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# Microbial Oxidation of Elemental Selenium in Soil Slurries and Bacterial Cultures

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The microbial oxidation of elemental selenium [Se(0)] was studied by employing $^{75}$Se(0) as a tracer. Live, oxic soil slurries demonstrated a linear production of mostly Se(IV), with the formation of smaller quantities of Se(VI). Production of both Se(IV) and Se(VI) was inhibited by autoclaving, formalin, antibiotics, azide, and 2,4-dinitrophenol, thereby indicating the involvement of microbes. Oxidation of Se(0) in slurries was enhanced by addition of acetate, glucose, or sulfide, which implied involvement of chemoheterotrophs as well as chemoautotrophic thiobacilli. Cultures of *Thiobacillus* ASN-1, *Leptothrix* MnB1, and a heterotrophic soil enrichment all oxidized Se(0) with Se(VI) observed as the major product rather than Se(IV). This indicated that microbial oxidation in soils is partly constrained by the adsorption of Se(IV) onto soil surfaces. Rate constants for unamended soil slurry Se(0) oxidation ranged from 0.0009 to 0.0117 day$^{-1}$ which were 3−4 orders of magnitude lower than those reported for dissimilatory Se(VI) reduction in organic-rich, anoxic sediments.

## Introduction

Se contamination of aquatic ecosystems can arise from mining activities, the combustion of fossil fuels (*1*), and from the cultivation of seleniferous soils. In the latter case, toxic and teratogenic effects upon wildlife were attributed to the introduction of Se oxyanions derived from agricultural drainage into man-made marshes such as the Kesterson Wildlife Refuge in California (*2, 3*). Because these marshes were underlain by anoxic sediments, research emphasized the reductive reactions whereby selenate ($SeO_4^{2-}$; or Se[VI]) and selenite ($SeO_3^{2-}$; or Se[IV]) became immobilized as elemental selenium (Se[0]). Elemental selenium is the major repository for selenium both in contaminated (*4*−*8*) as well as pristine (*9*) sediments. Although Se(0) is bioavailable to suspension feeders (*10*), the bulk of the Se(0) remains immobilized within sediments so long as anoxic conditions persist (*5*). Important agents of the reductive portion of the Se cycle are the selenate-respiring bacteria which oxidize organic matter by coupling electron flow to the reduction of selenate to Se(0) (11−15). Field studies with $^{75}$Se-selenate have demonstrated that dissimilatory selenate reduction occurs in a variety of anoxic sediments (*16, 17*) and rapidly immobilizes selenium oxyanions in evaporation ponds (*18*). However, hydrologic changes which lower water tables can dry out Se(0)-contaminated marsh sediments or alter the Se(0)-containing subsurface to the extent that buried soils are reexposed to atmospheric oxygen. Under such conditions, Se(0) may be reoxidized to form toxic and mobile selenite and selenate (*8, 19*).

It is not clear, however, if the reports of Se(0) oxidation in soil and sediments is a chemical or a biological reaction. *Bacillus megaterium* was reported to oxidize Se(0) (*20*), but its activity in soils has not been demonstrated. In this paper, we report on our novel use of $^{75}$Se(0) as a tracer to carry out manipulative experiments designed to resolve this question. We found evidence that oxidation of $^{75}$Se(0) to $^{75}$Se-selenate and $^{75}$Se-selenite in soils is carried out by a number of different aerobic microbes. Taken together with the discovery of dissimilatory selenate reduction, the finding that the reoxidation of Se(0) in soils is under microbial control validates the salient concept put forth over 30 years ago by Shrift (*21*) and proves that a complete biogeochemical cycle of selenium exists in nature.

## Materials and Methods

Biologically generated $^{75}$Se(0) was made by incubating washed cell suspensions of nitrate-grown *Sulfurospirillum barnesii* strain SES-3 (*13*−*15*) with 400 $\mu$Ci $^{75}$Se(IV) (carrier-free; Amersham Inc., Arlington Hts., IL) plus 500 $\mu$M $Na_2SeO_3$ for 48 h. The resulting $^{75}$Se(0) + unlabeled Se(0) precipitate was centrifuged at 7000 rpm, and the pellet went through repeated washings and centrifugations, first in 50% ethanol, followed by pH 7.3 phosphate buffer, and finally with deionized water. The pellet was resuspended in 20 mL of deionized water, with a final specific activity of $^{75}$Se(0) of ∼8 $\mu$Ci $\mu$mol$^{-1}$, followed by pasteurization at 70 °C for 1 h. Scanning electron microscopy (SEM) coupled with X-ray energy dispersive spectroscopy (EDS) was done on samples using fixation protocols published elsewhere (*22*) with unlabeled Se(0) formed by the above method. Preparation for the SEMs involved fixation in a glutaraldehyde, phosphate buffer and deionized water rinses and dehydration in ethanol and ethanol plus amyl acetate. The final critical point drying of the sample with $CO_2$ was eliminated (see below). The pellet was composed of small (usually < 1 $\mu$m) spheres and cylinders (Figure 1A) with an EDS signal containing peaks for both Se and S, but no other elements (Figure 1B). The absence of a C peak indicated that the above method eliminated cell material; however, the S peak showed that the selenium particles were impure. The S content of the precipitate was 30% as determined by combustion (*23*) and may have associated with the particles as a consequence of the cysteine-sulfide reducing agent used to grow *S. barnesii*. However, the EDS signal for S was lost upon critical point drying the SEM sample in $CO_2$ (not shown). This suggests that the S was not chemically bound to the Se (e.g, a selenylsulfide) and was possibly present as coprecipitated S(0). We conducted most of our experiments using this biologically formed $^{75}$Se(0) particles generated by this method because we felt that it is most representative of the "refractory" selenium [e.g., mostly Se(0)] which accumulates in sediments by bacterial Se(VI) reduction (*19*). However, to ensure that these results were reproducible, we also ran experiments using $^{75}$Se(0) made from $^{75}$Se(IV) by chemical reduction with ascorbic acid (*24*). This red-colored precipitate had a much different morphology (framboidal clusters), possibly indicating it was a different allotrope of Se(0) (Figure 2A). However, both its EDS signal (Figure 2B) and S combustion analysis lacked any S which could have been introduced into the matrix from bacterial metabolism, thereby indicating it was composed of 100% Se(0). Unless stated otherwise, the experiments outlined below were conducted with the

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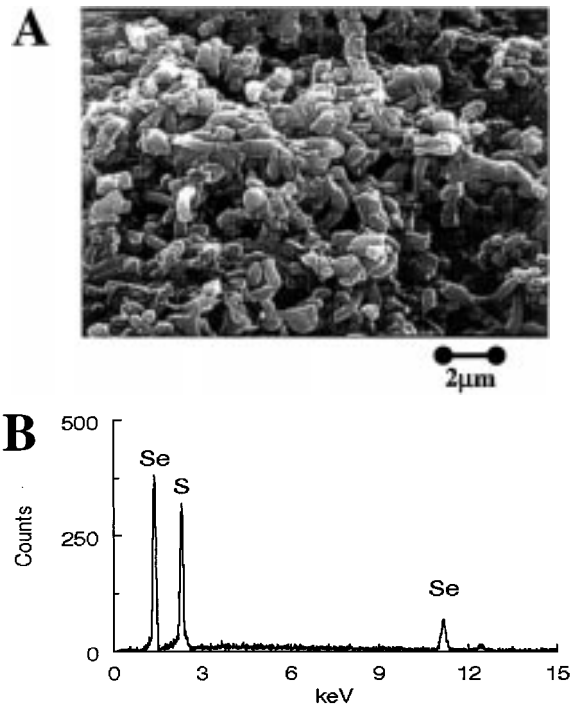


**FIGURE 1. Scanning electron micrograph (A) and energy dispersive spectrum (B) of Se(0) particles formed by reduction of Se(IV) with washed cell suspensions of *S. barnesii*.**

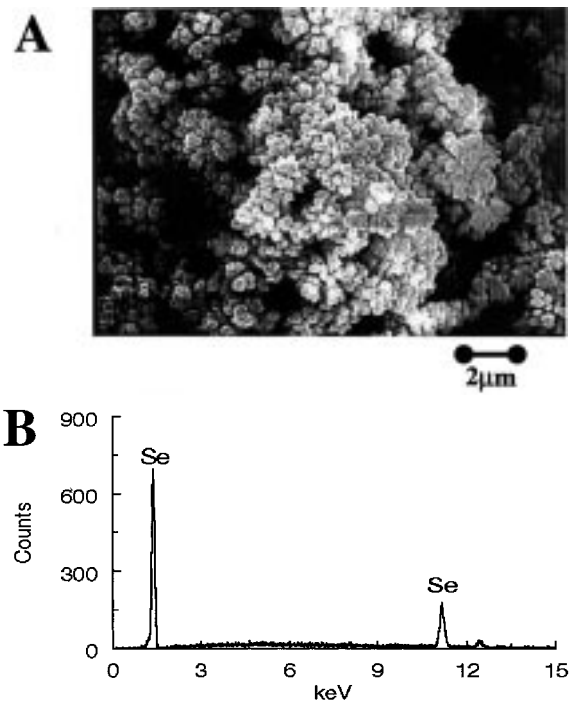


**FIGURE 2. Scanning electron micrograph (A) and energy dispersive spectrum (B) of Se(0) particles formed by chemical reduction of Se(IV) with acsorbate.**

biologically generated $^{75}$Se(0). All the radiotracer experiments outlined below were incubated in the dark in a lead-shielded cave at room temperature (23 °C).

Soils were recovered by hand-digging the dried former freshwater lakebed of pond no. 2 of the Kesterson National Wildlife Refuge. The soils were taken from beneath the surface layer (~5 cm depth) which consisted of dark-colored landfill which overlies the lighter tan soils of the lakebed sediments. Se(0) concentrations in these soils are ~1 mmol per kilogram (*19*). We purposely conducted experiments with highly diluted soil slurries which were maintained under well-aerated conditions in order to avoid the development of anoxic microsites. Soils were slurried with deionized water (ratio of soil:water = 1:10; pH = 7.5), and 15 mL was dispensed into 50 mL centrifuge tubes to which 250 $\mu$L (1.5 $\mu$Ci) of the $^{75}$Se(0) was added. For each triplicate set of slurry samples, 0.25 mL of the radiotracer was dispensed into each sample from a syringe which contained 1 mL of the radiotracer stock. The fourth 0.25 mL radiotracer aliquot was extruded into a filter-microfuge tube, and after centrifugation, the liquid and solid phases were counted. No counts were associated with the liquid phase, and the solid phase count was used to calculate the amount of radiotracer added to each triplicate subset of samples. The tubes containing the soil slurries were capped under an air atmosphere and placed on a rotator which was run with constant end-over-end rotation (12 rpm). This assured continuous aeration of the slurry. In addition, since the tubes were opened to the atmosphere every few days for sampling, the headspace was frequently freshened which further assured the prevention of suboxic conditions. Tubes were sampled by pipet (0.5 mL withdrawn) and filter-centrifuged, and the pellet was washed with 0.5 mL of 0.1 N NaOH to remove any adsorbed $^{75}$Se(IV). The resulting samples were either run immmediately or refrigerated until analyzed (within 1 month of collection). Separation of Se(IV) from Se(VI) was performed on a high performance liquid chromatograph (*14*) with the collection of eluted fractions for determination of $\gamma$ counts by a gamma spectrometer (*18*). Additions of poisons, inhibitors, antibiotic mixtures, inorganic electron donors, and organic substrates were made in various experiments, and the concentrations employed are given in the text.

The enrichment culture was established by inoculating Difco nutrient broth supplemented with 10 mM glucose with 1 mL of live soil slurry previously maintained in the presence of Se(0) for 2 months. Cultures contained ~1 mmol/L of red Se(0) and were transferred every 1−2 months for a period of 1 year. The enrichment was grown at 25 °C with rotary shaking (150 rpm) in 50 mL serum bottles containing 20 mL of broth and sealed with a porous foam rubber stopper to maintain sterility but which allowed for the penetration of air into the culture. The amount of Se(0) added to all the cultures at the outset of the experiments was 21−29 nmol mL$^{-1}$. The *Thiobacillus* ASN-1 was incubated as indicated above differing only by employing a medium for marine thiobacilli (*25*). Thiosulfate was used as an electron donor at 0, 5, and 10 mM concentrations. *Leptothrix* MnB1 was incubated as above only employing the heterotrophic medium of Boogerd and de Vrind (*26*) supplemented with or without 100 $\mu$M $MnSO_4$.

Apparent turnover rate constants for Se(0) oxidation were calculated from linear regressions of progress curves of Se(IV) + Se(VI) formed from the amount of Se(0) added:

$$k = \frac{\text{Se(IV)} + \text{Se(VI)}}{\text{Se(0)}} \div \text{time}$$

No attempt was made to determine the order of the oxidation reaction because we added high levels of Se(0) to our slurries and cultures and usually measured formation of product rather than the decline of reactant.

## Results and Discussion

**Soil Slurries.** There was a linear production of selenite from Se(0) in live soils but not in autoclaved or formalin-killed controls (Figure 3A). Selenate was produced in small quantities and accounted for less than 5% of the selenite production (Figure 3B). Although there was roughly an equivalent amount of selenate produced in the autoclaved

**TABLE 1. Soil Slurry Experiments Using Bacterially Produced $^{75}Se(0)$[a]**

| addition/condition | nmol $mL^{-1}$ (std dev) Se(IV) | Se(VI) | % oxidized | % inhibition/ stimulation | *k*, rate constant ($day^{-1}$) |
|---|---|---|---|---|---|
| **Exp 1:** [Se(0)] ~ 27 nmol $mL^{-1}$, incubation time (*T*) ~ 25 days | | | | | |
| none | 4.29 (0.65) | 0.21 (0.03) | 16.9 | | 0.006 55 |
| autoclaved | 0.21 (0.14) | 0.31 (0.16) | 2.0 | −88.4 | 0.000 84 |
| formalin (3.7% vol/vol) | 0.07 (0.01) | 0.01 (0.00) | 0.3 | −98.0 | 0.000 01 |
| **Exp 2:** [Se(0)] ~ 29 nmol $mL^{-1}$, *T* ~ 3 days | | | | | |
| none | 0.87 (0.07) | 0.11 (0.01) | 3.1 | | 0.010 45 |
| NaCl (2 mg $mL^{-1}$) | 1.01 (0.06) | 0.11 (0.05) | 3.6 | +14.9 | 0.012 00 |
| chloramphenicol and tetracycline (1 mg $mL^{-1}$) | 0.39 (0.13) | 0.01 (0.01) | 1.3 | −58.3 | 0.004 36 |
| cycloheximide and nystatin (1 mg $mL^{-1}$) | 0.21 (0.00) | 0.06 (0.02) | 0.9 | −72.4 | 0.002 88 |
| **Exp 3:** [Se(0)] ~ 25 nmol $mL^{-1}$, *T* ~ 3 days | | | | | |
| none | 0.85 (0.07) | 0.14 (0.09) | 3.5 | | 0.011 69 |
| ethanol (3.3% vol/vol) | 0.79 (0.43) | 0.09 (0.01) | 3.1 | −10.3 | 0.010 49 |
| sodium azide (1.0 mM) | 0.22 (0.05) | 0.16 (0.03) | 1.4 | −61.4 | 0.004 52 |
| 2,4-dinitrophenol (250 $\mu$M) | 0.19 (0.04) | 0.09 (0.00) | 1.0 | −71.0 | 0.003 39 |
| **Exp 4:** [Se(0)] ~ 25 nmol $mL^{-1}$, *T* ~ 12 days | | | | | |
| none | 2.50 (0.03) | 0.15 (0.02) | 11.2 | | 0.008 86 |
| $(NH_4)_2SO_4$ (0.5 mM) | 2.69 (0.05) | 0.14 (0.02) | 11.8 | +4.7 | 0.009 19 |
| $FeSO_4$ (1.0 mM) | 1.47 (0.25) | 0.14 (0.04) | 6.8 | −46.1 | 0.004 64 |
| $MnSO_4$ (1.0 mM) | 2.67 (0.01) | 0.16 (0.02) | 12.6 | +10.4 | 0.009 77 |
| $Na_2S$ (1.0 mM) | 11.13 (1.22) | 0.22 (0.02) | 47.1 | +325.2 | 0.035 22 |
| glucose (5.0 mM) | 3.31 (0.05) | 0.28 (0.01) | 17.1 | +57.4 | 0.022 15 |
| sodium acetate (5.0 mM) | 6.50 (2.01) | 0.86 (0.24) | 26.5 | +147.5 | 0.013 67 |

[a] Final concentrations of selenite [Se(IV)] and selenate [Se(VI)] produced in all of the Se(0) oxidation experiments conducted with soil slurries. The radiotracer used in these incubations was bacterially-precipitated $^{75}Se(0)$. Final concentrations of $^{75}Se(0)$ added to the slurries as well as the days duration of the incubation are given in the headings for each experiment. Sodium chloride was added as a placebo in experiment no. 2, and ethanol was the solvent used to add 2,4-dinitrophenol to the slurries. Neither substance had any effect on Se(0) oxidation compared to the unamended, live samples. All data represent the mean (±1 std dev) of three soil slurries.

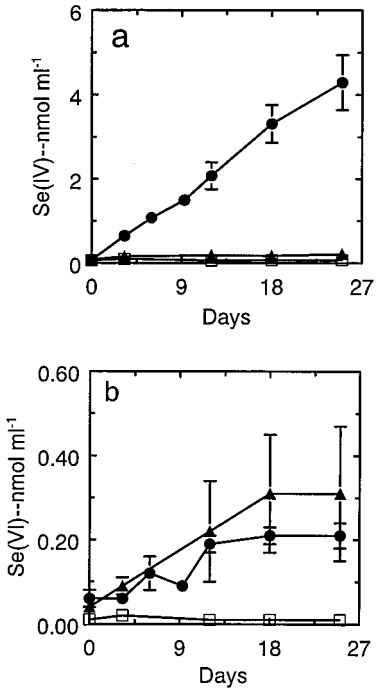


**FIGURE 3. Oxidation of Se(0) to (A) selenite [Se(IV)] and (B) selenate [Se(VI)] in soil slurries. Radiotracer employed was bacterially precipitated $^{75}Se(0)$. Symbols: (●), live; (▲), autoclaved twice at 1 h at 250 kPa and 120 °C; (□) poisoned with 3.7% formalin. Results represent the mean of three slurries and bars indicate + 1 std dev. Absence of bars indicates error was smaller than symbols.**

slurries as in live slurries, none was formed in the formalin-killed controls, indicating that formalin was more effective blocking the biological oxidation of selenite. Small amounts of abiotic selenate production from Se(0) in sterilized systems have also been noted by others (*20*). The $\gamma$ counts of the $^{75}Se(IV) + {}^{75}Se(VI)$ resolved by HPLC and fraction collection represented the recovery of 94.2 ± 7.2% (±1 std dev; $n = 7$ time points of 3 live slurries) of the total soluble counts in solution. Hence, Se(IV) and Se(VI) were the only oxidation products detected over the duration of the incubation. The total amount of Se(IV) + Se(VI) formed over the 25 day incubation represented an oxidation of 17% of the initially added Se(0), which balanced the amount of solid-phase Se(0) counts lost from the live slurries (17%). There was no significant loss of solid-phase counts from the formalin controls. This Se(0) oxidation in live slurries resulted in an apparent turnover rate constant (*k*) of 0.006 65 $day^{-1}$ (Table 1).

The production of selenite in slurries was inhibited by sodium azide (a respiratory inhibitor) as well as by the metabolic uncoupler 2,4-dinitrophenol (Table 1). Similarly, combinations of bacterial antibiotics (chloramphenicol + tetracycline) or fungal antibiotics (cycloheximide + nystatin) repressed both selenite and selenate formation. Collectively, these results demonstrate that soil Se(0) oxidation primarily proceeds to selenite and that it is carried out by microbes which include bacteria as well as fungi. We also observed $^{75}Se(0)$ oxidation in preliminary experiments with oxic sediment slurries from San Francisco Bay (*27*), indicating that this phenomenon is not confined to Se-contaminated soils.

We were able to qualitatively reproduce the salient aspects of the above results using the chemically precipitated $^{75}Se(0)$ in which biological oxidation was blocked by 2,4-dintrophenol (Figure 4). In this experiment Se(IV) accounted for ~85% of the oxidation products after 20 days incubation (Table 2). However, using this form of $^{75}Se(0)$, the rate constant for live samples was ~0.000 97 $day^{-1}$, which was >6-fold slower than the rates determined with the biologically formed $^{75}Se(0)$ (Table 2). The slower rates may have been due to the framboidal matrix of the Se(0) tracer imposing structural constraints on bacterial access.

The oxidation of selenite to selenate is probably limited by the adsorption of Se(IV) onto soil particles, making it less available to microbes. Selenite adsorbs strongly at neutral pH but not at highly alkaline pH (*19*), and to support this we

**TABLE 2. Soil Slurry Experiment Using Chemically Produced $^{75}Se(0)$[a]**

| addition/condition | nmol mL$^{-1}$ (std dev) Se(IV) | Se(VI) | % oxidized | % inhibition/ stimulation | *k*, rate constant (days$^{-1}$) |
|---|---|---|---|---|---|
| **Exp 1:** [Se(0)] ~ 20 nmol mL$^{-1}$, *T* ~ 20 days | | | | | |
| none | 0.29 (0.04) | 0.05 (0.02) | 1.66 | | 0.000 97 |
| $Na_2S$ (2.0 mM) | 0.42 (0.06) | 0.07 (0.06) | 2.42 | +46.3 | 0.000 97 |
| sodium acetate (10.0 mM) | 0.39 (0.10) | 0.13 (0.06) | 2.59 | +56.5 | 0.001 21 |
| 2,4-dintrophenol (0.5 mM) | 0.05 (0.05) | 0.05 (0.05) | 0.52 | −68.8 | 0.000 06 |

[a] Final concentrations of selenite [Se(IV)] and selenate [Se(VI)] formed in soil slurry experiments performed with chemically-precipitated $^{75}Se(0)$. All data represent the mean (±1 std dev) of three slurries.

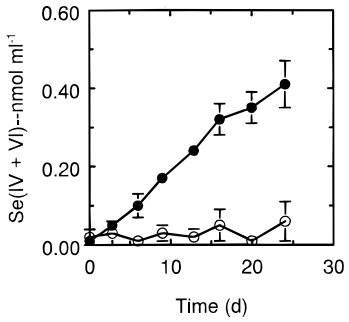


**FIGURE 4. Oxidation of Se(0) to Se(IV) + Se(VI) in soil slurries. Radiotracer used of chemically precipitated $^{75}Se(0)$. Symbols: (●), live; (○), inhibited with 0.5 mM 2,4-dinitrophenol. Symbols represent the mean of three slurries and bars indicate ± 1 std dev. Absence of bars indicates error was smaller than symbols.**

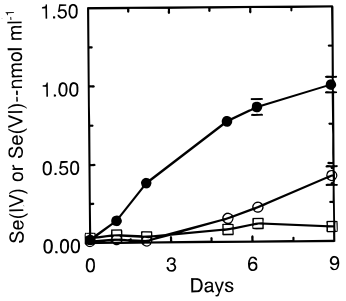


**FIGURE 5. Oxidation of Se(0) by an enrichment culture of heterotrophic bacteria. Radiotracer used was bacterially precipitated $^{75}Se(0)$. Symbols: (●), selenate [Se(VI)]; (○), selenite [Se(IV)]; (□), Se(IV) plus Se(VI) in a sterile control. Results represent the mean of three cultures and bars indicate ± 1 std dev, except for the sterile control where *n* = 1. Absence of bars indicates error was smaller than symbols.**

noted that ~80% of our $^{75}$Se-selenite counts were released from the soil particles after washing with 0.1 N NaOH. Although chemical oxidation of selenite occurs on the surface of birnessite (*28*) or titania (*29*), these conditions proceed very slowly at physiological pH and, in the latter case, also require light. Our experiments were conducted in the dark.

To determine the types of microbes involved in the oxidation of Se(0), we amended the soil slurries with substrates designed to enhance the activity of either lithotrophic bacteria or heterotrophic bacteria and fungi (Table 1; experiment no. 4). Incubation in the presence of sulfide stimulated selenite formation over that exhibited by unamended samples, while no stimulation was observed with $NH_4^+$, Fe(II), or Mn(II). This suggested the involvement of reduced sulfur-oxidizing chemoautotrophs such as thiobacilli, but not that of nitrifiers or lithotrophic Fe(II)- or Mn(II)-oxidizers. Fe(II) actually decreased the amount of selenium oxyanions produced, probably by the adsorption of selenite onto the surfaces of FeOOH formed from the oxidation of Fe(II). Mn(II) did not enhance the production of selenite, suggesting that there was no direct chemical oxidation of Se(0) by bacterially formed Mn(IV), as can occur with sulfides (*30*). Such oxidation would implicate the indirect involvement of chemolithotrophic bacteria which form Mn(IV) from oxidation of Mn(II) (*31*). Finally, there was a clear enhancement of oxidation with acetate but only a slight amount with glucose (Table 1). This can be interpreted as evidence for the involvement of heterotrophic microbes which have a degree of substrate specificity and prefer acetate over glucose, as was observed for selenate reduction in sediments (*11*). When we repeated some of the above experiments with chemically precipitated $^{75}Se(0)$, we also observed stimulation of Se(0) oxidation by addition of sulfide or acetate (Table 2).

**Bacterial Cultures.** The heterotrophic enrichment culture grown in the presence of Se(0) formed selenate as the major product, although some selenite was also produced (Figure 5; Table 3). Sterile controls did not oxidize Se(0). Therefore, the formation of selenate and selenite from Se(0) was entirely due to bacterial metabolism. These results differed from the soil slurries which mostly formed selenite. Hence, the absence from the enrichment culture of adsorptive soil surfaces which strongly bind selenite resulted in enhanced selenite oxidation.

The formation of selenate as the major oxidation product was also found for *Leptothrix* strain MNB-1 and *Thiobacillus* ASN-1, thereby reinforcing this interpretation (Table 3). *Leptothrix* strain MNB-1 is a heterotrophic bacterium which can oxidize Mn(II) or Fe(II) (*26*). Under growth conditions, *Leptothrix* strain MNB-1 oxidized Se(0) to Se(VI) + Se(IV), but there was no difference between cells grown with or without Mn(II) (Table 3). This suggests that Mn(II)-oxidizing enzymes were not involved in the oxidation of Se(0). Because of the chemical similarity of selenium with sulfur and the enhancement of soil Se(0) oxidation with sulfide (Table 1), we also tested the ability of the sulfur-oxidizing bacterium *Thiobacillus* strain ASN-1 (25) to oxidize Se(0). This organism oxidized Se(0) to selenate and selenite, and thiosulfate enhanced oxidation compared to cells incubated without this energy source (Figure 6). Cells with 10 mM thiosulfate formed only selenate as the oxidation product, while there was negligible Se(0) oxidation in the sterile control (Table 3).

Collectively, the above results indicate that there are at least two physiological types of microbes which may carry out Se(0) oxidation. The enrichment culture, *Leptothrix* strain MNB-1, *B. megaterium* (*20*), and perhaps fungi typify the first type, which is composed of heterotrophic microorganisms. The mechanism(s) for their oxidation is not known, but it appears to be constitutive and depends on provision of an electron donor like acetate or glucose rather than Mn(II) or Fe(II). The second type includes bacteria of the aerobic sulfur cycle (e.g., thiobacilli) which carry out the oxidation of Se(0) with enzymes that are used for generating energy from reduced sulfur compounds.

On the reductive side of the selenium cycle, the ability of sulfate-respirers to reduce selenate to selenide (Se[-II]) is greatly constrained by competition with sulfate (*32*), and the

**TABLE 3. Final Concentrations of Selenite [Se(IV)] and Selenate [Se(VI)] Produced in the Se(0) Experiments Conducted with the Enrichment Culture, *Leptothrix* MnB1, and *Thiobacillus* ASN-1[a]**

| addition/condition | nmol $mL^{-1}$ (std dev) Se(IV) | Se(VI) | % oxidized | *k*, rate constant $(days)^{-1}$ |
|---|---|---|---|---|
| enrichment culture growth [Se(0)] ~ 21 nmol $mL^{-1}$, *T* ~ 9 days | | | | |
| sterile (uninoculated media) | 0.06 | 0.05 | 0.5 | 0.000 49 |
| live | 0.42 (0.06) | 1.00 (0.05) | 6.9 | 0.007 80 |
| ***Leptothrix* MnB1 growth** [Se(0)] ~ 24 nmol $mL^{-1}$, *T* ~ 6 days | | | | |
| sterile (uninoculated media w/$Mn^{2+}$) | 0.04 | 0.04 | 0.3 | 0.000 52 |
| no $Mn^{2+}$ | 0.12 (0.02) | 0.20 (0.00) | 1.3 | 0.002 89 |
| 100 $\mu$M $Mn^{2+}$ | 0.15 (0.05) | 0.24 (0.02) | 1.6 | 0.003 66 |
| ***Thiobacillus* ASN-1 growth** [Se(0)] ~ 21 nmol $mL^{-1}$, *T* ~ 7 days | | | | |
| sterile (uninoculated media) | 0.01 | 0.13 | 0.6 | 0.000 14 |
| no $S_2O_3^{2-}$ | 0.27 (0.01) | 0.18 (0.02) | 2.1 | 0.002 96 |
| 5 mM $S_2O_3^{2-}$ | 0.12 (0.03) | 1.06 (0.04) | 5.6 | 0.006 51 |
| 10 mM $S_2O_3^{2-}$ | 0.05 (0.00) | 1.28 (0.05) | 6.3 | 0.007 20 |

[a] Final concentrations of $^{75}Se(0)$ were added, and the duration of the incubation are given in the headings for each experiment. Data for live samples represent the mean of three cultures (±1 std dev). Sterile controls represent single samples.

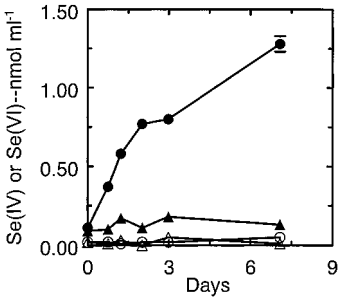


**FIGURE 6. Oxidation of Se(0) by *Thiobacillus* strain ASN-1. Radiotracer used was bacterially precipitated $^{75}Se(0)$. Symbols: (●), selenate [Se(VI)] formed by cells incubated with 10 mM thiosulfate; (○), selenite [Se(IV)] formed by cells incubated with thiosulfate; (▲), Se(VI) in a sterile control; (△), Se(IV) in a sterile control. Results for live sample represent the mean of three cultures and error was smaller than the symbols.**

degradation of dimethylselenide by methanogens competes with dimethyl sulfide (*33, 34*). Thus the reduction of selenate to Se(0), because it is carried out independently of sulfate, dominates the biogeochemistry of selenium because sulfate is prevalent in aquatic ecosystems (*11*). Our results with soil slurries (Table 1) and *Thiobacillus* strain ASN-1 suggest that the aerobic selenium cycle differs from the reductive side in one important aspect: provision of a reduced sulfur electron donor can stimulate, rather than inhibit, the overall oxidation of Se(0).

**Rate Constants.** A reported rate constant for Se(0) oxidation by soils taken from the same region of Kesterson that we sampled and incubated statically for ~2 years at 25 °C was 0.002 $day^{-1}$ (*19*). This value was roughly 2-fold faster than our results with abiotically precipitated $^{75}Se(0)$ (Table 2) but about 3−6-fold lower than our other slurry experiments with biogenic $^{75}Se(0)$ tracer and is over an order of magnitude lower than what we observed when we added sulfide or acetate to stimulate Se(0) oxidation in those slurries (Table 1). The reason for our observation of higher rate constants partly lies in the fact that we encouraged the activity of aerobic microbes by purposely maintaining a well-aerated environment or, in some cases, by also providing them with various stimulatory nutrients. In contrast, the penetration of oxygen into the statically incubated soils was likely to severely constrain Se(0) oxidation. In addition, the allotropic form of Se(0) appears to be a consideration since the biogenic, amorphous Se(0) underwent a more rapid oxidation than did the chemically precipitated Se(0). This type of an effect was seen with cultures of *B. megaterium* in which the rate constant for oxidation of amorphous, red Se(0) oxidation was ~0.002 $day^{-1}$, while that for the gray allotrope was only 0.0007−0.001 $day^{-1}$. These values are below those we observed for our bacterial cultures (Table 2), which could be explained by our enrichment being comprised of heterotrophs which were capable of more efficient Se(0) oxidation than *B. megaterium*. Additionally, the fact that *Thiobacillus* strain ASN-1 proved so facile at Se(0) oxidation argues that it is better adapted to the task because its enzymes oxidize the analogous sulfur compounds (*25*).

It is of interest to compare the rate constants for Se(0) oxidation with those obtained from field studies of selenate reduction in anoxic sediments. Selenate reduction rate

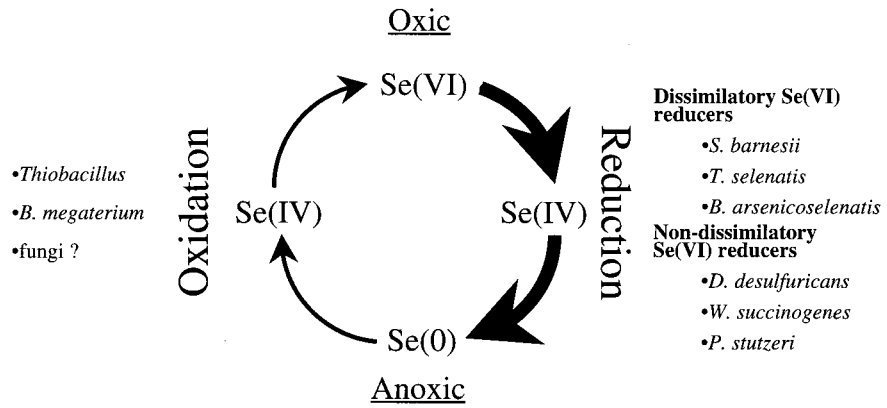


**FIGURE 7. Redox cycle of selenium in nature as updated from Shrift (*20*). Because the rate constants for the reduction of Se(VI) are 3−4 orders of magnitude greater than those for the oxidation of Se(0), thicker arrows on the reductive side of the cycle are used to emphasize this fact.**

constants taken from 8 depths in a vertical core profile (0−28 cm) ranged from 2.1 to 52.8 $day^{-1}$ for an evaporation pond in the San Joaquin Valley of California (mean = 26.3 ± 16.2 std dev; $n = 24$ samples) (*18*). Selenate reduction rate constants for core profiles taken in western Nevada (*17*) were (mean ± 1 std dev): 7.5 ± 10.3 $day^{-1}$ ($n = 9$), 5.8 ± 1.1 $day^{1}$ ($n = 9$), and 21.1 ± 20.4 $day^{-1}$ ($n = 9$), respectively for South Lead Lake, Hunter Drain, and Massie Slough. These values for reduction are 3 to 4 orders-of-magnitude greater than what we measured for oxidation of Se(0) by live soils and bacterial cultures (Tables 1 and 2). Thus, microbial oxidation of Se(0) is a very slow process when compared with the dissimilatory reduction of selenate. This observation agrees with field data which show that the selenate pool of some contaminated anoxic sediments can have turnover times of less than 1 h (*16*−*18*). In contrast, our studies indicate that the turnover rate for Se(0) oxidation is hundreds of days, a finding which reinforces observations of slow oxidation rates in stored soils (*19*) and for *B. megaterium* (*20*). Bacterial dissimilatory selenate reduction is a highly exergonic reaction which couples anaerobic growth to the oxidation of electron donors such as lactate (*13*−*15*). It is logical that the rate constants for this process can be very high in anoxic environments rich in decomposing organic matter. In contrast, the benefit microbes gain from oxidizing Se(0) is unclear, but the much lower rate constants that we report (Table 1) as well as those published (*19, 20*) would suggest this is a cometabolic process rather than one which yields energy.

**The Redox Cycle of Selenium.** Although pursuit of the biochemical basis for Se(0) oxidation is an area ripe for future investigation, its biogeochemical significance is such that we propose that the full redox cycle of selenium speciation observed in nature is controlled by microoganisms (Figure 7). This cycle is a refinement of that first proposed by Shrift (*21*), its salient point being that microbes control both the reduction and the oxidation of Se. The cycle ignores the assimilatory reduction of selenate [Se(VI)] into cellular material (e.g., selenomethionine) and the formation of volatile products such as dimethylselenide (*34, 35*). The reductive side occurs in anoxic environments where bacteria like *Sulfurospirillum barnesii* (*13*−*15*), *Thauera selenatis* (*12, 36*), and *Bacillus arsenicoselenatis* (*37*) oxidize organic matter coupled with the quantitative dissimilatory reduction of selenate [Se(VI)] and selenite [Se(IV)] to Se(0) to achieve growth. Nondissimilatory reducers include *Wolinella succinogenes* (*38*), *Desulforvibrio desulfuricans* (*39*), and *Pseudomonas stutzeri* (*40*) which can reduce Se(IV) and Se(VI) to Se(0) but do not couple this reduction to growth. The oxidative side carries out the oxidation of Se(0) to Se(IV) and Se(VI) and appears to involve heterotrophic bacteria, thiobacilli, and possibly fungi.

Recognition of the existence of this cycle, and that the relative rates of selenate reduction greatly exceed those for Se(0) oxidation, should lead to better quantification of the dynamics and mass balances of Se in contaminated ecosystems (*7*−*9*) and result in improved strategies for their management and remediation. Hence, the hydrologic introduction of contaminant Se(VI) into anoxic, organic-rich sediments will result in its rapid immobilization as Se(0). The rate limitation of this process will be that of physical diffusion of Se(VI) into the sediments, rather than that associated with its biological reduction (*41*). When hydrologic changes are made which dry these sediments out, a slow microbially-catalyzed reoxidation of Se(0) to Se(IV) and Se(VI) can occur, and the biological oxidation, combined with the adsorption of Se(IV) to soil surfaces, will be the rate-limiting steps. This will ensure that such soils will leach selenium oxyanions into the surrounding surface waters for many years.


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## Literature Cited


(1) Lemly, A. D. *Ecotoxicol. Environ. Saf.* **1997**, *36*, 275.
(2) Presser, T. S. *Environ. Manage.* **1994**, *18*, 437.
(3) Ohlendorf, H. M.; Hoffman, D. J.; Saiki, M. K.; Aldrich, T. W. *Sci. Total Environ.* **1986**, *52*, 49.
(4) Long, R. B. H.; Benson, S. M.; Tokunaga, T. K.; Lee, A. J. *Environ. Qual.* **1990**, *19*, 302.
(5) White, A. F.; Benson, S. M.; Yee, A. W.; Wollenberg, H. A., Jr.; Felxser, S. *Water Resour. Res.* **1991**, *27*, 1085.
(6) Tokunaga, T. K.; Sutton, S. R.; Bajt, S. *Soil Sci.* **1994**, *158*, 421.
(7) Zhang, Y.; Moore, J. M. *Environ. Sci. Technol.* **1990**, *30*, 2613.
(8) Tokunaga, T. K.; Pickering, I. J.; Brown, G. E., Jr. *Soil Sci. Am. J.* **1996**, *60*, 781.
(9) Velinsky, D. J.; Cutter, G. A. *Geochim. Cosmochim. Acta* **1991**, *55*, 179.
(10) Luoma, S. N.; Johns, C.; Fischer, N. S.; Steinberg, N. A.; Oremland, R. S.; Reinfelder, J. R. *Environ. Sci. Technol.* **1992**, *26*, 485.
(11) Oremland, R. S.; Hollibaugh, J. T.; Maest, A. S.; Presser, T. S.; Miller, L. G.; Culbertson, C. W. *Appl. Environ. Microbiol.* **1989**, *55*, 2333.
(12) Macy, J. M.; Michel, T. A.; Kirsch, D. G. *FEMS Microbiol. Lett.* **1989**, *61*, 195.
(13) Oremland, R. S.; Switzer Blum, J.; Culbertson, C. W.; Visscher, P. T.; Miller, L. G.; Dowdle, P.; Strohmaier, F. E. *Appl. Environ. Microbiol.* **1994**, *60*, 3011.
(14) Laverman, A. M.; Switzer Blum, J.; Schaefer, J. K.; Phillips, E. J. P.; Lovley, D. R.; Oremland, R. S. *Appl. Environ. Microbiol.* **1989**, *61*, 3556.
(15) Stolz, J. F.; Gugliuzza, T.; Switzer Blum, J.; Oremland, R.; MartinJz Murillo, F. *Arch. Microbiol.* **1997**, *167*, 1.
(16) Steinberg, N. A.; Oremland, R. S. *Appl. Environ. Microbiol.* **1990**, *56*, 3550.
(17) Oremland, R. S.; Steinberg, N. A.; Presser, T. S.; Miller, L. G. *Appl. Environ. Microbiol.* **1991**, *57*, 615.
(18) Oremland, R. S.; Steinberg, N. A.; Maest, A. S.; Miller, L. G.; Hollibaugh, J. T. *Environ. Sci. Technol.* **1990**, *24*, 1157.
(19) Zawislanski, P. T.; Zavarin, M. *Soil Sci. Soc. Am. J.* **1996**, *60*, 791.
(20) Sarathchandra, S. U.; Watkinson, J. H. *Science* **1981**, *211*, 600.
(21) Shrift, A. *Nature* **1964**, *201*, 1304.
(22) Smith, R. L.; Strohmaier, F. E.; Oremland, R. S. *Arch. Microbiol.* **1985**, *141*, 8.
(23) Jackson, L. L.; Brown, F. W.; Neil, S. T. *USGS Bulletin* **1985**, *1770*, G1.
(24) Combs, G. G., Jr.; Garbisu, C.; Yee, B. C.; Yee, A.; Carlson, D. E.; Smith, N. R.; Magyarosy, A. C.; Leighton, T.; Buchanan, B. B. *Biol. Trace Elem. Res.* **1996**, *52*, 209.
(25) Visscher, P. T.; Taylor, B. F. *Appl. Environ. Microbiol.* **1993**, *59*, 3784.
(26) Boogerd, F. C.; de Vrind, J. P. M. *J. Bacteriol.* **1987**, *169*, 489.
(27) Oremland, R. S.; Dowdle, P.; Culbertson, C. W.; Foster, A. *Abstr. Annu. Meet. Am. Soc. Limnol. Oceanogr., Santa Fe* **1997**, 257.
(28) Scott, M. J.; Morgan, J. J. *Environ. Sci. Technol.* **1991**, *30*, 1990.
(29) Gruebel, K. A.; Davis, J. A.; Lecke, J. O. *Environ. Sci. Technol.* **1995**, *29*, 586.
(30) Aller, R. C.; Rude, P. D. *Geochim. Cosmochim. Acta* **1988**, *52*, 751.
(31) Tebo, B. M. *Genetic Eng.* **1995**, *17*, 231.
(32) Zehr, J. P.; Oremland, R. S. *Appl. Environ. Microbiol.* **1987**, *53*, 1365.
(33) Oremland, R. S.; Zehr, J. P. *Appl. Environ. Microbiol.* **1986**, *52*, 1031.
(34) Ansede, J. H.; Yoch, D. C. *FEMS Microbiol. Ecol.* **1997**, *23*, 315.
(35) Reamer, D. C.; Zoller, W. H. *Science* **1980**, *208*, 500.
(36) Macy, J. M.; Rech, S.; Auling, G.; Dorsch, M.; Stackebrandt, E.; Sly, L. I. *Int. J. Syst. Bacteriol.* **1993**, *43*, 135.
(37) Switzer Blum, J.; Burns Bindi, A.; Buzzeli, J.; Stolz, J. F.; Oremland, R. S.; *Arch. Microbiol.* **1998**, *168*, in press.
(38) Tomei, F. A.; Barton, L. L.; Lemanski, C. L.; Zocco, T. G. *Can. J. Microbiol.* **1992**, *38*, 1328.

(39) Tomei, F. A.; Barton, L. L.; Lemanski, C. L.; Zocco, T. G.; Fink, N. H.; Sillerud, L. O. *J. Ind. Microbiol.* **1995**, *14*, 329.

(40) Lortie, L.; Gould, W. D.; Rajan, S.; McCready, R. G. L.; Cheng, K.-J. *Appl. Environ. Microbiol.* **1992**, *58*, 4043.

(41) Tokunaga, T. K.; Brown, G. E., Jr.; Pickering, I. J.; Sutton, S. R.; Bajt, S. *Environ. Sci. Technol.* **1997**, *31*, 1419.

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