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Stable isotope fractionation of selenium by natural microbial consortia

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Abstract

The mobility and bioavailability of Se depend on its redox state, and reduction of Se oxyanions to less mobile, reduced species controls transport of this potentially toxic element in the environment. Stable isotope fractionation of Se is currently being developed as an indicator of Se immobilization through reduction. In this study, Se isotope fractionation resulting from reduction of Se(VI) and Se(IV) oxyanions by natural microbial consortia was measured in sediment slurry experiments under nearly natural conditions, with no substrate added. Experiments were conducted with a wide range of initial Se concentrations and with sediment and water from three locations with contrasting environmental settings. The products of Se(VI) and Se(IV) reduction were enriched in the lighter isotopes relative to the reactants. Shifts of −2.6‰ to −3.1‰ and −5.5‰ to −5.7‰, respectively, were observed in the $^{80}Se/^{76}Se$ ratio. These isotopic fractionations did not depend significantly on initial Se concentrations, which were varied from 22 μg/l to 8 mg/l, or on geochemical differences among the sediments. These results provide estimates of Se isotope fractionation in organic-rich wetland environments but may not be appropriate for substrate-poor aquifers and marine sediments.

Keywords: Selenium; Stable isotopes; Water contamination; Bacteria

1. Introduction

Selenium poses a challenge to scientists seeking to unravel its complex biogeochemistry. Accurate characterization of Se-contaminated sites and design of remediation schemes depend on knowledge of the speciation and biogeochemical transformations of Se. Since irrigation-induced Se contamination was determined as the primary cause for waterfowl deformities seen in Kesterson, CA (Ohlendorf and Santolo, 1994; Presser, 1994), the toxic effects of Se on migrating waterfowl in Se-contaminated natural wetlands have been a concern (Seiler, 1998). Se-stable isotope ratios have the potential to provide valuable insights into the biogeochemical transformations that occur among Se species.

Selenium exists in four valence states in nature: VI, IV, 0 and −II (Elrashidi et al., 1987). In oxidized natural waters, it is found in the form of oxyanions,

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with Se(VI) occurring as selenate and Se(IV) occurring as selenite or biselenite. Dissolved organic Se compounds are commonly observed as well (Elrashidi et al., 1987; Cutter and Cutter, 1995). Se(IV) tends to adsorb strongly when pH is less than 8 (Bar-Yosef and Meek, 1987). Se(0), or elemental Se, is insoluble and commonly found in sediments, as are a variety of Se(−II)-bearing organic compounds (Tokunaga et al., 1991; Zhang and Moore, 1996). As Se(IV) adsorbs strongly and Se(0) is insoluble, Se mobility in natural environments is dependent on its oxidation state, which elevates the importance of understanding Se redox chemistry and transformations (Elrashidi et al., 1987; McNeal and Balistrieri, 1989; Tokunaga et al., 1994). Abiotic Se reduction is slow under natural conditions and is, thus, generally mediated by bacteria. This has been demonstrated in laboratory experiments wherein Se uptake by bacteria in pure cultures and sediment slurries was monitored (Oremland, 1994; Herbel et al., 2000).

Se isotopes are useful as indicators of redox reactions and biogeochemical processes that control Se distribution and speciation. Alternatively, Se isotope ratios may be used to distinguish different sources of Se in the environment, but possible modification of original isotope signatures by biogeochemical reactions must be understood. As for sulfur-stable isotopes (Kaplan and Rittenberg, 1964; Strebel et al., 1990; Bruchert et al., 2001; Canfield, 2001; Detmers et al., 2001; Habicht and Canfield, 2001), Se-stable isotopes are sensitive to reduction reactions. Se has six stable isotopes: ^{74}Se, ^{76}Se, ^{77}Se, ^{78}Se, ^{80}Se and ^{82}Se (Wachsmann and Heumann, 1992). All isotope values reported here are expressed as ^{80}Se/^{76}Se ratios relative to a standard as described below. A recent study of Se oxyanion reduction by pure cultures of bacteria (Herbel et al., 2000) showed that the reduced products are enriched in the lighter isotopes by 1.1–9.1 ‰, and unreduced reactant Se in solution becomes enriched in the heavier isotopes as reduction proceeds. In a field setting, such isotopic fractionation could be used to indicate reduction. For example, enrichment of ^{80}Se relative to ^{76}Se in Se(VI) along a flow path would indicate that reduction is actively occurring.

The present study was designed to bridge the gap between recent experiments and our understanding of natural settings. The experiments by Herbel et al. (2000) were conducted with abundant electron donor (e.g., lactate) and initial Se concentrations higher than those of most contaminated sites. Natural settings are more complex than these experiments, and it is likely that isotopic fractionation varies with the microbial environment. Sulfur isotope studies have shown that fractionation during bacterial sulfate reduction depends on the specific bacterial community and the specific type and availability of substrate used by that community, along with variability linked to specific metabolic pathways (Kaplan and Rittenberg, 1964; Bruchert et al., 2001; Detmers et al., 2001). Ideally, we would measure isotope fractionations in situ, but achieving mass balance from field measurements is difficult. The sediment slurry experiments of the present study provide us with a close alternative by approximating natural conditions and natural microbial populations with no electron donor added. We report here determinations of Se isotope fractionation during Se(VI) and Se(IV) reduction by natural microbial consortia from three different sediments.

2. Methods and materials

2.1. Sediments

We conducted experiments using three sediment types from differing environments. The first sediment was collected in June 1999 from an intertidal mud flat in the northern reach of the San Francisco Estuary at Martinez Regional Park, just north of Martinez, CA. The upper 2 cm of sediment was discarded, and the underlying sediments were sampled to a depth of approximately 15 cm. The material was brown and sandy (approximately 70% slit and sand) with clumps of organic matter, and an average total organic carbon (TOC) of 0.92%. Sediments in this area had an E_h of approximately 100 mV and were not strongly reducing because of tidal cycling (Zawislanski and McGrath, 1998). The brackish water of the estuary at this location was approximately one-half seawater. Overlying water samples for the experiments were collected at the time of sediment sampling. Because Se concentrations in the water samples were less than 1 μg/l, it was necessary to augment the natural Se levels for both Se(IV) and Se(VI) experiments.

Sediment and water samples were also collected from the Se-contaminated San Luis Drain (SLD), a

drainage ditch south of Dos Palos, CA, in September 1999. The top 10 cm of sediment was collected from the side of the drain. The sediment was dark and clayey with an average TOC of 0.94% and was covered with water for several months prior to sampling. The saline SLD water is derived from agricultural subsurface drains, and sulfate concentrations vary between 1300 and 3000 mg/l (Robert TerBerg, personal communication).

The third sediment samples were collected in January 2000 from a wetland operated by the Tulare Lake Drainage District (TLDD) and constructed in 1996 at Corcoran, CA, to determine if wetland systems remove Se from agricultural drainage water where Se(VI) predominates (Gao et al., 2000). The sediment collected was from Cell 1, one of 10 cells with different plant species in the cells. Cell 1 was vegetated with deep-rooted saltmarsh bulrush (*Scirpus maritimus*), and average TOC for this sediment was 0.90%. Redox potentials were between 0 and −100 mV (Gao et al., 2000). Under these conditions, elemental Se is the stable form (Elrashidi et al., 1987). The water is saline, with sulfate concentrations varying between 2000 and 3000 mg/l.

2.2. Experimental methods

We conducted experiments using two concentrations: approximately 8 mg/l (0.1 mM) and less than or equal to 36 μg/l. The 8 mg/l experiments were conducted using sediments from San Francisco Estuary. Se was added to the natural waters for most experiments, but in two cases, no Se was added (Table 1). The experimental design was similar to that of Oremland et al. (1989), except that no electron donor was added. Sediment slurries were prepared by mixing sediment and water collected at the various sites in a 1:10 ratio for the high-Se concentration experiments and in a 1:20 ratio for the remainder. The slurries were transferred into 20–1200-ml serum bottles (depending on the total volume of slurry used), crimp-sealed and purged with oxygen-free nitrogen to create an anaerobic head-space. The bottles were placed on an orbital shaker (125 oscillations/min) at room temperature (20–24 °C). This temperature range was within the range of ambient field temperatures for the SLD and TLDD sediments but was 5–8 °C above water temperatures measured for the sediments from San Francisco Estuary. Fluid samples from the bottle incubations were collected at set time intervals via syringes and filtered through 0.45-μm pore-size syringe filters. The filtrate was acidified with HCl to pH ≤ 2 and stored at 4 °C to maintain the Se speciation present at the time of sampling.

For the experiments with added Se(IV), it was necessary to recover both solution and sediment at several points in time, as Se(IV) adsorbs strongly onto the sediment. A single bottle could not be sampled several times in series because representative sediment samples of the slurries could not be collected without compromising the bottle seals. Thus, multiple experiments were conducted in parallel; several bottles were prepared and opened at different intervals along the time series. As discussed below, the conditions were not perfectly identical in the various bottles used for the given experiment, and this apparently led to minor

Table 1
Compiled table of experimental details and corresponding ε values

Sediment (water)	Initial Se concentrations	Se oxyanion reduced	ε (‰)
High Se concentration			
San Francisco Estuary intertidal	8.4 mg/l (added)	VI	−2.8 ± 0.3
sediment (brackish water)	8 mg/l (added)	IV	−5.6 ± 0.5
Low/natural Se concentration			
San Francisco Estuary intertidal	22 μg/l (added)	VI	−2.6 ± 0.5
sediment (brackish water)	19 μg/l (added)	IV	−5.5 ± 0.5
San Luis Drain (agricultural wastewater)	34 μg/l (natural concentration)	VI	−2.7 ± 0.3
Tulare Lake Drainage District	18.4 μg/l (natural concentration)	VI	−3.1 ± 0.3
(saline agricultural wastewater)	36 μg/l (added)	IV	−5.7 ± 0.5

differences in calculated adsorption or reduction rates. The dissolved Se(IV) fraction was recovered by centrifuging, and the adsorbed Se(IV) fraction was recovered via a phosphate extraction procedure (Martens and Suarez, 1997). Twenty milliliters of a phosphate buffer solution at pH 7 was added to the sediment, and the mixture was shaken at room temperature for 1–2 h at 125 oscillations/min. The buffer solution containing the desorbed Se(IV) was separated by centrifugation. This extraction was tested on sediments with known amounts of adsorbed Se(IV); recoveries were between 85% and 100%, and fluid volumes left in pore spaces of the pellets at the bottoms of the centrifuge tubes were insignificant. For the Se(IV) experiments with SLD and TLDD sediments, Se(VI) in the slurry was removed via natural reduction before the experiments were started. The slurries were prepared and the bottles were allowed to stand until all the natural Se in solution was precipitated and removed by microbial reduction. Se(IV) was then added to bring the concentration to approximately 35–40 μg/l.

Autoclaved experiments were also conducted to determine if the reduction was microbial or abiotic. Slurries of all three sediments were prepared in the same manner as for the experiments outlined above, except that they were autoclaved at 121 °C for 20 min. Dissolved Se concentrations were monitored for several days as before.

2.3. Sample preparation and analyses

Se concentrations were analyzed using hydride-generation atomic absorption spectrometry, which detects only Se(IV). Se(IV) concentrations were obtained by analyzing fluid subsamples directly with no preparation. Interference by dissolved organic compounds was monitored and, when necessary, corrected for via the standard additions method. Concentrations of Se(VI) + Se(IV) were obtained by analyzing fluid subsamples digested with 6 M HCl for 45 min at 90 °C to convert Se(VI) to Se(IV) (Martens and Suarez, 1997). Se(VI) concentration was calculated by difference. In the Se(VI) reduction experiments, Se(IV) concentrations were usually much smaller than Se(VI) concentrations and, thus, this scheme does not introduce large uncertainties. To remove interference in solutions with abundant dissolved organic compounds, 0.9–2 ml of 2% $K_2S_2O_8$ was added prior to an equal volume of sample prior to HCl digestions. Results for standard additions and duplicates indicate that our analyses are precise to ± 10% (2σ), except for the 8.4 mg/l Se(VI) experiment, where uncertainty was ± 5% (2σ).

Se isotope ratios were measured using the double-spike NTIMS technique of Johnson et al. (1999). Se has six stable isotopes; we measure the $^{80}Se/^{76}Se$ ratio. Briefly, a double isotope spike containing ^{74}Se and ^{82}Se in a known ratio, and in the same form as the target Se species, was added to each sample. This spike was later used to correct for instrumental discrimination and any isotopic fractionation resulting from Se purification. Se(VI) and Se(IV) were separated by anion exchange if both Se(VI) and Se(IV) were needed for analysis. A subsample was loaded onto a 1.3-ml pore volume AG1-X8 resin column. Se(IV) was eluted with 10 ml of 0.1 M HCl, and Se(VI) was then eluted with 10 ml of 6 M HCl. If only Se(VI) was required for analysis, Se(IV), if present, was removed via coprecipitation with ferric hydroxide (Chau and Riley, 1965).

In previous efforts, purification was done using a batch reactor (Tanzer and Heumann, 1991; Johnson et al., 1999). Recently, however, we have developed a continuous-flow hydride generation system to facilitate purification of large-volume samples, reduce cleaning time between samples and reduce the effect of Fe(III) and other interferences (Brindle et al., 1992). If Se(VI) is to be purified, it is first converted to Se(IV). The sample, in a 3–6 M HCl matrix, is pumped at 8 ml/min and mixed with a 1% $NaBH_4$ solution pumped at 1 ml/min (Fig. 1). H_2Se is stripped out of the solution by an N_2 carrier gas in a liquid–gas separator modified after Brindle et al. (1992) (Fig. 1). The H_2Se is passed to a fluoropolymer tube containing concentrated nitric acid, where it is absorbed into solution via oxidation to Se(IV). The nitric acid is then evaporated to dryness. The overall recovery of Se from this process is about 85%, with the major loss occurring through incomplete reaction and flow of unreacted Se out of the liquid–gas separator. Dissolved organic substances that strongly interfere with mass spectrometry (e.g., ionization) can be transferred from sample solutions to the oxidation trap. The organic compounds are removed by oxidation with several additions of 100 μl concentrated HNO_3 and 50 μl 30% H_2O_2 to the dried samples, each followed by evaporation to dryness.

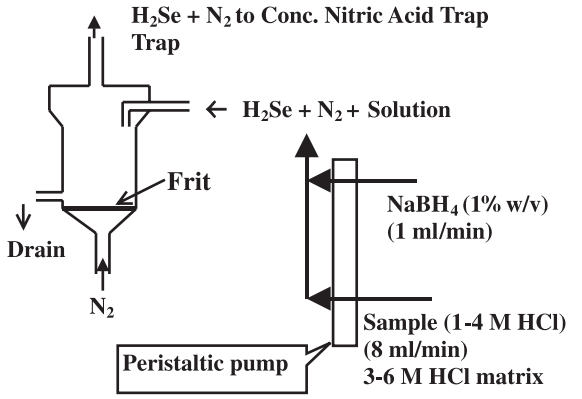

Fig. 1. Schematic diagram of the new Continuous Flow Hydride Generation System developed for purification of Se before TIMS analysis. The frit is a fine-glass frit (4–5.5 μm).

Mass spectrometry was carried out as previously described (Johnson et al., 1999). Briefly, 1 μl of a saturated $Ba(OH)_2$ solution is dried on rhenium filaments. Se samples of 0.3–0.7 μg and 0.2 μg colloidal graphite were suspended in water and loaded on top of the $Ba(OH)_2$ and dried. Three ratios, $^{82}Se/^{80}Se$, $^{76}Se/^{80}Se$ and $^{74}Se/^{76}Se$, were then measured on a Finnigan MAT 261 TIMS configured for negative ions. $\delta^{80/76}Se$ values were calculated using an iterative double-spike data reduction routine (Johnson et al., 1999), where

$$\delta^{80/76}\mathrm{Se}(‰) = \frac{(^{80}\mathrm{Se}/^{76}\mathrm{Se})_{\mathrm{sam}} - (^{80}\mathrm{Se}/^{76}\mathrm{Se})_{\mathrm{std}}}{(^{80}\mathrm{Se}/^{76}\mathrm{Se})_{\mathrm{std}}} 1000. \quad (1)$$

These $\delta^{80/76}Se$ values have a precision of $\pm 0.2‰$.

2.4. Calculation of ε, the instantaneous isotopic fractionation

The instantaneous fractionation, ε, is the difference between the $\delta^{80/76}Se$ value of the reactant pool and the $\delta^{80/76}Se$ of the reduced product formed at an instant in time. Values for ε can be calculated by directly measuring the $\delta^{80/76}Se$ of the first-formed reduced product. However, recovering Se(0) produced in the Se(IV) reduction experiments was not feasible because the sediments already contained Se(0), and the Se(IV) produced by Se(VI) reduction was greatly affected by simultaneous sorption and reduction to Se(0). We, thus, chose to calculate the ε values from the observed $\delta^{80/76}Se$ increases in the reactant pools. For Se(VI) reduction experiments, sorption of Se(VI) was negligible, and ε for Se(VI) reduction to Se(IV) was calculated using a Rayleigh fractionation model.

$$\varepsilon = \frac{\mathrm{d}(\delta^{80/76}\mathrm{Se})}{\mathrm{d}(\ln(f))}, \quad (2)$$

where f is the fraction of unreduced Se(VI) remaining (Herbel et al., 2000).

A more complex model was needed to extract ε from the Se(IV) reduction data because of sorption. The adsorbed and dissolved Se did not behave as a single pool. We created a numerical model to simulate simultaneous reduction of dissolved Se(IV) and exchange between dissolved and adsorbed Se(IV) fractions. The exchange component includes a mass flux onto the solid and a flux from the solid into the solution. The numerical model was constructed based on the following differential equations and was used to calculate the isotopic evolution of the dissolved and adsorbed fractions.

The $\delta^{80/76}Se$ of the dissolved fraction is calculated from

$$\frac{\mathrm{d}\delta_s}{\mathrm{d}t} = \frac{1}{m_s}R_r(\delta_r - \delta_s) + \frac{1}{m_s}R_a(\delta_a - \delta_s) + \frac{1}{m_s}R_d(\delta_d - \delta_s), \quad (3)$$

where δ_s and m_s are the $\delta^{80/76}Se$ and mass of the dissolved fraction, respectively; R_r, R_a and R_d are the rates of Se reduction, adsorption and desorption (mass per unit time), respectively; and δ_r, δ_a and δ_d are the $\delta^{80/76}Se$ values of the Se transferred by reduction, adsorption and desorption, respectively. This equation can be understood intuitively as follows. The rate of change in $\delta^{80/76}Se$ for a modeled dissolved Se fraction equals the rate of Se transfer, relative to the mass present, multiplied by the difference in the $\delta^{80/76}Se$ values of the transferred Se and the dissolved fraction pool. Two simplifications can be made to Eq. (3): (1) $\delta_r - \delta_s = \varepsilon$ and (2) as sorption itself does not fractionate the isotopes (Johnson et al., 1999), $\delta_a = \delta_s$, and the second term is zero.

The $\delta^{80/76}$Se of the adsorbed fraction is obtained using

$$\frac{d\delta_{sorb}}{dt} = \frac{1}{m_{sorb}} R_a(\delta_a - \delta_{sorb}) + \frac{1}{m_{sorb}} R_d(\delta_d - \delta_{sorb}), \quad (4)$$

where δ_{sorb} and m_{sorb} are the $\delta^{80/76}$Se and the mass of the adsorbed fraction, respectively; R_a and R_d are the rates of Se adsorption and desorption, respectively; and δ_a and δ_d are the $\delta^{80/76}$Se values of the adsorbing and desorbing Se fluxes, respectively. We introduce an approximation by assuming $\delta_d = \delta_{sorb}$ and, thus, the second term becomes zero.

The concentrations of the dissolved and adsorbed fractions were approximated using first order reaction rates. Then, for each time step, the $\delta^{80/76}$Se of each fraction was calculated using finite difference approximations of Eqs. (3) and (4). The adsorption rate constant was chosen so that the model reproduced the rate of adsorption observed in the first few hours of the experiment. The desorption rate constant was chosen so that the ratio of adsorbed Se to dissolved Se in the model approximated that observed late in the experiment. The reader will note that in this formulation, the equilibrium distribution coefficient for sorption is the adsorption rate constant divided by the desorption rate constant. $\delta^{80/76}$Se values for the dissolved and adsorbed fractions were plotted as a function of the total Se remaining unreduced, and the ε value was determined by fitting the data.

3. Results

In the Se(VI) reduction experiments, Se(VI) concentrations decreased while $\delta^{80/76}$Se values increased in the Se(VI) remaining in solution (Fig. 2). An initial lag phase varying from 2 to 4 days was observed, during which there was no decrease in Se(VI) concentrations. In experiments with pure cultures, a similar lag phase occurs before the growth phase where bacterial cells increase exponentially. No decrease in Se(VI) was seen in the autoclaved controls (Fig. 2); this confirms that reduction was biologically mediated. In Fig. 3, the $\delta^{80/76}$Se values for one representative experiment are plotted vs. ln(f), where f is the fraction of unreduced Se(VI) remaining. The slope of this plot

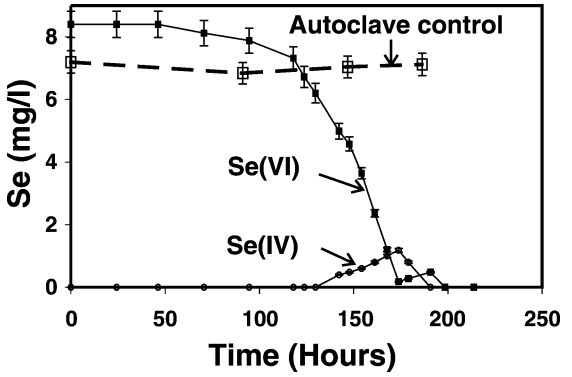

Fig. 2. The 8 mg/l Se(VI) experiments. Plot of Se remaining in solution vs. time. The apparent appearance of Se(VI) after reduction is complete may be other species of Se.

yields ε, the instantaneous difference in $\delta^{80/76}$Se values between the reduced Se formed at one moment in time and the pool from which it was reduced (Eq. (1)). Values for ε appear to be constant over time, and the slope of the best fit line gives an ε of -2.8 ± 0.3 ‰. ε values from the other experiments of the present study, generally with fewer data but calculated using the same slope method, are given in Table 1.

Concentrations of Se(IV) formed via Se(VI) reduction increased to a maximum value well below that of the initial Se(VI) concentration, then decreased as Se(IV) was reduced to elemental Se. The $\delta^{80/76}$Se values for Se(VI) increased during the course of the reaction. Most of the Se(IV) formed during reduction of Se(VI) adsorbs onto the sediment. Because sorption, Se(VI) reduction and Se(IV) reduction all affected the Se(IV) concentration and isotopic values simultaneously, it was impossible to establish an adequate mass balance and extract precise ε values for Se(IV) reduction in the Se(VI) reduction experiments. ε values for Se(IV) were obtained only from experiments with Se(IV) added.

The Se(IV) experiments showed little or no time lag before reduction began, in contrast to the Se(VI) reduction experiments. As no reduction was observed in the autoclaved controls, we conclude that reduction was microbially mediated but not subject to the same lag phase. Complete reduction occurred within 6 days. Fig. 4 shows the fraction of the added Se(IV) remaining vs. time for the various experiments conducted in parallel. Concentrations of both dissolved and ad-

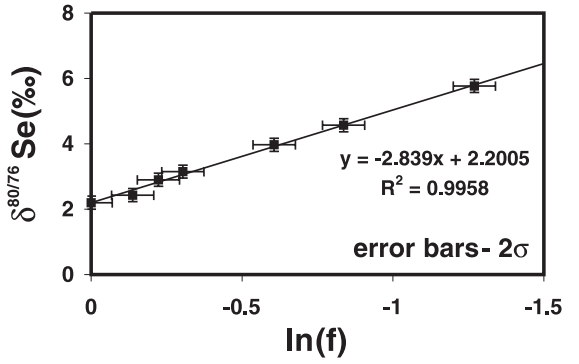

Fig. 3. The 8 mg/l Se(VI) experiments. Plot of $\delta^{80/76}$Se of unreduced Se(VI) remaining in solution vs. ln(f), where f = fraction of Se remaining in solution. The slope gives an ε of 2.8 ± 0.3 ‰.

sorbed Se(IV) decreased as the reaction progressed. Assuming that the adsorbed fraction is not bioavailable, the decrease in adsorbed Se(IV) concentration reflects net desorption in response to decreasing dissolved Se(IV) concentrations. Significant scatter in this plot arose from minor variations in reduction rates and sorption between bottles.

$\delta^{80/76}$Se data are plotted in Fig. 5; both dissolved and adsorbed fractions showed increasing $\delta^{80/76}$Se values as reduction progressed. However, the $\delta^{80/76}$Se value of the adsorbed fraction was lower than that of the dissolved fraction. This isotopic disequilibrium indicates that exchange between the solution and the sorption sites was not very rapid, and that the isotopic evolution of the adsorbed Se pool lagged behind that of the dissolved fraction. Initially, $\delta^{80/76}$Se values increased rapidly in the dissolved fraction, but as the difference between the dissolved and adsorbed $\delta^{80/76}$Se values increased, desorption began to exert a lowering effect on the solution's $\delta^{80/76}$Se value. The rate of change of the dissolved $\delta^{80/76}$Se value, thus, decreased, which is reflected as a decrease

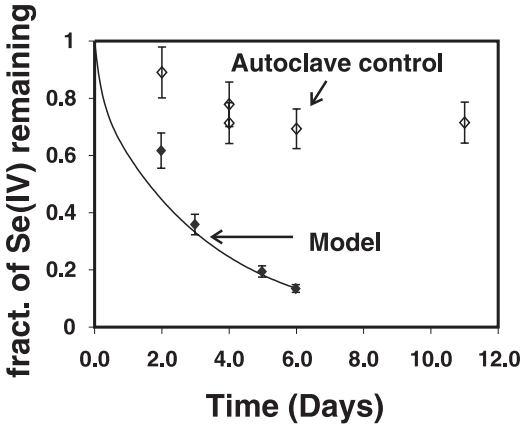

Fig. 4. High-concentration Se(IV) experiments. Fraction of total Se(IV) unreduced vs. time. Each point represents a different bottle. The continuous line is the fraction of Se remaining vs. time modeled as first order reactions for reduction and sorption.

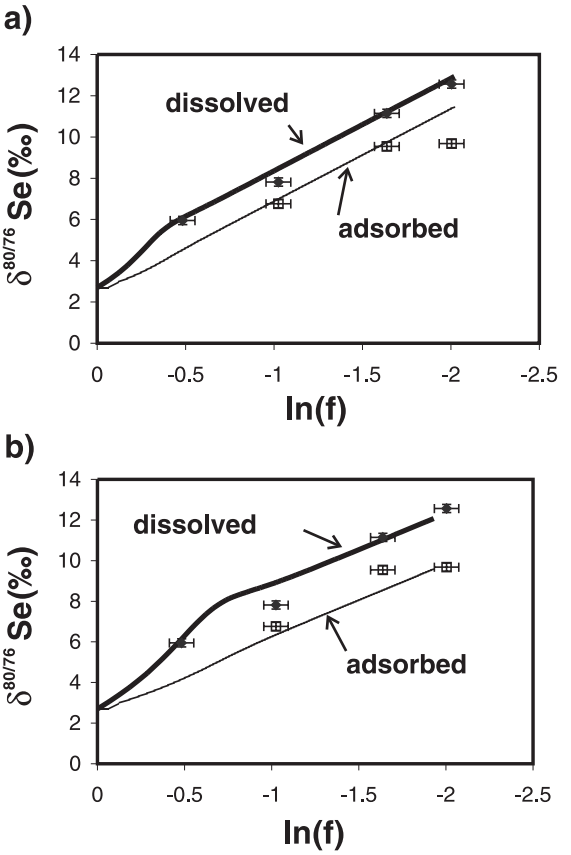

Fig. 5. High-concentration Se(IV) experiments. Plots of $\delta^{80/76}$Se of solution (filled squares) and adsorbed Se(IV) remaining (open squares) vs. ln(f), where f = fraction of total unreduced Se(IV). The thick line is fitted to the solution fraction while the thin line is fitted to the adsorbed fraction. The scatter of points is caused by differences between the slurries of individual bottles, resulting in different reduction and adsorption rates. An ε of 5.6 ‰ was obtained fitting the data with a reduction rate of 0.5/h. (a) An adsorption rate of 0.1/h was used. (b) An adsorption rate of 0.05/h would fit the last adsorbed data-point without affecting ε.

in the slope of the plot of $\ln(f)$ vs. $\delta^{80/76}$Se at $\ln(f) = -0.5$ (Fig. 5). Accordingly, this slope is not equal to ε. In order to determine the true value of ε, we developed the model described above. The model was fit to the data (Fig. 5), and the ε value obtained is -5.6 ± 0.5 ‰. Useful data were not obtained from SLD Se(IV) reduction experiments because sorption and interaction between Se(IV) and organic species were both very strong, resulting in large uncertainties in concentration analyses.

4. Discussion

4.1. Factors controlling Se isotope fractionation

Reduction of Se by natural sediment slurries has been studied previously (Oremland et al., 1989; Zhang and Moore, 1997). However, the present study is the first to measure Se isotope fractionations using such media. Two pure-culture studies have measured Se isotope fractionation by microbes (Rashid et al., 1978; Herbel et al., 2000). The recent study by Herbel et al. (2000) obtained Se isotope fractionations in pure cultures of bacteria isolated from natural environments. The ε values from these cultures ranged from 1.1 ‰ to 5.0 ‰ for Se(VI) to Se(IV) transformations and from 6.0 ‰ to 9.1 ‰ for Se(IV) to Se(0) reactions and depended on the experimental conditions. These results are important in providing an initial understanding of microbial fractionation, but extending them to field settings is difficult. Although isolated from natural settings, the bacteria used in the Herbel et al. (2000) study were pure cultures and, thus, not natural populations. Se concentrations used were between 10 and 20 mM, which, though possible, are not common in Se-contaminated areas. In addition, electron donors in the culture media are not necessarily similar to those found in sediments.

By contrast, the present study used natural microbial populations within the original host sediment, though some selection of species might have occurred during sediment handling. Initial concentrations ranged between 0.004 and 0.1 mM Se(VI) or Se(IV); this range is reasonably close to the range of concentrations seen in field settings. Also, no external electron donor was added. The type of organic substrate available is known to affect isotope fractionation during sulfate reduction (Detmers et al., 2001) and may have similar effects during Se reduction. For example, sulfate reduction experiments with hydrogen as electron donor produced fractionations that were significantly lower than those with butyrate.

In general, isotopic fractionation by microbes is expected to vary according to environmental conditions and biochemical pathways. Microbial sulfate reduction experiments have yielded isotope fractionations that varied between -2 ‰ and -46 ‰ (Kaplan and Rittenberg, 1964; Habicht and Canfield, 1997; Bruchert et al., 2001; Detmers et al., 2001). Because these microbial processes consist of multiple steps, each with a particular isotopic discrimination, the isotopic fractionation for the overall process is variable. Sulfate reduction has been simplistically modeled as a process with two major steps (Rees and Thode, 1966; Canfield, 2001). The first step is the uptake of S into the cell, with a small isotopic fractionation. The second is reduction of the S oxyanions, involving breaking of the S–O bond and greater fractionations. Either step can be rate-limiting, and the rate-limiting step controls the isotopic fractionation. Some studies on this topic concluded that the substrate availability and, hence, overall rate of reduction per bacterial cell, controls the extent of fractionation (Kaplan and Rittenberg, 1964; Habicht and Canfield, 1997, 2001; Canfield, 2001). Under conditions of abundant substrate availability or optimal substrate utilization, the overall reduction rate is somewhat limited by sulfate uptake into the cell and lower overall fractionations are expected. However, other studies by Detmers et al. (2001) and Bruchert et al. (2001) found no direct relationship between reaction rates and isotope fractionation and attributed the variability in isotope fractionation values to physiological differences among bacteria, the type of substrate utilized by the bacterial community, and variations in metabolic pathways. Extending these results to selenium reduction, we expect isotopic fractionation to vary according to availability and type of substrate and the bacterial species present.

Although S and Se reducers are physiologically different (Oremland et al., 1989), and the magnitude of Se isotope fractionation may not be related to physiological differences among Se reducers, it is somewhat surprising that the results from our three sediment slurries were similar. It is certainly possible

that this is fortuitous, but we tentatively suggest that the obtained values can be applied in other settings with similar sediments. Based on data obtained in this study, we speculate that the bacteria populations that reduce Se at all three sites are similar physiologically and that the availability and type of substrate used by the bacteria community are also sufficiently similar. The environmental conditions encountered at the SLD and TLDD sites are similar to a number of Se-contaminated wetlands. An example would be the natural wildlife refuge at Benton Lake, Montana. The refuge is a wetland that has dissolved Se concentrations as high as 1880 mg/l, total carbon values between 1.3% and 3.6%, and dissolved sulfate concentrations ranging from 10^4 to 10^5 mg/l (Zhang and Moore, 1996, 1997).

The fractionations observed with SFB sediment were not significantly different from those of the experiments with TLDD and SLD sediments, and we suggest that Se isotope fractionations may be similar under a range of natural conditions, provided labile organic matter is present as an electron donor. Stable groundwater regimes or ocean sediments, where organic electron donor supply may be limited, could induce larger isotope fractionations.

4.2. Modeling sorption and reservoir effects on Se isotope fractionation

Although determining ε for the Se(VI) reduction experiments was straightforward, the model used to estimate ε values for Se(IV) reduction contains assumptions that may affect the accuracy of the results. The adsorbed fraction is treated as one homogenous pool, whereas the slurry probably contained a variety of sorption sites with varying exchange rates. However, the model presented here captures the essential features of the data, and incorporating multiple sorption sites with varying exchange rates would likely not change the results greatly. The difference in ε values between the model results presented here and a simple Rayleigh model that ignores the lack of complete isotope exchange between the dissolved and adsorbed fractions is 1‰. Further refinement of our model would, thus, be expected to improve results by a small fraction of this 1‰. Another approximation made is that the entire data set was fit using a single ε and a single adsorption rate for different bottles. However, our estimated uncertainty of 0.5‰ in ε includes these uncertainties.

A potential source of uncertainty for all the experiments is incomplete contact between the microbes and the solution. The slurries contain some particles of 1-mm diameter and larger and, thus, diffusive exchange between the solution and pore spaces near microbes in particle interiors is not instantaneous. Sorption of Se(IV) took a few hours to equilibrate at the beginning of the experiments; this provides an indication of the rate of exchange. Incomplete exchange between particle interiors and the exterior solution could cause the effective ε values observed in the experiments to be smaller than the fractionation induced by the microbes. A similar model was described by Jorgensen (1979) to explain reservoir effects on the distribution of sulfur isotopes in marine sediments. Dissolved Se in interior pore spaces can be isotopically heavy relative to the bulk solution if the reduction rate is sufficiently large compared to the exchange rate because of the preferential removal of light isotopes by reduction. Microbes living in particle interiors draw from this isotopically heavy pool and, thus, their reduced products are isotopically heavier than if they were in contact with the more open exterior solution. If this reservoir effect does indeed occur, the effective fractionation observed between the exterior solution and the reduced products is less than the true microbial fractionation. Accordingly, actual microbial fractionations by natural populations may be somewhat larger than the values given in Table 1. Incomplete isotope exchange between pores and particles or between sediments and surface waters is important in field studies and is in need of further exploration.

5. Conclusions

In situ measurements of isotope fractionations are difficult to obtain and the sediment slurry experiments of this study presently provide the best estimates of Se isotope fractionations that occur in nature. In sediment slurry experiments under nearly natural conditions and using natural microbial populations, reduction of Se(VI) to Se(IV) yielded a fractionation of -2.6‰ to -3.1‰, while reduction of Se(IV) to Se(0) yielded a larger fractionation of -5.5‰ to -5.7‰. The fractionations did not vary significantly between ex-

periments despite initial Se concentrations that varied within a wide range of 0.004–0.1 mM and the inclusion of sediments from three different environments.

Se isotope fractionations obtained in this study did not decrease with decreasing dissolved Se concentrations and were significantly greater than our precision of 0.2 ‰. This included two experiments that were at ambient Se concentrations with no Se added. We therefore suggest that Se isotopes may be used to indicate and quantify Se reduction and attenuation. The lack of variation in isotope fractionations for the different experiments points toward the possibility that the bacterial populations reducing Se in the various sediments may be physiologically similar and follow similar reductive pathways. If this is so, the values for fractionation obtained here should be applicable to natural settings. Specifically, it would apply to a range of environments between that of a wetland and an estuary, with conditions similar to those of our experiments.

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