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Bioavailability of Particle-Associated Se to the Bivalve *Potamocorbula amurensis*

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Elemental selenium, Se(0), is a prevalent chemical form in sediments, but little is known about its bioavailability. We evaluated the bioavailability of two forms of Se(0) by generating radioisotopic ^{75}Se(0) through bacterial dissimilatory reduction of $^{75}SeO_3^{2-}$ by pure bacterial cultures (SES) and by an anaerobic sediment microbial consortium (SED). A third form was generated by reducing $^{75}SeO_3^{2-}$ with ascorbic acid (AA). Speciation determinations showed that AA and SES were >90% Se(0), but SED showed a mixture of Se(0), selenoanions, and a residual fraction. Pulse-chase techniques were used to measure assimilation efficiencies (AE) of these particulate Se forms by the bivalve *Potamocorbula amurensis*. Mean AE values were 3 ± 2% for AA, 7 ± 1% for SES, and 28 ± 15% for SED, showing that the bioavailability of reduced, particle-associated Se is dependent upon its origin. To determine if oxidative microbial processes increased Se transfer, SES ^{75}Se(0) was incubated with an aerobic sediment microbial consortium. After 113 d of incubation, 36% of SES Se(0) was oxidized to SeO_3^{2-}. Assimilation of total particulate Se was unaffected however (mean AE = 5.5%). The mean AE from the diatom *Phaeodactylum tricornutum* was 58 ± 8%, verifying the importance of Se associated with biogenic particles. Speciation and AE results from SED suggest that selenoanion reduction in wetlands and estuaries produces biologically available reduced selenium.

Introduction

Selenium (Se) is both a necessary micronutrient and, at environmentally realistic concentrations, a potent reproductive toxicant. Because Se can accumulate progressively through trophic orders, predators are particularly susceptible to Se toxicity (*1*). Adverse effects of Se on upper trophic level wildlife have been demonstrated in several instances, most notably in wildfowl in the Kesterson Reservoir, CA (*2*), and in fish in Belews Lake, NC (*3*). To quantify the Se bioaccumulation by upper trophic level organisms and to predict subsequent Se-related toxicity, it is necessary to identify factors controlling the initial transfer of Se into prey organisms. In estuarine systems, where diving ducks and bottom-feeding fish are at risk for selenium toxicity, benthic invertebrates are considered the most important and relevant prey organisms (*4*).

Ultimately the degree to which benthic invertebrates bioaccumulate Se depends on the chemical form in which it is present. In aquatic environments, Se can be present in several valence states, including reduced selenide (Se(−II)), elemental selenium (Se(0)), and two oxidized forms, Se(IV) and Se(VI), that occur as the selenoanions selenite (SeO_3^{2-}) and selenate (SeO_4^{2-}), respectively. Most valence states can be present in both dissolved and particulate phases (*5*). Dietary uptake can be the dominant pathway for Se bioaccumulation by invertebrates, including bivalves (*4*) and copepods (*6*), so the concentration and form of particulate selenium is particularly important. To date, most studies have examined only a few forms of particulate Se. For example, Wang and Fisher (*6*) measured the efficiency with which copepods assimilated Se that was associated with phytoplankton. Luoma et al. (*4*) found that the bivalve *Macoma balthica* assimilated elemental Se associated with sediment particles at 22% and phytoplankton associated Se at 88%, demonstrating the variability in bioavailability that can exist among different combinations of Se chemical species and particle types.

Elemental selenium is often the dominant Se species in sediments of estuarine (*7*) and freshwater (*8*) marshes. It can be precipitated in sediments by both bacterial dissimilatory reduction (*9*−*11*) and abiotic geochemical processes (*12*). The occurrence of multiple biogeochemical processes is of potential significance to sediment-ingesting infauna because each process occurs in different microenvironments and may yield different Se(0) particulate combinations and different Se(0) allotropes (*13*). The influence of these processes on Se bioavailability to food webs is important for evaluation of remediative procedures that remove soluble selenooxyanions from wastewater via Se(0) precipitation.

The effectiveness of remediative procedures lies in the notion that Se(0) remains immobile as long as anaerobic conditions persist. The consequences of dynamic biological and mechanical processes that could expose Se(0) to aerobic conditions were addressed recently by Dowdle and Oremland (*14*). By showing that oxidative microbial processes are capable of reoxidizing Se(0) to selenoanions, they demonstrated that Se(0) reduction is a reversible process. The majority of the reoxidized selenoanions remain sorbed to sediment particles (*14*), but the consequences of this phenomenon to particle-ingesting invertebrates is unknown.

We performed a series of experiments to determine the potential contribution of different forms of Se(0) to elevated selenium tissue concentrations (10−20 μg of Se g^{-1} dry wt) that are observed in the bivalve *Potamocorbula amurensis* in portions of San Francisco Bay (USGS, unpublished data). *P. amurensis* dominates many benthic habitats in San Francisco Bay and serves as food for fish and wildfowl in these areas (*15*). To determine the influence of the origin of Se(0) on its bioavailability to *P. amurensis*, we generated two forms of radioisotopic ^{75}Se(0) through bacterial dissimilatory reduction by reducing $^{75}SeO_3^{2-}$ using both a specific bacterial isolate and an anaerobic sediment microbial consortium. A third form was generated by reducing $^{75}SeO_3^{2-}$ with ascorbic acid. A second goal was to determine if oxidative microbial processes increased the transfer of selenium to invertebrates from sediments. To this end, ^{75}Se(0) was incubated in microbially active aerobic sediment slurries, which reoxidizes Se(0) to SeO_3^{2-}. The bioavailability of total particle-associated

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^{75}Se in these slurries to *P. amurensis* was measured at several time intervals.

Experimental Section

Experimental Animals. *Potamocorbula amurensis* were collected by hand from a mudflat in the Carquinez Strait of San Francisco Bay near Martinez, CA. Clams, ranging in shell length from 6.4 to 21.7 mm, were collected within 1 week of initiation of each of four experiments. Clams were maintained in aerated 12‰ seawater at 13−15 °C, and they received a suspension composed of sediment particles and the diatom *Phaeodactylum tricornutum* each day.

Particulate Forms of Selenium. Three basic categories of particle-associated selenium were generated and used in a series of four assimilation efficiency experiments with *P. amurensis*. Particle categories included (i) selenium associated with the diatom *Ph. tricornutum*, (ii) amorphous elemental selenium created through abiotic and microbial selenite reduction, and (iii) reoxidized elemental selenium. Elemental selenium was generated by two reductive mechanisms: chemical reduction using ascorbic acid and two microbially mediated reductive pathways. The first utilized a defined bacterial strain, and the other used endemic salt marsh microbial communities. The source of selenium for all particles was radiolabeled selenite ($Na_2{}^{75}SeO_3$) (Amersham, specific activity = 14.8 μCi/μg). Specific labeling procedures are described below. Assimilation efficiency of Se from diatoms was measured in three of the four experiments to determine inter-experimental variability.

Preparation of Particulate Selenium. *Phaeodactylum tricornutum*. Radiolabeled selenite was introduced to log-phase cultures of *Ph. tricornutum* in f/2 media that was prepared with 0.45 μm of filtered 12 ‰ seawater. Cultured algae were grown under 24 h light for 4−6 d and were suspended 2−3 times/d. Prior to each experiment, cell density was measured using a hemocytometer. Then, cells were filtered over a 0.45-μm membrane filter, washed with 10 mL of 0.2-μm filtered seawater, and resuspended in 200 mL of 0.2-μm filtered seawater. Subsamples of this suspension were filter-centrifuged (0.45 μm) to determine the partitioning of selenium between diatom cells and the dissolved phase.

Chemically Reduced Se(0). Abiotically generated elemental selenium was produced by reducing 0.5 L of a 3 μM solution of selenite (which included 2 μCi (3.1 μmol) of $Na_2{}^{75}SeO_3$) with 0.5 L of a 0.5 M solution of ascorbic acid. This process produced approximately 120 mg of a dark red precipitate, which was suspended in 30 mL of Milli-Q water. Partitioning of ^{75}Se in this suspension, measured by centrifuge filtration (0.45 μm), showed that >99% of activity was present in the particulate phase (*14*).

Pure Culture Bacterially Reduced Se(0). The first form of biologically generated elemental selenium was achieved by the reduction of selenite by the bacterium *Sulfurospirillum barnesii* following the methods described by Dowdle and Oremland (*14*). Briefly, a mixture of unlabeled 5 mM Na_2SeO_3 and 4.5 μCi (7 μmol) of $Na_2{}^{75}SeO_3$ was added to a 200-mL suspension of washed, nitrate-grown *S. barnesii* (SES-3 strain) cells in a crimped serum vial maintained under anaerobic conditions. Lactate (5 mM) was added as the electron donor. After 24 h, bacterial cells were removed by centrifuging the contents of the serum vial and resuspending the pellet sequentially in 0.1 M NaOH, 50% EtOH, pH 7.3 phosphate buffer, and Milli-Q water. The resulting bright orange pellet was resuspended in 30 mL of Milli-Q water and maintained under anaerobic conditions until use. Partitioning measurements, which were accomplished by centrifuge filtration (0.45 μm), showed that >99% of ^{75}Se activity was in the particulate phase.

Microbial Reduction with Anoxic Sediment. The second biological mechanism used the reductive potential of naturally occurring microorganisms endemic to salt marsh sediments. Sediment from the redox interface was collected from a San Francisco Bay mud flat near Palo Alto, CA, and press-sieved through a 63-μm screen to remove large organic particulate matter. A suspension consisting of 2 g (dry wt) of sediment and 50 mL of 15‰ seawater was placed into a crimped serum vial, and the headspace was evacuated with nitrogen. After shaking for 24 h, 1 μCi (50 μmol) of $Na_2{}^{75}SeO_3$ was added. Filter centrifugation (0.45 μm) showed that >99% of ^{75}Se activity was present in the particulate phase, demonstrating that soluble selenite was reduced to insoluble selenium.

Reoxidized Elemental Selenium. A second batch of *S. barnesii*-generated ^{75}Se(0) was produced following the methods described above, with the exception that a higher concentration of radioactive selenite was added to the cell suspensions. Specifically, a 25-mL cell suspension received 250 μL of 0.1 M Na_2SeO_3 and 2.2 mL of 0.9 μM Na_275SeO_3 (total radioactivity added = 287 μCi). The purification process described earlier yielded 2 mg of precipitate (specific activity = 0.14 μCi/μg), which was resuspended in 20 mL of Milli-Q water.

Two experiments were conducted to quantify the reoxidation of Se(0) to SeO_3^{2-} and SeO_4^{2-} (experiment 1) and to determine effects of this reoxidation on the bioavailability of total particle associated Se (experiment 2) (Table 1). Both experiments used aerobic sediment slurries following methods described by Dowdle and Oremland (*14*). Slurries were made by adding 1.5 g of Palo Alto salt marsh sediment (63 μm sieved) to a 30-mL Oakridge tube, adding 13.5 mL of 12‰ seawater, and resuspending until homogeneous. After 24 h, 15 μCi of ^{75}Se(0) was added to each tube. All tubes were provided with continuous end-over-end rotation in air at 12 rpm, which assured continuous aeration.

Experiment 1 consisted of triplicate unamended slurries and one autoclaved (1 h at 120 °C) slurry (Table 1). To tentatively identify the microbes involved in Se(0) oxidation, additional slurries were prepared as described above and amended with the following inorganic electron donors and organic substrates: Na_2S (20 mM), $MnSO_4$ (10 mM), $FeSO_4$ (10 mM), Na_2CO_3 (10 mM), sodium acetate (10 mM), and glucose (10 mM).

Experiment 2 consisted of two unamended slurries (ReOx1 and ReOx2) that were initiated 2 weeks apart (Table 1). These slurries were used to measure the effect of the Se(0) reoxidation process on total particle-associated Se bioavailability. Bioavailability was determined by measuring the efficiency with which the bivalve *P. amurensis* assimilated ^{75}Se from slurry particles (see feeding protocols below).

Speciation of Particle-Associated Selenium. The chemical species of particle-associated selenium was measured using two protocols. Protocol 1 measured reoxidation of ^{75}Se(0) in oxidative sediment slurries, and protocol 2 measured selenium speciation within all particles fed to *P. amurensis* except for diatoms.

For protocol 1, slurry samples (0.5 mL) were filter centrifuged to separate soluble and insoluble selenium. Pellets were washed with 0.1 N NaOH and filter centrifuged again. Separation of SeO_3^{2-} and SeO_4^{2-} from other forms of soluble Se was performed by measuring the activity of eluted fractions from a high-performance liquid chromatograph (*14*).

For protocol 2, three 1-mL samples were removed from each feeding suspension, filtered on a 0.45-μm glass fiber filter, and rinsed with 10 mL of seawater. Filters were immediately frozen at −80 °C until analyzed (within 2 months). Methods originally described by Cutter (*16*) and Velinsky and Cutter (*7*) were used to separate particle-associated radiolabeled selenium into three operationally defined species: selenoanions (i.e., SeO_3^{2-} + SeO_4^{2-}), ele-

TABLE 1. Description of Treatments Used in Sediment Slurry Experiments with Bacterially Produced $^{75}Se(0)$[a]

treatment	*n*	incubation duration (d)	purpose/use	speciation protocol[b]
		Time Course Experiment		
time course slurries	3	38	measurement of microbiologically mediated selenoanion oxidation over time	1
autoclaved slurry	1	38	control	1
amendment slurries	1/substrate	4	stimulation of microbial reoxidation by Na_2S (20 mM), $MnSO_4$ (10 mM), $FeSO_4$ (10 mM), Na_2CO_3 (10 mM), sodium acetate (10 mM), and glucose (10 mM)	1
		Assimilation Efficiency Experiments		
ReOx1 slurry	1	113	measurement of Se speciation and ^{75}Se assimilation efficiency by *P. amurensis* after 29, 56, and 113 d of oxidation	2
ReOx2 slurry	1	99	measurement of Se speciation and ^{75}Se assimilation efficiency by *P. amurensis* after 15, 42, and 99 d of oxidation	2

[a] Sediment slurries were prepared by adding bacterially precipitated $^{75}Se(0)$ to 1.5 g of oxidized sediment in 13.5 mL of seawater. [b] Speciation protocol 1 used 0.1 N NaOH to extract particle-associated selenoanions followed by separation of Se(IV) and Se(VI) by high-performance liquid chromatography. Speciation protocol 2 used 0.1 N NaOH to extract particle-associated selenoanions and 1 M Na_2SO_3 to extract Se(0).

mental selenium (Se(0)), and organoselenium. Selenoanions and Se(0) were determined directly by measuring the fraction of total Se in filtered particles that was extracted with NaOH (0.1 N) and Na_2SO_3 (1 M), respectively. For each extraction, between 1.0 and 2.7 mg of particles were placed within the filter portion of a 0.45-μm centrifuge filter device. After 0.4 mL of either 0.1 N NaOH or 1 M Na_2SO_3 was added to triplicate tubes per particle type, the devices were vortexed for 2 min until a homogeneous suspension was achieved. The vortexing was repeated several times over the course of 24 h. Dissolved and particulate phases were separated by filter centrifugation, and the activity within each phase was measured. For Na_2SO_3 extractions, the dissolved phase contained $^{75}Se(0)$. To separate any organoselenium that may have been solubilized by NaOH, the dissolved phase of this extraction was diluted with 5 mL of Milli-Q water (adjusted to pH 1.6) and passed through a mini-prep column packed with XAD-8 resin. Columns were rinsed with pH 1.6 Milli-Q water, and the combined eluent and rinse volumes were measured for radioactivity. This measurement represented sorbed selenoanions. Organoselenium was measured indirectly as the difference between total selenium and the sum of the selenoanions and Se(0) (*7*).

Bioavailability Measurements. Bioavailability of particle-associated ^{75}Se to *P. amurensis* was measured using a pulse-chase procedure modified from methods described by Decho and Luoma (*17, 18*). For each treatment, approximately 30 clams were placed in a 1-L beaker containing 800 mL of filtered (0.45 μm) seawater ($T = 13-15$ °C, $S = 12$‰). Clams were fed by adding 200 mL of a suspension of experimental particles so that the final concentration was 100 mg/L for abiotic particles and 1.5×10^5 cells/mL for *Ph. tricornutum*. After 40 min, clams were removed from radiolabeled food and placed into scintillation vials as replicates that were composed of two (large clams, shell length = 17.0−21.7 mm) or three (small clams, shell length = 6.5−13.0 mm) clams per replicate. The activity of ^{75}Se ingested by clams was measured for 1 min at 75−450 keV using a γ-counter. After γ-counting, clams were distributed into two groups denoted T_0 and T_F. Each group was composed of at least five replicates (including three small and two large clam replicates) for a minimum of 19 clams per group. T_0 clams were immediately frozen at −80 °C. T_F clams were placed as replicates in separate depuration chambers within 5 min of counting.

All T_F clams were measured for ^{75}Se activity at $T = 3$, 6, 9, 12, 24, and 48 h or until ^{75}Se activity stabilized. Activity of ^{75}Se in feces was also measured at each time point. Throughout the depuration period, clams received a ration consisting of *Ph. tricornutum* and suspended sediment. After clam activity stabilized (indicating that all of the ingested radioactive particles had been egested as feces), clams were frozen at −80 °C.

After clams were shucked, tissues and shells and were placed in separate scintillation vials (vials holding tissue were preweighed). Subsequent γ-counting yielded shell:tissue ratios of ^{75}Se activity for T_0 clams. Shell:tissue ratios were applied to initial whole clam T_F ^{75}Se values to provide an estimate of tissue activity at T_0.

Assimilation Efficiency. Assimilation efficiency was calculated in two ways. The first approach used the following formula:

$$AE_1 = (A_{T=F}/A_{T=0}) \times 100 \quad (1)$$

where $A_{T=F}$ is the final measured tissue activity and $A_{T=0}$ is the estimated initial tissue activity.

The second approach used the following formula:

$$AE_2 = (A_{T=F}/(A_{T=F} + A_{feces})) \times 100 \quad (2)$$

where $A_{T=F}$ is the final measured tissue activity and A_{feces} is the summary of feces activity collected throughout the depuration period.

Results

Speciation. Operationally defined measurements of Se speciation (i.e., Na_2SO_3 and NaOH extractions) showed that compositions of selenium within the three types of Se(0) particles were distinctly different (Table 2). Over 94% of activity in abiotically generated particles (AA) was present in Na_2SO_3 extractions and was therefore operationally defined as Se(0). Likewise, <1% of AA particles were soluble in NaOH (operationally defined as selenoanions). Compared to AA particles, the fraction of Se(0) in particles generated by pure bacterial cultures (SES) was lower (81.6%), but these particles also contained a very small fraction of selenoanions (0.01%) (Table 2). From these results, AA and SES appear to be composed of pure Se(0), but the lower Na_2SO_3 solubility of SES suggests that SES may be comprised of a more recalcitrant Se(0) allotrope. The sediment slurry generated mostly Se(0), but other forms were also present. The greatest difference among the forms was the more pronounced residual phase

TABLE 2. Selenium Extraction and Assimilation Efficiency from Three Forms of Elemental Selenium Formed by Reducing Selenite with Ascorbic Acid (AA), by the Bacterium *Sulfurospirillum barnesii* (SES), and in Anaerobic Sediment Slurries (SED)[a]

particle type	SeO_3^{2-} + SeO_4^{2-} [b]	Se(0)[c]	residual Se[d]	assimilation efficiency
ascorbic acid-reduced Se(0)	0.5 (0.1)	94.7 (0.7)	4.8	2.6 (1.5)^A
SES-reduced Se(0)	0.01 (0)	81.6 (2.1)	18.4	3.3 (1.2)^A
sediment-reduced Se(0)	7.5 (0.8)	60.1 (2.6)	32.4	28.3 (14.8)^B

[a] Separate aliquots of each particle type were extracted with 0.1 N NaOH or 1 M Na_2SO_3. Mean percent of extracted SeO_3^{2-} + SeO_4^{2-} (from 0.1 N NaOH extraction) and Se(0) is presented with standard deviation ($n = 3$) in parentheses. Mean assimilation efficiency (%) by the bivalve *Potamocorbula amurensis* is presented for each particle with standard deviation ($n = 5$) in parentheses. Assimilation efficiency was compared among particles using Tukey's honestly significant difference test. Mean values with the same letter are not significantly different at $\alpha = 0.05$. [b] Obtained by initial extraction with 0.1 N NaOH followed by cleanup using XAD-8 resin column. [c] Obtained by extraction with 1 M Na_2SO_3. [d] Difference between total mean selenium minus the combination of mean SeO_3^{2-} + Se(VI) and mean Se(0).

TABLE 3. Selenium Speciation and Assimilation Efficiency from Two Aerobic Sediment Slurries (ReOx 1 and 2) over Three Time Periods[a]

particle type	time of oxidation (d)	SeO_3^{2-} + SeO_4^{2-} [b]	Se(0)[c]	residual Se[d]	assimilation efficiency
ReOx 2	15	18.9 (0.2)	84.3 (0.7)	0	4.5 (2.3)
ReOx 1	29	24.4 (0.5)	81.0 (0.4)	0	3.7 (2.5)
ReOx 2	42	22.5 (1.3)	80.9 (0.3)	0	3.6 (0.6)
ReOx 1	56	27.3 (0.5)	80.2 (0.4)	0	4.8 (2.2)
ReOx 2	99	30.3 (1.5)	75.5 (1.9)	0	5.9 (1.3)
ReOx 1	113	36.0 (1.0)	75.7 (0.7)	0	5.5 (0.1)

[a] Separate aliquots of each particle type were extracted with 0.1 N NaOH or 1 M Na_2SO_3. Mean percent of extracted SeO_3^{2-} + SeO_4^{2-} (from 0.1 N NaOH extraction) and Se(0) are presented with standard deviation ($n = 3$) in parentheses. Mean assimilation efficiency (%) by the bivalve *Potamocorbula amurensis* is presented for each time period with standard deviation ($n = 5$) in parentheses. Assimilation efficiency was not significantly different across time within each slurry. [b] Obtained by initial extraction with 0.1 N NaOH followed by cleanup using XAD-8 resin column. [c] Obtained by extraction with 1 M Na_2SO_3. [d] Difference between total mean selenium minus the combination of mean SeO_3^{2-} + Se(VI) and mean Se(0).

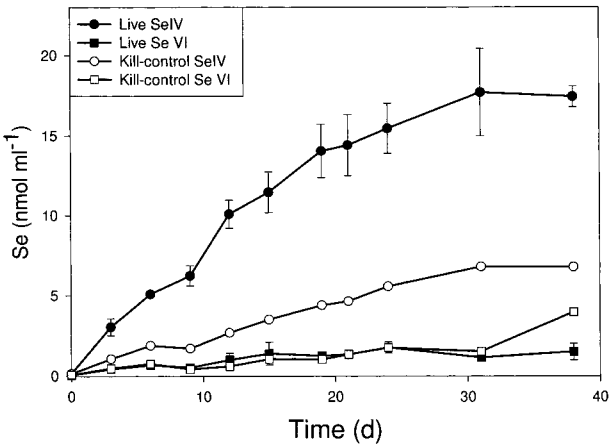

FIGURE 1. Oxidation of $^{75}Se(0)$ to $^{75}SeO_3^{2-}$ and $^{75}SeO_4^{2-}$ in aerobic salt marsh sediment slurries. Triplicate "live" slurries and one autoclaved "kill-control" slurry were subjected to continuous end-over-end rotation for up to 38 d. $^{75}SeO_3^{2-}$ and SeO_4^{2-} were separated with HPLC, and concentrations were measured radiometrically.

in the sediment slurry. A total of 32% of the Se in the slurry was soluble in neither NaOH or Na_2SO_3 (Table 2).

Speciation measurements conducted on aerobic sediment slurries that were fed to *P. amurensis* indicated that Se(0) reoxidation progressed over time. The proportion of selenoanions in these slurries increased to 19% at 15 d and 36% at 113 d. The proportion of Se(0) decreased accordingly (Table 3).

Measurements of selenooxyanion concentrations in aerobic sediment time course slurries also demonstrated Se(0) reoxidation. The proportion of sorbed SeO_3^{2-} increased over the 38-d incubation period at a higher rate in live slurries than in the autoclaved slurry (Figure 1), showing that this was a biologically accelerated process. In live slurries, production of selenate was lower than that of selenite. This indicates that selenite, an intermediate in the production of selenate, was unavailable to microbes, probably because it was tightly sorbed to iron oxides on sediment particles (*19*). In amendment experiments conducted with live slurries, the production of soluble selenium increased more than 2-fold with the addition of sulfide (data not shown), but no change was observed with the addition of other inorganic and organic stimulants. Stimulation by sulfide addition indicates that sulfide oxidizers were responsible for the oxidation of Se(0) (*14*).

Variability among Experiments. Time course measurements during egestion showed that clams had egested the majority of radiolabeled food after 24 h and that clam activity at 48 h consisted of assimilated ^{75}Se. Activity in clam tissues stabilized by 48 h, and ^{75}Se activity in feces was minimal during the last 24 h period (Figure 2). Selenium assimilation efficiency from *Ph. tricornutum* was not measured in one of the four experiments as diatoms grew poorly. Assimilation efficiencies from *Ph. tricornutum* were not significantly different among the three remaining experiments regardless of how assimilation efficiency was calculated ($F_{1,13} = 2.98$, $p = 0.086$ for AE_1, $F_{1,13} = 0.932$, $p = 0.418$ for AE_2). The inter-experimental agreement for Se shows that the assimilation process was consistent throughout all experiments for *P. amurensis* and indicates that inter-experimental comparisons of assimilation efficiencies are warranted. For the AE_1 calculation method, mean assimilation efficiencies in these experiments ranged from 49.5 to 59.0% (Table 4). Mean assimilation efficiencies were consistently higher for the AE_2 calculation method, ranging from 61.9% to 68.3% (Table 4). The difference between AE_1 and AE_2 is probably due to desorption of Se from fecal material and is not unusual for Se and other weakly bound elements (*17*, *18*). For this reason, AE_1 will be used for interparticle comparisons. No relationship was found between mass per clam and Se assimilation from phytoplankton or Se(0) particles. Therefore, no attempt was made to normalize bioavailability results to clam size.

Se(0) Bioavailability. Time course measurements of ^{75}Se activity in clams showed that clams continued to egest ascorbic acid- and *S. barnesii*-reduced Se(0) particles for up to 96 h (Figure 2). *P. amurensis* assimilated ^{75}Se with significantly different efficiencies among treatments ($F_{2,13} = 15.663$, $p < 0.001$) (Table 2). Selenium assimilation efficiencies were statistically similar from Se(0) particles generated in isolation by ascorbic acid (mean Se AE = 2.7% ± 1.5) and *S. barnesii* (mean Se AE = 3.3 ± 1.2) reduction. Assimilation efficiencies were much greater for sediment-reduced Se(0)

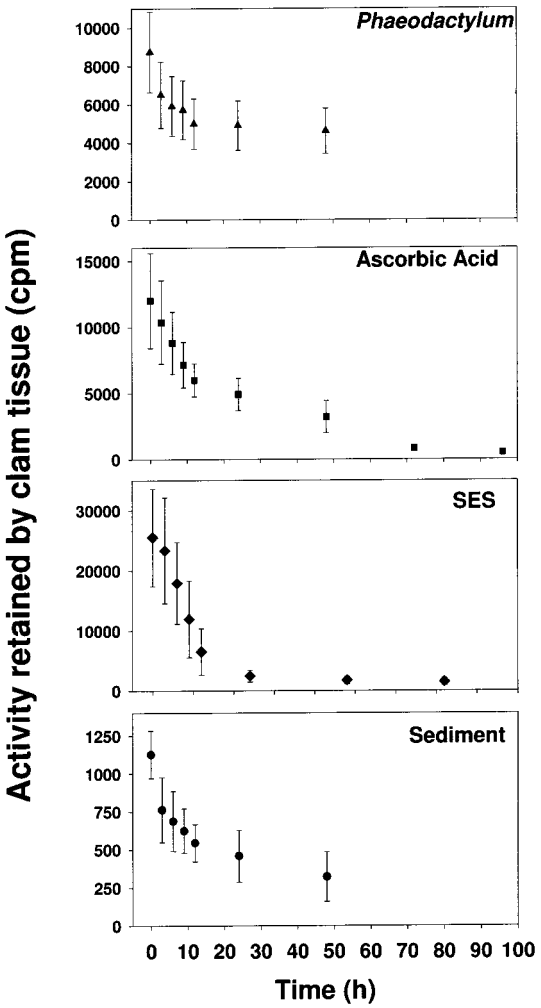

FIGURE 2. Time courses showing ^{75}Se activity in the bivalve *Potamocorbula amurensis* that were fed four ^{75}Se-enriched particle types. Particle types included (a) the diatom *Phaeodactylum tricornutum*, (b) ascorbic acid reduced Se(0), (c) Se(0) reduced through dissimilatory reduction by pure cultures of the bacterium *Sulfurospirillum barnesii* (SES) and (d) Se(0) reduced by anaerobic salt marsh sediment slurries.

(mean Se AE = 28.3% ± 14.8) than for the other two treatments (Table 2).

Bioavailability of Reoxidized Se(0). Although the proportion of oxidized selenoanions associated with sediment particles in oxidized sediment slurries increased substantially from the first to the third feeding experiment, ^{75}Se assimilation efficiencies from these experiments were not significantly different ($F_{5,24}$ = 2.09, p = 0.101) (Table 3). Assimilation efficiency did not show a significant relationship with the proportion of selenooxyanions in particulate matter ($F_{1,4}$ = 3.61, p = 0.130). Assimilation efficiencies ranged from 3.7% (SD = 0.2) after 29 d reoxidation to 5.9% (SD = 1.3) for 99 d of reoxidation (Table 3). The small variation observed for assimilation efficiency indicates that the biogeochemical transformation of Se(0) to selenoanions had little consequence to bulk selenium bioavailability and that the selenoanions must be tightly sorbed to sediment particles.

Discussion

Bioavailability of Particulate Se. Differences in the efficiency with which the bivalve *P. amurensis* assimilated Se from different particle types were explained by the chemical form of selenium within each particle. Two forms of pure elemental selenium showed the lowest bioavailabilities, whereas selenium associated with the diatom *Ph. tricornutum* was the most bioavailable. Bioavailability of sediment-reduced selenium, which exhibited a transitional composite of selenium chemical forms, was between these two extremes. In aerobic sediment slurries, approximately 40% of elemental selenium was oxidized to adsorbed selenoanions after a period of 113 d, but this biotransformation did not affect total particle-associated assimilation efficiency.

TABLE 4. Mean Selenium Assimilation Efficiency (AE) from the Diatom *Phaeodactylum tricornutum* by the Bivalve *Potamocorbula amurensis* from Three Experiments[a]

date	AE_1 (%)	AE_2 (%)
2/19/99	53.2 (7.9)	68.3 (10.9)
4/27/99	59.0 (5.7)	66.3 (5.1)
7/20/99	49.5 (3.8)	61.9 (4.5)

[a] AE was calculated two ways. AE_1 was calculated as the ratio of assimilated to ingested ^{75}Se activity. AE_2 used a mass balance approach as the ratio of assimilated ^{75}Se activity to assimilated activity plus activity in feces. Numbers in parentheses represent the standard deviation (n = 5). Assimilation efficiency was not significantly different (α = 0.05) among the three experiments for each method.

Bacterial dissimilatory reduction is coupled to the oxidation of organic material by a range of bacteria in anoxic sediments and is probably responsible for the majority of Se(0) that is present in sediments (*10*, *14*, *20*). However, Se(0) can differ in chemical form and bioavailability, depending upon the pathways by which it is reduced. Chemical reduction by ascorbic acid produced a precipitate that was chemically defined as pure elemental selenium; low assimilation efficiencies by clams suggest that if this abiotically reduced elemental selenium occurs in nature (*12*), it is of little biological significance. Interestingly, particulate selenium that was reduced by pure cultures of *S. barnesii* was similar to the abiotically reduced elemental selenium in terms of chemical and biological characterizations. Assimilation efficiencies were low and SES Se(0) was also insoluble in NaOH. Thus, pure elemental selenium was not biologically available regardless of how it was generated.

Assimilation efficiencies of 28% were found when Se was transformed in anaerobic slurries composed of San Francisco Bay sediment. These results are close to those measured in the one other study of Se(0) bioavailability from slurries. Luoma et al. (*4*) determined that the bivalve *Macoma balthica* assimilated 22% of ^{75}Se from anaerobic salt marsh sediment slurries. Hansen et al. (*21*) suggested that (constructed) wetlands comprise "an environmentally friendly way of cleaning up selenium from effluents". Most of the selenium removed from an effluent in a wetland is trapped in sediments, probably by dissimilatory reduction. Although pure Se(0) may not be an important source of Se for consumer organisms such as *P. amurensis*, the particle-associated selenium produced in wetlands is not pure Se(0). If sediment particle ingestion rates for bivalves range from 0.25 to 1.0 g of sediment (g of tissue)$^{-1}$ day^{-1} (*4*), sediment-associated Se can be an important source for food webs (e.g., bivalves) associated with these systems. Thus, wetland-trapped selenium should be viewed as a potential source of selenium for biota rather than an "immobile" form.

The mixture of chemical species of selenium in the slurries included selenoanions, elemental selenium, and a residual fraction. This speciation mix of Se(0) = 60.9%, selenoanions = 7.5%, and organoselenium = 31.6% matched well with speciation of Se within natural sediments sampled in estuarine (*7*, *22*) and freshwater (*8*) marshes. The residual fraction in this speciation scheme is defined to be organoselenium (*7*). In slurries, sediment-associated microbes might biotransform a portion of the original selenite as organoselenide through assimilatory mechanisms. However,

the presence of extractable quantities of selenoanions might also indicate that reduction of SeO_3^{2-} in these slurries was incomplete. Another alternative is that the residual selenium may have been a recalcitrant allotrope of elemental selenium.

The efficiency with which *P. amurensis* assimilated Se from the sediment slurry might be used as a probe to independently test these possibilities. If assimilation efficiency from different selenium species is assumed to be additive, then assimilation efficiency of total sediment-associated selenium in the sediment slurry can be calculated as

$$AE_{Se-total} = (AE_{Se(0)} \times \%\ Se(0)) + (AE_{selenoanions} \times \%\ selenoanions) + (AE_{organoselenium} \times \%\ organoselenium) \quad (3)$$

We can employ AE values for individual Se species of Se(0) = 4% (AE from AA and SES), selenoanions = 11% (AE from ReOx 2; 99 d old, assuming 100% of Se assimilated from this ReOx sediment was selenoanions), and organoselenium = 53% (*Ph. tricornutum*). On the basis of the proportional chemical speciation measured in this study, overall Se AE is predicted to be 20%, a value that is 8% less than the measured value of 28%. On the other hand, if the proportion of residual selenium is considered to be a recalcitrant form of elemental selenium, it would not be assimilated efficiently. An AE value of 4% would be appropriate for that fraction (AE from AA and SES). The predicted AE for total selenium from this scenario is 6.7%. Thus, the bioavailability of the residual selenium in the slurries more closely resembles organoselenium than it does an allotrope of elemental selenium. The calculation also is consistent with the organoselenium being the predominant bioavailable form in the mix.

Consequences of Se(0) Reoxidation. Results from aerobic sediment slurries show that Se(0) reintroduced to atmospheric oxygen will be subject to microbial and abiotic oxidation, which will result in net selenite production. These findings are consistent with Dowdle and Oremland's (*14*) proposed selenium cycle for sediments, where they demonstrated that the chemical form of Se is controlled by the nature of the ambient microbial community (i.e., either selenooxyanion reducers or elemental selenium oxidizers), which in turn is dictated by redox conditions.

Dowdle and Oremland (*14*) showed that Se(0) oxidation by pure bacterial cultures (i.e., in the absence of mineral particles) culminates in the oxidation of SeO_3^{2-} to SeO_4^{2-}. However, the reaction was limited to Se(0) oxidation to SeO_3^{2-} in soil slurries. Absorption of oxidized SeO_3^{2-} onto sediment particles appeared to limit Se bioavailability to microbes and to prevent the production of SeO_4^{2-}. One explanation is that SeO_3^{2-} is unavailable to microbes when it strongly sorbs to iron and manganese oxyhydroxides (*19*). Extractions showed that Se(0) oxidation products were generated in our reoxidation experiment. Direct tests of assimilation efficiency showed that those products were unavailable to bivalves, consistent with a low bioavailability of sorbed SeO_3^{2-}. In contrast, bivalves appear to more efficiently assimilate particle-associated SeO_3^{2-} after short-term incubations. *P. amurensis* assimilated 40% of SeO_3^{2-} that was incubated with aerobic sediment (B.-G. Lee, personal communication). *Mytilus edulis* assimilated 28% of SeO_3^{2-} that was incubated with glass beads (*23*) and from 28 to 34% of SeO_3^{2-} incubated with Long Island Sound seston (*24*). The form of Se was not determined in those experiments, but it does appear that the bioavailability of particle-associated selenoanions may depend on the original transformation product.

Application of Results Observed Se Tissue Concentrations in *P. amurensis*. *P. amurensis* exhibits high selenium concentrations in northern San Francisco Bay (SFB). Consolidation of assimilation efficiency results from all experiments (Figure 3) provides some insight into the source of this selenium and illustrates the importance of understanding the biogeochemical forms of Se. High assimilation efficiencies from the diatom *Ph. tricornutum* (Figure 3) verifies the potential importance of phytoplankton as a source, as reported in other studies (*4*, *25*, *26*). However, primary productivity is thought to be insufficient to account for rates of secondary production exhibited by *P. amurensis* in northern SFB (*27*). Assimilation efficiency results appear to rule out pure Se(0) and adsorbed selenoanions as significant sources. Assimilation efficiency results from selenium generated in anaerobic sediment (wetlands) show that this is another possible source, primarily during periods of low primary productivity; but links between wetlands and suspended material in the Bay are not well-known. Selenium bioavailability to *P. amurensis* from bacterioplankton, microzooplankton, and nonliving organic matter have not yet been investigated.

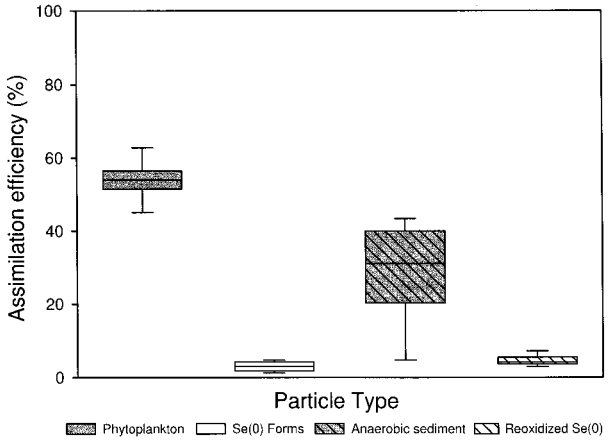

FIGURE 3. Summary of selenium assimilation efficiencies (AE) by *Potamocorbula amurensis* from all experimental particles. Particles include the diatom *Phaeodactylum tricornutum*, pure elemental selenium particles, reduced selenium in an anaerobic sediment slurry, and reoxidized elemental selenium in an aerobic sediment slurry. Data from three experiments with *Ph. tricornutum* was pooled for the phytoplankton plot. The pure elemental selenium plot represents pooled AE data from both ascorbic acid- and *Sulfurospirillum barnesii*-reduced particles. The reoxidized selenium plot represents pooled AE data from six experiments with two separate aerobic sediment slurries. The anaerobic sediment-reduced plot uses data from one experiment. Median, upper and lower quartile, and the upper and lower range of AE values are represented by lines within box plots, box boundaries, and whisker bars, respectively.

Implications for Se Remediation. Strategies to prevent, remediate, or minimize broad-scale selenium contamination and toxicity would be most effective if they could trap selenium in a form that is not bioavailable. Several bioremediative approaches have been proposed, including volatilization of Se via salt marsh microbes (*28*) and vascular plants (*29*) and precipitation via anaerobic bacteria (*20*). Reduction of selenoanions by pure cultures of *S. barnesii* might be another Se minimization strategy to consider, if a suitable engineering approach could be found. First, the process through which mobile SeO_3^{2-} and SeO_4^{2-} are precipitated as immobile Se(0) is more rapid than volatilization via plants (*20*). Second, this process produces Se in its least bioavailable form; even if it is reoxidized, the Se(0) precipitate produced by *S. barnesii* should remain nonbioavailable to invertebrates based upon our assimilation efficiency results.

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