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Oxygen kinetic isotope effects in selenate during microbial reduction

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ABSTRACT

The redox cycling of selenium oxyanions, elemental selenium, and selenides within water resources has implications for Se bioavailability and ecotoxicity. Dual stable isotope analysis of Se and O may provide important information for interpreting environmental Se transformations. Stable Se isotope systematics within the Se redox cycle has been well characterized within the literature, however concomitant oxygen isotope composition requires additional investigation. This study reports the O isotope fractionation of selenate (SeO_4^{2-}) during microbial reduction by the dissimilatory Se-reducing bacterium *Sulfurospirillum barnesii* SES-3. Microbial reduction experiments were conducted under various conditions in order to investigate the range of ^{18}O enrichment factors (ε_O) in selenate. The reduction of selenate to selenite coupled to the oxidation of lactate to acetate resulted in an ^{18}O kinetic isotope effect with ε_O values 1.5 −5.8‰. Greater ^{18}O enrichment was observed with increasing pH, but no correlation was found between ε_O and reduction rates, lactate availability, or cell density. ε_O values from biotic reduction by *S. barnesii* here are significantly less than those observed for abiotic reduction with Fe(II)-rich minerals reported in the literature, and this difference could be explained by a diffusion limitation during enzymatic reduction. Our results expand the isotope systematics of the selenium redox cycle and suggest ε_O has potential usefulness as indicators for *in situ* selenate reduction.

1. Introduction

Selenium (Se) within lakes, wetlands, and estuaries poses a concern because Se exposure may lead to Se bioaccumulation and toxic health effects in wildlife (Lemly, 1985, 2002; Ohlendorf et al., 1986; Presser, 1994). Se presence in natural waters may arise from natural sources such as erosion from seleniferous soils or ores, or from anthropogenic inputs such as deposition from combustion or waste disposal from petroleum processing (Lemly, 2004; Mast et al., 2014; Tuttle et al., 2014). Consequently, considerable effort has been made to understand Se source identification, its transport through environmental compartments, and the biogeochemical processes that control its fate (Seiler et al., 1999). As part of these investigations, stable isotope analysis has been a particularly useful tool to interpret Se behavior in nature by providing a means to measure source-dependent isotopic signatures and to observe reaction-induced isotopic changes (Schilling et al., 2011a; Clark and Johnson, 2010).

Interpretations of Se isotope measurements in nature have been supported by laboratory experiments that evaluate stable Se isotope systematics within the Se biogeochemical cycle (Johnson, 2004). Se may cycle among four redox states across the aqueous, solid, and gaseous phases. The dissolved oxyanions selenate (Se(VI), SeO_4^{2-}), selenite (Se(IV), SeO_3^{2-}), and biselenite (Se(IV), $HSeO_3^{2-}$) are mobile and more bioavailable, whereas elemental Se (Se(0)) and mineral and organic selenides (Se(−II)) may remain fixed among soils and sediments, and biomethylated selenides may become volatilized. Fractionation of Se isotopes on the order of ~3–12‰ have been shown to occur during microbial and mineralogical reduction of selenate to selenite and from selenite to elemental Se (Johnson, 2004). This is caused by the preference for lighter Se isotopes during the Se–O bond breaking and results in the products becoming isotopically lighter and the reactants becoming enriched with heavier Se isotopes. Smaller but significant enrichments (~−3–−6‰, i.e. enriched in lighter isotopes) in methylselenide have been observed during biomethylation of selenate and selenite by fungi and soil, and fractionation extents

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were indicative of the Se oxyanion source (Schilling et al., 2011b, 2013). Oxidation reactions of Se(0) to Se(IV) or Se(IV) to Se(VI) so far reveal little or no fractionation in Se isotopes (Johnson, 2004).

The present study and our previous work (Schellenger and Larese-Casanova, 2013) contribute to Se oxyanion isotope systematics by expanding observations to include fractionations imprinted upon stable oxygen isotopes during redox reactions. The use of stable oxygen isotopes can be combined with Se isotope measurements for a dual isotopic analysis approach that may strengthen lines of evidence for Se oxyanion transformation or source identification. As is typical for light element fractionation, $^{18}O/^{16}O$ isotope ratios may provide a more sensitive measurement and therefore a larger fractionation compared to the heavier $^{82}Se/^{76}Se$ ratios owing to the greater percent mass difference (~13% and ~7%, respectively). Like Se, a kinetic isotope effect (KIE) for O can be induced when the Se–O bond breaking reactions involving the Se oxyanions preferentially consume the oxyanions containing the lighter isotope (for instance O^{16} rather than O^{18}), enriching the remaining reactant pool with the heavier isotope (O^{18}) similar to other oxyanions such as phosphate (Blake et al., 2005), nitrate (Kendall, 1998), and sulfate (Turchyn et al., 2010). This enrichment may be measured as long as the isotopes are preserved within the oxyanion and enriched selenate mixes back with the reservoir of bulk dissolved selenate. Although the oxygen isotopes of selenite readily equilibrate with water molecules and thus erase any enrichment for lighter isotopes (Okumura and Okazaki, 1973a), selenate oxygen does not exchange isotopes within the pH range of natural waters (6–9), and may therefore be preserved within the water column or in transport (Okumura and Okazaki, 1973b; Kaneko and Poulson, 2012). The KIE associated with the oxygen isotope during mineralogical selenate reduction with the Fe(II) layered double hydroxide green rust has been identified (Schellenger and Larese-Casanova, 2013), and previous work has explored the KIE for the Se isotope during both mineralogical and microbial reduction of selenate (Clark and Johnson, 2010; Johnson and Bullen, 2003; Ellis et al., 2003; Herbel et al., 2000).

The objective of this work, therefore, is to examine how the microbial reduction process influences the stable oxygen isotope values of selenate oxyanions. Bacteria capable of coupling Se oxyanion reduction to organic matter oxidation have been observed within diverse soil, sediment, fresh water, and saline water environments. (Switzer Blum et al., 1998; Switzer Blum et al., 2001; Fan et al., 1998; Macy et al., 1993; Oremland et al., 1989; Stolz et al., 1999). Isolates have been identified across various genera, with notable species including *Thauera selenatis*, *Bacillus selenitireducens*, *Pseudomonas stutzeri*, and *Sulfirospirillum barnesii* SES-3, which are typically capable of dissimilatory reduction or cometabolism of several inorganic oxyanions as electron acceptors (Switzer Blum et al., 1998; Stolz et al., 1999; DeMoll-Decker and Macy, 1993; Lortie et al., 1992; Oremland et al., 1994; Macy et al., 1989; Macy et al., 1989; Maiers et al., 1988). The prevalence and flexibility of Se-respiring microorganisms in nature consequently allows for their harnessing for bioremediation of Se-contaminated sites under a wide range of natural conditions (Frankenberger and Arshad, 2001), during which the processes of microbial Se oxyanion reduction to insoluble elemental Se or to volatile organic selenides plays an important role in lessening Se bioavailability. Mineralogical reduction to Se(0) by Fe(II) (Myneni et al., 1997), might occur only within Fe-rich, reducing subsurface environments (Christiansen et al., 2009), whereas microbial reduction may be more widespread. Stable isotope analysis of selenate during microbial reduction may assist in characterizing the prevailing modes of Se redox cycling in Se-impacted environments.

The goals of this work are (i) to identify whether microbial reduction induces a kinetic isotope effect for oxygen in selenate and produces a quantifiable enrichment factor (ε_0) for the reaction, and (ii) to determine the range of observed ε_0 values as a function of microbial suspension conditions and determine if these ε_0 values differ from those of chemical reduction processes. Enzymatic reduction by selenate-respiring bacteria could be described to involve a selenate diffusion step to enzyme sites followed by electron transfer and reduction to selenite. The relative rates of selenate diffusion to reactive sites and reduction to selenite may influence the extent of O isotope fractionation, with any mass transfer limitation in microbial systems possibly leading to lower observable enrichments (Herbel et al., 2000), compared to chemical reduction by chloride green rust with no diffusion limitation (Schellenger and Larese-Casanova, 2013). Such differences in element enrichments have been observed for chemical and microbial reduction of organic pollutants (e.g., (Liang et al., 2007)). If the observed difference is large enough oxygen isotopes may have a role to play in reaction differentiation in subsurface systems. This question has implications to whether both Se and O enrichment values can be used simultaneously in a dual KIE approach as indicators of subsurface selenate reduction and possibly as a distinct identifier of biological reduction in particular.

The anaerobic freshwater bacterium *Sulfurospirillum barnesii* SES-3 was chosen as a representative selenate-respiring microorganism for observing kinetic isotope effect in selenate oxygen. *S. barnesii* is an environmental opportunist, capable of using a number of electron acceptors to facilitate respiration including arsenate, thiosulfate, nitrate, and selenate, and thrives under neutral to slightly alkaline pH (Laverman et al., 1995), which are conditions similar to many surface or subsurface waters with elevated Se (Seiler et al., 1999). Like many Se reducing microbes, *S. barnesii* utilizes membrane bound enzymes to couple the reduction of selenate to selenite and Se(0) with the oxidation of organic carbon sources such as lactate (Oremland et al., 1994, 1999). Finally, this strain has been shown previously to produce a measurable KIE in the Se isotopes when conducting this reduction at circumneutral pH (Herbel et al., 2000). Because microbial isotope enrichment may be affected by reduction rate (Harrison and Thode, 1958) and because microbial reduction rates may be affected by alterations in environmental conditions, we surveyed the ^{18}O-KIE in cell suspensions under a range of geochemical conditions. Differences in pH, cell density, and electron donor/acceptor ratios were evaluated for their effect on ^{18}O enrichment during reduction.

2. Experimental methods

2.1. *Sulfurospirillum barnesii SES-3 cultivation*

Anoxic conditions were maintained for all cell growth and cell suspension experiments within a COY vinyl chamber with a 1% H_2 and 99% N_2 atmosphere. All solutions were prepared with deionized water (>18 MΩ) deoxygenated by boiling in an autoclave (121 °C) and immediately placed inside the anoxic chamber.

Sulfurospirillum barnesii SES-3 cells were purchased freeze-dried from the American Type Culture Collection (ATCC, Cat. No. 700032) and stored at −80 °C. A portion of these purchased cells were thawed and grown on the DSMZ-recommended growth medium (Medium 771 containing lactate as a carbon source and nitrate as a terminal electron acceptor, sterilized by autoclaving or sterile filtering) within autoclaved glass serum bottles under anoxic conditions at 30 °C. To prepare starting cultures for experiments, the suspension was preserved by adding autoclaved glycerol (to a concentration of 15%) and dividing into autoclaved 2-mL crimp-sealed vials for storage at −80 °C.

To identify the time intervals for growth phases under operating conditions here, a vial of preserved *S. barnesii* cells were grown over