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Sulfur and oxygen isotope fractionation during sulfate reduction coupled to anaerobic oxidation of methane is dependent on methane concentration

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ABSTRACT

Isotope signatures of sulfur compounds are key tools for studying sulfur cycling in the modern environment and throughout earth's history. However, for meaningful interpretations, the isotope effects of the processes involved must be known. Sulfate reduction coupled to the anaerobic oxidation of methane (AOM-SR) plays a pivotal role in sedimentary sulfur cycling and is the main process responsible for the consumption of methane in marine sediments – thereby efficiently limiting the escape of this potent greenhouse gas from the seabed to the overlying water column and atmosphere. In contrast to classical dissimilatory sulfate reduction (DSR), where sulfur and oxygen isotope effects have been measured in culture studies and a wide range of isotope effects has been observed, the sulfur and oxygen isotope effects by AOM-SR are unknown. This gap in knowledge severely hampers the interpretation of sulfur cycling in methane-bearing sediments, especially because, unlike DSR which is carried out by a single organism, AOM-SR is presumably catalyzed by consortia of archaea and bacteria that both contribute to the reduction of sulfate to sulfide.

We studied sulfur and oxygen isotope effects by AOM-SR at various aqueous methane concentrations from 1.4 ± 0.6 mM up to 58.8 ± 10.5 mM in continuous incubation at steady state. Changes in the concentration of methane induced strong changes in sulfur isotope enrichment (ε^{34}S) and oxygen isotope exchange between water and sulfate relative to sulfate reduction (θ_O), as well as sulfate reduction rates (SRR). Smallest ε^{34}S (21.9 ± 1.9‰) and θ_O (0.5 ± 0.2) as well as highest SRR were observed for the highest methane concentration, whereas highest ε^{34}S (67.3 ± 26.1‰) and θ_O (2.5 ± 1.5) and lowest SRR were reached at low methane concentration. Our results show that ε^{34}S, θ_O and SRR during AOM-SR are very sensitive to methane concentration and thus also correlate with energy yield. In sulfate–methane transition zones, AOM-SR is likely to induce very large sulfur isotope fractionation between sulfate and sulfide (i.e. >60‰) and will drive the oxygen isotope composition of sulfate towards the sulfate–water oxygen isotope equilibrium value. Sulfur isotope fractionation by AOM-SR at gas seeps, where methane fluxes are high, will be much smaller (i.e. 20 to 40‰).

1. Introduction

1.1. Sulfur and oxygen isotopes as tracers of present and past environmental processes

Sulfur isotopes are essential for the reconstruction of sulfur cycling through earth's history. They have been used to explore

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themes such as Earth's early atmosphere (Farquhar et al., 2000; Ono et al., 2005), the antiquity of sulfate reduction (Philippot et al., 2007; Shen et al., 2009), the evolution of atmospheric oxygen content over the Phanerozoic (Berner et al., 2000), and biogeochemical sulfur cycling over a broad range of geological time periods (e.g. Burdett et al., 1989; Canfield and Teske, 1996; Paytan et al., 1998; Ohkouchi et al., 1999; Kampschulte et al., 2001; Strauss et al., 2001; Paytan et al., 2004; Johnston et al., 2005; Wortmann and Chernyavsky, 2007; for a review see Bottrell and Newton, 2006). In recent years, it has become evident that the oxygen isotope composition of sulfate provides information on oxidative processes within the sulfur cycle that cannot be elucidated by the analysis of sulfur isotopes alone (Turchyn and Schrag, 2004). The study of the oxygen isotope composition of sulfate has turned out to be crucial for the understanding of deep biosphere sulfur cycling (Böttcher et al., 1998, 2000; Wortmann et al., 2007; Riedinger et al., 2010; Antler et al., 2013), oxidative sulfur cycling at the sediment-water interface (Ku et al., 1999; Böttcher and Thamdrup, 2001; Böttcher et al., 2001), the exploration of the formation of phosphatic laminites (Arning et al., 2009) and diagenetic gypsum (Pierre, 1985; Pirlet et al., 2010). Furthermore, the oxygen isotope composition of sulfate has been found to be a useful tool for evaluating sulfur cycling in soils and aquifers contaminated with aromatic hydrocarbons (Knöller et al., 2006, 2008), as well as for fingerprinting of microbial activity in acid mine drainage (e.g. Balci et al., 2007; Brunner et al., 2008; Heidel and Tichomirowa, 2011; Müller et al., 2013).

1.2. Sulfate reduction coupled to the anaerobic oxidation of methane

Sulfate reduction coupled to the anaerobic oxidation of methane (AOM-SR) is considered a process of worldwide relevance, because it is responsible for methane consumption in anoxic marine environments and thereby limits the escape of this potent greenhouse gas from the seabed (Reeburgh, 2007). Availability of dissolved methane regulates AOM-SR, with maximum solubility of methane being dependent mainly on pressure and temperature conditions. Consequently, methane availability varies strongly between different sites where AOM-SR is active, which can be gas seeps, mud volcanoes, hydrate fields or marine sediments rich in organic matter (Knittel and Boetius, 2009). Additionally, at sites where AOM-SR occurs, methane concentrations can vary strongly depending on spatial and temporal variations in methane supply and microbial turnover rates. Allegedly, AOM-SR is catalyzed by syntrophic consortia of methanotrophic archaea (ANME) that generally appear to operate in concert with *Desulfosarcina*-like (DSS) bacterial partners belonging to the *Deltaproteobacteria* class (for review, see Knittel and Boetius, 2009) according to the net reaction:

$$CH_4 + SO_4^{2-} \rightarrow HCO_3^- + HS^- + H_2O$$
$$\Delta G^0 = -16.6\ \mathrm{kJ/mol} \qquad (1)$$

Recently Milucka et al. (2012) proposed that AOM-SR may be carried out by the ANME and not (only) by their bacterial partners and suggested that the ANME reduce sulfate to zero-valent sulfur, which is disproportionated to sulfate and sulfide by the DSS (Fig. 1b). Immuno-assay studies of the same AOM consortium found no indication that the ANME possess ATP sulfurylase (Sat) or dissimilatory sulfite reductase (Dsr), key enzymes of the classic pathways to activate sulfate to APS and to produce the final product, sulfide (Milucka et al., 2012).

The possibility that ANME use an alternative sulfate reduction pathway to canonical DSR and partner with sulfur disproportionating DSS that likely use the canonical DSR pathway by partially operating it in reverse direction (Frederiksen and Finster, 2003; Finster et al., 2013; Fig. 1) raises the question if the relationship between sulfur and oxygen isotope fractionation observed for AOM-SR may be similar to the observed relationship for DSR, despite the differences in metabolic pathways.

1.3. Sulfur and oxygen isotope effects by DSR – the links between reversibility, energy yield and sulfate reduction rate

Sulfur isotope enrichment effects ($\varepsilon^{34}S$) reported for DSR cover a wide range from observations of $-3‰$ to the theoretical sulfur isotope equilibrium between sulfate and sulfide at $+75‰$, or even higher (for a review, see Sim et al., 2011a). The oxygen isotope effects by DSR are somewhat more subtle, driving the oxygen isotope composition of sulfate towards the isotope equilibrium fractionation value between sulfate and water (Mizutani and Rafter, 1969; Fritz et al., 1989; Brunner et al., 2005) of approximately $\sim 28‰$ at 4 °C (Zeebe, 2010). There are also cases where no oxygen isotope fractionation was detected (Turchyn et al., 2010).

Sulfur and oxygen isotope effects during DSR are the result of a sequence of isotope effects intrinsic to enzymatically-catalyzed steps in the sulfate reduction cascade and of the reversibility of this process (Fig. 1a). The extent to which these intrinsic isotope effects are expressed as overall isotope fractionation by DSR depends on how much back flux relative to the forward flux (i.e. reversibility) occurs in single steps (e.g. Rees, 1973; Brüchert, 2004; Brunner and Bernasconi, 2005; Farquhar et al., 2008; Bradley et al., 2011). The effects of increasing reversibility are two-fold. First, the sulfur isotope offset (expressed as $\varepsilon^{34}S$) between substrate sulfate and produced sulfide increases. Second, the oxygen isotope exchange between sulfate and water, mediated by rapid oxygen isotope exchange between sulfur-oxy intermediates in the DSR pathway, relative to the sulfate reduction rate (overall expressed as θ_O, see Brunner et al., 2012 and references therein) also increases.

The reversibility of DSR has long since been recognized as the main controlling factor for the expression of sulfur and oxygen isotope effects, while the parameters that control the reversibility of the DSR network and its individual steps remain less certain. Nevertheless, it is evident that ultimately, thermodynamics control the reversibility of individual biochemical reactions, and thereby the reversibility of a sequence of such steps. This can best be exemplified for a simple enzymatic reaction from a substrate to a product ($A \rightarrow P$). The reversibility is given by the relationship

$$\frac{f_-}{f_+} = \frac{[P]}{K_e \cdot [A]} = e^{\Delta G/(R \cdot T)} \qquad (2)$$

where f_- and f_+ denote back and forward flux, K_e the equilibrium constant ($K_e = e^{-\Delta G/(R \cdot T)}$), and ΔG the free energy of the reaction (for a discussion, see Holler et al., 2011a). The net flux (f_{net}) is given as

$$f_{net} = f_+ - f_- = f_+ \cdot \left(1 - \frac{[P]}{K_e \cdot [A]}\right) = f_+ \cdot \left(1 - e^{\Delta G/(R \cdot T)}\right) \qquad (3)$$

Eqs. (2) and (3) show that if the free energy of the reaction is small, which is the case when the reaction approaches thermodynamic equilibrium, reversibility becomes large (i.e. close to unity) while f_{net} becomes small (i.e. close to zero). The above equations do not imply that thermodynamics govern kinetics; they merely state that for a certain reaction rate (i.e., f_+), thermodynamics determine the rate in the opposite direction (i.e., f_-).

Intuitively, one also expects that reaction rates control the expression of isotope effects: reaction steps in DSR that proceed rapidly are assumed to deplete internal sulfur-oxy pools and