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A H_2S microsensor for profiling biofilms and sediments: application in an acidic lake sediment

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ABSTRACT: We developed a microsensor for the amperometric detection of dissolved hydrogen sulfide, H_2S, in sediments and biofilms. The microsensor exhibits a fast (t_{90} <0.2 to 0.5 s) and linear response to H_2S over a concentration range of 1 to >1000 µmol H_2S l^{-1}, and has a low stirring dependency of the microsensor signal (<1 to 2%). We used the new microsensor to obtain the first microprofiles of H_2S in an acidic lake sediment with a several cm thick flocculant surface layer. Despite the low pH of 4.6, a relative low SO_4^{2-} level in the lake water, and a broad O_2 respiration zone of ca 6 mm, we were able to measure H_2S depth profiles in the sediment at a good resolution, that allowed for calculation of specific sulfate reduction and H_2S oxidation activities. Such calculations showed highest sulfate reduction activity in the anoxic sediment down to ca 20 mm depth. A comparison of calculated areal rates of O_2 consumption and sulfate reduction indicated that sulfate reduction accounted for up to 13% of total organic carbon mineralization in the acidic sediment. All produced H_2S was reoxidized aerobically with O_2 at the oxic-anoxic interface. In addition to its good performance in acidic environments, the new H_2S microsensor has proven useful for sulfide measurements in neutral and moderate alkaline (pH < 9) biofilms and sediments, and thus is a true alternative to the traditionally used potentiometric Ag/Ag_2S microelectrode for most applications in aquatic ecology and biogeochemistry.

KEY WORDS: Microsensor · Hydrogen sulfide · Acidic sediment · Sulfate reduction · Sulfide oxidation · Freshwater

INTRODUCTION

Fine scale measurements of sulfide concentration gradients in combination with microsensor measurements of other chemical parameters such as O_2 (Revsbech 1989), pH (Revsbech & Jørgensen 1986), NO_2^- and NO_3^- (de Beer & Sweerts 1989, de Beer et al. 1997) allow detailed studies of sulfate reduction and various sulfide oxidation reactions in biofilms (Nelson et al. 1986, Kühl & Jørgensen 1992), microbial mats (Jørgensen & Revsbech 1983, Revsbech et al. 1983, Fenchel & Bernard 1995), and sediments (Sweerts et al. 1990, Visscher et al. 1991, Wetzel et al. 1995). Hitherto, sulfide microprofiles have been measured in near neutral or alkaline biofilms and sediments with ion-selective Ag/Ag_2S microelectrodes, originally adapted for aquatic sediment studies by Revsbech et al. (1983), or with a combined O_2-Ag/Ag_2S needle electrode, by Visscher et al. (1991).

The construction and use of well-functioning Ag/Ag_2S electrodes can, however, be problematic due to e.g. non-ideal responses (Frevert 1980, Revsbech & Jørgensen 1986, Camman & Galster 1996), signal drift and very long response times at low sulfide levels (Harsanyi et al. 1984, Kühl & Jørgensen 1992), susceptibility of the polycrystalline Ag_2S membrane to physical damage, or mixed potentials due to coprecipitates in the membrane (van Staden 1988, De Marco et al. 1990). Furthermore, the large uncertainty in the determination of the second dissociation constant of H_2S (Meyer et al. 1983, Myers 1986, Millero et al. 1988, Millero & Hershey 1989) makes it problematic to directly convert microprofiles of S^{2-} activity measured with the Ag/Ag_2S electrode to total sulfide concentrations, even if a good alignment with a measured pH

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profile exists. In practice such conversions thus often rely on interpolation from calibration curves obtained at various pH. The very high pK_2 of the sulfide system also precludes the application of Ag/Ag_2S electrodes in acidic environments, where S^{2-} is practically non-existent.

Brendel & Luther (1995) reported on a miniature voltammetric electrode method that allows for direct measurements of sulfide species and various other biogeochemically important chemical species. This method requires some conditioning steps during the measurement, which is therefore relative slow. The approach of Brendel & Luther (1995) is, however, interesting as it in principle allows direct and almost simulataneous measurements of total sulfide along with other important porewater solutes, and a comparison of this method with other microsensor techniques for sulfide measurements would be highly relevant. Alternatively, optical means of detecting sulfide have been investigated, but no optical microsensor for sulfide has so far been realized (Kohls et al. 1996). Recently, we used an amperometric measuring principle for H_2S (Jeroschewski et al. 1994, Jeroschewski & Braun 1996) to construct and characterize the first H_2S microsensor for use in well-defined aqueous solutions (Jeroschewski et al. 1996). Here we report on further developments of this sensor and show its application for sulfate reduction and sulfide oxidation studies in an acidic sediment. A detailed account of the chemical principles of the microsensor, and a comparison with other measuring techniques for sulfide is outside the scope of this paper and can be found elsewhere (Steuckart 1997). With the presented H_2S microsensor, a fast and reliable measurement of sulfide is now possible in various aquatic sediments and biofilms under acidic to moderate alkaline (pH < 9) conditions.

THE H_2S MICROSENSOR

Construction of microsensor

The H_2S microsensor is based on the basic design of a previously described Clark-type oxygen microelectrode with a guard cathode (Revsbech 1989). The microsensor consists of (1) an outer casing made out of a Pasteur pipette and sealed with a thin silicone rubber membrane (Medical Adhesive Type A, Dow Corning), (2) a tapered platinum measuring electrode coated with well-insulating and alkaline-resistant glass (Schott 8516) except for the distal few µm of the tapered tip, (3) an uncoated tapered platinum guard electrode, and (4) a platinum counter electrode (Fig. 1A). The technical procedures for making the casing and the measuring and guard electrodes are identical to those published for O_2 and combined O_2/N_2O microsensors (Revsbech & Jørgensen 1986, Revsbech et al. 1988, Revsbech 1989) and will not be described in detail here. The individual parts were positioned inside the outer casing while observing the microsensor tip under a microscope and then fixed in position by a droplet of fast-curing epoxy resin, connecting the electrodes to the outer casing of the microsensor. In order to avoid interference with the electrolyte, both the silicone and epoxy were allowed to cure for a minimum of 5 to 7 d before filling the assembled microsensor with electrolyte. Care was also taken to avoid direct contact between the cured epoxy and the electrolyte.

Conditioning of measuring electrode

In order to minimize the zero current of the H_2S microsensor, we conditioned the platinum surface of the measuring electrode before mounting it in the outer casing by repetitive cyclic polarization sweeps from +0.5 to −1.5V (>100 cycles at a speed of ca 0.5 V s^{-1}). It is important to finish this treatment at the reducing potential to avoid formation of platinum oxides on the electrode surface. This procedure cleaned the platinum surface and solved the problem of sensors with a high zero current, which was frequently observed using the earlier described construction procedure (Jeroschewski et al. 1996).

Measuring principle

The measuring principle of the H_2S microsensor is based on a previously described amperometric detection principle for H_2S (Fig. 1) (Jeroschewski et al. 1994). Dissolved H_2S diffuses via the silicone membrane into the microsensor tip, which is filled with an alkaline solution of 0.05 M $K_3Fe(CN)_6$ (ferricyanide) buffered with 0.5 M carbonate buffer (pH 10). Behind the silicone membrane, H_2S is deprotonated to HS^-, which is oxidized to S^0 by $K_3Fe(CN)_6$. The reduced $K_4Fe(CN)_6$ (ferrocyanide) formed by this process is then reoxidized to ferricyanide at the polarized platinum measuring anode (+85 to +150 mV vs the counter electrode) situated 20 to 30 µm behind the silicone membrane of the microsensor tip (Fig. 1A). The electrons generated from this process are used in the simultaneous reduction of $K_3Fe(CN)_6$ to $K_4Fe(CN)_6$ at the platinum counter electrode (Fig. 1B), which is situated in the bulk volume of the electrolyte-filled shaft of the microsensor (not depicted in Fig. 1A). The guard electrode is polarized like the measuring anode and serves to shield the measuring anode from reduced

components, e.g. $K_4Fe(CN)_6$ produced at the counter electrode, that diffuse towards the sensor tip. The guard electrode, thus, keeps a constant high ratio of $K_3Fe(CN)_6$ to $K_4Fe(CN)_6$ in the sensor tip, which is a prerequisite for a low zero current and good signal stability. More details of the reaction scheme can be found elsewhere (Jeroschewski et al. 1994, Steuckart 1997).

Geometric parameters of the sensor tip

Best performance of the H_2S microsensor was found when the measuring electrode was positioned 25 to 30 µm behind the silicone membrane. Due to the formation of S^0 when sulfide reacts with the electrolyte, the measuring anode should be >15 to 20 µm away from the silicone membrane to avoid interference of sulfur species with the reaction at the measuring electrode surface. In case of a short term contact and, therefore, direct oxidation of sulfide on the platinum electrode, this leads to formation of platinum sulfide (PtS) on the electrode surface, which will, however, still be able to reoxidize ferrocyanide (Gerischer 1950). After longer exposure times to sulfide, PtS_2 and elemental sulfur can form a coating on the measuring electrode surface (Ramasubramanian 1975, Kapusta et al. 1983), which leads to a blocking of the electrode surface and, therefore, a deterioration of the sensor signal.

In order to avoid signal drift due to local changes in pH and/or the ratio of ferri-/ferrocyanide in the tip region, it is necessary to allow for a good exchange between the tip region and the bulk electrolyte by avoiding a close fit of the outer casing around the measuring electrode. Thus, the ratio of the measuring anode tip diameter to the inner tip diameter of the casing should be <0.5. Best sensor performance is obtained with a distance of 100 to 200 µm between measuring anode and the guard electrode. Furthermore, glass beads (40 to 70 µm diameter) were added to the electrolyte. The beads accumulated near the sensor tip and prevented precipitation of dirt from the bulk electrolyte to the tip, which would otherwise affect the sensor signal and result in a higher residual current.

Effects of light

The electrolyte containing the redox mediator ferri-/ferrocyanide is an alkaline yellow-colored solution. Alkaline ferricyanide solutions are relatively stable and, in contrast to acidic solutions, do not degrade rapidly. However, the electrolyte can be decomposed by high levels of light due to a photodegradation of the hexacyanoferrate complex to pentacyano-aquoferrate(III), by removal of 1 CN^- (Hollemann & Wiberg 1985). This leads to an increased zero current and, therefore, a higher microsensor signal in light than in darkness for the same H_2S concentration. In order to minimize this interference of light when the H_2S microsensor is used in e.g. illuminated sediments and biofilms, we painted the finished H_2S microsensors with a black optical insulation, covering the outer casing with the exception of the outermost <10 to 50 µm of the sensor tip. Such an optical isolation was obtained by use of black enamel paint with xylol as a solvent, or by applying a fine carbon paste containing a mixture of solvents (Planocarbon N650, W. Plannet GmbH, Germany). An even better adhesion of the black coating can be obtained by siliconization of the outer casing prior to application of the black isolation.

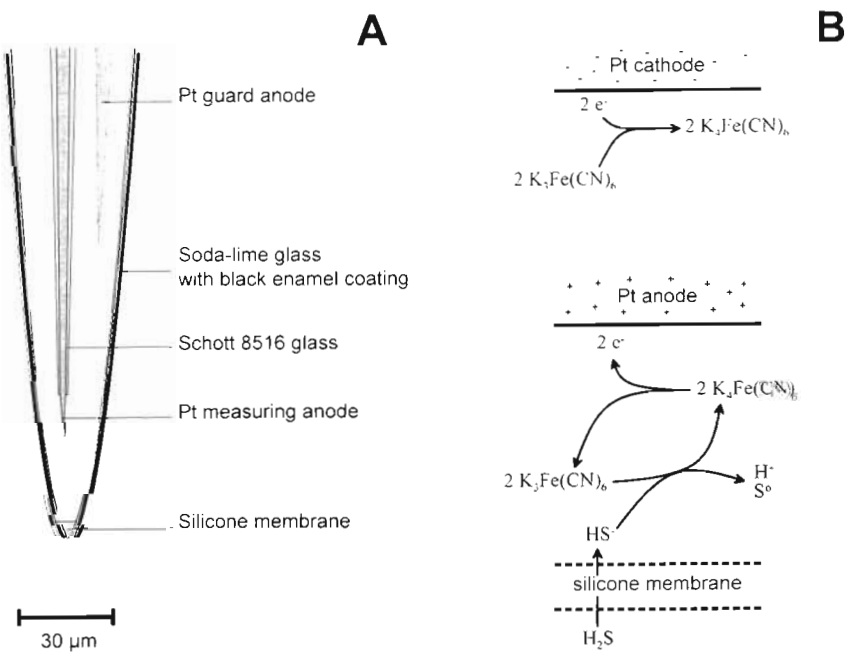

Fig. 1. H_2S microsensor. (A) Detailed view of the sensor tip. (B) Schematic drawing of the measuring principle. Upper part shows the reaction at the counter electrode situated in the bulk electrolyte, while the lower part shows the reaction at the measuring electrode in the microsensor tip. The reaction on the guard electrode is equal to that at the measuring electrode

Calibration

Dilute sulfide solutions are relative unstable and readily react with oxidants such as trace amounts of oxygen. In acidic solutions, H_2S gas may also escape from the calibration solution. In this study we calibrated the H_2S microsensors under acidic conditions in a flow cell, which was connected to a coulometric sulfide generator (Jeroschewski & Schmuhl 1993) and a peristaltic pump. In the generator system, H_2S is generated by cathodic reduction of HgS (which is a component of the generator electrode) in O_2-free acidic carrier solution (0.005 M H_2SO_4). Depending on the actual flow rate and applied current, acidic standard solutions ranging from <1 µM to >2 mM H_2S can be realized. If necessary, neutral or alkaline calibration solutions can be realized with the same system, where the acidic calibration standard is then mixed after the generator with a flow of oxygen-free buffer via a second peristaltic pump. As the microsensor exhibits a linear response to H_2S, a simple 2 or 3 point calibration using anoxic buffer solutions with a known H_2S concentration and pH can also be used under more alkaline conditions.

When measuring at pH >5, the signal of the H_2S microsensor for total sulfide, $\Sigma H_2S = [H_2S] + [HS^-] + [S^{2-}]$, depends on the ambient pH according to the protolytic equilibrium of H_2S:

$$[H_2S] = \Sigma H_2S \Big/ \left(1 + \frac{K_1}{[H_3O^+]} + \frac{K_1 K_2}{[H_3O^+]^2}\right) \quad (1)$$

where K_1 and K_2 are the dissociation constants of the hydrogen sulfide system. Thus, total sulfide in most natural systems can only be determined from simultaneous microsensor measurements of H_2S and pH.

As the H_2S microsensor measures dissolved H_2S, the measuring signal for the same total sulfide concentration decreases with increasing pH due to the increasing speciation of sulfide into ionic forms that cannot penetrate the silicone membrane of the microsensor. In an earlier study (Jeroschewski et al. 1996), we showed that the signal decrease actually follows the decrease, which can be predicted from Eq. (1). This would e.g. not be the case if the H_2S consumption by the microsensor were so large that it disturbs the local protolytic equilibrium at the measuring tip. Besides this inherent decrease in sensitivity towards total sulfide at pH > 5, there is no change in sensor performance at higher pH, i.e. the calibration curve towards H_2S remains the same, while the calibration curve towards total sulfide becomes less steep with increasing pH. We have successfully measured ΣH_2S levels down to a few µM with the new H_2S microsensor in defined neutral and moderately alkaline solutions (Jeroschewski et al. 1996) as well as in sediments and biofilms up to a pH of 8.5 (Santegoeds et al. 1997, Pringault et al. 1998).

Oxygen microelectrodes (Revsbech 1989) and pH glass microelectrodes (Revsbech et al. 1983) were used in combination with the new H_2S microsensor in an acidic sediment (see below). Oxygen microelectrodes were linearly calibrated from readings in the air-saturated water above the sediment and the zero current obtained in the anoxic parts of the sediment. Millivolt readings of the pH microelectrodes were calibrated in standard pH buffers with a calomel electrode (Radiometer, Denmark) as a reference electrode. Oxygen and H_2S microsensors were connected to custom-built pA-meters with a variable polarization voltage set at −800 and +85 mV, respectively. pH microelectrodes were connected to a custom-built high impedance mV-meter. All meters were connected to strip chart recorders.

Measuring characteristics

The new H_2S microsensor exhibited a linear response to H_2S (from 1 to >1000 µM) and a low zero current. An example of a calibration curve measured under acidic conditions is presented in Fig. 2A. Upon first exposure to sulfide the sensitivity decreases to a stable level, which enables the use of the microsensor for several weeks. The operational sensitivity is typically 0.2 to 3 pA μM^{-1} H_2S with zero currents of <5 pA. This allows for reliable measurements down to 1 µM H_2S with the microsensor connected to a sensitive pA-meter.

The operational lifetime of the H_2S microsensor is several weeks. Most H_2S microsensors exhibit a low zero current of a few pA when first connected, but older sensors may exhibit an increase in zero current, especially if impurities aggregate in the microsensor tip. A high zero current can sometimes be lowered significantly by keeping the electrodes polarized over a long time. This conditioning can be further enhanced by increasing the polarization voltage up to +200 mV For short term storing of the H_2S microsensor it seems advantageous to keep the sensor polarized, i.e. connected to the pA-meter. Long term storage at low (<5 to 10°C) temperature should be avoided, as this may lead to a precipitation of the electrolyte salts in the sensor.

Due to the geometric proportions of the sensor tip, the construction of sensors with a very low stirring dependency of the sensor signal can be realized (Heinze 1993). By keeping the diameter of the silicone-filled opening of the outer casing, i.e. the membrane diameter, small (<3 to 5 µm) in combination with a membrane thickness of 5 to 10 µm and a distance to the

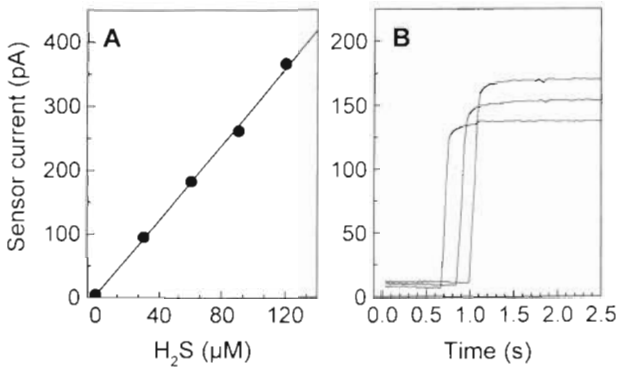

Fig. 2. (A) Example of a calibration curve for the H_2S microsensor obtained under acidic (pH < 5) conditions. The curve represents a linear regression through the data points ($r^2 > 0.99$). The zero current of the microsensor was 4 pA and the sensitivity was 2.9 pA μM^{-1} H_2S. (B) Three representative measurements of the response time of the H_2S microsensor The sensor was moved quickly from air into an acidic H_2S solution (pH 2.2, 186 µM H_2S)

measuring electrode of 20 to 30 µm, it was possible to construct H_2S microsensors with a stirring sensitivity of <1 to 2% and a response time (t_{90}) of <0.2 to 0.5 s (Fig. 2B). If a higher sensitivity for H_2S is necessary, this can be realized by increasing the opening of the outer casing, but this can result in a higher stirring dependency, which may lead to artefacts when measuring in a gradient of flow and/or diffusivity, e.g. in the diffusive boundary layer and upper layers of sediments (Revsbech 1989, Klimant et al. 1995). Furthermore, a H_2S microsensor with a too high consumption of H_2S, i.e. a high stirring sensitivity, may also create local disturbances of the redox equilibria. For very fast sensors, where the membrane diameter is further reduced and the diffusional distances are minimized, the stirring sensitivity can be kept low but this can result in a loss of sensitivity.

Interference

Only uncharged molecules can pass the silicone membrane and may interfere with the H_2S measurement. Under acidic conditions (pH < 6.5) SO_2 is the main interfering agent. Methyl- and ethylthiols may also interfere as they can penetrate silicone and react with ferricyanide. Earlier tests showed that these compounds give rise to a 20-fold smaller signal as compared to a similar concentration of H_2S (Steuckart 1997). Organic sulfides, e.g. dimethylsulfide, may penetrate the silicone membrane but do not interfere in the buffered alkaline electrolyte as they only react with ferricyanide under strong acidic conditions (Gurumurthy & Karunakaran 1995). Thiosulfate only interferes indirectly as it can be generated by a slow oxidation with oxygen of elemental sulfur accumulated in the sensor tip over time. This can result in a slow increase of the zero current over time, which is, however, insignificant for the practical use of the microsensor.

As part of the measuring process, elemental sulfur is formed that accumulates as colloidal sulfur in the sensor tip. Sulfur granules were, however, only visible in the tip after prolonged exposure of the microsensor to very high H_2S concentrations (>2 to 5 mM H_2S). At these high sulfide levels, we also observed a significant drift over time, which may be caused by sulfur accumulation, e.g. by a change of the membrane permeability due to sulfur. In practice, as long as the measuring anode is not too close to the membrane, accumulation of elemental sulfur seems of no significance for the practical use of the H_2S microsensor.

APPLICATION IN ACIDIC LAKE SEDIMENT

We tested the new H_2S microsensor in a sediment core sample from the acidic Lake Fuchskuhle, Germany. The lake water has a pH ranging from 4.2 to 4.6 and a sulfate content of ca 125 µmol SO_4^{2-} l^{-1} (Babenzien et al. 1991). The sediment sample was taken by a Jenkin sediment sampler with a ca 50 cm long core tube (inner diameter 50 mm) and transported to our laboratory, where measurements of oxygen, pH, and H_2S microprofiles were performed at room temperature (22 ± 1°C). Measurements were done in the core tubes with ca 10 cm lake water (pH 4.6) above the sediment. The water was aerated and gently stirred via a glass microcapillary connected to an air pump. The upper 3 to 5 cm of the sediment was very flocculent and easily resuspended by more vigorous stirring. The microsensors were mounted in a manually operated micromanipulator and inserted into the sediment in steps ranging from 0.1 to 1 mm. Smallest steps were used close to the sediment-water interface, in the oxic part of the sediment, and at the oxic-anoxic interface, where the gradients were steepest. The position of the microsensors relative to the sediment-water interface was determined visually under a dissection microscope while the microsensors were advanced toward the sediment surface. An accurate determination was, however, difficult due to the pronounced surface topography of the flocculent sediment surface.

Measurements with a pH microelectrode in the lake water and sediment exhibited an offset relative to the potential expected from the calibration curve using the independently measured lake water pH of 4.6. The pH microelectrode, however, exhibited a relatively constant potential throughout the water and sediment, i.e.

equivalent to changes of <0.2 pH units. We do not know the reason for the offset but speculate that the relatively high amount of humic substances might have affected the microsensor, the electrolytic conjunction to the reference electrode, or the reference electrode itself. The microelectrode showed the same calibration curve before and after measurements in the lake water and sediment, and the pH microelectrode was thus not irreversibly damaged by the measurements. In the following, we assume a constant pH in the sediment. However, more detailed studies of the porewater pH should be done as earlier studies in acidic lakes have shown that the porewater pH in some systems with a low buffering capacity can be several pH units higher than the pH of the overlying water, due to alkalinity production by microbial activity, e.g. by sulfate reduction (Cook et al. 1986, Herlihy & Mills 1986). In case there was a significant pH gradient towards more alkaline conditions in deeper sediment layers present in the system we only accounted for H_2S in our measurements.

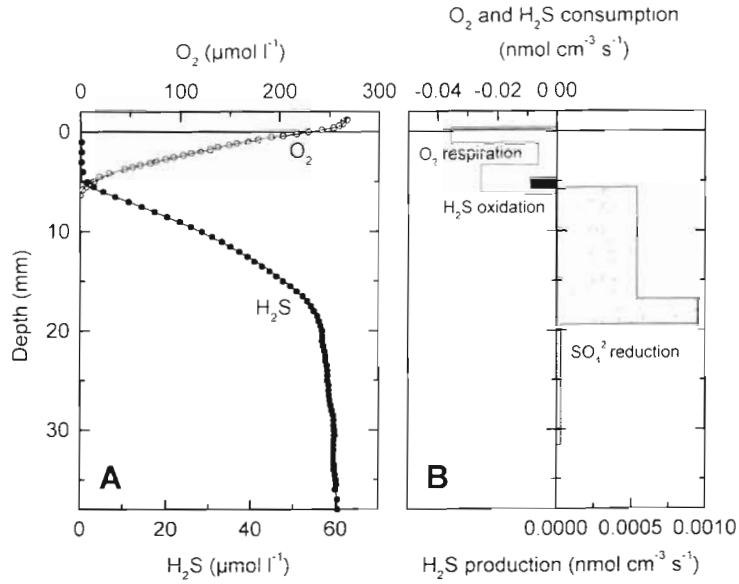

Fig. 3. (A) Oxygen and H_2S concentration profiles measured in acidic (pH 4.6) sediment from Lake Fuchskuhle, Germany. (B) Calculated activity distribution of O_2 respiration, H_2S oxidation, and SO_4^{2-} reduction (see details in text). Note the different scales in (B)

Oxygen diffused into the sediment over a ca 500 µm thick diffusive boundary layer and penetrated to a depth of 6 mm into the flocculant surface layer of the sediment (Fig. 3A). In deeper sediment layers, sulfate reduction resulted in the formation of H_2S, which was oxidized with oxygen at the oxic-anoxic interface. The measured H_2S profile does not indicate significant precipitation of metal sulfides due to reaction with e.g. iron. From the measured microprofiles, depth distributions of O_2 respiration, SO_4^{2-} reduction, and H_2S oxidation were calculated (Fig. 3B). Specific net production or consumption rates at depth z, $R(z)$, can be calculated from steady state concentration profiles as:

$$R(z) = \phi D_s \frac{d^2C(z)}{dz^2} \quad (2)$$

where ϕD_s is the sediment diffusivity of O_2 or H_2S, and d^2C/dz^2 is the curvature of the concentration profile, i.e. the second derivative of the profile (Nielsen et al. 1990, Kühl & Jørgensen 1992). We assumed zero-order kinetics and a constant diffusivity of O_2 and H_2S in the sediment (Kühl & Jørgensen 1992).

As the measured microprofiles consist of discrete data points with inherent small variations, differentiation of the raw data often leads to a very noisy pattern. A possible solution is to fit one or more functions to the data before differentiation, such as the simple method based on the fit of parabolic curves (Nielsen et al. 1990, Rasmussen & Jørgensen 1992). Alternatively, it is possible to carefully smooth and interpolate the measured data (Ramsing 1992). However, by doing so, one inevitably loses spatial resolution and precise zonation. We chose a different approach in which theoretical concentration profiles are calculated from activity profiles by integration of Eq. (2). The activity profile consists of a number of zones, with constant activity in each zone. A Simplex optimization method was used to find the activity profile that minimized the difference between the theoretically calculated and the measured concentration profile. This procedure was used to find a set of activity profiles, with an increasing number of zones, that described the concentration profile best. Each of these solutions was tested by evaluating the sensitivity of the solution to additions of noise to the measured concentration profile. The activity profile which was most robust towards noise addition was finally chosen.

Volumetric activities of O_2 respiration, sulfate reduction, and H_2S oxidation were calculated from the measured O_2 and H_2S microprofiles, respectively, assuming a constant sediment diffusivity of 0.8 times the diffusivity of O_2 and H_2S in water. Sweerts et al. (1991) studied the diffusivity of ^3H_2O in freshwater sediments and found an average ratio of the molecular diffusion coefficient (D_0) to the sediment diffusivity of 1.4 ± 0.2 in flocculent sediments (n = 9). According to Broecker & Peng (1974), $D_0(O_2) \approx 2.2 \times 10^{-5}$ cm^2 s^{-1} and $D_0(H_2S) \approx 1.7 \times 10^{-5}$ cm^2 s^{-1} at 22°C. Thus, in the analysis of the profiles it was assumed that $\phi D_s(O_2) \approx 1.8 \times 10^{-5}$ cm^2 s^{-1} and $\phi D_s(H_2S) \approx 1.4 \times 10^{-5}$ cm^2 s^{-1}.

The depth distribution of oxygen respiration showed an enhanced activity near the surface and at the oxic-anoxic interface, while the zone in between exhibited a low activity (Fig. 3B). The lower maximum of O_2 respiration overlapped with the zone of H_2S oxidation around 5.5 to 6 mm depth. Comparison of the specific oxygen respiration and sulfide oxidation rates in this zone yielded a H_2S to O_2 molar consumption ratio of ca 0.4, indicating a complete oxidation of H_2S. A relatively high abundance of the colorless sulfur bacteria *Achromatium* spp. has been observed in the investigated sediment (Babenzien et al. 1991, J. Kuever pers. comm.), and these sulfide oxidizers may thus play an important role in the reoxidation of produced H_2S at the oxic-anoxic interface within the sediment.

Hydrogen sulfide was produced in the anoxic parts of the sediment, with the highest sulfate reduction activity from 6 mm down to ca 20 mm below the sediment surface, where sulfate became depleted or reached a rate-limiting concentration. Areal rates of O_2 respiration, H_2S oxidation, and SO_4^{2-} reduction were calculated by depth integration of the specific conversion rates. The areal rates of O_2 respiration and SO_4^{2-} reduction were 1.215 and 0.076 µmol cm^{-2} d^{-1}, respectively. The areal H_2S oxidation rate was calculated to be 0.078 µmol cm^{-2} d^{-1}. Taking the O_2 uptake as a measure of total mineralization, and taking into account that the reduction capacity of 1 mol of SO_4^{2-} is equivalent to 2 mol of O_2 in terms of electron flow, it can thus be estimated that sulfate reduction can account for up to 13% of total organic carbon mineralization in the acidic sediment. This is in contrast to similar calculations made on data obtained in biofilms and marine sediments, where sulfate reduction can account for >50% of the organic carbon mineralization (Jørgensen 1982, Kühl & Jørgensen 1992).

CONCLUSIONS

To our knowledge, we present here the first combined microprofiles of O_2 and H_2S measured in acidic sediments. Oxygen and pH microsensors have previously been applied in an acidic microbial mat from a hot spring (Revsbech & Ward 1983). Sweerts et al. (1986) used oxygen microsensors in flocculant lake sediments of an experimentally acidified lake and Sweerts (1990) also investigated several other freshwater sediments from acidic environments. However, the use of microsensors in acidic environments still seems an underexplored area of research despite the fact that a relative large number of suitable microsensors exist (reviewed in Kühl & Revsbech 1998).

The new H_2S microsensor now allows detailed studies of the sulfur cycle in acidic environments. Used in combination with other available microsensors, detailed measurements of the zonation and specific activity of sulfate reduction and sulfide oxidation in acidic biofilms and sediments can be performed at high spatial resolution, and then related to other respiratory processes, i.e. O_2 respiration, nitrification, denitrification, and methanogenesis. Due to the almost non-invasive measurements with microsensors, it also becomes possible to study the regulation and dynamics of sulfate reduction and sulfide oxidation in intact samples by varying e.g. electron donor and acceptor availability along the lines of previous studies in neutral or alkaline biofilms and sediments (Nielsen et al. 1990, Dalsgaard & Revsbech 1992, Kühl & Jørgensen 1992).

While the new H_2S microsensor is obviously ideal for sulfide measurements in acidic systems it is also a good alternative to traditional S^{2-} microelectrode measurements in most neutral to moderately alkaline (pH < 9) systems. In our laboratory, the new H_2S microsensor has now totally replaced the S^{2-} microelectrode for most applications in natural systems, and we have had good first experiences with the use of the sensor in wastewater biofilms, sediments, and hypersaline microbial mats (de Beer et al. 1997, Santegoeds et al. 1997, Pringault et al. 1998, A. Wieland & M. Kühl unpubl. results). Furthermore, first preliminary results from SCUBA-diver-collected measurements and test measurements with benthic lander instruments (W. Ziebes & F. Wenzhöfer unpubl. results) indicate that the new H_2S microsensor is also suitable for *in situ* field applications.

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