Microscale effects of oxygen and light on bacterial sulfate reduction in organic-rich lacustrine sediments

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Abstract. Biogeochemical cycling involves the exchange of electrons between chemical species through redox reactions. Microorganisms can utilize energy released by redox reactions for their metabolism following a thermodynamic sequence. Among these reactions, the dissimilatory reduction of sulfate (SO$_4^{2-}$) to produce hydrogen sulfide (H$_2$S) is one of the most important microbially-mediated pathways. The use of microelectrodes at the water-sediment interface provided a better understanding of sulfate reduction processes in the suboxic and anoxic zones. The goal of this study was to assess the microzonation of H$_2$S and O$_2$ under different conditions of light and oxygen in the water column. For this purpose, organic-rich lacustrine sediments from Pétrola Lake (SE Spain) were used. Sediment incubations were performed in mesocosm devices. The highest production of H$_2$S (up to 0.36 nmol/cm$^3$ s$^{-1}$) was observed under anoxic and dark conditions. Production under those conditions was several orders of magnitude higher than those measured when oxygen was present in the water column. Furthermore, the absence of O$_2$ in the water column significantly altered the microzonation of H$_2$S in depth. The absence of light seems not to affect the dynamics of O$_2$ and H$_2$S in depth. The study contributes to our understanding of microzonation in organic-rich sediments.

1 Introduction

Biogeochemical cycling involves the exchange of electrons between chemical species through reduction and oxidation (redox) reactions. Microorganisms can utilize chemical energy released from redox reactions by using the terminal electron acceptors that have the highest reduction potential (Eh) available following a redox reaction sequence [1]. The thermodynamic sequence starts by using oxygen (O$_2$) as an electron acceptor, followed by nitrate (NO$_3^-$), manganese and iron oxides, sulfate (SO$_4^{2-}$) and carbon dioxide (CO$_2$) in the different redox zones (oxic, suboxic, sulfidic, and methanic). This redox sequence establishes a vertical succession of redox reactions driven by microbial respiration beyond oxic-anoxic interfaces (e.g. water-sediment interface). The location of the oxic-anoxic

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interface plays a key role to understanding the redox sequence, and therefore, a good knowledge of the processes modifying redox sensitive reactions (e.g. oxygen production) is necessary for a complete biogeochemical perspective.

The dissimilatory reduction of \( \text{SO}_4^{2-} \) drives the formation of reduced sulfur, mainly as hydrogen sulfide (H\(_2\)S). Most of the H\(_2\)S is oxidized again to \( \text{SO}_4^{2-} \) coupled to the reduction of electron acceptors such as O\(_2\), NO\(_3^-\), or Fe-Mn-oxides, contributing to S recycling [2]. The development of high-resolution electrochemical techniques has enabled monitoring of chemical microenvironments, in sediments, with minimal disturbances [3]. Microelectrodes are used for the analysis of selective physico-chemicals parameters such as H\(_2\)S. These devices consist of small needle-shaped probes with a tip diameter of 1–20 mm that minimally disturb sediments or microenvironments [3]. As the profiles of reactants are determined by microbial conversion rates and mass transport resistance, microbial conversion rates can be calculated from the measured profiles when the mass transfer rates are quantified [4]. In fine-grained sediments, mass transfer is governed by diffusion, and local activities can be calculated using diffusion-conversion models [5].

The goal of this study was to assess the microzonation of H\(_2\)S and O\(_2\) under different conditions of light and oxygen in the water column. For this purpose, organic-rich sediments from Pétrola Lake (SE Spain), a saline eutrophic lake where high sulfate-reduction rates have been previously reported (Valiente et al., 2017), were used.

2 Material and methods

2.1 Sediment incubations

Recent organic matter-rich sediments cores were collected from Pétrola Lake (38° 50' 14” N, 1° 33' 40” W), 35 km southwest of Albacete (Spain), in July 2015. Pétrola Lake is a terminal lake occupying about 1.76 km\(^2\) in the lowest topographic position of the Pétrola endorheic basin (43 km\(^2\)), which consists mainly of Mesozoic materials. Lake water hydrofacies vary between Mg-Cl-SO\(_4\) (early spring) and Mg-Na-Cl-SO\(_4\) (early fall) (Valiente et al., 2017). Sediment incubations were performed by means of mesocosm devices (n=3). Mesocosms preparation for core incubations was adapted from previous works (Welti et al., 2012). For this purpose, acrylic mesocosms (40 cm in length, 20 cm in inner diameter, containing a total volume of 12.6 L) were used for sampling and incubation to guarantee minimal disturbances of the sediment during sampling. Moreover, initial natural conditions (IC) were evaluated by sampling sediment, using a sediment core of 20 cm length and 5 cm in inner diameter. The mesocosm were acid-prewashed and then drilled into the sediment down to approximately 20 cm depth. Then, mesocosms were filled with 2 L of lake water to maintain sediment saturation during transportation. Additional lake water was collected from sampling points and stored at 4 °C to fill mesocosms in the lab. Black plastic sheets were used to cover the mesocosms to prevent light penetration during transport. Mesocosms were placed in a temperature-controlled room (25 °C) with no exposure to direct sunlight. Small pump was installed in the inner wall, to maintain water circulation inside each mesocosm. Three different treatments were considered. First treatment (oxygen + light; OL) represented field conditions using atmospheric air bubbling, to provide oxygen, and normal dark-light cycles (= 14 h of light per day, natural sunlight source was used). The second treatment (oxygen + darkness; OD) simulated oxic conditions in the water column, which were preserved via atmospheric air bubbling. In OD treatment, each mesocosm was covered with aluminum foil to prevent exposure to light. Finally, the third treatment (anoxia + darkness; AD) maintained anoxic conditions in the
water column and sediment-water interface by bubbling argon and shielding mesocosms from light by aluminum foil. Sediment incubations were kept for 120 h.

2.2 H$_2$S and O$_2$ microprofiles

H$_2$S and O$_2$ activities in recent organic matter-rich sediments were evaluated by means of H$_2$S and O$_2$ microprofiles. Microprofiles were measured using an H$_2$S and O$_2$ amperometric microelectrode (Unisense A/S, Denmark) with a tip diameter of 100 μm. Microprofiles measurements were carried out at 100 and 50 μm intervals respectively to reach a depth of 2.5 cm in the fresh sediment mesocosms. H$_2$S and O$_2$ activity calculations were performed using dedicated software (SensorTrace Pro v.2.8.2, Unisense A/S, Denmark). To calculate H$_2$S and O$_2$ consumption and production, the method published by [4] was used assuming steady-state conditions where transport of solutes occurs by diffusion.

3 Results and discussion

Differences in the rates of production/consumption and depth were assessed among treatments (IC, OL, OD, and AD) in the upper 25 mm of sediment (Table 1; Figure 1). The highest H$_2$S production and consumption rates were observed in AD treatment reaching values of 0.36 and -0.35 nmol/cm$^2$s$^{-1}$, respectively. These results were up to two orders of magnitude larger than those measured in the initial conditions (IC) and in treatments with dissolved O$_2$ in the water column (OL and OD). The highest production of H$_2$S (Table 1) observed in AD treatment occurred at much shallower depths (1 – 2 mm in depth) than in the rest of treatments. Under these conditions, H$_2$S accumulates in both sediment and water column because the reoxidation of SO$_4^{2-}$ is not completed, most probable due to the lack of electron acceptors (O$_2$) in the sediment-water interface.

Table 1. For each treatment: medium H$_2$S in water at the sediment-water interface (SWI) in μM; maximum H$_2$S in porewater in μM; H$_2$S production and O$_2$ consumption rates in sediment (nmol/cm$^2$s$^{-1}$), as well as the maximum depth (mm) from the SWI of both processes. Suboxic zone also in mm. IC: initial conditions. OL: oxygen and light. OD: oxygen and darkness. AD: anoxia and darkness.

| Treatment | [H$_2$S]$_{\text{water}}$ (μM) | [H$_2$S]$_{\text{max-pore}}$ (μM) | H$_2$S production (nmol/cm$^2$s$^{-1}$) | Depth max. H$_2$S production (mm) | O$_2$ consumption (nmol/cm$^2$s$^{-1}$) | Depth max. O$_2$ consumption (mm) | Suboxic zone (mm) |
|-----------|------------------|------------------|-------------------------------|------------------|------------------|-------------------------------|------------------|
| IC        | 9.88             | 1.01             | 0.007                         | 21.5 – 25.5      | 0.223            | 0.1 – 0.2                     | 2.0              |
| OL        | 8.98             | 0.84             | 0.020                         | 18.8 – 20.0      | 0.317            | 1.0 – 1.2                     | 0.0              |
| OD        | 0                | 0.09             | 0.002                         | 24.4 – 25.5      | 0.013            | 1.2 – 1.8                     | 1.9              |
| AD        | 1.468            | 18.4             | 0.360                         | 1.0 – 2.0        | 0.015            | 0.2 – 0.3                     | 0.0              |

In comparison with previous studies carried out in marine environments (Preisler et al., 2007; Schauer et al., 2011; Weber et al., 2001), H$_2$S production data showed higher rates in Pétrola Lake sediment, even in treatments with the lowest rates (i.e. IC and OD). The large amount of dissolved SO$_4^{2-}$ in the system (up to 123,000 mg/L) and the high availability of organic carbon (Valiente et al., 2017) help to explain such high sulfate-reduction rates. In OL and OD treatments, O$_2$ penetrated between 1 and 1.8 mm in depth. The maximum H$_2$S concentration was reached at 13 and 25 mm for OL and OD treatments, respectively. Below the subsurface peak a quick decrease was observed for OL treatment. The zone of H$_2$S formation was less than 20 mm thick for OL treatment and there was different H$_2$S production zones in OD treatment (6 - 12 mm and 24 - 25 mm; Figure 1).
Fig. 1. H$_2$S concentrations (curved line) and rates of production (green bars) vs consumption (red bars) for the different treatments studied. IC: initial conditions. OL: oxygen and light treatment. OD: oxygen and darkness treatment. AD: anoxia and darkness.

H$_2$S and O$_2$ concentrations below the SWI show parallel trends for OL and AD treatments, and a transitional suboxic zone is not observed. On the contrary, a suboxic zone where both H$_2$S and O$_2$ are absent can be observed for IC and OD treatments at depths of 2.0 mm and 1.9 mm, respectively. The high sediment O$_2$ uptake combined with stagnant conditions in the overlying water promoted the disappearance of the suboxic zone in treatments OL and AD. Moreover, the oxic/anoxic interface with free H$_2$S present just below the oxic zone moved upwards to the SWI or even into the overlying water (Kristensen et al., 2000). In this suboxic zone, the metal oxides serve as oxidants in mineralization of organic matter, either directly by heterotrophic iron/manganese reducing bacteria, or indirectly by reaction with sulfide formed by sulfate-reducing bacteria (Nelson et al., 2004).

4 Conclusions

The results of this investigation show that the absence of oxygen in the water column significantly alter the microzonation of H$_2$S in depth. In contrast to a well-oxygenated water column, the absence of O$_2$ promoted H$_2$S production with rates up to two orders of magnitude higher (0.36 nmol/cm$^2$ s$^{-1}$). The layer of highest H$_2$S production was revealed in the upper part of the sediment, close to the water-sediment interface, instead of the bottom horizon in initial conditions (IC). The absence of light, despite the influence of primary producers, seems not to affect the dynamics of O$_2$ and H$_2$S in depth. The study contributes to our understanding of microzonation in organic-rich sediments from eutrophic saline lakes. Nevertheless, further research should be carried out to establish the conceptual model of sulfur recycling including other redox sensitive species (i.e. nitrogen compounds).
This work was funded by the projects SBPLY/17/180501/000296 from the Castilla-La Mancha Government and CGL2017-87216-C4-2-R from the Spanish Government.

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