor in seawater were published by *Reimers et al.* [5]. The profiler was later extended for other chemical parameters and used by other research groups. New profilers were built later on, such as 'Profilur' [18] and were applied to oceanographic systems.

The low electrolyte concentration of freshwater allows the detection of NO_3^-, NO_2^-, NH_4^+, Ca^{2+}, and CO_3^{2-} with liquid membrane ISEs in addition to the 'classic' parameters O_2, pH, and S^{2-}. For this pur-

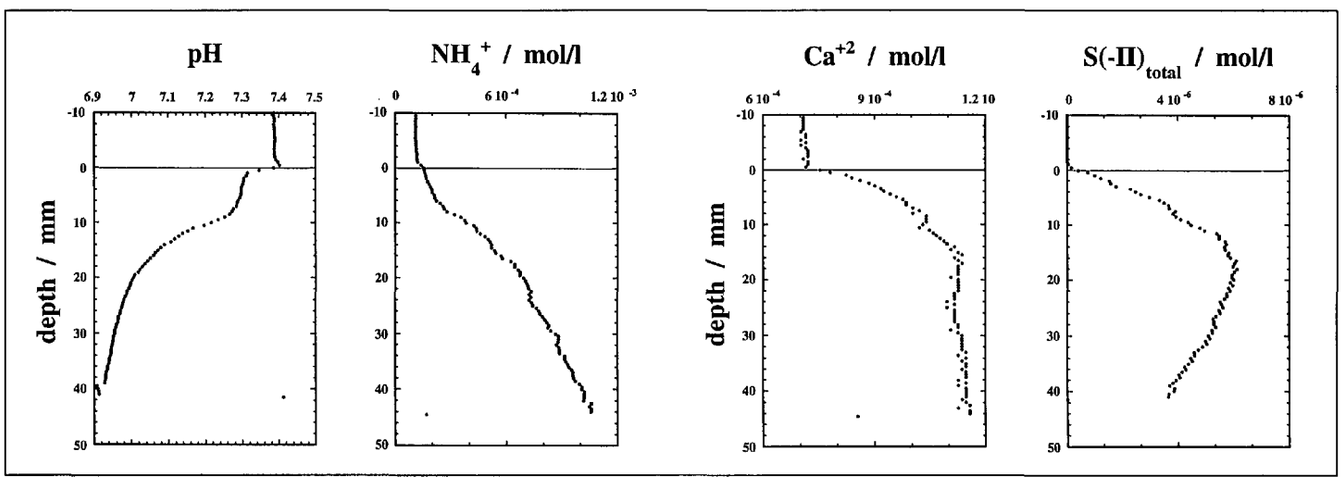

Fig. 2. *ISE profiles in a sediment core of Rotsee (29.8.96).* Cores were taken at the deepest point in 15 m depth. The line at zero depth marks the sediment-water interface. The residual signal of the NH_4^+ sensor above the sediment originates from the detection of K^+ interfering with the electrode at very low levels of NH_4^+ and can be corrected for mathematically. $S(-II)_{total}$ was calculated from the S^{2-} and pH concentration profiles. The last dot in the profile is the value measured in the overlying water after the measurement and serves as a control of the sensor.

CHIMIA 51 (1997) Nr. 12 (Dezember)

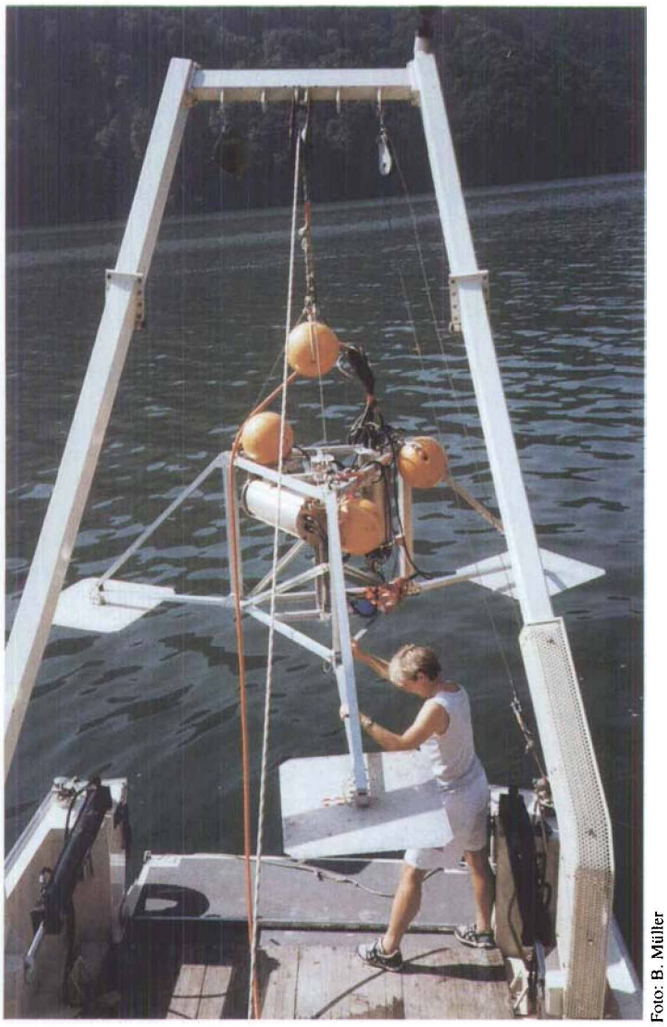

Foto: B. Müller

Fig. 3. *The lander for ion-selective analysis (LISA)*

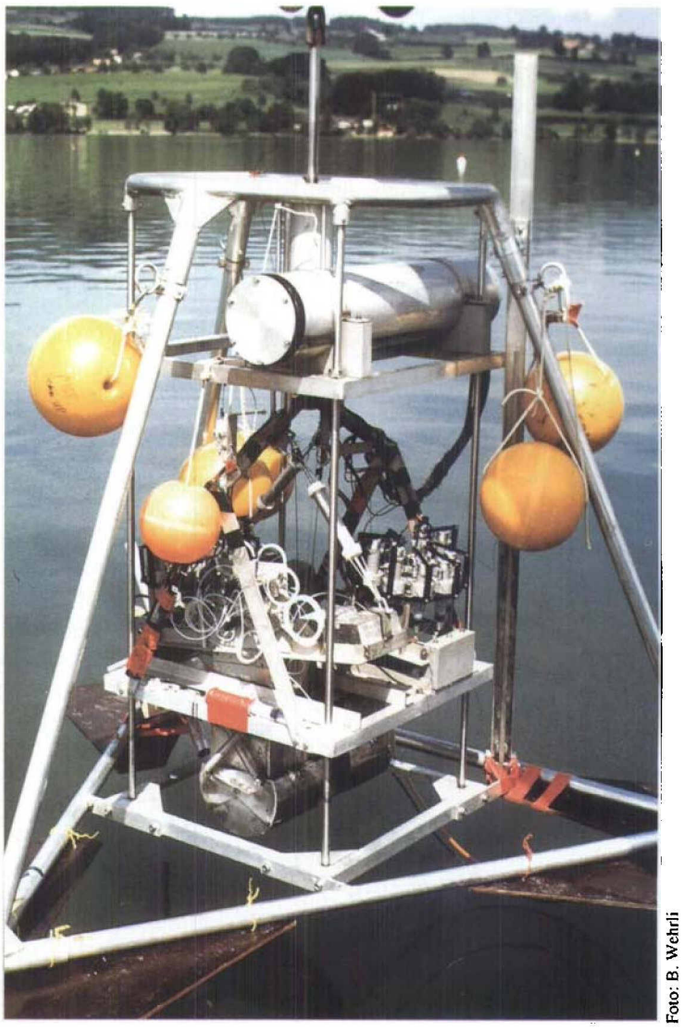

Foto: B. Wehrli

Fig. 4. *The benthic lander of EAWAG*

pose we constructed an underwater manipulating and measuring device to be operated in lakes of up to 400 m depth: LISA (Lander for Ion-Selective Analysis, *Fig. 3* and front page of this issue), described in [19]. The benthic lander system consists of a vertically positioned stepping motor which moves the electrodes mounted at the lower end. The resolution of the stepping motor is in the lower µm range. Two pressure casings contain power supply and data acquisition electronics. Three feet spread apart 2 m with large bearing surfaces prevent the device from sinking into the soft sediment prevalent in eutrophic lakes. Position of the sensors can be observed with an endoscope. The LISA can be handled in remote control. Data are acquired with a data logger system after signal conditioning and can be watched on-line on the computer onboard ship.

For underwater use evaluation of temperature and pressure dependence was investigated. To avoid complications with the reference electrode due to high water pressure, we apply a miniaturized Ag/AgCl solid-state reference electrode [20].

Investigations in progress are the comparison of mineralization rates in the littoral and profundal zones of lakes and the influence of light, explanation of the influence of sediment processes on the fate of the spawn of whitefish (*Coregonus* sp.) during development on the littoral sediment, to trace seasonal variation of the electron flow through the sediment-water interface, influence of mixing and sedimentation of organic material on the transport of electron acceptors to the sediment surface, and the investigation of the patchiness on lake bottoms.

In situ Incubation Techniques and Isotope Tracers

Denitrification in Lakes

Denitrifying microorganisms reduce nitrate to N_2 to respire organic carbon. This process is an important 'self-cleaning' reaction in natural waters with high nitrate loading. In a survey of major Swiss lakes a total elimination rate in the order of 15 kt nitrogen per year was estimated [21], which is *ca.* 20% of the amount of nitrogen exported *via* the main rivers towards the coastal seas. Thus, it seems to be important to evaluate the transformation reactions of nitrogen at the sediment-water interface in detail. In order to work under natural conditions, *in situ* incubation experiments were performed with the help of a benthic chamber. One of the major goals of these experiments was the question whether denitrification or ammonification

$$NO_3^- + 6H^+ + 5e^- \rightarrow \tfrac{1}{2} N_2(g) + 3H_2O$$

denitrification

$$NO_3^- + 10H^+ + 8e^- \rightarrow NH_4^+ + 3H_2O$$

ammonification

controls nitrate losses at the sediment-water interface. While denitrification transfers nitrogen back into the atmosphere as N_2, ammonification merely recycles this nutrient.

CHIMIA 51 (1997) Nr. 12 (Dezember)

In situ *Incubation*

The benthic chamber technique is now widely used in oceanography to analyze benthic microbial processes [22–24]. The benthic chamber used at EAWAG (see *Fig. 4*) consists of a tripod with two grab chambers, of which each can incubate a sediment area of 20 × 20 cm together with about 4–6 l of overlying water. The whole device is lowered to the sediment by a winch. After 'landing' the chambers operate automatically. Different functions such as deployment of the grab, closing of the lids, injection of tracers, and sampling with a series of ten syringes are released by a timing mechanism and burning wires. The device allows the use of stable isotope tracers to analyze pathways of microbial transformations. In the course of a field study in Baldeggersee, we combined this technique with other methods to obtain a 'snapshot' of nitrogen fluxes and transformation rates at the deepest part of the lake. The benthic lander experiment was performed to distinguish between denitrification and ammonification. *Fig. 5* summarizes the results of this study. $^{15}NO_3^-$ was injected into the benthic chamber and the isotope ratios were analyzed by mass spectrometry at different time steps during an incubation of 24 h [25]. A decrease of the isotope ratio in NO_3^- indicates that the tracer is diluted with nitrate formed by nitrification of ammonia which is released by the sediment. The absence of this effect in Baldeggersee indicates that nitrification is not important in the zone close to the sediment. The ratios of $^{30}N_2$, $^{29}N_2$, and $^{28}N_2$ liberated are an indication of a coupling of nitrification and denitrification. In Baldeggersee no evidence for this coupling effect was found. Finally, the ratios of ^{15}N in N_2 and NH_4^+ yield direct information about the relative importance of nitrification and nitrate reduction to ammonia. We found that more than 80% of the consumed nitrate was transformed to N_2 indicating that this self-cleaning effect which eliminates nitrate from the environment is very active in Baldeggersee. This results are compared in *Fig. 5* with other flux estimates obtained from sediment traps and sediment cores. Diffusion of nitrate into the sediment is of the same magnitude as the settling flux of particulate organic nitrogen arriving at the sediment surface in the form of dead biomass. However, only *ca.* 20% of this organic nitrogen accumulate within the sediment, a major fraction is released as ammonia by various degradation processes ranging from oxic decomposition to methanogenesis. Ammonia is released to the water column where it is mixed into oxic waters. The cycle is closed when ammonia is reoxidized to nitrate by nitrifying microorganisms.

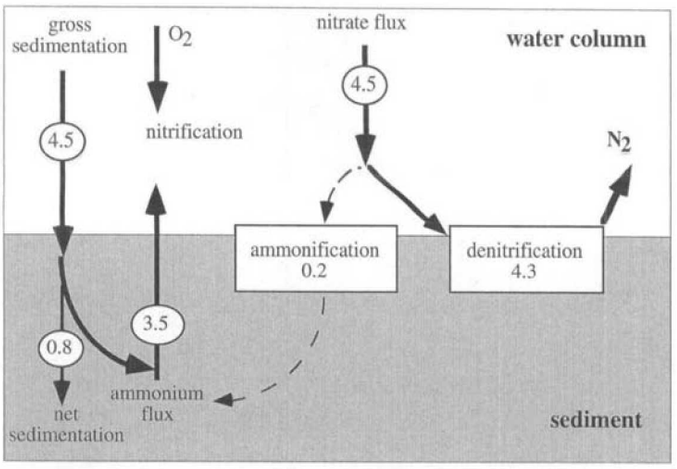

Fig. 5. *Graphical representation of estimated benthic fluxes and process rates in* [mmol nitrogen m^{-2} d^{-1}]. The scenario for nitrogen cycling is based on different types of flux measurements which are subject to analytical errors as well as spatial and temporal variabilities.

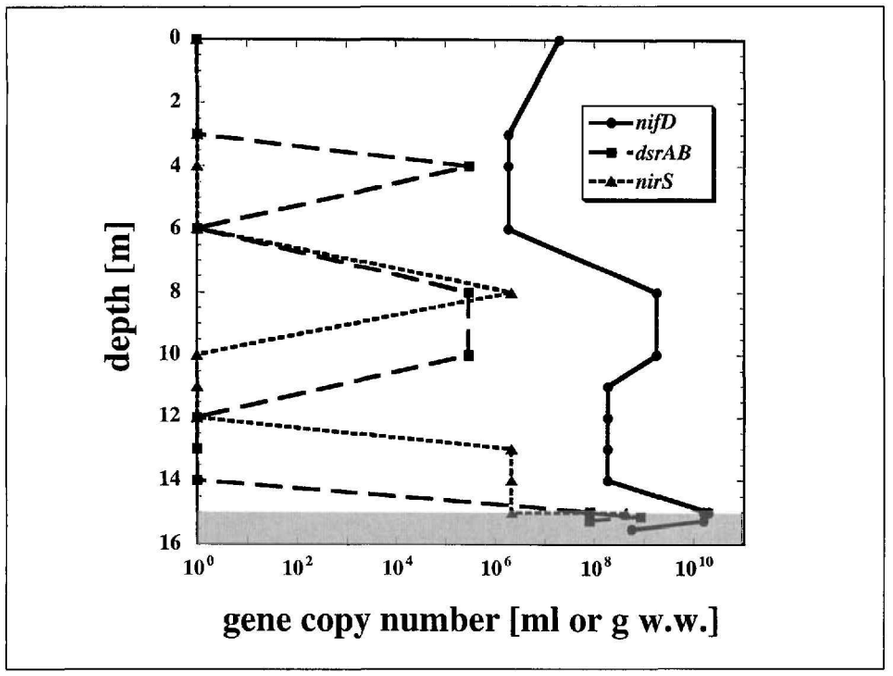

Fig. 6. *Vertical abundance of gene fragments for nitrogenase (nifD), dissimilating sulfite reductase (dsrAB), and nitrite reductase (nirS) in Rotsee in summer 1996.* The white area depicts the water column, whereas the shaded zone exemplifies samples taken from the sediment. Gene copy numbers are presented per ml of water (for the water column) or per g wet weight (for the sediment). Values equal to 10^0 are below the detection limit of the PCR.

Molecular Genetic Methods for the Study of Microbial Population Dynamics and Activity in Anaerobic Sediments

Polymerase Chain Reaction (PCR)

The PCR is an *in vitro* performed reaction in which short stretches of DNA molecules present in a sample are enzymatically copied to very high numbers, at which they become detectable [8][26]. By adding two oligonucleotides (with a length of between 18 and 25 nucleotides) to the reaction mixture, which each bind to complementary sites on the target DNA molecule, the DNA region encompassed by them will be copied by the DNA polymerase. How can the PCR then be used to describe and quantify the relevant populations of microorganisms which contribute to the nitrogen and sulfur cycle?

One attempt to follow is to look for the genetic material encoding critical enzyme steps, such as nitrite reductase (for denitrifying microbes), dissimilatory sulfite reductase [27] (for sulfate reducers), ammonia monooxygenase [28] (for nitrifiers) or

CHIMIA 51 (1997) Nr. 12 (Dezember)

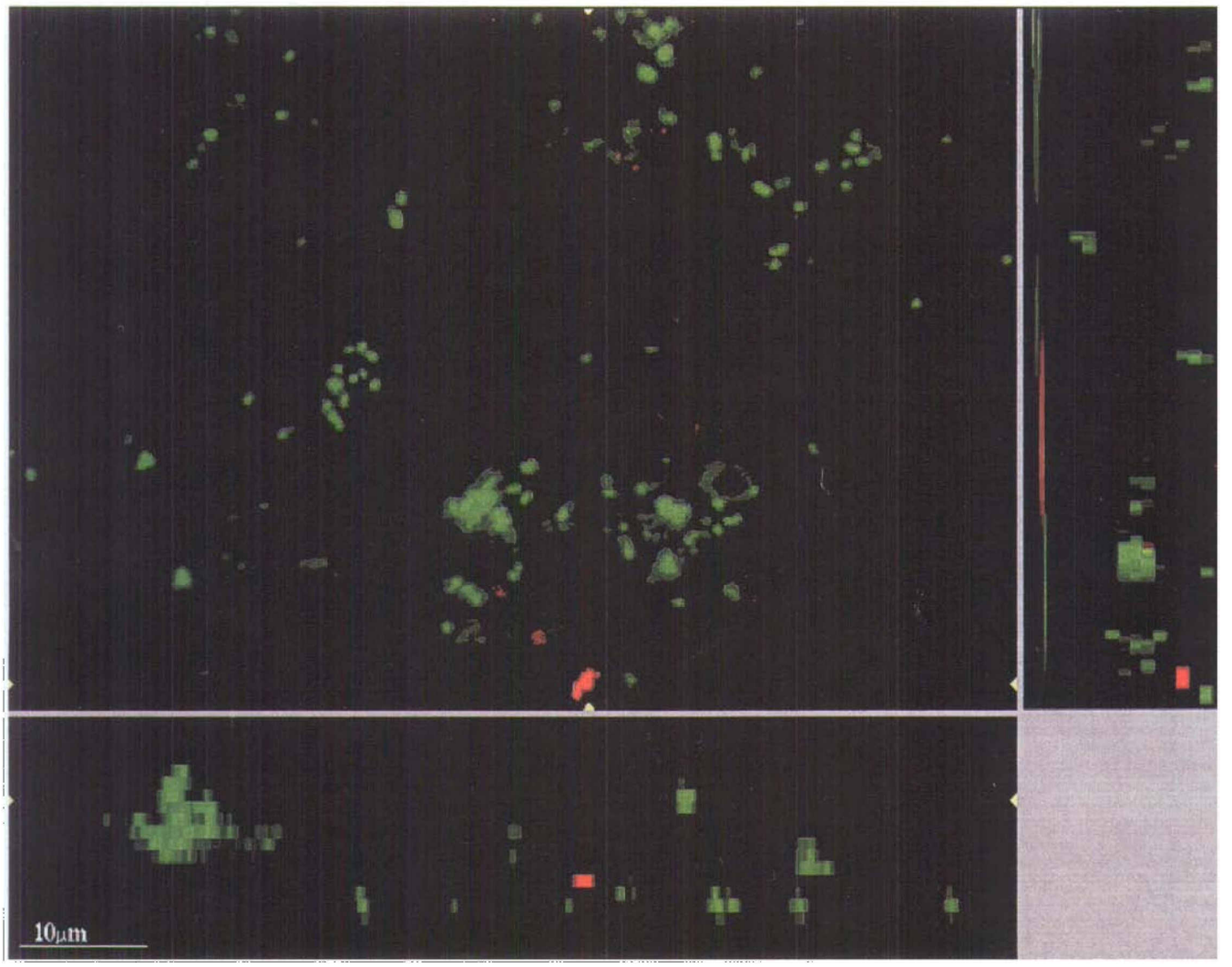

Fig. 7. *A laser confocal image of a heterotrophic biofilm on a stainless steel coupon 24 hr after inoculation with SRB. In situ* hybridization was performed with an oligonucleotide probe for SRB 16S rRNA labelled with Cy3 fluorescent dye (red). The biofilm bacteria were nonspecifically stained with PicoGreen DNA stain (green). The center panel depicts a number of cells at a specific depth of the biofilm. The xz plane (lower inset) and the yz plane (inset right) show the location of the SRB cell with respect to the depth of the biofilm.

nitrogenase [29] (for nitrogen fixers). The genes for such enzymes appear to be relatively well conserved among different microorganisms. In alignments of the related gene sequences, therefore, stretches can be found in which most base pairs are identical among the aligned sequences (see *e.g.* [30]). Oligonucleotides can be synthesized complementary to those highly conserved stretches which function as primers in the PCR. When DNA is isolated from environmental samples, the PCR with such primers will lead to amplification of the target sequences, allowing judgement of their abundance. No isolation or cultivation of the microbes is necessary. Unfortunately, quantification of initial target numbers by the PCR is tricky, since the amplification process in itself is nonlinear [31], and the original relative composition of the gene sequences is distorted during amplification [32]. By adding internal DNA standards and/or performing limited dilution series, we attempted to obtain quantitative results. First measurements were performed along the water column and in the first centimeters of the sediment of Rotsee, a small eutrophic lake near Lucerne, Switzerland, in 1996 (*Fig. 6*). Although quantities of target gene numbers were overestimated in some samples, the overall vertical distribution was comparable with that obtained by counting groups of microorganisms with *in situ* hybridization (*Hahn* [33]).

In situ *Hybridization*

In whole cell and *in situ* hybridization, bacterial membranes are permeabilized for single-stranded gene probes, which are labelled with, *e.g.*, a fluorescent molecule to enter the cell and (mostly) bind to the ribosomal RNAs [34]. Binding of the gene probes can be detected microscopically, usually by epifluorescence microscopy. Specific strains or groups of microbial strains can be detected with this technique in their natural environment without (much) disturbance of their spatial distribution [7]. We have been studying the penetration and distribution of a sulfate-reducing bacterium (SRB), *Desulfovibrio desulfuricans*, within biofilms of other heterotrophic bacteria. By using different gene probes for all Eubacteria, for the α, β and γ-subgroups of the proteobacteria, and for SRBs, the composition of the biofilm could be determined. With the help of confocal laser scanning microscopy and *in situ* hybridization, the spatial location of the SRBs in the biofilm was investigated (*Fig. 7*). Current work focuses on the question whether SRBs can penetrate already established heterotrophic biofilms, and under which conditions this will take place most optimally.

Activity Measurements of Microorganisms

One way of determining whether or not a microbe is in a metabolically active state, is to look for specific mRNA molecules present in the cells. Unfortunately, bacterial mRNA molecules have mostly rather short half-life times (2–5 min), and can reach very different copy numbers in the cell (from one up to several hundreds). In addition, their presence in the cell during different activity phases can be very different. For example, we determined that the mRNAs for the denitrification enzymes of *Paracoccus denitrificans* can only be measured in cells during a relatively short transition phase after a change from aerobic to anaerobic conditions [30]. The relative numbers of mRNA molecules during that phase can reach up to a hundred, but decreases to a mean value of *ca.* five copies when the cells enter the new stable situation. When conditions are continuously fluctuating between aerobicity and anaerobicity relatively fast, some mRNAs will not be synthesized in larger quantities anymore. They 'remember' the conditions that were prevailing before the last transition stage. *P. denitrificans* cells, *e.g.*, do not actively degrade the once synthesized denitrification enzymes. Somehow this prevents a renewed mRNA synthesis. We observed that from the denitrification enzymes, only the mRNA for the nitrite reductase will be continued to be synthesized after many fluctuations; those for nitrate and nitrous oxide reductase are no longer [35].

Several techniques can be applied when studying the prevalence of mRNA molecules in microbial systems. We have mostly used a direct extraction of the RNA fraction from bacterial samples, followed by an immobilization on a nylon support and hybridization with nonradioactive gene probes. This method is relatively fast and easy, but requires that large numbers of the bacterial cells from which the mRNA molecule should be detected are present. We estimated that at least around 10^7 cells should be available. For environmental samples, this is usually not the case, although we could detect mRNA for the nitrite reductase in wastewater treatment systems [35]. The detection limit could be lowered when a PCR is carried out with the isolated RNA, but this makes quantification even more difficult [36]. Another approach which is currently tested in our laboratory, is to detect mRNA molecules directly in the cell by *in situ* hybridization. At this moment, we focus on detection of the mRNA for the dissimilatory sulfite reductase of SRBs.

Outlook

It is obvious that a combination of such pathway measurements with high-resolution sensor studies and molecular genetic techniques has the potential to understand the black box of microbial transformation in great detail. At EAWAG the cooperation between microbial ecologists and environmental chemists has been initialized. In the framework of the nitrogen example outlined above there are many scientific and practical questions which we will address in the near future:

- Where are the active zones for nitrification and denitrification? Under which conditions is a close spatial coupling of both processes possible?
- How is microbial activity influenced by rapid changes in oxygen gradients induced by mixing processes?
- What are the effects of the decreasing phosphorus concentration and the declining biomass production in most Swiss lakes on the intensity of denitrification? How much phosphorus is needed to maintain this self-cleaning effect?

With several new quantitative approaches around the corner the collaboration between microbiology and chemistry could transform descriptive microbial ecology into a more quantitative science. Such a step could greatly reduce the error bars associated with most estimates of regional and even global biogeochemical cycles.

Received: September 26, 1997

[1] R.H. Hesslein, *Limnol. Oceanogr.* **1976**, *21*, 912.
[2] P.N. Froelich, G.P. Klinkhammer, M.L. Bender, N.A. Luedtke, G.R. Heath, D. Cullen, P. Dauphin, D. Hammond, B. Hartmann, V. Maynard, *Geochim. Cosmochim. Acta* **1979**, *43*, 1075.
[3] R.A. Berner, 'Early diagenesis: A theoretical approach', Princeton University Press, 1980.
[4] N.P. Revsbech, B.B. Jørgensen, in 'Advances in Microbial ecology', Ed. K.C. Marshall, Plenum, New York, 1986, Vol. 9.
[5] C.E. Reimers, K.M. Fischer, R. Merewether, K.L. Smith, R.A. Jahnke, *Nature (London)* **1986**, *320*, 741.
[6] L.P. Nielsen, *FEMS Microbiol. Lett.* **1992**, *86*, 357.
[7] R.I. Amann, W. Ludwig, K.-H. Schleifer, *Microbiol. Rev.* **1995**, *59*, 143.
[8] R.J. Steffan, R.M. Atlas, *Annu. Rev. Microbiol.* **1991**, *45*, 137.
[9] B. Müller, K. Buis, R. Stierli, B. Wehrli, *Limnol. Oceanogr.* **1997**, in press.
[10] A. Lerman, 'Geochemical processes: water and sediment environments', Wiley, New York, 1979.
[11] B. Müller, B. Wehrli, in 'Mikrobiologische Charakterisierung aquatischer Sedimente', Oldenbourg Verlag, 1997, accepted.
[12] B. Müller, P.C. Hauser, R. Stierli, B. Wehrli, Jahresbericht EAWAG, 1995.
[13] B. Müller, P.C. Hauser, *Analyst (Cambridge U.K.)* **1996**, *121*, 339.
[14] K. Kelts, U. Briegel, K. Ghilardi, K. Hsü, *Schweiz. Z. Hydrol.* **1986**, *48*, 104.
[15] J. Hunziker, Diplomarbeit, ETH-Zürich, 1997.
[16] N.R. Urban, C. Dinkel, B. Wehrli, *Aquatic Sci.*, submitted.
[17] C.E. Reimers, *Deep-Sea Res.* **1987**, *34–12*, 2019.
[18] J.K. Gundersen, B.B. Jørgensen, *Kieler Meeresforsch.* **1991** Sonderh., *8*, 376.
[19] B. Wehrli, C. Dinkel, B. Müller, *Mineral. Magazine* **1994**, *58A*, 961.
[20] B. Müller, P.C. Hauser, *Anal. Chim. Acta* **1996**, *320*, 69.
[21] M. Mengis, R. Gächter, B. Wehrli, *Gas Wasser Abwasser* **1997**, *5/97*.
[22] A. Tengberg, F. De Bovee, P. Hall, W. Berelson, D. Chadwick, G. Ciceri, P. Crassous, A. Devol, S. Emerson, J. Gage, R. Glud, F. Graziottin, J. Gundersen, D. Hammond, W. Helder, K. Hinga, O. Holby, R. Jahnke, A. Khripounoff, S. Lieberman, V. Nuppenau, O. Pfannkuche, C. Reimers, G. Rowe, A. Sahami, F. Sayles, M. Schurter, D. Smallman, B. Wehrli, P. De Wilde, *Prog. Oceanog.* **1995**, *35*, 253.
[23] A. Devol, *Deep-Sea Res.* **1987**, *34*, 1007.
[24] A. Devol, *Nature (London)* **1991**, *349*, 319.
[25] M. Mengis S. Bernasconi, R. Gächter, B. Wehrli, *Limnol. Oceanogr.* **1997**, in press.
[26] N. Arnheim, H. Erlich, *Ann. Rev. Biochem.* **1992**, *61*, 131.
[27] R.R. Karkhoff-Schweizer, D.P.W. Huber, G. Voordouw, *Appl. Environ. Microbiol.* **1995**, *61/1*, 290.
[28] V. Degrange, R. Bardin, *Appl. Environ. Microbiol.* **1995**, *61/6*, 2093.
[29] Y.-T. Chien, S.H. Zinder, *J. Bacteriol.* **1996**, *178/1*, 143.
[30] B. Baumann, M. Snozzi, A.J.B. Zehnder, J.R. van der Meer, *J. Bacteriol.* **1996**, *178/15*, 4367.
[31] P.D. Siebert, J.W. Larrick, *Nature (London)* **1992**, *359*, 557.
[32] R.F. Wang, W.W. Cao, C.E. Cerniglia, *Appl. Environ. Microbiol.* **1996**, *62/4*, 1242.
[33] D. Hahn, Institute for terrestrial ecology, Schlieren, Switzerland, 1996, unpublished results.
[34] R.I. Amann, L. Krumholz, D.A. Stahl, *J. Bacteriol.* **1990**, *172/2*, 762.
[35] B. Baumann, M. Snozzi, J.R. van der Meer, A.J.B. Zehnder, *Water Res.* **1997**, *31/8*, 1947.
[36] R.E. Hodson, W.A. Dustman, R.P. Garg, M.A. Moran, *Appl. Environ. Microbiol.* **1995**, *61/11*, 4074.