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Biogeochemical sulfur cycling in the water column of a shallow stratified sea-water lake: Speciation and quadruple sulfur isotope composition

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ABSTRACT

Concentrations of sulfate, sulfide and intermediate sulfur species as well as quadruple sulfur isotope compositions of sulfate, sulfide and zero-valent sulfur (ZVS) were analyzed in the water column of Lake Rogoznica (Croatia), a stratified marine euxinic lake. The chemocline in the lake, which was located at 8.5–9.5 m depth, supports a dense population of purple phototrophic sulfide oxidizing bacteria from the genus *Chromatium*. The highest ZVS (5.42 μmol L^{-1}) and sulfite (1.13 μmol L^{-1}) concentrations were detected at the chemocline. Thiocyanate concentrations up to 288 nmol L^{-1} were detected near the bottom of the lake. The thiocyanate profile suggests that it diffuses up from the sediment, where it may be produced by the reaction of cyanide with sulfide oxidation intermediates. Multiple sulfur isotope fractionations between sulfate and sulfide were consistent with a model finding that disproportionation is not a dominant process below the chemocline. Microbial sulfide oxidation was found to be the dominant process of the reoxidative part of the sulfur cycle. Despite the absence of a clear signal for sulfur disproportionation in multiple sulfur isotope values, $\delta^{34}S$ fractionations between sulfate and sulfide were in the range of 43.8–45.2‰, is relatively large in comparison to most laboratory culturing studies. Our results suggest that such fractionation is achieved by microbial sulfate reduction alone, which is in agreement with metabolic models and recent laboratory studies.

1. Introduction

The formation and reduction of sulfate is one of the most significant biogeochemical processes regulating the redox state of the oceans both presently and over Earth history. In modern seawater and near-surface sediment porewaters, sulfate is the most abundant soluble electron acceptor, with concentrations of 28.24 mmol L^{-1} at salinity of 35.00. Reduction of sulfate in the absence of dissolved oxygen is an important energy source for microorganisms (Eq. (1)).

$$SO_4^{2-} + 2CH_2O + 2H^+ \rightarrow H_2S + 2CO_2 + 2H_2O \quad (1)$$

A complicated web of sulfide oxidation reactions partially replenishes the pool of available sulfate, and maintains sulfate reducing activity. This oxidative part of the sulfur cycle proceeds, in part, through the formation of various intermediate sulfur compounds (inorganic polysulfides and their protonated forms, particulate and dissolved elemental sulfur, thiosulfate, polythionates and sulfite) utilizing different electron acceptors (oxygen, nitrate, iron(III), manganese(III), manganese(IV)) (Zopfi et al., 2004, and references therein).

In stratified water columns hydrogen sulfide can be oxidized to zero-valent sulfur (ZVS) and other intermediate sulfur compounds by phototrophic sulfur oxidizing bacteria (Eq. (2)) (e.g., Overmann et al., 1991; Canfield et al., 2005)

$$2H_2S + CO_2 \rightarrow CH_2O + H_2O + 2S^0 \quad (2)$$

These organisms (primarily green and purple sulfur bacteria) can also reoxidize ZVS and a number of other reduced sulfur compounds (including thiosulfate) to sulfate.

Bacteria can also mediate the disproportionation of elemental sulfur (Bak and Cypionka, 1987; Thamdrup et al., 1993; Canfield and Thamdrup, 1994) (Eq. (3)) as well as thiosulfate and sulfite (Bak and Cypionka, 1987; Cypionka et al., 1998; Jørgensen, 1990; Habicht et al., 1998) (Eqs. (4) and (5)). At sulfide concentrations of >1 mmol L^{-1}, the bacterial disproportionation of elemental sulfur (Eq. (3)) only

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occurs in the presence of an external sulfide sink (Thamdrup et al., 1993).

$$4S^0 + 4H_2O \rightarrow 3H_2S + SO_4^{2-} + 2H^+ \quad (3)$$

$$S_2O_3^{2-} + H_2O \rightarrow H_2S + SO_4^{2-} \quad (4)$$

$$4SO_3^{2} + 2H^+ \rightarrow H_2S + 3SO_4^{2-} \quad (5)$$

In sulfide-rich waters polluted by cyanide, formation of thiocyanate occurs by reaction of cyanide with sulfide oxidation intermediates such as colloidal sulfur (Kamyshny, 2009a), polysulfides (Luthy and Bruce, 1979; Kamyshny et al., 2009), thiosulfate and tetrathionate (Szekeres, 1974; Dzombak et al., 2006; Kamyshny et al., 2011a) (Eqs. (6)–(9)) via reactions such as:

$$S_n^{2-} + (n-1)CN^- + H^+ \rightarrow (n-1)SCN^- + HS^- \quad (6)$$

$$S_2O_3^{2-} + CN^- \rightarrow SO_3^{2-} + SCN^- \quad (7)$$

$$S_nO_6^{2-} + (n-3)CN^- + H_2O \rightarrow S_2O_3^{2-} + SO_4^{2-} + 2H^+ + (n-3)SCN^- \quad (8)$$

$$S_{8(colloidal)} + 8CN^- \rightarrow 8SCN^- \quad (9)$$

Formation of thiocyanate has never been previously documented in stratified non-polluted aquatic systems.

Typically, speciation and isotope composition analyses alone are not sufficient to understand the relative importance of various processes in element cycling. The chemical and microbial transformations of sulfur species lead to measurable fractionations of sulfur isotopes between reactants and reaction products, and these fractionations provide insight into reductive and oxidative sulfur pathways, considered in the context of chemical speciation. Sulfide produced in experiments with cultures and natural populations of sulfate reducers has been found to be up to 70‰ depleted in ^{34}S with respect to sulfate (Harrison and Thode, 1958; Bolliger et al., 2001; Canfield et al., 2010; Sim et al., 2011). Li et al. (2010) recently argued that the differences between sulfate and sulfide $\delta^{34}S$ (~54‰) in the Cariaco Basin water column were consistent with the activity of sulfate reduction alone, based on multiple sulfur isotope fractionation.

The magnitude of sulfur isotope fractionation by sulfate reduction is known to depend on substrate type and substrate concentrations. Oxidation of hydrogen as well as incomplete oxidation of lactate, propionate, or pyruvate to acetate leads to relatively small fractionation (Kaplan and Rittenberg, 1964; Brüchert et al., 2001). Oxidation of acetate to CO_2 leads to larger sulfur isotope fractionations, possibly due to lower cell specific sulfate reduction rates (Brüchert et al., 2001). The largest fractionations were observed during oxidation of complex substrates, such as crude oil (Bolliger et al., 2001). Lower, but still non-limiting electron donor concentrations lead to larger fractionations (Chambers et al., 1975; Canfield, 2001).

In some natural aquatic systems, large fractionations between sulfide and sulfate greater than 48‰ (Fry et al., 1991; Canfield and Teske, 1996; Neretin et al., 2003) have been explained by repeated step-wise fractionations imparted during oxidative sulfur cycling, including sulfide oxidation and the disproportionation of product sulfur intermediates (especially ZVS) to produce more ^{34}S-depleted sulfide and ^{34}S-enriched sulfate (e.g., Canfield and Thamdrup, 1994). Unidirectional sulfate reduction in sediments, however, was also argued to create sulfur isotope fractionation exceeding 65‰ (Wortmann et al., 2001; Rudnicki et al., 2001) as has been shown in recent experiments (Sim et al., 2011).

Nevertheless, even analysis of ^{34}S to ^{32}S ratios is often insufficient to quantify the oxidative part of the sulfur cycle, since the majority of the fractionation is imparted during sulfate reduction (e.g. Brunner and Bernasconi, 2005). Analysis of multiple sulfur isotope values is an emerging tool for the study of biogeochemical sulfur cycling in modern aquatic (Canfield et al., 2010; Li et al., 2010; Zerkle et al., 2010) and ancient sedimentary (Johnston et al., 2005a; Philippot et al., 2007; Shen et al., 2009; Wacey et al., 2010) systems. Small variations in mass dependent fractionation of ^{33}S and ^{36}S isotopes were shown to have a diagnostic pattern for biogeochemical transformations of sulfur compounds, such as sulfate reduction (Farquhar et al., 2003, 2007; Ono et al., 2006), sulfide oxidation (Zerkle et al., 2009, 2010) and sulfur compound disproportionation (Johnston et al., 2005b). Although a limited amount of data for quadruple sulfur isotope fractionation by various biological and chemical processes is available, this approach has already been successfully applied to determine the origin of sulfide (sulfate reduction alone vs. sulfide oxidation and sulfur disproportionation), elemental sulfur (chemical vs. microbial sulfide oxidation) and sulfate (consumption by sulfate reduction vs. production by sulfur disproportionation and/or sulfide oxidation) in natural aquatic systems (Canfield et al., 2010; Li et al., 2010; Zerkle et al., 2010). Patterns in minor sulfur isotopes (^{33}S in particular) have revealed that very large fractionations between sulfate and sulfide may be produced via sulfate reduction in natural systems, both with and without the additional overlay of oxidative sulfur cycling (Li et al., 2010; Zerkle et al., 2010).

In this paper we studied the sulfur cycle in the stratified water column of seasonally anoxic seawater Lake Rogoznica, Croatia, a unique marine anoxic environment on the Adriatic coast (Fig. 1). Lake Rogoznica provides the opportunity to study sulfur dynamics in a stratified anoxic basin where the deep basin is sulfidic, and discernible rates of sulfur cycling dominate at the chemocline, in an otherwise normal seawater environment. We were especially interested to see how such a close to marine situation compares with other well-studied sulfidic lakes such as Green Lake or Lago di Cadagno. Here we present concentrations of sulfur species, including sulfur intermediates such as thiocyanate, sulfite, thiosulfate, and ZVS in the lake. Moreover, we present data on the distribution of quadruple sulfur isotopes in a stratified sea-water lake, in order to understand the processes which control isotopic fractionation between sulfur species.

2. Materials and methods

2.1. Study site

Lake Rogoznica is a seawater lake located on the Gradina Peninsula (43°32′N, 15°58′E) approximately 100 m from the open Adriatic Sea, close to village Rogoznica on the Dalmatian coast (Fig. 1). The lake has a circular shape with an area of 10,276 m^2, maximum length of 143 m, and a maximum depth of 15 m. It is sheltered from the wind by 4–23 m high cliffs that prevent wind-shear mixing. Lake Rogoznica can be characterized as a typical euxinic environment (Ciglenečki et al., 2006). Very limited water exchange with the open sea through porous karsts leads to a visible push-and-pull water movement associated with Adriatic Sea tidal cycle (Žic and Branica, 2006 and references therein). Compared with the open Adriatic Sea, Lake Rogoznica is eutrophic. Nitrate, phosphate and silicate concentrations are up to 19 μmol L^{-1}, 22 μmol L^{-1}, 400 μmol L^{-1} respectively (Ciglenečki et al., 2005). Lake Rogoznica is thermo-haline stratified from spring to autumn (Ciglenečki et al., 1998, 2005). In years with cold and dry autumn and winter, mixing of the lake water layers takes place (Ciglenečki et al., 1998, 2005).

We can define three geochemically-distinct water layers in the lake. The epilimnion is the upper, oxic water layer. At the chemocline the hydrogen sulfide concentration is below 1 μmol L^{-1} and oxygen concentration is below 5 μmol L^{-1}. In Lake Rogoznica this layer is c.a. 50 cm thick. The chemocline of the lake hosts a dense population of purple phototrophic sulfur bacteria (up to 4.3×10^8 cells mL^{-1} in July 1997) *Chromatium* that comprises up to 51% of total bacteria