Mass-dependent Selenium Isotopic Fractionation during Microbial Reduction of Seleno-oxyanions by Phylogenetically Diverse Bacteria

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Abstract

Selenium (Se) isotope fractionation has been widely used for constraining redox conditions and microbial processes in both modern and ancient environments, but our knowledge of the controls on fractionation during microbial reduction of Se-oxyanions is based on a limited number of studies. Here we complement and expand the currently available pure culture data for Se isotope fractionation by investigating for the first time six phylogenetically and physiologically non-respiring bacterial strains that reduce Se-oxyanions to elemental Se [Se(0)]. Experiments were performed with either selenate [Se(VI)] or selenite [Se(IV)] at lower, more environmentally-relevant concentrations (9 to 47 μM) than previously investigated. *Enterobacter cloacae* SLD1a-1, *Desulfitobacterium chlororespirans* Co23 and *Desulfitobacterium sp.* Viet-1 were incubated with Se(VI) and Se(IV). *Geobacter sulfurreducens* PCA, *Anaeromyxobacter dehalogenans* FRC-W and *Shewanella sp*. (NR) were examined for their ability reducing Se(IV) to Se(0). Our data confirm that microbial reduction of both Se-oxyanions is accompanied by large kinetic isotopic fractionation (reported as $^{82/76}\varepsilon = 1000*(^{82/76}\alpha - 1)$ ‰). Under our experimental conditions, microbial reduction of Se(VI) shows consistently greater isotope fractionation (ε= -9.2‰ to -11.8‰) than reduction of Se(IV) (ε= -6.2 to -7.8‰) confirming the difference in metabolic pathways for the reduction of the two Se-oxyanions. For Se(VI), the inverse relationship between normalized cell specific reduction rate (cSRR) and Se isotope fractionation suggests that the kinetic isotope effect for Se(VI) reduction is governed by an enzymatically-specific pathway related to the bacterial strain-specific physiology. In contrast, the lack of correlation between normalized cSRR and isotope fractionation for Se(IV) reduction indicates a non-enzyme specific pathway which is dominantly extracellular. Our study highlights the importance to understand microbially-mediated Se isotope fractionation depending on Se species, and cell-specific reduction rates before Se isotope ratios can become a fully applicable tool to interpret Se isotopic changes in modern and ancient environments.

1. Introduction

Selenium (Se) isotope fractionation has been widely described in modern environments as well as in ancient settings preserved in sedimentary rocks (Herbel et al., 2002; Mitchell et al., 2012; Wen and Carignan, 2011; Wen et al., 2014; Schilling et al., 2015; Stüeken et al., 2015a, b; Basu et al., 2016; Kipp et al., 2017). Selenium stable isotopes are particularly sensitive to redox reactions, which determine chemical Se speciation in the environment. The most mobile and bioavailable forms of Se are the water-soluble oxyanions selenate [Se(VI)] and (hydro)selenite [Se(IV)]. Selenate is the dominant redox state in modern surface waters (Martin et al., 2011) while Se(IV) is present in ocean surface water, but adsorbs strongly onto iron, manganese and aluminum oxides (Parida et al., 1997; Peak and Sparks, 2002; Peak, 2006) as well as clays. Sparingly soluble elemental Se [Se(0)] and selenide [Se(-II)] are the dominant redox states in anoxic environments. Typically, environmental Se concentrations are at sub-micromolar levels (Conde and San Alaejos, 1997; Fordyce, 2013) but can locally be elevated in sulfide ores and roll-front type uranium ores (Howard, 1977; Basu et al., 2016), shales (Pogge von Strandmann et al., 2015; Stüeken et al., 2015a) or by anthropogenic pollution. (*e.g.,* Presser and Ohlendorf, 1987; Dreher and Finkelman, 1992; Lemly, 2004; Muscatello et al., 2008). Because the mobility and environmental impact of Se are determined by its chemical speciation, it is important to understand the environmental processes that control Se speciation and transitions between oxidation states.

Microbial reduction of Se-oxyanions is the primary set of reactions generating solid Se(0) in natural settings. Both Se-oxyanions are energetically favourable electron acceptors because the reduction of Se(IV) or Se(VI) can provide 90 to 150 times more free energy ‘G (- 8.9 to - 15.5 kcal $mol^{-1}e^{-1}$) for bacteria than the reduction of sulfate to sulfide (Stolz and Oremland, 1999). Bacteria able to reduce Se-oxyanions are phylogenetically diverse, have different metabolic strategies, and have been isolated from both oxic and anoxic environments (*e.g.,*

Macy et al., 1993; Herbel et al., 2000; Stolz et al., 2006; Yee and Kobayashi, 2008; Pearce et al., 2009). All microorganisms are facultative and it has been shown that non-specific metabolic Se reduction can involve different enzyme systems, e.g. for reduction of nitrite, nitrate, arsenate, sulfate and glutathione (*e.g.*, Switzer Blum et al., 1998; Sabaty et al., 2001; Kessi and Hanselmann, 2004; Basaglia et al., 2007). Few Se(VI)-reducing bacteria have been identified to catalyze Se(VI) reduction by the Se-specific enzyme selenate reductase (Schröder et al. 1997; Bébien et al., 2002; Ridley et al., 2006, Theissen and Yee, 2014) and only two bacterial strains (*Tetrathiobacter kashmirensis* and *Pseudomonas sp.*) have a Se(IV)-specific enzyme (Hunter and Manter, 2008, 2009).

Limited published data on Se isotope fractionation during microbial Se reduction have revealed large variations (Herbel et al., 2000; Ellis et al., 2003, Clark and Johnson, 2008). A previous study with pure cultures (*Bacillus selenitireducens, Bacillus arsenicoselenatis* and *Sulfurospirillum barnesii*) was restricted to dissimilatory Se-reducing bacteria grown with very high millimolar levels of Se (10 – 20 mM) and with only lactate as electron donor at high concentrations (10 – 40 mM). For these conditions, the reported Se isotope fractionation varied greatly between -1.7 and -13.7‰ for the reduction of Se(IV) to Se(0) and -1.7 and -7.5 ‰ for reduction of Se(VI) to Se (IV) (Herbel et al., 2000). At contaminated sites, however, Se concentrations are two to three orders of magnitude lower with Se concentration up to 150 µM (= 12,000 µg/L) in agricultural drainage and irrigation water (Deverel and Fujii, 1988; Meseck and Cutter, 2011; Schilling et al., 2015) and up to 12 µM (= 955 µg/L) in waste water from Se-bearing phosphorite mining (Mars and Crowley 2003; Stiling and Amacher, 2010). Even lower Se concentrations occur in modern ocean and aquifers with average values of 0.002 µM and 0.5 µM Se, respectively (*e.g.*, Conde and San Alaejos, 1997; Pearce et al., 2009; Basu et al., 2016).

Another study using sediments slurries reported Se isotopic fractionation by resident Se-reducing microbial community between -8.3 and -8.6‰ for the reduction of Se(IV) to Se(0) and -3.9 and -4.7‰ for the reduction of Se(VI) to Se(IV) (Ellis et al., 2003). Resident Se-reducing microbial communities comprise diverse groups of bacteria with different metabolic strategies. Although the magnitude of Se isotope fractionation by microbial reduction of Se-oxyanions has been previously studied, the cause of such large variation in ε values and their relevance for environmental settings remains unclear.

In this study, we extend the currently available experimental data by determining the isotopic fractionation during microbial reduction of Se-oxyanions [Se(VI), Se(IV)] that includes previously unexplored groups of mesophilic bacteria (Fig. 1), with the first results for bacteria that perform non-catabolic reduction of Se-oxyanions. To investigate whether different Se metabolic strategies affect the magnitude of isotopic fractionation, we selected bacterial strains based on their ubiquitous distribution and their well-studied metabolisms. For Se(VI) reduction, we conducted experiments with the Gram-negative bacterium *Enterobacter cloacae* SLD1a-1, and the Gram-positive bacteria *Desulfitobacterium sp.* Viet-1, and *Desulfitobacterium chlororespirans* Co23. For the reduction of Se(IV), we used isolates of three Gram-negative bacteria namely *Geobacter sulfurreducens* PCA, *Anaeromyxobacter dehalogenans* FRC-W and *Shewanella sp.* (NR) in addition to the three Se(VI)-reducing bacterial strains. Studying pure bacterial cultures has the advantage to eliminate any complexity from natural microbial communities such as competing strains with possibly different reduction rates and to determine isotopic fractionation linked to a single reduction mechanism. Further, we used lower electron acceptor and electron donor concentrations compared to previous studies to achieve slow Se reduction rates as it is well established that rapid reductions are transport limited and suppress the overall isotopic fractionation (Clark and Johnson, 2010). These experimental conditions are very close to Se

concentrations reported for Se contaminated groundwater, soils, porewater (*i.e*., Meseck and Cutter, 2011; Stiling and Amacher, 2010; Schilling et al., 2015; Basu et al., 2016).

2. Materials and Methods

2.1 Microbial culture

The strains, *Desulfitobacterium sp.* Viet-1, *Desulfitobacterium chlororespirans* Co23, *Geobacter sulfurreducens* PCA, *Aneromyxobacter dehalogenans* FRC-W and *Shewanella sp.* (NR) (Table 1) were supplied by Sanford, and *Enterobacter cloacae* SLD1a-1 (Table 1) was supplied by Pallud. For initial growth of bacterial cultures and for Se reduction experiments, we used the mineral-salt medium described by He and Sanford (2002). One liter of test medium was prepared with 10 mL buffer (12.5 g KH_2PO_4, 20.0 g K_2HPO_4 per liter), 10 mL trace salt (1.17 g $CaCl_2$, 2.00 g $MgCl_2 \times 6H_2O$, 0.70 g $FeSO_4 \times 7H_2O$, 0.50 g Na_2SO_4 per liter), 1 mL trace metals (0.05 g $ZnCl_2$, 0.5 g $MnCl_2 \times 4H_2O$, 0.03 g $CuCl_2 \times 2H_2O$, 0.05 g $CoCl_2 \times 6H_2O$, 0.05 g H_3BO_3, 0.05 g $NiSO_4 \times 6H_2O$, 0.01 g $Na_2MoO_4 \times 2H_2O$, 0.004 g Na_2WO_4), 1 mL ammonium chloride, 1 mL selenium-tungsten and 0.84 g $NaHCO_3$. The growth medium was supplemented with 0.03 g L-cysteine while the reductant L-cysteine was omitted for the test medium used for Se batch experiments. Anaerobic condition in both growth and test media was generated by boiling and degassing with N_2/CO_2 mix, the subsequent transfer into 120 mL glass serum bottles sealed with butyl rubber stoppers, and autoclaving at 121°C for 30 min.

In the growth medium, the bacterial strains *Desulfitobacterium chlororespirans* Co23 and *Desulfitobacterium sp.* Viet-1 were initially grown under fermentative conditions using 10 mM pyruvate and *Enterobacter cloacae* SLD1a-1 using 2 mM glucose. *Shewanella sp*. (NR) was incubated with 1 mM nitrate and 2.5 mM lactate. *Anaeromyxobacter dehalogenans* FRC-W was incubated with 2.5 mM acetate and 1.25 mM nitrate. *Geobacter sulfurreducens* PCA was grown with 3 mM acetate as electron donor and 10 mM fumarate as electron acceptor.

All anaerobic cultures were incubated at 30°C for 3 to 5 days to achieve high cell densities (~10^{10} cells mL^{-1}) and complete consumption of the growth substrates.

2.2 Seleno-oxyanions reduction experiments

To test the reduction of Se-oxyanions, 10 mL of inoculum from the growth cultures, corresponding to bacterial densities of 10^5 -10^7 cells mL^{-1} (Table 2) were transferred to the test medium. The microbial cultures were amended with 10 to 47 μM Se(VI) or Se(IV) as the sole terminal electron acceptor. Depending on the bacterial strain, 500 μM (2,000 and 10,000 μM for *Geobacter sulfurreducens PCA*) acetate or lactate as electron donor was added to each reactor (Table 1, 2). The experiments containing Se-oxyanions were conducted without any chemical reducing agent to minimize any cell reproducibility and to avoid any abiotically mediated Se reduction. The "no-cell" control experiments were carried out using identical concentrations of electron donor and Se(VI) or Se(IV), but without any cell suspension. To identify if viable cells were responsible for Se reduction, a "heat-killed cell" control of *Desulfitobacterium chlororespirans* Co23 (autoclaved for 30 minutes) was inoculated with 18 μM Se(VI) and 500 μM lactate as electron donor. All cultures were incubated anaerobically at 30°C under continuous shaking, and sampled at regular time intervals for periods ranging from 60 to 800 hours, depending on the bacterial strains. Subsamples were filtered through 0.2 μm nylon filters.

2.3 Determination of cell-specific reduction rate (cSRR)

A 1 mL aliquot of each culture was sampled for cell counting at the beginning of the experiment (t = 0). Cells were prefixed in 8% formaldehyde and stored at 4°C until analyzed. Bacterial cells were stained with 1 μL of the STYO bacterial stain and 10 μL of microsphere standard (bacteria counting kit, Invitrogen). The cell counting was performed by flow cytometry analysis using a LSR II analyzer (BD Biosciences). The cell density was

169 determined from the cell-counts for a known number of microspheres in each sample. Culture
170 cell density values were used to calculate the initial cell density in the batch experiments and
171 to calculate the cell specific Se reduction rate (cSRR) using the following expression:

172 $$cSRR = \Delta c / t_{1/2} \times d_0 \quad \text{(Eq. 1)}$$

173 where Δc is the decrease in Se(VI) or Se(IV) concentration at the half-life $t_{1/2}$, and the initial
174 cell density d_0. All cSRR values were normalized relative to the initial Se concentrations.

175 *2.4 Transmission electron microscopy (TEM)*

176 Microbial cultures of *Enterobacter cloacae* SLD1a-1 were incubated with Se(VI) or Se(IV)
177 for 24 hours. Afterwards the bacterial cells were pre-concentrated by centrifugation and fixed
178 with 2% glutaraldehyde in a 0.04 M phosphate buffer. After the second cell fixation using 1%
179 osmium tetraoxide, the samples were dehydrated with different concentrated ethanol
180 solutions and embedded in resin. After sectioning of the bacterial cells, micrographs were
181 taken with a Technai 12 transmission electron microscope (University of California,
182 Berkeley).

183 *2.5 Se isotope and concentration analysis*

184 Initially, the concentrations of dissolved Se species [Se(VI) and/or Se(IV)] were measured
185 using mass ^{78}Se by hydride generation-inductively coupled plasma-mass spectrometry (ICP-
186 MS). Prior to measurement, Se(VI) was converted to Se(IV) by heating at 105°C degrees for
187 60 minutes in a 5 M HCl matrix. The reported Se concentrations were calculated using an
188 isotope dilution double-spike method by adding ^{74}Se + ^{77}Se double spike of known isotope
189 ratio and concentration to the sample with unknown Se concentration (*e.g.*, Heumann, 1992).
190 We also used double spike isotope technique with an approximate sample spike proportion of
191 2 to correct for isotopic fractionation during sample purification and instrumental mass bias
192 during the isotope measurement.

193 Selenium species separation from matrix solutes was performed using 1 mL AG1-X8 anion-
194 exchange resin (Eichrom). Prior to anion-exchange, all subsamples of Se(IV) were oxidized
195 to Se(VI) with a 20 mM solution of the strong oxidizer potassium persulfate (Schilling et al.,
196 2014, 2015) prior to heating at 90°C for 1h. The sample purification procedure (including an
197 oxidation step for Se(IV) and chromatographic separation for all samples) resulted in
198 recoveries of >90%.
199 Selenium isotope ratios were measured using a Nu Plasma high resolution multiple collector-
200 ICP-MS, connected to a custom-built hydride generation system described in previous studies
201 (*e.g.*, Clark and Johnson, 2008; Mitchell et al. 2012; Zhu et al., 2014; Schilling et al., 2015;
202 Mitchell et al., 2016). All $^{82}Se/^{76}Se$ ratios are reported as δ notation relative to the NIST SRM
203 3149 inter-laboratory standard:

204 $$\delta \qquad \text{———————} \qquad \text{(Eq. 2)}$$

205 Blank solutions processed through the same sample purification procedure contained an
206 average of 4.4 ± 2.9 ng Se (n=12), less than 0.7% of the total sample. The uncertainty on
207 $\delta^{82/76}Se$ was estimated by calculating the root mean square difference (RMS) for samples
208 prepared and analyzed in duplicate (n = 25). The in-house standard MH-495 was measured
209 with an average value of -3.35 ± 0.1‰ (2σ, n = 12) relative to SRM-3149 within excellent
210 agreement of previously reported values (Carignan and Wen, 2007; Zhu et al., 2008).
211 Average external $^{82/76}Se$ precision was ±0.16‰ based on repeated analysis of SRM-3149
212 standards (n = 145) over two years. The external reproducibility for $^{82/76}Se$ of the samples,
213 determined as twice root mean square, was ±0.17‰ (n = 25) across a range of $\delta^{82/76}Se$ values
214 between -0.3‰ and +29.3‰.

215 *2.6 Determination of the magnitude of isotopic fractionation (ε)*

As all experiments were conducted in sealed batch reactors, the experiments were assumed to follow closed system behavior. Positive $\delta^{82/76}$Se values of the remaining, unreacted Se thus indicate enrichment in heavy isotopes relative to the standard, whilst negative values represent depletion of heavy isotopes. Changes in $\delta^{82/76}$Se can be directly related to the extent of Se(VI) or Se(IV) reduction. The magnitude of Se isotope fractionation was determined for each experiment by fitting the measured $\delta^{82/76}$Se values to Rayleigh distillation models following the method described by Scott et al. (2004):

— (Eq.3)

where *c(t)* and *δ(t)* are the concentration and the isotopic composition of the remaining reactant (Se(VI) or Se(IV)) in solution as a function of reaction time. The fractionation factor (α) is defined as defined as $\alpha = R_{product}/R_{reactant}$, where R is the measured $^{82}Se/^{76}Se$, and often expressed in terms of ε (a per mil quantity) as

(Eq. 4)

The magnitude of isotopic fractionation, ε, was calculated from the corresponding slope from the linear regression of $\ln(\delta^{82}Se + 1000)$ *versus* $\ln(c(t)/c_0)$.

2.7 Statistical analysis

One-way analysis of variance (ANOVA) with Tukey-HSD test ($\alpha = 0.05$) was used to evaluate the potential difference in the magnitude of Se isotope fractionation (ε) between Se(IV) or Se(VI) reduction by the different bacterial strains and among the different bacterial strains. The statistical analyses were performed using JMP software 13.1.0. with a statistical probability of $P < 0.05$.

3. Results

3.1 Microbial reduction of Se(VI)

Figure 2 shows Se(VI) removal over time during anaerobic microbial Se(VI)-reduction experiments by three bacterial strains (*Enterobacter cloacae* SLD1a-1, *Desulfitobacterium chlororespirans* Co23, *Desulfitobacterium sp.* Viet-1). In the presence of Se(VI)-reducing bacteria, the decrease in Se(VI) concentration ranged between 51% to 99% relative to the initial Se(VI) concentrations in the batch reactors. In all experiments, the decrease in Se(VI) concentration with time follows a first-order kinetics except for the latest sampling points. A single first-order rate constant reasonably fit all data from each experiment. The heat-killed control with bacterial cells from *Desulfitobacterium chlororespirans* Co23 did not show any measurable Se(VI) removal after ca. 4 days of incubation (Figure 2A). We observed in the batch reactors an initial increase in Se(IV) resulting from the reduction of Se(VI), followed by Se(IV) reduction to Se(0) (Table A1).

In all Se(VI) experiments, an enrichment of ^{82}Se occurred in the remaining unreacted Se(VI) with progressive Se(VI) reduction (Figure 3). The largest $\delta^{82/76}Se$ value of +38.6‰ was observed at 99% reduction of Se(VI) for the experiment with *Desulfitobacterium sp.* Viet-1 (Figure 3C). The $\delta^{82/76}Se$ values for the intermediate Se(IV) varied between -9.6‰ for 1.5% Se(IV) and 16.6‰ for 15% Se(IV) relative to the initial Se(VI) concentration (9 µM) (Table A1). The magnitudes of Se isotopic fractionation (ε) for microbial Se(VI) reduction, obtained by fitting $\delta^{82/76}Se$ data to Eq. 3, are illustrated in Figure 3 and reported in Table 3. Among the three Se(VI)-reducing pure bacterial cultures, the ε values varied between -9.2‰ and -11.8‰ with a mean value of -10.6 ± 1.3‰. The ε values did not deviate in duplicate reactors within a 2σ uncertainty limit. The initial cell densities varied by two orders of magnitude in the Se(VI) incubation with the highest cell density of 1.9×10^8 cell ml^{-1} for the batch of *Enterobacter cloacae* SLD1-1a. The normalized cSRR ranged from 0.11×10^{-17} to 1.30×10^{-17} mol Se(VI) $cell^{-1}$ d^{-1} (Table 2). We observed a strong inverse relationship ($r^2 = 0.91$) between

normalized cSRR and ε for Se(VI) reduction with decreasing ε values for increasing values of cSRR (Figure 4A).

Transmission electron microscope images of a washed cell suspension of *Enterobacter cloacae* SLD1a-1 after the reduction of Se(VI) showed intracellular Se(0) precipitates (Figure 5A). The particle sizes were spherical, <0.2 μm in diameter and located in the periplasmic space.

3.2. Microbial reduction of Se(IV)

Anoxic batch incubations of six Se-reducing bacterial strains with Se(IV) showed a decrease in Se(IV) concentration as a function of time (Figure 6 and Table A2). The Se(IV) removal ranged between 56% to 92% over a period of 0.4 to 6.1 days. The no-cell control experiments did not show any change in Se(IV) concentrations with time. In each experiment, a first order kinetic model with a single rate constant fits all Se(IV) concentration data. The time interval for 50% removal of the initial Se(IV) concentration varied from 0.42 to 3.1 days. *Geobacter sulfurreducens* PCA assayed with varying electron donor concentrations (2,000 to 10,000 μM) exhibited an approximately 50% Se(IV) removal after similar time periods ($t_{1/2}$ 0.49 to 0.66 days).

The initial cell densities varied by approximately two-orders of magnitudes depending on the volume of inoculum. The highest cell density was found for the Se(IV) batch of *Anaeromyxobacter dehalogenans* FRC-W (1.3×10^8 cell ml^{-1}) and the lowest for *Enterobacter cloacae* SLD1a-1 (8.8×10^6 cell ml^{-1}). The calculated cell-specific reduction rate ranged from 0.28 to 5.7 $\times10^{-17}$ mol $cell^{-1}$ d^{-1} with no correlation between normalized cSRR and ε values (r^2 = 0.13; Figure 3B). We observed no correlation between normalized cSRR and ε for Se(IV) reduction (Figure 4B).

While the Se(IV) concentration in the batch reactors decreased, $\delta^{82/76}$Se progressively increased with time (Figure 7) relative to the starting Se(IV). The duplicate batch reactors

showed similar ε values within the 2σ uncertainty level of ±0.12‰ to ±0.43‰. (Table 3). The largest $\delta^{82/76}$Se value of +15.7‰ was observed for *Shewanella sp.* (NR) after 91% Se(IV) removal. The ε values for Se(IV) reduction span a narrow range of -6.2‰ to -7.8‰ with a mean value of -7.0 ± 0.6‰. We observed no significant difference in ε values among the six different bacterial strains (p = 0.376; Figure 7).

Transmission electron microscope images of *Enterobacter cloacae* SLD1a-1 incubated with Se(IV) showed exogenous precipitates, presumably Se(0) (Figure 5A) which are smaller than the intracellular Se(0) particles observed as a product of Se(VI) reduction (Figure 5B).

4. Discussion

Our results demonstrate that the magnitude of Se isotope fractionation by microbial reduction of Se-oxyanions depends mainly on two factors (1) growth conditions and (2) the Se-reduction by cometabolic or Se-respiring pathway. Below we discuss variation in Se isotope fractionation based on these two factors, specifically in the context of previous studies. Differences in the magnitude of Se isotope fractionation in our study compared to the previous studies (Herbel et al., 2000; Ellis et al., 2003; Clark and Johnson, 2008) is attributed to differences in the experimental approach and selection of bacterial strains.

4.1. Effect of experimental conditions on ε

In the following section we discuss the reasons behind differences in the magnitude of Se isotope fractionation between our study and previous studies (Herbel et al., 2000; Ellis et al., 2003; Clark and Johnson, 2008).

4.1.1 Comparison to pure culture studies

Environmentally-relevant conditions, more specifically much lower Se substrate concentrations, result in a narrower-range Se isotope fractionation factors for the reduction of both Se(VI) and Se(IV) compared to previously published values obtained for *Bacillus*

312 *selenitireducens*, *Bacillus arsenicoselenatis* and *Sulfurospirillum barnesii* (Herbel et al.
313 2000). Indeed, Herbel et al. (2000) experiments were conducted under highly optimum
314 growth conditions for anaerobic Se respiration *i.e.*, using high initial Se-oxyanion
315 concentrations (10 - 20 mM) and high carbon concentrations (10 - 40 mM). This led to fast
316 reduction rates and 100-fold increase in cell density during the experiment, which might
317 explain the large span in isotopic fractionation they observed for Se(VI) reduction (ε = -1.7 to
318 -7.5‰; Herbel et al., 2000). In addition, Se reduction rates in the previous study were one-
319 order of magnitude (10^{-16} mol $cell^{-1}$ d^{-1}) higher than the cSRRs observed in our experiments
320 (10^{-17} mol $cell^{-1}$ d^{-1}). In contrast to the large increase in cell density in the experiment by
321 Herbel et al (2000), natural microbial consortia generally maintain a rather steady state
322 population where cell decay balances cell growth (Brock, 1971). The more uniform ε values
323 in our study correspond to minor cell growth or decay evident from single first-order rate
324 constants fitting the time series from each experiment and thus more consistent cSRRs
325 throughout the experiment. Moreover, three-orders of magnitude lower initial Se-oxyanions
326 concentrations, together with 20 times lower carbon concentrations as electron donor (10 mM
327 *vs.* 0.5 mM), led to significantly larger ε values (ε = -9.2‰ to -11.8‰). Likewise, our data on
328 microbial Se(IV) reduction result in an overall narrow distribution of ε values (ε_{mean} = -7 ±
329 0.6‰) compared to the previous study (ε = -1.7 to -13.7‰; Herbel et al., 2000). This narrow
330 range of ε values is likely when the tested strains are not actively respiring Se. Instead, the
331 reduction of Se-oxyanions is a response to cope with the element's toxicity. Lower initial
332 Se(IV) concentration also affect the results in two different ways by producing lower
333 reduction rates and lower Se concentrations are less toxic for the cells and thus maintains the
334 cell viability.
335 Whether bacteria reduce Se as respiration pathway (Herbel et al., 2000) or as a cometabolic
336 pathway determines the magnitude of isotope fractionation. All bacterial strains (*Bacillus*

selenitireducens, Bacillus arsenicoselenatis and *Sulfurospirillum barnesii)* used in Herbel et al. (2000) are capable of actively metabolizing Se oxyanions. This means that the bacteria are able to harness the energy derived from coupling reduction of Se(VI)/Se(IV) and oxidation of lactate to synthesize biomass. This leads to bacterial growth (increase of cell density by about two orders of magnitude) during the experiments which changes the cSRR and thus affects the ε values. For example, isotope fractionation by *Bacillus selenitireducens* varied between -2.6 and -13.7‰ (Herbel et al., 2000). This also explains why the relationship between cSRR *vs*. ε breaks down for Se-respiring bacteria. Further, both Se(VI)-respiring bacterial strains, *Bacillus arsenicoselenatis* and *Sulfurospirillum barnesii*, only reduce a very small amount of Se(IV) to Se(0) (Herbel et al., 2000), while our tested bacterial strains reduce Se(VI) all to Se(0) (Table A1).

Although the tested Se-reducers are not confined to any particular group of bacteria (Figure 1), we demonstrate that not the phylogenetic differences but the metabolic mechanisms control Se isotope fractionation. There is no systematic difference in ε between the tested non-respiring Gram-positive (*Desulfitobacteria*) and Gram-negative bacteria (*Enterobacter cloacae* SLD1a-1). Gram-negative bacteria possess two membranes separated by the periplasmic space. The selenate reductase of Gram-negative bacterium *Enterobacter cloacae* SLD1a-1 is a membrane-bound enzyme situated in the cytoplasmic (inner) membrane (Schröder et al., 1997; Bébien et al., 2002; Ma et al., 2009). The location for the Se(VI)-reducing enzymes of *Desulfitobacterium chlororespirans* Co23 and *Desulfitobacterium sp.* Viet-1 are not known but are probably also membrane-bound as described for other Se(VI)-reducing Gram positive bacteria (Kuroda et al., 2011). Hence, the diffusive transport across the outer membrane for Gram-negative bacteria seems not to affect the reaction rate and Se isotope fractionation for Se(VI) reduction.

In bacterial strains with no Se-specific enzymatic pathway, the reduction is carried out by various enzyme systems, e.g. nitrite reductase, nitrate reductase, arsenate and sulfate reductases, or the reduction of Se(IV) by glutathione (*e.g.,* Tomei et al., 1995; Switzer Blum et al., 1998; Sabaty et al., 2001; Kessi and Hanselmann, 2004; Basaglia et al., 2007; Nancharaiah and Lens, 2015). These enzyme systems have mainly a detoxifying function and the energy released by the redox reaction is not generally utilized to synthesize biomass. Therefore, we assume that the obtained isotope fractionation factors can be extrapolated to a much wider group of microorganisms because the studied pure cultures include bacteria with different cell membranes (Gram positive and Gram negative), different enzymes in the electron transfer chain (*e.g.,* selenate reductase for *Enterobacter cloacae*) and carbon substrates (acetate and lactate) but show nearly identical isotope fractionations for the reduction of the respective Se-oxyanion.

4.1.2 Comparison to natural microbial consortia in sediment slurries and cores

Our pure culture microbial Se(VI) reduction experiments induced significantly larger Se isotope fractionation than experiments with sediment slurries and sediment cores involving complex microbial communities (Ellis et al., 2003; Clark and Johnson, 2008). Here reported ε values relate to suspensions of free-living cells with maximized mass transfer and accessibility of Se-oxyanions for each bacterial cell. Mass transfer limitation in sediment slurries and cores is expected to decrease the exchange between Se(VI) in solution and the particle-bound bacteria. This in turn should reduce selectivity for an isotopologue (heavy vs. light). Generally, mass transfer is faster for the isotopically light ^{76}Se-oxyanions than for the isotopically heavy ^{82}Se-oxyanions. If the probability for Se(VI) selectivity of an isotopologue is limited for the particle-bound bacteria the mass transfer affects the Se isotope fractionation. This presumably explains the relatively small Se isotope fractionation observed in sediment slurries (Ellis et al., 2003) where the contact between bacteria and Se-oxyanions in solution is

limited but still higher than for sediment cores (Clark and Johnson, 2008). Selenium isotope signals in sediments controlled by the diffusion of Se from the overlying water have the highest mass transfer limitation determined by incomplete solution exchange at the water-sediment interface and the lowest Se isotope fractionation of 0.4‰ for microbial Se reduction. In contrast, pure cultures in our study are more selective to a particular Se isotopologue (heavy vs. light) as they are not particle-bound. However even if in porewaters, diffusion limitation yields a smaller Se isotope fractionation the reduction of Se(VI) and Se(IV) are still detectable because the shift in δ values from the initial value is significant.

4.2. Effect of metabolic pathway on Se isotope fractionation

Reduction of Se-oxyanions occurs intracellularly in the periplasmic space for Se(VI) (*i.e.*, Ridley et al., 2006; Nancharaiah and Lens, 2015) or extracellularly for Se(IV) (*e.g.,* Pearce et al., 2009; Nancharaiah and Lens, 2015). Our data and the pure culture study by Herbel et al., (2000) confirm that the pathways for reduction of Se(VI) or Se(IV) determine the Se isotope fractionation. Microbial Se(VI) reduction induces significantly larger fractionation than Se(IV) reduction for the six tested bacterial strains ($p > 0.01$).

4.2.1 Intracellular Se(VI) reduction

The reduction Se(VI) to Se(0) is a sequential two-step reaction which leads to significantly larger fractionation than Se(IV) reduction for the tested bacterial strains ($p > 0.01$). The reduction of Se(VI) to Se(IV) *via* two electron transfer is followed by a four electron transfer to form Se(0). Heat-killed control experiments with *Desulfitobacterium chlororespirans* Co23, which did not show Se(VI) reduction and any concomitant Se isotopic fractionation, confirms that Se(VI) reduction is enzymatically-mediated by viable cells. Correlation between normalized cSRR and ε for Se(VI) reduction indicates that the rate of electron transfer depends on the abundance of bacteria and their enzymes (*e.g.*, selenate reductase)

(Yee and Kobayashi, 2008). Mechanistically, diffusion transport brings Se(VI) to the reduction site of the bacterial cell where Se(VI) is then reduced intracellularly. As diffusive transport of Se(VI) does not involve changes in coordination of oxygen around Se, any discrimination between the isotopologes in the Se-oxyanions will be minor compared to enzymatic reduction. If the reduction rate of Se(VI) at these reduction sites is very slow either due low abundances of bacteria and their enzymes it is expected that ε reaches a maximum value. Future studies can help determining the maximum value as well as the more in-depth understanding of the relevant enzymes involved in the cometabolic Se(VI) reduction and the related Se isotope fractionation.

4.2.2 Extracellular Se(IV) reduction.

The four electron transfer by only one reduction step for Se(IV) explains the smaller Se isotope fractionation compared to the reduction of Se(VI). Electron transfer for the reduction of Se(IV) is driven by either an exogenous electron shuttle, extracellular proteins or possibly pili structures (Pearce et al., 2009). This also explains why the magnitude of Se isotope fractionation does not correlate with normalized cSRRs (Figure 4C) because an exogenous electron transfer does not require a direct contact between the bacterial cell and the substrate *via* a specific enzyme. Such extracellular reaction is also most likely decoupled from electron donor oxidation, so that different donor types or concentrations do not affect the isotope fractionation factor. This is clearly shown for the experiments with *Geobacter sulfurreducens* PCA, known for reducing Se(IV) extracellularly by outer membrane cytochromes (Pearce et al., 2009). Varying concentrations of electron donor (500 to 10,000 µM) have no effect on ε values (Table 3) for Se(IV) reduction by *Geobacter sulfurreducens* PCA. This is consistent with the observation for microbial Cr(VI) reduction where an extracellular Cr reduction pathway results in uniform Cr isotope fractionation at different electron donor concentrations (*i.e.,* Sikora et al., 2008; Basu et al., 2014; Zhang et al., 2019). Further, the extracellular

435 reduction pathway for Se(IV) is not impacted by mass transfer limitation of Se(IV) to the
436 bacterial cell and this explains the relatively good agreement for isotope fractionation
437 between sediment slurry experiments for particle-bound natural microbial consortia (ε = -
438 8.4‰; Ellis et al., 2003) and our pure culture study with free-living cells (ε = -6.2 to -7.8‰).

439 **5. Implications**

440 Given the distinctive amounts of Se isotope fractionation for microbial reduction of the two
441 Se-oxyanions, Se isotope ratios can, in principle, shed light on the processing of Se-
442 oxyanions in both modern and ancient environments.

443 *5.1. Modern environments.*

444 Selenium stable isotope ratios have been previously used as indicators for Se sources and
445 cycling in aquifers, lakes, soils and sediments (Clark and Johnson 2010; Schilling et al.,
446 2015; Basu et al., 2016). Microbial reduction of Se reduces the mobility of Se from soluble
447 poorly adsorbed Se(VI), to soluble strongly adsorbed Se(IV), to solid Se(0).

448 Our ε values can be understood as reference values to estimate the extent of Se
449 reduction for bacterial groups most commonly found in the environment. A clear indicator of
450 the reduction of Se(VI) to Se(IV) is the enrichment of ^{82}Se in Se(VI) or Se(IV) in
451 groundwater or porewater while the reduced Se species in sediments and soils is enriched in
452 ^{76}Se. If microbial Se reduction is the dominant reaction mechanism in nature, this should be
453 reflected by shifts in $\delta^{82/76}$Se ratios according to the aqueous speciation of Se and the ε for
454 that particular reaction.

455 In groundwater systems, we can infer the ε for Se removal mechanism from $\delta^{82/76}$Se of
456 Se(VI) and Se(IV) measured in the same sample (Schilling et al., 2015; Basu et al., 2016).
457 The Microbial reduction of Se-oxyanions in a groundwater plume moving through a redox
458 gradient will fractionate $\delta^{82/76}$Se of both the reactant and the product. This fractionation

459 combined with the reactive transport of Se should lead to a systematic pattern of $\delta^{82/76}$Se
460 induced by a distillation effect in the groundwater. With progressive reduction along the flow
461 path, Se-oxyanions will become isotopically heavy. For instance, Basu et al. (2016) observed
462 increasing $\delta^{82/76}$Se with decreasing Se(VI) concentrations in an aquifer along the redox
463 gradient at an *in-situ* recovery mining site. However, along the flow path of groundwater the
464 rate of microbial Se reduction may vary depending on the organic carbon content of the
465 aquifer, and the bacterial population density. This variation in the Se reduction rates can
466 systematically affect the ε, which is determined by the difference in $\delta^{82/76}$Se between Se(VI)
467 and Se(IV) of the same sample.

468 The dependence of ε on the Se(VI) reduction rate has important implications for predicting
469 Se removal/accumulation patterns in modern settings based on Se isotope ratios. Our
470 experimental results suggest a higher ε at low Se(VI) reduction rates generally found in
471 terrestrial sediments with low organic carbon. In contrast, lower ε is expected in geochemical
472 settings with high Se(VI) reduction rate commonly found after organic carbon amendment
473 (*i.e.*, acetate) at active bioremediation sites. Our results suggest that the ε inferred from water
474 samples may be used to estimate the Se(VI) reduction rate, which is difficult to determine
475 accurately in open systems. Similarly, if the Se(VI) reduction rate is known, an appropriate
476 ε can be determined for calculating the extent of remediation for active remediation sites
477 using rate-ε relationship. Therefore, any quantitative interpretation of the groundwater Se
478 isotope ratios is predicated on the knowledge of the size of the intrinsic ε and the factors that
479 control ε at a geochemical setting.

480 Sedimentary Se isotope ratios may provide a complimentary view of the relationship
481 between reduction rate of Se oxyanions and ε. The $\delta^{82/76}$Se values of different Se soil pools in
482 agricultural seleniferous soils vary up to 13‰ (Schilling et al., 2015). The isotopically heavy

adsorbed Se(IV) in the agricultural seleniferous soils suggest Se isotope fractionation by microbial reduction of Se(VI) in irrigation water prior to scavenging of the reaction product Se(IV) by reactive minerals in the soil. Therefore, our laboratory-derived ε values for microbial reduction are essential to quantitative determination of the extent of microbial reduction in the field. Nevertheless, site-specific ε values should still be obtained from experiments using the resident Se-reducing microbial community

5.2. *Ancient environments*

The Se isotope signature preserved in rocks and sediments can be used to constrain the evolution of the biosphere and redox conditions in near surface environments through time (Wen and Carignan, 2011; Mitchell et al., 2012, 2016; Wen et al., 2014; Stüeken et al., 2015a, b; Kipp et al., 2017). However, interpreting Se isotope signature preserved in rocks and sediments is still difficult as (1) bulk rock Se isotope data mask the variability in Se isotope ratio of various Se phases (*i.e.*, organically-bound, pyritic, adsorbed) in the rocks and (2) the effects of local versus global controls on Se cycling due to the short oceanic residence time (10^3 years) of Se species and their low concentrations (<1nM). Therefore, the application of Se isotopes as a paleoredox proxy relies on experimentally determined ε values for microbial reduction of Se-oxyanions. High-resolution isotope analyses with an analytical precision of 0.2‰ allows to resolve different reaction pathways and fingerprint Se sources preserved within the rock record.

Selenium isotope signature in bulk shales range between -1.5 and +2.2‰ over geological time (Wen and Carignan, 2011; Mitchell et al., 2012; Pogge von Strandmann et al., 2014; Stüeken et al., 2015a, b; Mitchell et al., 2016). The sequestration of isotopically light Se has been reported for shale deposits (Wen and Carignan, 2011; Mitchell et al., 2012; Pogge von Strandmann et al., 2014; Stüeken et al., 2015a, b) suggesting the partial reduction of Se-

oxyanions in suboxic basins. Our results suggest a lower ε during very rapid removal of Se(VI), the dominant Se species in the ocean (Conde and Alaejos, 1997), during anoxia which is consistent with small changes in $\delta^{82/76}$Se in the black shales. However, it is necessary to extract phase-specific Se (*i.e.*, adsorbed, organic, pyritic) from rocks and sediments and determine their Se isotope compositions to disentangle microbial reduction of Se-oxyanions from other reaction pathways (*i.e.,* adsorption and assimilation). It should be noted that muting of ε values are expected in semi-closed or open flow through systems (Shrimpton et al., 2018) like microbial Se reduction in porewater in ancient oceans compared to ε values typically observed during Rayleigh distillation in a closed system.

In our study, we demonstrate that decreasing microbial activity results in smaller cSRR, which causes larger Se isotope fractionation and this could ultimately be reflected in lighter Se isotopic signature in sedimentary Se reservoirs. Despite uncertainties about the Se concentrations and its speciation in ancient oceans, our results imply a systematic relationship between the rate of Se reduction and the observed Se isotope fractionation which must be considered when interpreting $\delta^{82/76}$Se signatures. This relationship can be of great importance because Se reduction rates may be derived from Se isotope signature preserved in the rock record. Thus, the Se isotope signatures recorded in ancient sediments should be re-examined given the influence of Se(VI) reduction rate on the magnitude of Se isotope fractionation.

Future studies should be designed to constrain Se isotope fractionation at microbial Se reduction rates relevant to ancient marine conditions and reconstruct $\delta^{82/76}$Se ratios imprinted in the rock record. Additionally, phase-specific Se isotope analysis for rocks and sediments, and quantitative modeling approaches can help to provide further insight into the microbial Se cycling in the ancient ocean.

6. Summary and Conclusions

This study provides the first insights on the variation of Se isotope fractionation for non-respiring Se reduction by six different bacterial strains (*Geobacter sulfurreducens* PCA, *Anaeromyxobacter dehalogenans* FRC-W, *Shewanella* sp. (NR), *Enterobacter cloacae* SLDa1-1, *Desulfitobacterium chlororespirans* Co23 and *Desulfitobacterium sp.* Viet-1). We demonstrate that under environmentally-relevant experimental conditions (*e.g.*, <42 µM Se; 500 µM electron donor), Se isotope fractionation factors reveal a relatively narrow range for both Se(VI) and Se(IV) reduction with consistently larger Se isotope fractionation for Se(VI) (ε_{mean} = -10.6 ± 1.3‰) than for Se(IV) reduction (ε_{mean} = -7 ± 0.6‰). Based on the present and previous studies on microbial reduction of Se-oxyanions, we conclude that Se isotopic fractionation during microbial reduction is controlled by the co-metabolic reaction pathway(s).

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720

Figure Caption

Figure 1. Phylogenetic tree showing currently described Se(VI) and Se(IV)-reducing bacteria Species names are shown in italics. Red marked species represent the bacterial strains studied in this work. Also indicated the taxonomic classes of bacteria.

Figure 2. Time series (batch experiments) of Se(VI) reduction at 30°C with 500 μM of acetate (*Enterobacter cloacae SLD1a-1*) or lactate (*Desulfitobacterium chlororespirans Co23, Desulfitobacterium sp. Viet-1*) as electron donor. Heat kill control with cells from *Desulfitobacterium chlororespirans* Co23 incubated at 30°C with 500 μM of acetate. The analytical uncertainty Se concentration is less than 1% and close to the size of the symbols.

Figure 3. Values of $\delta^{82/76}$Se of Se(VI) versus remaining Se(VI) during microbial reduction in closed system (batch experiments) Modelled lines (dashed) follow a predicted Rayleigh fractionation process. Uncertainties (±2 SD) are close to the size of the symbols. For the heat-kill control the error bars show the root mean square error (RMS) of replicate measurements as described in section 2.5.

Figure 4. Normalized cell-specific reduction rate (cSRR) and Se isotopic fractionation (ε) by phylogenetically diverse bacteria **(A)** reduction of Se(VI) and **(B)** reduction of Se(IV). The error bars correspond to standard deviation (±2 SD) of ε's from duplicate batch experiments (x-axis), and error (%) of cSRR calculated from repeated cell-counting measurements (y-axis). For some data points, the error bars are within the size of the symbol.

Figure 5. TEM images of *Enterobacter cloacae SLD1a-1* grown at 30°C under anoxic conditions in presence of acetate as electron donor and **(A)** Se(VI))or **(B)** Se(IV). Red arrows indicate the presence of intracellular (A) or extracellular (B) Se(0) as reduction product. Scale bars represent 0.2 μM.

Figure 6. Time series (batch experiment) of Se(VI) reduction at 30°C with 500 μM of lactate (*Desulfitobacterium chlororespirans Co23, Desulfitobacterium sp. Viet-1*, Shewanella sp. (NR)) or acetate (*Enterobacter cloacae SLD1a-1, Anaeromyxobacter* FRC-W, *Geobacter sulfurreducens* PCA,) as electron donor. The analytical uncertainty Se concentration is less than 1% and close to the size of the symbols.

754 **Figure 7**. Values of $\delta^{82/76}$Se of Se(IV) versus remaining Se(IV) during microbial reduction in
755 closed system (batch experiments). Modelled lines (dashed) show predicted Rayleigh
756 fractionation. Uncertainties (±2 SD) are close to the size of the symbols.

Table 1

Table 1. List of bacterial strains investigated in this study

Bacterial strain	Gram strain	Electron acceptor	Electron donor
Enterobacter cloacae SLD1a-1	-	Se(VI)/Se(IV)	Acetate
Desulfitobacterium chlororespirans Co23	+	Se(VI)/Se(IV)	Lactate
Desulfitobacterium sp. Viet-1	+	Se(VI)/Se(IV)	Lactate
Geobacter sulfurreducens PCA	-	Se(IV)	Acetate
Anaeromyxobacter dehalogenans FRC-W	-	Se(IV)	Acetate
Shewanella sp. (NR)	-	Se(IV)	Lactate

Table 2

Table 2. Reduction rate [$t_{50\%}$ in days] and cell-specific reduction rate (cSRR) of investigated bacterial strains

Bacterial strain	Electron acceptor	Initial Se (μM)	Time for 50% reduction ($t_{50\%}$) (d)	Normalized cSRR (10^{-17} mol cell^{-1} d^{-1})
Enterobacter cloacae SLD1a-1	Se(VI)	30	1.13	0.24
	Se(IV)	9	1.03	5.68
Desulfitobacterium chlororespirans Co23	Se(VI)	42	41.25	0.11
		9	2.31	0.29
			1.27	0.45
	Se(IV)	9	0.42	0.51
			0.71	0.31
Desulfitobacterium sp. Viet-1	Se(VI)	47	1.10	0.34
			0.79	1.22
		13	3.80	0.98
			3.85	1.30
	Se(IV)	9	3.10	0.65
			2.49	0.85
Geobacter sulfurreducens PCA	Se(IV)	13	3.10	0.28
		8	0.49	0.92
			0.66	1.14
		15	0.63	0.81
			0.55	1.32
Anaeromyxobacter dehalogenans FRC-W	Se(IV)	13	0.51	0.78
Shewanella sp. (NR)	Se(IV)	19	2.37	0.53
		13	1.60	2.17

Table 3

Table 3. Magnitude of isotopic fractionation ε of Se(VI) and Se(IV) reduction by various bacterial strains

Bacterial strain	Electron acceptor	Initial Se concentration (μM)	Electron donor concentration (μM)	ε (‰) $^{82/76}$Se ± 2s.e.	Number of experiments
Enterobacter cloacae SLD1a-1	Se(VI)	30	500	-11.5±0.5	2
	Se(IV)	9	500	-7.6±0.3	1
Desulfitobacterium chlororespirans Co23	Se(VI)	42	500	-11.8±0.6	1
		9	500	-11.3±0.2	2
	Se(IV)	9	500	-7.8±0.8	2
Desulfitobacterium sp. Viet-1	Se(VI)	47	500	-9.3±0.5	2
		13	500	-9.2±0.2	2
	Se(IV)	9	500	-7.3±0.2	2
Geobacter sulfurreducens PCA	Se(IV)	13	500	-6.3±0.6	2
		8	2000	-7.0±0.4	3
		15	10000	-7.3±0.5	3
Anaeromyxobacter dehalogenans FRC-W	Se(IV)	13	500	-6.3±0.5	1
Shewanella sp. (NR)	Se(IV)	19	500	-6.9±0.2	1
		13	500	-6.2±0.4	1

Figure 1

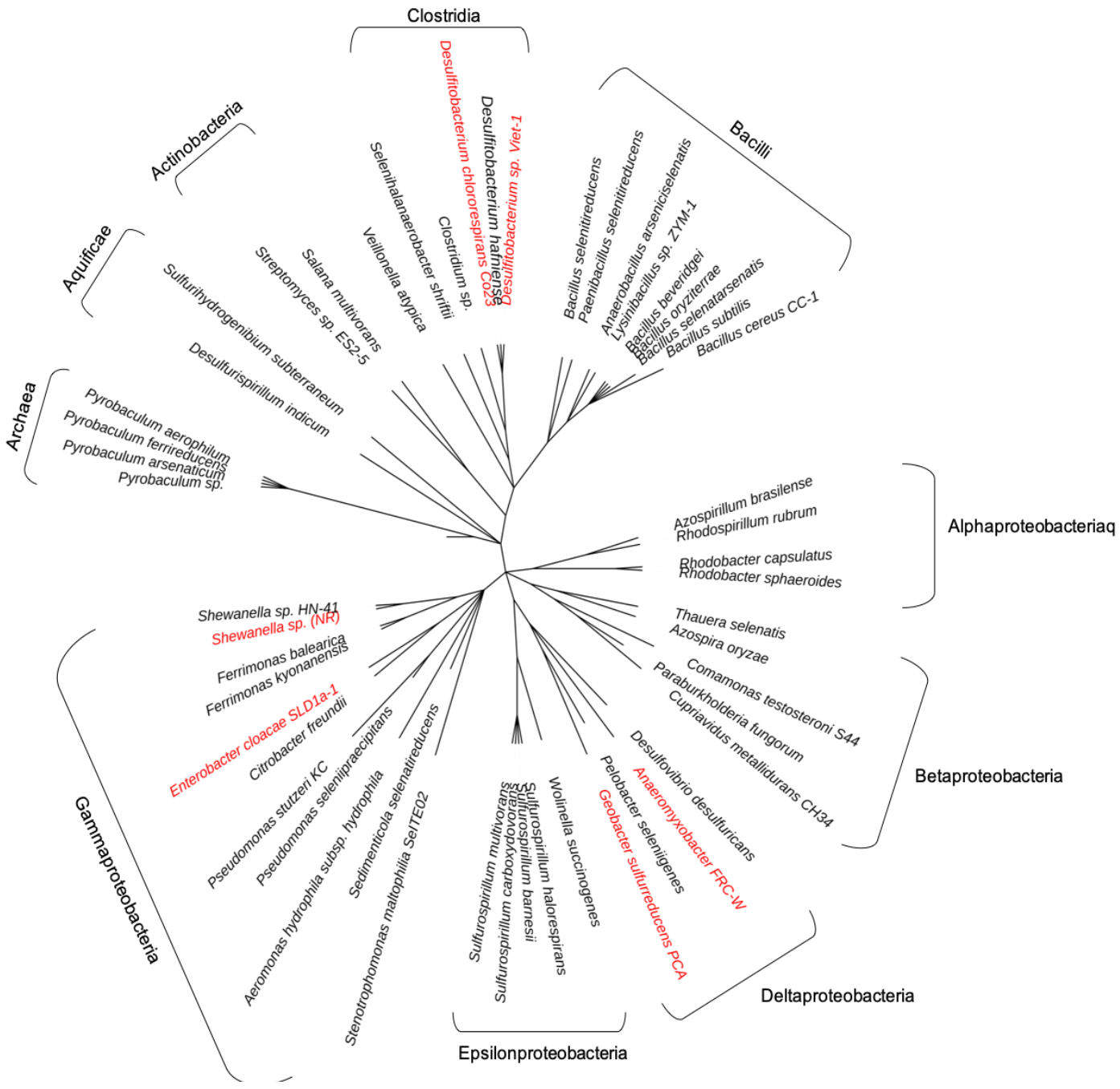

Clostridia
Desulfitobacterium chlororespirans Co23
Desulfitobacterium hafniense
Desulfitobacterium sp. Viet-1
Actinobacteria
Selenihalanaerobacter shriftii
Clostridium sp.
Veillonella atypica
Bacilli
Bacillus selenitireducens
Paenibacillus selenitireducens
Anaerobacillus arseniciselenatis
Lysinibacillus sp. ZYM-1
Bacillus beveridgei
Bacillus oryziterrae
Bacillus selenatarsenatis
Bacillus subtilis
Bacillus cereus CC-1
Aquificae
Salana multivorans
Streptomyces sp. ES2-5
Sulfurihydrogenibium subterraneum
Desulfurispirillum indicum
Archaea
Pyrobaculum aerophilum
Pyrobaculum ferrireducens
Pyrobaculum arsenaticum
Pyrobaculum sp.
Alphaproteobacteriaq
Azospirillum brasilense
Rhodospirillum rubrum
Rhodobacter capsulatus
Rhodobacter sphaeroides
Betaproteobacteria
Thauera selenatis
Azospira oryzae
Comamonas testosteroni S44
Paraburkholderia fungorum
Cupriavidus metallidurans CH34
Deltaproteobacteria
Desulfovibrio desulfuricans
Anaeromyxobacter FRC-W
Pelobacter seleniigenes
Geobacter sulfurreducens PCA
Epsilonproteobacteria
Wolinella succinogenes
Sulfurospirillum halorespirans
Sulfurospirillum barnesii
Sulfurospirillum carboxydovorans
Sulfurospirillum multivorans
Gammaproteobacteria
Shewanella sp. HN-41
Shewanella sp. (NR)
Ferrimonas balearica
Ferrimonas kyonanensis
Enterobacter cloacae SLD1a-1
Citrobacter freundii
Pseudomonas stutzeri KC
Pseudomonas seleniipraecipitans
Aeromonas hydrophila subsp. hydrophila
Sedimenticola selenatireducens
Stenotrophomonas maltophilia SeITE02

Figure 2

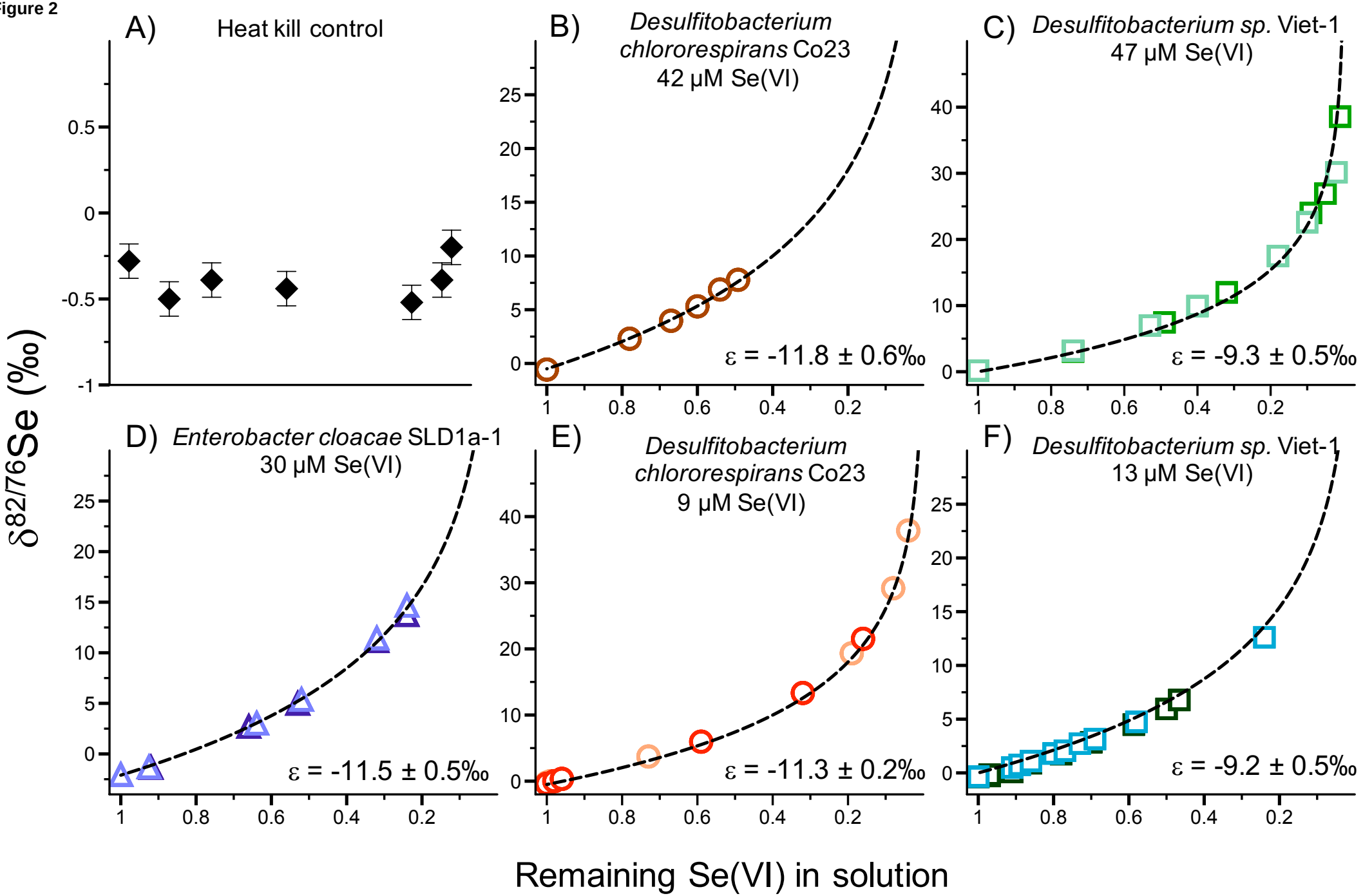

A)
Heat kill control
B)
Desulfitobacterium chlororespirans Co23
42 µM Se(VI)
ε = -11.8 ± 0.6‰
C)
Desulfitobacterium sp. Viet-1
47 µM Se(VI)
ε = -9.3 ± 0.5‰
D)
Enterobacter cloacae SLD1a-1
30 µM Se(VI)
ε = -11.5 ± 0.5‰
E)
Desulfitobacterium chlororespirans Co23
9 µM Se(VI)
ε = -11.3 ± 0.2‰
F)
Desulfitobacterium sp. Viet-1
13 µM Se(VI)
ε = -9.2 ± 0.5‰
δ82/76Se (‰)
Remaining Se(VI) in solution

Figure 3

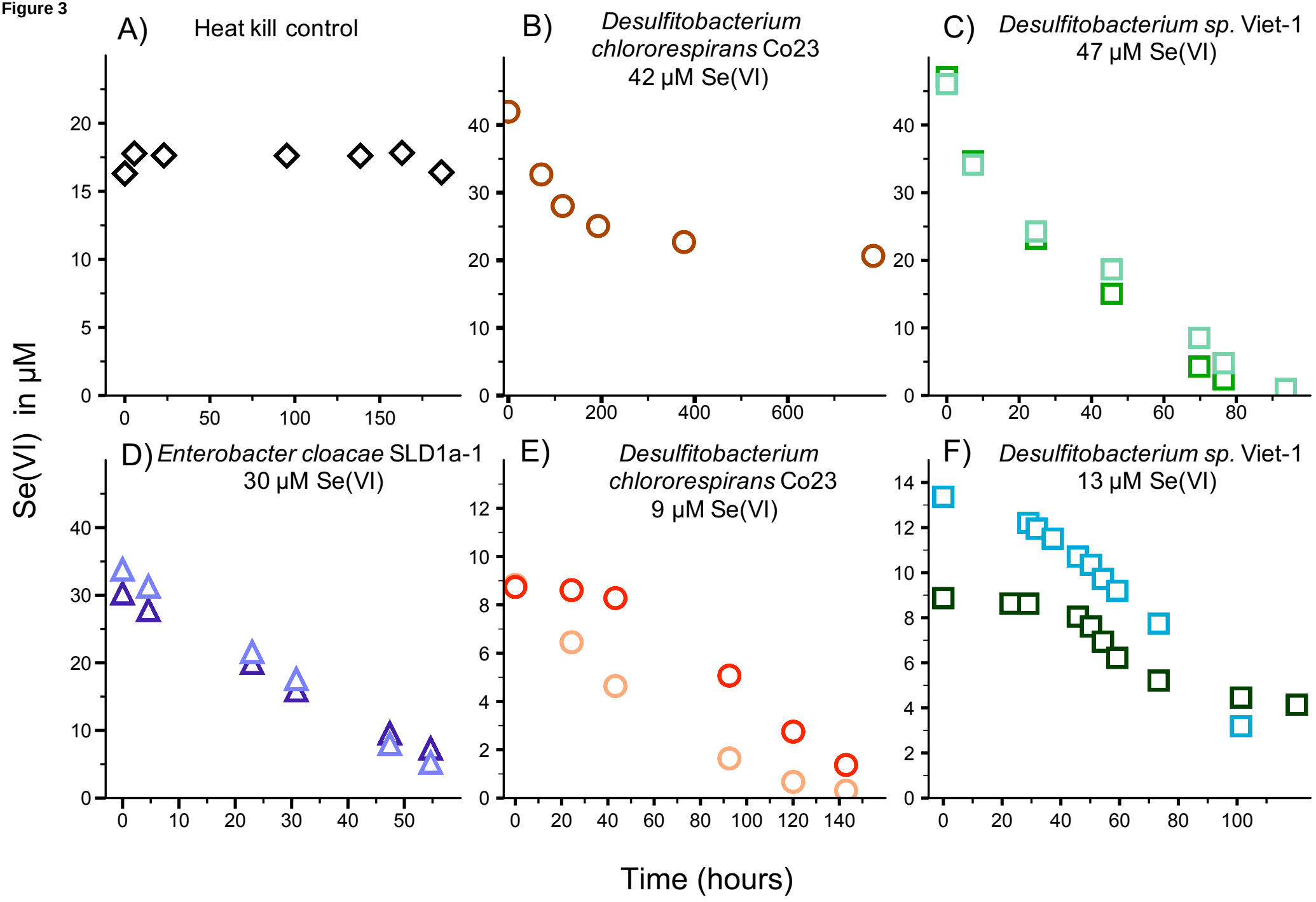

A) Heat kill control
B) Desulfitobacterium chlororespirans Co23
42 µM Se(VI)
C) Desulfitobacterium sp. Viet-1
47 µM Se(VI)
D) Enterobacter cloacae SLD1a-1
30 µM Se(VI)
E) Desulfitobacterium chlororespirans Co23
9 µM Se(VI)
F) Desulfitobacterium sp. Viet-1
13 µM Se(VI)
Se(VI) in µM
Time (hours)

Figure 4

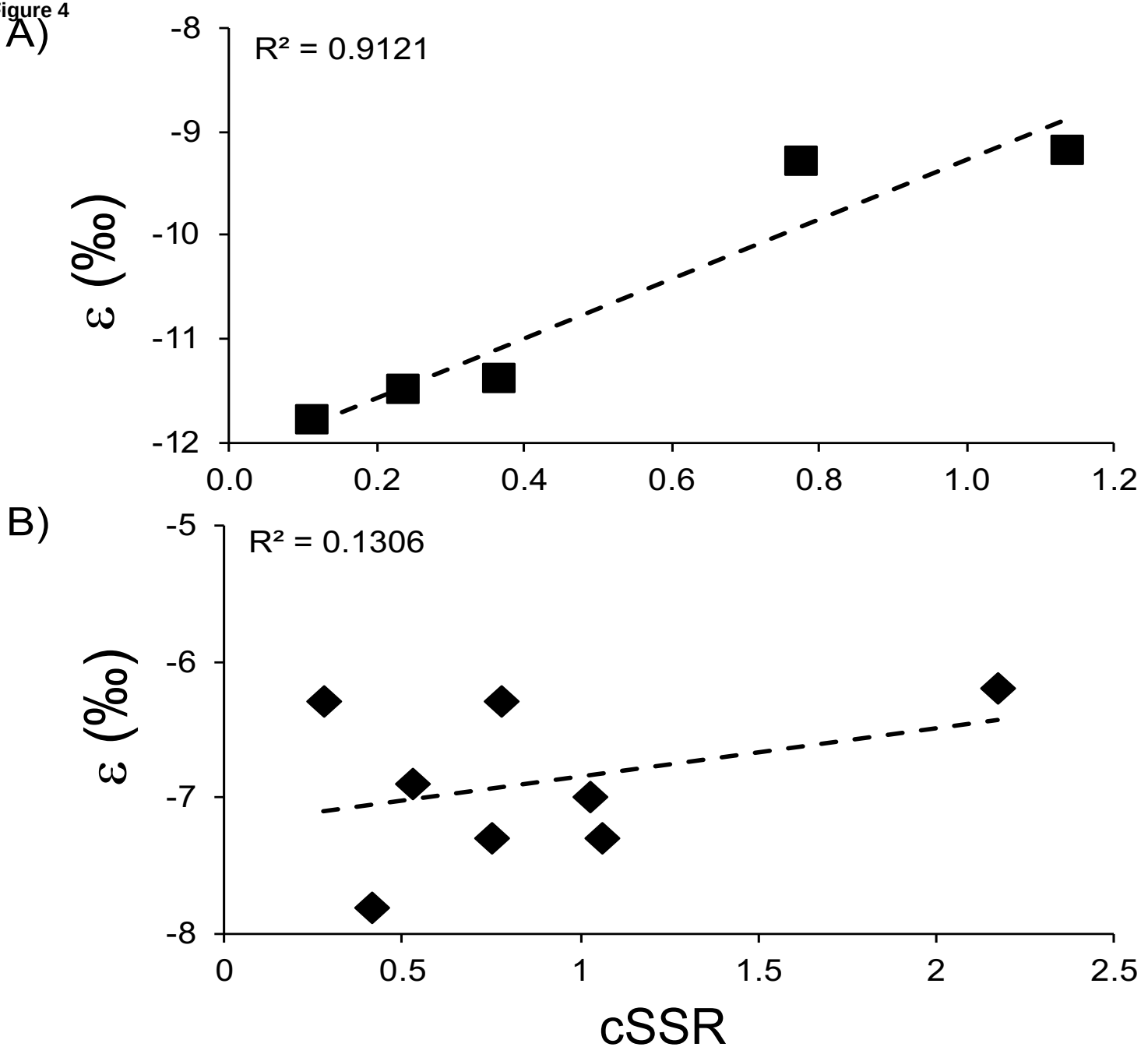

A)
R² = 0.9121
ε (‰)
-8
-9
-10
-11
-12
0.0
0.2
0.4
0.6
0.8
1.0
1.2
B)
R² = 0.1306
ε (‰)
-5
-6
-7
-8
0
0.5
1
1.5
2
2.5
cSSR

Figure 5

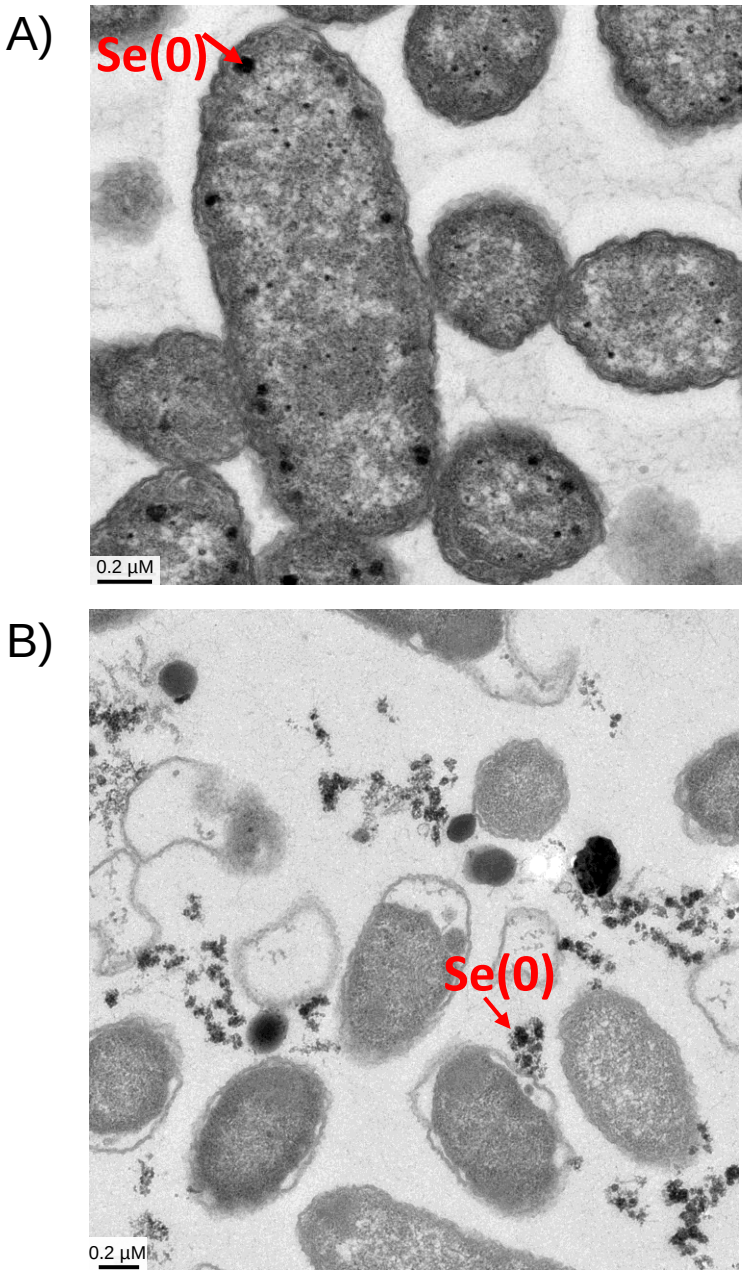


Figure 6

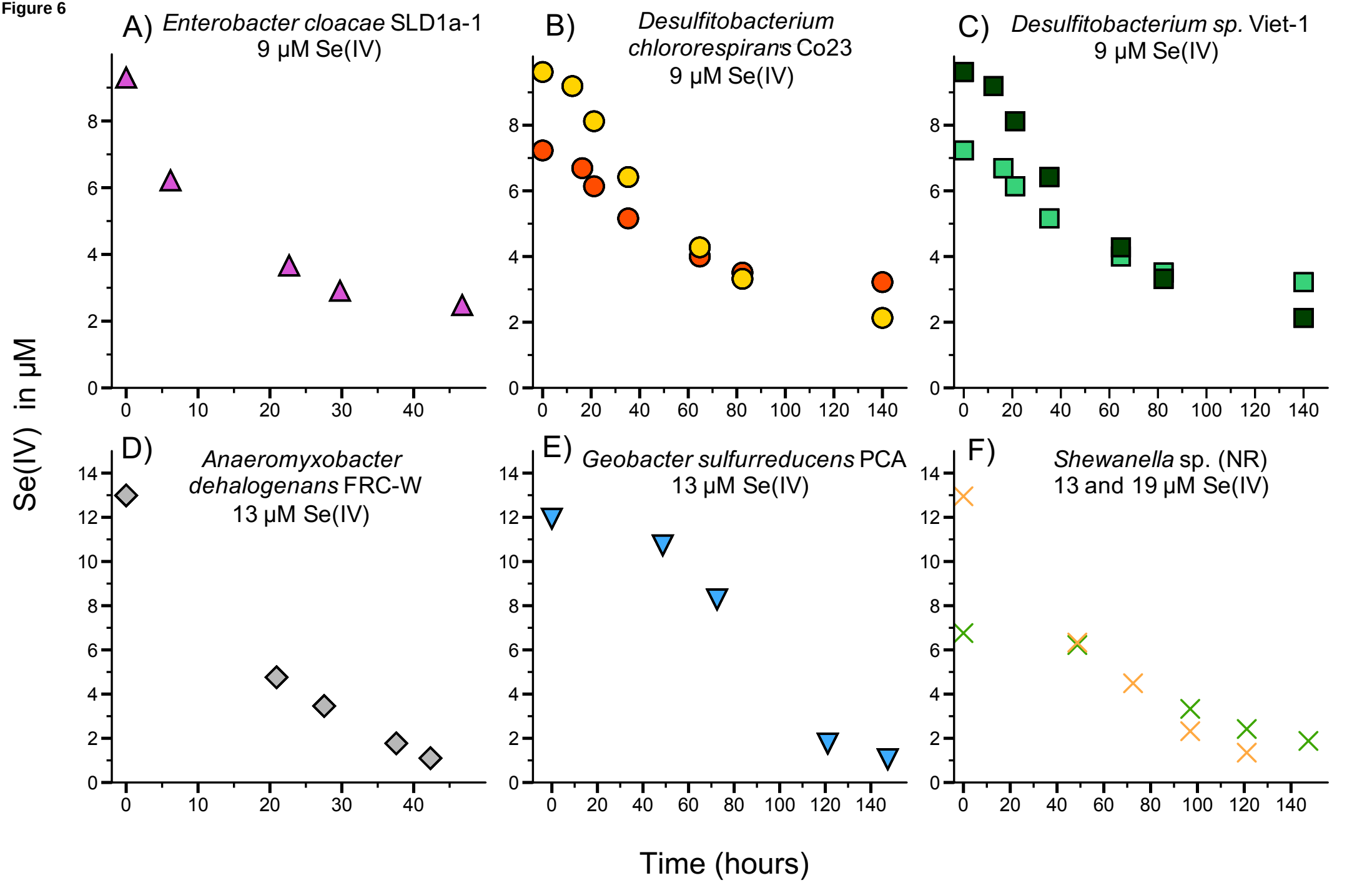
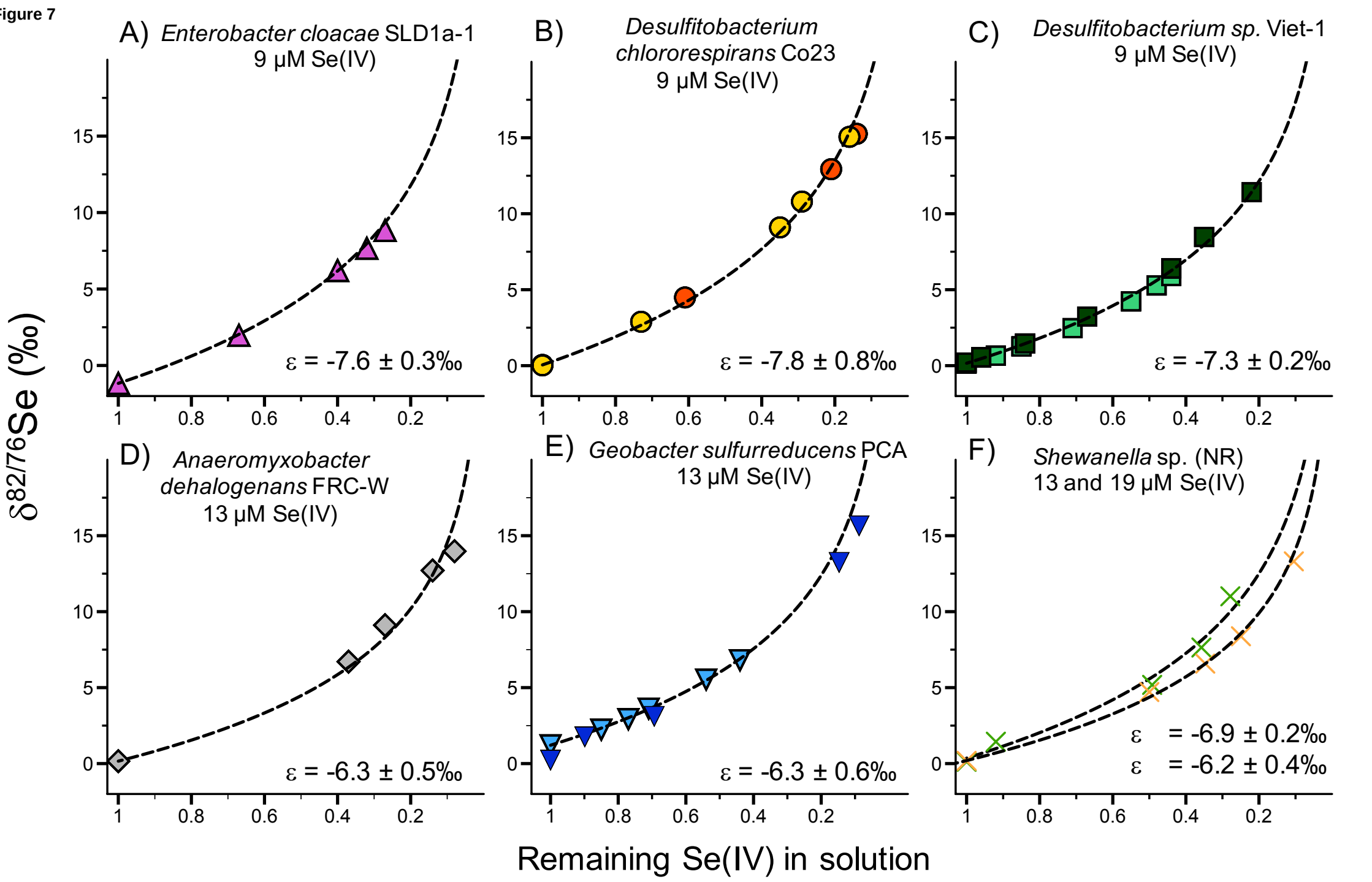

A) Enterobacter cloacae SLD1a-1
9 µM Se(IV)
B) Desulfitobacterium chlororespirans Co23
9 µM Se(IV)
C) Desulfitobacterium sp. Viet-1
9 µM Se(IV)
D) Anaeromyxobacter dehalogenans FRC-W
13 µM Se(IV)
E) Geobacter sulfurreducens PCA
13 µM Se(IV)
F) Shewanella sp. (NR)
13 and 19 µM Se(IV)
Se(IV) in µM
Time (hours)

Figure 7


A) Enterobacter cloacae SLD1a-1
9 µM Se(IV)
ε = -7.6 ± 0.3‰
B) Desulfitobacterium chlororespirans Co23
9 µM Se(IV)
ε = -7.8 ± 0.8‰
C) Desulfitobacterium sp. Viet-1
9 µM Se(IV)
ε = -7.3 ± 0.2‰
D) Anaeromyxobacter dehalogenans FRC-W
13 µM Se(IV)
ε = -6.3 ± 0.5‰
E) Geobacter sulfurreducens PCA
13 µM Se(IV)
ε = -6.3 ± 0.6‰
F) Shewanella sp. (NR)
13 and 19 µM Se(IV)
ε = -6.9 ± 0.2‰
ε = -6.2 ± 0.4‰
δ82/76Se (‰)
Remaining Se(IV) in solution

Electronic Annex

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Appendix

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