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Sulfate biosensor for environmental applications

Ugo Marzocchi[1,2]* **Niels Peter Revsbech**[1]

[1]Center for Water Technology (WATEC), Department of Biology, Aarhus University, Aarhus, Denmark
[2]Center for Electromicrobiology, Department of Biology, Aarhus University, Aarhus, Denmark

Abstract

Sulfate is present in all freshwater and marine environments and is reduced to toxic and corrosive hydrogen sulfide by anaerobic bacteria. By measuring depth profiles of sulfate in sediments, it is possible to obtain estimates of sulfate reduction rates (SRRs). A whole-cell microscale biosensor for sulfate was constructed by placing a mixture of sulfate-reducing and aerobic bacteria in front of a hydrogen sulfide microsensor. The bacteria were supplied with electron donors from a reservoir behind the bacterial biomass. The aerobic bacteria ensured anaerobic conditions in deeper parts of the 150-μm-thick bacterial layer, so that the sulfate reducers could be active. A typical tip diameter of a sulfate biosensor is 50 μm. The calibration curve was close to linear in the 0–2 mM range, with a detection limit of about 10 μM. The 90% response time varied between 90 and 220 s, slowest response at low concentrations. Oxygen and ferrous iron at environmental concentrations gave no interference, but there was a 6% signal difference between stirred and stagnant liquid medium. The sensitivity of the biosensor could be modulated by applying a charge between sensor interior and an external electrode. The lifetime is usually a few weeks. The biosensor was used to measure sulfate profiles in a freshwater sediment.

Sulfate is a very important oxidant for the degradation of organic matter in the environment, and the sulfate-reducing bacteria mediating the oxidation are present in many anoxic environments such as sediments (Widdel 1988; Muyzer and Stams 2008) and sewers (Ito et al. 2002). The reduction of sulfate leads to the formation of H_2S that is toxic for higher organisms and also causes corrosion in, for example, sewers and offshore oil installations with following astronomical annual figures of cost for damages. Just the damages to US sewers have been estimated to US$ 14 billion per year (Koch et al. 2002).

In both environmental investigations and in industrial systems, there is a high interest in analyzing the process of sulfate reduction. There are two different avenues of quantifying sulfate reduction: (1) to measure the production of H_2S and (2) to measure the consumption of sulfate. The production of H_2S is by far the most commonly used approach. For some biofilms, it has been possible to quantify sulfate reduction by analysis of H_2S profiles, with following diffusion–reaction modeling of the profiles to obtain profiles of rates (Kühl and Jørgensen 1992). However, in most systems, H_2S reacts with iron minerals in the matrix, and such direct analysis is not possible. Sulfate reduction in sediments has therefore been analyzed in great detail by injection of radioactive $^{35}SO_4^{2-}$ with subsequent analysis of the formed radioactive H_2S during an incubation. By such a radiotracer approach it is possible to quantify the reaction even when a large pool of sulfide is present in the form of metal sulfides. Such analyses have resulted in a profound knowledge about the regulating factors for sulfate reduction in sediments, including the depth distribution of the process (Jørgensen 2021). However, the method works best for marine sediments with high-sulfate concentrations, whereas it is very difficult to get reliable estimates for sulfate reduction in low-sulfate freshwater sediments (Bak and Pfennig 1991) where sulfate may penetrate only a few millimeters below the oxic horizon of the sediment. At such low-sulfate penetration, it is difficult to estimate the relative proportion of radioactive vs. nonradioactive sulfate during an incubation. Reoxidation of sulfide within the sediment is also a problem with all ^{35}S determinations of sulfate reduction (Fossing 1995),

*Correspondence: ugomar@bio.au.dk

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and due to the proximity of the oxic surface layer this issue is most important in freshwater sediments (Jorgensen 1990; Roden and Tuttle 1993; Sandfeld et al. 2020).

Analysis of sulfate profiles in sediments has also been used to estimate rates of sulfate reduction (Berner 1964; Sinke et al. 1992). It is now widely recognized that such analyses do not result in realistic rates in layers affected by burrowing animals (Jørgensen et al. 2019), that for some species may extend to > 1 m depth (Ziebis et al. 1996). However, for sulfate profiles in layers below the depth of bioturbation rates seem to correlate well with rates determined by radiotracers. Difficulties in obtaining high resolution sulfate profiles over a few millimeters have until now prevented estimates of sulfate reduction from profile analysis in freshwater sediments.

In this paper, we describe how a biosensor for sulfate can be constructed, and how this sensor can be used to determine profiles of sulfate in freshwater sediments. When sulfate only penetrates to about 1 cm depth, quasi-steady-state profiles are established within few hours after disturbances such as burrowing activity of animals, and in contrast to the situation for marine sediments, it is thus possible to determine reduction rates from the profiles.

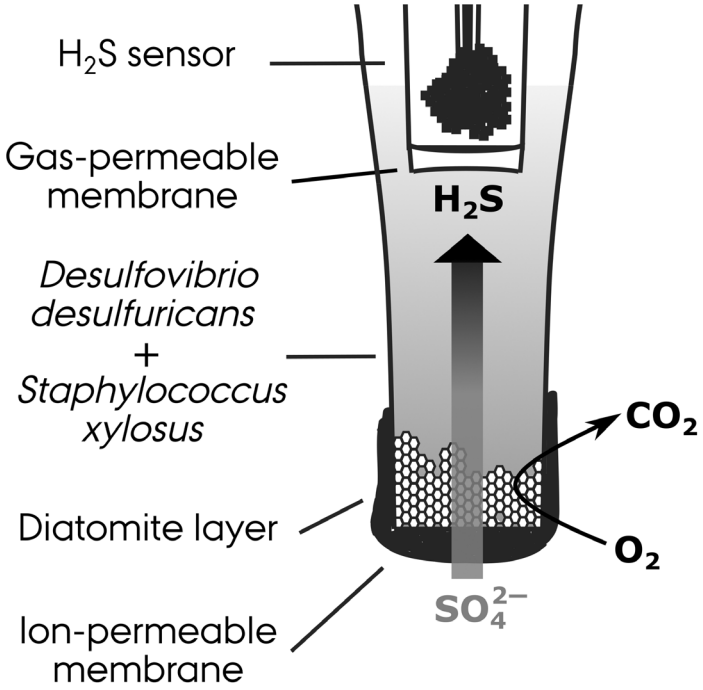

Fig. 1. Tip of microscale SO_4^{2-} biosensor showing the bacterial chamber where sulfate is converted to hydrogen sulfide by the anaerobic bacterium *Desulfovibrio desulfuricans*. Aerobic bacteria (*Staphylococcus xylosus*) maintain anoxic conditions within the chamber by consuming oxygen in proximity of the sensor opening. The tip diameter typically ranges between 50 and 100 μm.

Materials and procedures

Sensor construction and calibration

The sulfate biosensor was constructed using the basic design described by Revsbech and Glud (2009) for a nitrate biosensor. The biosensor is based on reduction of sulfate to H_2S by sulfate-reducing bacteria, and subsequent detection of the formed H_2S by a microsensor (Fig. 1). The outer casing containing the sulfate-reducing bacterial culture was made as described by Revsbech and Glud (2009) with a diatomite-supported polycarbonate–polyether dialysis membrane (Gambrane, Gambro AB, Sweden) in the tip. The membrane is out of production, but can be obtained from the authors upon request. As it is essential that the sulfate-reducing bacterium has a low half-saturation constant (K_m) for sulfate, and also is able to survive exposure to oxygen, we used the versatile, freshwater strain *Desulfovibrio desulfuricans* (DSM 9104) that can even respire with oxygen (Krekeler and Cypionka 1995). However, to increase the O_2 consuming potential within the biosensor, *Desulfovibrio* biomass was mixed with an equal amount of *Staphylococcus xylosus* that is a fast growing, biofilm forming, and immotile aerobic heterotroph.

Both bacteria were grown on agar plates. The agar medium for the sulfate reducer was designed to contain only little iron as excess iron minerals in the biosensor could affect the sensor response due to H_2S binding. Initially, the following chemicals were mixed (amounts for 0.2 liters final vol.) 3.0 g Agar, 0.2 g KH_2PO_4, 0.2 g NH_4Cl, 0.5 g $NaSO_4$, 1.3 g Na–lactate solution (60% w : w), 0.25 g yeast extract, 0.3 mL trace element solution (TMS3, Ingvorsen and Jorgensen 1984), 0.1 mL resazurin, adjusted to pH 7.0, and autoclaved in a blue-cap bottle. After cooling to 45°C, a mixture of $CaCl_2$ dihydrate (0.015 g) and $MgCl_2$ (0.2 g) in 5 mL of water that had been autoclaved separately was added to the blue-cap. Finally, 0.2 g of cysteine hydrochloride in 5 mL of water was sterile filtered into the medium, and the medium was poured rapidly into Petri plates and inserted into anaerobic jars (Anaerocult®, Millipore) containers. An AnaeroGen™ (Thermo Scientific) envelope was added before the anaerobic jars was closed. It is essential that the time span from addition of cysteine to closure of anaerobic jars is very short to avoid excessive cysteine oxidation. The next day, the resazurin should have turned colorless and the plates are ready for inoculation from liquid medium or from older agar-grown cultures. Suitable liquid media for growth of DSM 9104 are recommended at the DSMZ homepage (www.DSMZ.de). The agar plates should be inoculated with 3–4 parallel well-separated streaks to optimize growth of concentrated *Desulfovibrio* biomass. After addition of a new AnaeroGen™ envelope and closure, the anaerobic jars were placed at 30°C for 3–5 d. *S. xylosus* was grown on nutrient broth (8 g L^{-1}) agar (15 g L^{-1}) plates incubated aerobically at 30°C for 1 d.

For inoculation of the outer casing of the biosensor, we mixed equal amounts of bacterial biomass from the two

cultures. Immediately after opening the anaerobic jars with the *Desulfovibrio*, the chosen plate was scraped with a sterile scalpel blade and the bacterial biomass transferred to a glass microscope slide. An equal amount of biomass was harvested from a *Staphylococcus* plate, and the combined biomass was mixed and rapidly sucked into the tip region of a flame-pulled Pasteur pipette with a tip diameter of about 50 μm. The bacterial biomass was subsequently injected into the tip of the membrane-equipped biosensor outer casing (Fig. 1). The tip of the outer casing had a slightly larger diameter than the injection capillary. Immediately after this, the H_2S microsensor constructed according to Kuhl et al. (1998) was inserted and fixed in place with UV curing cement (Loctite® 3494). "SULF-type" H_2S sensors acquired from Unisense A/S (Denmark) that function by direct oxidation of H_2S were also tested. The lumen behind the tip of the H_2S sensor could now be filled with a suitable growth medium. The best growth medium should ensure a stable pH below 7, so that most sulfide is found as H_2S, and of course promote growth of both bacteria. The chosen medium contained (amounts for 0.2 liters final vol.) 0.05 g KH_2PO_4, 0.1 g NH_4Cl, 1.4 mL Na–lactate solution (60% w : w), 1.1 g Na–citrate dihydrate, 0.3 mL TMS 3, adjusted to pH 6.9, and the solution autoclaved in blue-cap bottle. After cooling to 45°C, a mixture of $CaCl_2$ dihydrate (0.3 g) and $MgCl_2$ (0.2 g) in 10 mL of water that had been autoclaved separately were added.

The H_2S microsensors functioning as transducers in the biosensors were polarized at +0.085 V vs. the internal platinum counter electrode. Sensor polarization and measurement of the resulting currents were done by a commercial multimeter (Unisense, Denmark).

Characterization of the sensor response

Calibration of the sensors were conducted both from low to high and from high to low concentration. It is our experience that ion biosensors based on the design shown in Fig. 1 do not function well in demineralized water, and calibration was therefore routinely performed in demineralized water with 0.3 g L^{-1} of NaCl corresponding to an ionic strength typical of Danish freshwater systems. Calibrations from low to high were done by addition of $NaSO_4$ from a concentrated stock solution, and the reversed calibration was subsequently performed by dilution of the thereby obtained solution. Calibrations were usually done under air saturation of the analyzed media, but the effect of shifts between oxic and anoxic conditions was also tested.

The sensitivity of the sensor can be modulated via the Electrophoretic Sensitivity Control (ESC) system (Kjaer et al. 1999; Marzocchi and Revsbech 2014), whereby the migration of charged species into the sensor can be stimulated by imposing an electric field across the membrane. Modulation of the sensor response via ionic migration was tested by repeated calibrations with an Ag/AgCl electrode inside the bacterial chamber during open circuit and polarizations of 0, +50, +100, and +150 mV vs. an external Ag/AgCl electrode (Supporting Information Fig. S1). Calibrations were performed from high to low concentrations.

Interference from O_2 was tested by two consecutive calibrations (low to high concentration), the first with aerated water and the second with the water sparged with nitrogen gas. In a second test, the sensor was incubated in 200 and 400 μM sulfate until a stable signal was obtained and subsequently the solution was sparged with air or nitrogen gas. Interference from ferrous iron was tested by incubation in 200 μM sulfate under anoxic conditions and subsequent addition of 0.1 and 1 mM $FeCl_2$.

The sensitivity to external turbulence (i.e., advectional transport of sulfate to the sensor tip) was tested by incubation in aerated 200 μM sulfate solution while exposed to alternating 10 min vigorously air bubbling and stagnant periods.

Sediment concentration profiles

Sulfate concentrations in natural waters was determined by ion chromatography (Dionex IC-2500, Thermo Fisher Scientific). Concentration profiles were measured in freshwater sediments from a small lake at the Aarhus University campus, Vennelyst Lake. The sediment cores were collected in 5.2 mm (I.D.) Plexiglas cylinders so that they had 5-cm water layer on top. The water was bubbled with air after transfer to the laboratory and profiles of O_2, SO_4^{2-}, H_2S, and pH were analyzed the following day. The water phase was then bubbled with N_2 to induce anoxic conditions and thereby stop infauna activity. After 24 h of anoxia a new set of profiles was measured. All analyses were conducted at room temperature (21–23°C). The microsensor tips were advanced by a motorized micromanipulator and the resulting currents in the measuring circuit were collected on a PC using the program SensorTrace PRO from Unisense A/S (Denmark). Oxygen, H_2S, and pH sensors were calibrated using standard procedures as described in Marzocchi et al. (2018). Total hydrogen sulfide ($\Sigma H_2S = [H_2S] + [HS^-] + [S^{2-}]$) concentrations were calculated at each depth from the measured H_2S and pH values (Jeroschewski et al. 1996). SRRs were estimated by modeling the concentration microprofiles with the algorithm developed by Berg et al. (1998). Porosity (vol/vol) was determined from density and water content of 5-mm-thick sediment slides. The diffusion coefficient of SO_4^{2-} was calculated according to Boudreau (1997).

Assessment

Zero current and sensitivity control

The first attempt for making a sulfate biosensor was by the use of the SULF H_2S microsensor of Unisense, as this sensor has superior long-term stability. Excellent calibration curves could be obtained by use of the SULF sensor, but the zero current was high and increasing with time (not shown). We finally concluded that *Desulfovibrio* fermented the medium constituents (probably the lactate) and produced H_2 to which the SULF sensor is sensitive, and for subsequent biosensors we

therefore used the H_2S microsensor developed by Kühl et al. (1998). A high capacity for fermentative metabolism by the applied *Desulfovibrio* strain has previously been documented (Cypionka 1989).

Well-functioning sensors exhibit a linear calibration curve when calibrated from high to low concentration at open ESC circuit, whereas prolonged exposure to sulfate-free conditions to get a stable signal resulted in poor sensitivity and long response times at low concentrations by calibration from low to high concentrations (Fig. 2a). The 90% response time for the sensor tested in Fig. 2 varied between 90 and 220 s, with the slowest response at low sulfate. Calibrations points were thereby recorded 5 min after each change in concentration. This sensor exhibited near linear calibration curve up to 2 mM at open ESC circuit, but the linear range depends on sensor geometry including the distance between H_2S microsensor tip and biosensor tip and on the density and activity of the bacterial biomass. Some sensors exhibited lowered sensitivity at low sulfate, even by calibration from high to low concentration, and the linearity should therefore be checked before environmental analysis.

When the ESC circuit was connected there were pronounced changes in sensitivity (Fig. 2b). An applied potential of 0 V resulted in a doubling of the sensitivity, and further increases in sensitivity were obtained by polarizations of +50 and +100 mV, while maximum concentration for linear response decreased to 0.5 mM. The sensitivity at +100 mV was five times higher than at open circuit. An about ninefold increase in sensitivity was obtained at +150 mV, but at this high polarization the limit for near-linear response decreased to about 250 μM.

Effect of environmental parameters on signal

By shifts between anoxic and oxic conditions there was no change in signal (Supporting Information Fig. S2). A change from stagnant to vigorously stirred 200 μM sulfate solution resulted in a 6% lower signal. Dissolved ferrous iron may be found in concentrations up to a few hundred μM in freshwater sediments (O'Connell et al. 2015), but is often found in the low μM range (Melton et al. 2012). It was thus necessary to check the biosensor for ferrous iron interference as the ferrous iron could precipitate FeS inside the sensor and thereby scavenge H_2S. We observed no change in signal upon addition of 100 μM and 1000 μM ferrous iron (anoxic conditions) when analyzing a 100 μM phosphate buffered sulfate solution at pH 7 (Supporting Information Fig. S3). H_2S does, of course, interfere and environmental analyses are therefore limited to near-H_2S free environments such as aerobic water or sediment rich in iron compounds that precipitates or oxidizes the H_2S. At open circuit the sensitivity to H_2S was 3–4 times higher than for SO_4^{2-}.

Sediment profiles

The Vennelyst Lake sediment was inhabited by a dense population of Chironomid larvae. Two sulfate profiles and an O_2 profile from a sediment core is shown in Fig. 3A. The sulfate profiles were separated by 2 cm. The sulfate concentration in the overlying water was 440 μM. There was a small peak in sulfate at the sediment surface in one of the profiles. Sulfate-free conditions were reached at about 1 cm depth. Secondary peaks of sulfate were present in both profiles at 2–3 cm depth, indicating ventilation of infaunal burrows. Oxygen only penetrated to 2 mm depth, and no secondary peaks of O_2 were seen in deeper layers. The general sulfate penetration was unchanged by defaunation (Fig. 3B), but no secondary peaks of sulfate were observed. SRR was calculated to be 30 nmol cm^{-3} h^{-1} in the top 0.74 cm of sediment. Only marginal sulfate reduction activity (< 0.4 nmol cm^{-3} h^{-1}) was

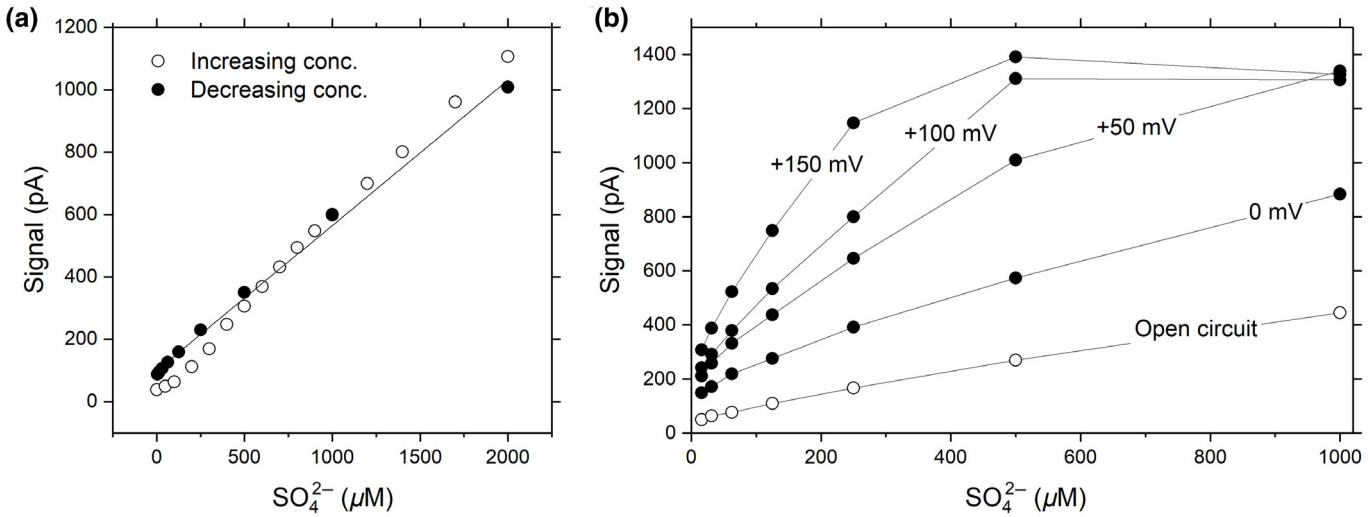

Fig. 2. (**a**) Sensor calibration performed at increasing and decreasing concentrations (open and closed circles, respectively). (**b**) Sensor calibration under increasing ESC potential (closed circles) and with the ESC circuit disconnected ("open circuit"; open circles). Calibrations were performed at decreasing concentrations.

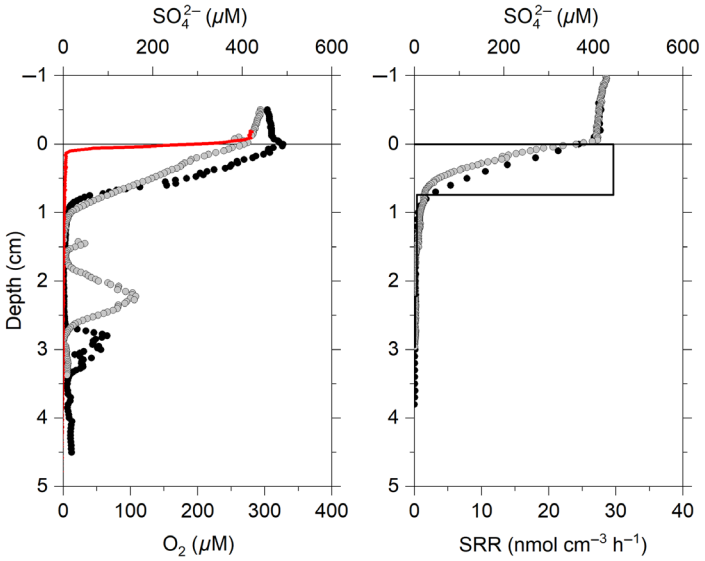

Fig. 3. Sulfate depth microprofiles (circles) measured with the biosensor in intact sediment cores inhabited by Chironomid larvae with aerated (left panel) and anoxic (right panel) overlying water. Red line in left panel indicates oxygen depth distribution. Black box in right panel indicates the SRR that can be calculated from the data.

detected below the top layer. The depth-integrated areal sulfate consumption rate was 5.4 mmol m^{-2} d^{-1}.

Discussion

The sulfate microscale biosensor described in this study is an analogue to the NO_x^- and NO_2^- biosensors previously described (Larsen et al. 1997; Nielsen et al. 2004), but instead of a pure culture it contains a mixture of two cultures so that both O_2 and SO_4^{2-} can be reduced. Accidental contamination with N_2O-reducing bacteria poses a problem with the NO_x^- and NO_2^- biosensors, as they rely on reduction to only N_2O that is quantified by a built-in electrochemical sensor. For the sulfate biosensor contamination may not be a problem, as H_2S cannot be reduced further. Contamination might, however, accelerate degradation of the nutrient medium within the sensor. We observed that lactate could be fermented by the *Desulfovibrio*, resulting in H_2 formation, so even without contamination there was degradation of the medium. The fermentation of lactate to acetate and H_2 is energetically favorable, even under standard conditions. However, the use of a mixed culture including possible "contaminants" from the environment should result in long-term functional sensors. Some sensors have actually been functioning for over 2 months, but there is still need for optimization of all aspects of the sensor construction to ensure reproducibly long-term functional sensors, including culture handling and nutrient medium composition. It should be possible to construct macroscale analogues of the sulfate biosensor using the basic macrosensor design of Nielsen et al. (2004), and such sensors could be valuable in, for example, sewer monitoring.

The calibration curve had a better linearity when the calibration was performed from high toward lower concentration. This is to be expected, as the sulfate-reducing bacteria have to accumulate sulfate to high concentrations intracellularly to enable the low-affinity ATP sulfurylase to be active. ATP sulfurylase is mediating the first energy-requiring activation of sulfate where sulfate reacts with ATP. An accumulation of sulfate by a factor of 20.000 has thus been observed at an external concentration of 0.1 μM, leading to an internal concentration of 2 mM (Kreke and Cypionka 1994). However, such an accumulation may take time and requires energy which may be in short supply after a long time of sulfate starvation. We chose a freshwater strain for our biosensor to ensure presence of high-affinity sulfate transporters (Marietou et al. 2018), but high affinity for sulfate (Km values of 2–8 μM) has also been found in a marine strain and in intact marine sediments (Tarpgaard et al. 2017). It is thus not surprising that we get linear calibration curves down to about 10–20 μM. It is difficult to define a detection limit of the biosensor, as the calibration curves tend to be somewhat nonlinear at low concentration. It is thus not appropriate to use the normal conventions for definition of detection limit. Our best estimate of detection limit is 10 μM.

The biosensor was very dependent on tip (i.e., interface) potential. There is a diffusive flux of ions between the sensor interior and the external environment, resulting in a charge at the sensor tip caused by differences in diffusivities of the individual ions. Most common bacterial nutrient media have faster diffusing cations than anions, resulting in a negatively charged tip when immersed into a low-salinity solution. We have previously tried to counteract build-up of a negative tip potential by adding a high concentration of LiCl to the inside medium of NO_x^- biosensors, as Li^+ due to high hydration has a low diffusivity of 8.7×10^{-6} cm^2 s^{-1} as compared to 17.1×10^{-6} $cm^2 s^{-1}$ for Cl^-, both values measured at 18°C (Li and Gregory 1974). Such Li^+ addition was not attempted here, and we instead used the ESC technique where the tip potential can be varied by applying a charge from an external source. It is evident from Fig. 2b that such applied potentials had huge effects on the sensitivity and dynamic range of the sensor. Even an applied potential of 0 V (i.e., short-circuit between internal and external electrodes) led to a doubling in sensitivity. With this configuration, the difference in Cl^- concentration between the inside of the sensor (34.6 mM) and in the outside water (5.1 mM) determined a cell potential of 0.049 V, thereby inducing a migrational flow of SO_4^{2-} into the sensor chamber. An applied potential of 100 mV gave a 6.5 times higher sensitivity than open circuit, but at the same time resulted in a four times lower near-linear range. The effect of moderate applied tip potential was much larger than previously observed for NO_x^- biosensors (Marzocchi and Revsbech 2014) as the migration of the double charged sulfate

ions in an electrical field is much more affected by the applied potential. It should be noted that the current in a similar ESC circuit was stable over hours (Marzocchi and Revsbech 2014), and use of a galvanostat was thus not necessary to ensure a stable sensitivity during the recording of a calibration curve. A commercial reference (REF201; Hach) with potential 200 mV inverted the cell potential annihilating the biosensor signal under short-circuit conditions. Higher ESC potentials would thus have to be applied to obtain amplification of the signal. Because of the possible reaction of the exposed Ag/AgCl wire with H_2S, reference electrodes with electrolyte bridge to the medium or even double junction reference electrodes are advisable for environmental applications.

The effect of stirring at open circuit was opposite of what we usually observe for other microsensors, with 6% lower signal when stirred. We interpret this as the result of an increased interface potential that counteracts sulfate diffusion into the sensor, while leaving H_2S free to diffuse out. Stirring of the external medium may help sulfide transport away from the sensor, resulting in a lower signal. The sensitivity to H_2S (pH 7) was 3–4 times higher than for sulfate at open circuit, probably again caused by electrostatic repulsion of sulfate.

Only iron rich freshwater sediments can be analyzed with the sensor due to the high free sulfide levels of marine sediments, but relatively high Fe^{2+} concentrations may build up due to microbial Fe^{3+} reduction. To completely rule out that this Fe^{2+} could interfere, the sensor was exposed to 1000 μM Fe^{2+}, which is much more than found in any neutral pH sediment. The addition had surprisingly absolutely no effect on the signal (Supporting Information Fig. S3). High Fe^{2+} concentrations should not co-exist with high H_2S, as FeS has a solubility product of 4.9×10^{-18}. The exposure to Fe^{2+} was only for 30 min, and it is possible that some FeS would form inside the sensor tip by longer exposure.

The sulfate profiles were largely as expected with depletion at 1 cm, and secondary presence in deeper layers caused by infauna. The sulfate concentrations in the secondary peaks were much lower than in the overlying water. Possibly due to the intermittent burrow ventilation by the larvae or because the sensor did not hit a burrow directly, but was passing the halo of sulfate around it. A small peak at the sediment–water interface was observed in one of the profiles in Fig. 3A. It is possible that it was a real peak due to oxidation of solid sulfides in the oxic horizon, but it could also be due to transition from stirred to stagnant conditions where the sensor exhibit about 6% higher sensitivity.

It is, of course, essential to know the background of dissolved sulfide in the sediment. When compensated for pH we found about 1 μM dissolved sulfide below 2 cm depth (Supporting Information Fig. S4). This would cause an interference corresponding to 3–4 μM sulfate, which is close to the zero-level stability of the sensor. It is thus not possible to see this H_2S background in the measured sulfate profiles.

Microprofiles modeling resulted in sulfate consumption rates of 5.4 mmol m^{-2} d^{-1}, which compare well with rates derived with radioactive tracers in lake sediments (Holmer and Storkholm 2001), indicating the viability of this method to obtain relatively fast estimates of net SRRs in freshwater benthic systems.

Conclusions

A mixture of the sulfate-reducing bacterium *D. desulfuricans* and the immotile aerobic bacterium *S. xylosus* was successfully used as mediator for SO_4^{2-} reduction to H_2S in a sulfate biosensor, where an electrochemical H_2S gas microsensor served as transducer. Near-linear calibration curves were obtained in the environmentally relevant range of 0–2 mM, and even better performance can probably be obtained by optimization of sensor design and composition of the internal nutrient/pH buffer medium for the bacteria. The sensor is insensitive to oxygen and ferrous iron at environmental relevant concentration, and the lifetime is up to two-months. The biosensor is now a powerful tool for research on sulfur cycling in freshwater environments. For instance, comparing net SRRs obtained by microsensor profiles with gross rates by radiotracers will provide insights on cryptic S cycling in freshwater systems. It should be possible, in addition, to construct macroscale analogues enabling long-term monitoring of for example sulfate in sewers. Main limits are the range of application which is constrained to iron-rich/sulfide-free freshwater systems and the relatively long response time.

Data availability statement

All data used for this work are available upon request from Ugo Marzocchi.

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