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Selenate Reduction to Elemental Selenium by Anaerobic Bacteria in Sediments and Culture: Biogeochemical Significance of a Novel, Sulfate-Independent Respiration†

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Interstitial water profiles of SeO_4^{2-}, SeO_3^{2-}, SO_4^{2-}, and Cl^- in anoxic sediments indicated removal of the seleno-oxyanions by a near-surface process unrelated to sulfate reduction. In sediment slurry experiments, a complete reductive removal of SeO_4^{2-} occurred under anaerobic conditions, was more rapid with H_2 or acetate, and was inhibited by O_2, NO_3^-, MnO_2, or autoclaving but not by SO_4^{2-} or FeOOH. Oxidation of acetate in sediments could be coupled to selenate but not to molybdate. Reduction of selenate to elemental selenium was determined to be the mechanism for loss from solution. Selenate reduction was inhibited by tungstate and chromate but not by molybdate. A small quantity of the elemental selenium precipitated into sediments from solution could be resolublized by oxidation with either nitrate or FeOOH, but not with MnO_2. A bacterium isolated from estuarine sediments demonstrated selenate-dependent growth on acetate, forming elemental selenium and carbon dioxide as respiratory end products. These results indicate that dissimilatory selenate reduction to elemental selenium is the major sink for selenium oxyanions in anoxic sediments. In addition, they suggest application as a treatment process for removing selenium oxyanions from wastewaters and also offer an explanation for the presence of selenite in oxic waters.

Bacterial mineralization of organic matter in anoxic sediments proceeds by reduction of electron acceptors like nitrate (denitrification), sulfate (sulfate reduction), and bicarbonate (methanogenesis). In addition, Fe^{3+} and Mn^{4+} can also be of significance in the oxidation of carbon compounds (14, 19, 22, 26). Although these dissimilatory processes oxidize deposited organic matter, they also have important environmental or geochemical effects such as the removal of nitrogenous fertilizer (denitrification), the production of hydrogen sulfide (sulfate reduction) or methane (methanogenesis), and the deposition of magnetite formed from iron reduction (23). Because of the relative abundance of the above oxidants in sediments, these processes have been well studied. However, oxyanions of less abundant elements may also serve as electron acceptors for bacterial respiration in anoxic sediments. Here, we report that selenate is one such oxyanion. Because it is present at only trace (nanomolar to micromolar) concentrations in the environment, selenate is unimportant as an oxidant for the mineralization of organic carbon when compared with more abundant (millimolar) electron acceptors. However, it is geochemically and environmentally significant that its dissimilatory reduction removes toxic selenate (and selenite) from water by forming nontoxic, insoluble elemental selenium [Se^0(s)].

Selenate and selenite are both constituents of agricultural wastewaters which drain from seleniferous soils, like those found in parts of the San Joaquin Valley in California (35). This phenomenon is apparently widespread in the western United States (42). These oxyanions were implicated as the cause of massive waterfowl mortalities in the Kesterson Wildlife Refuge, a man-made salt marsh which received the agricultural wastewaters of the San Joaquin Valley. However, selenate was not chemically conservative in this environment (35), and the sediments may have acted as a sink for selenium by processes mediated by microorganisms (O. Weres, A. R. Jaouni, and L. Tsao, Appl. Geochem., in press).

Because of their proximity as group VIA elements, sulfur and selenium share many chemical and biochemical properties (12, 40). In this regard, sulfate has been identified as an antagonist for selenate transport or toxicity in various microorganisms (7, 8, 37, 39). However, less is known about the biogeochemistry of selenium, although a microbial "selenium cycle" has been proposed which is analogous to that for sulfur (38). Recent investigations have revealed that with regard to demethylation of dimethylselenide by methanogens (33) and reduction of selenate to selenide by sulfate reducers (51), such a cycle is at least conceptually possible. However, an important caveat upon this latter reaction was that sulfate acted as a competitive inhibitor, which thereby precluded significant selenate reduction to selenide in sulfate-rich environments. We now report on our observations of a novel bacterial dissimilatory reduction of selenate which occurs by pathways unrelated to those for sulfate. We therefore conclude that Se and S have different reductive biogeochemical cycles. These results suggest the feasibility of a microbial water treatment process and, in addition, help to explain the commonly observed relative abundance of selenite ions in oxygenated seawater.

MATERIALS AND METHODS

Core profiles of SeO_4^{2-}, SeO_3^{2-}, SO_4^{2-}, and Cl^-. A core (1 m by 8.25 cm) was taken from an agricultural wastewater evaporation pond from a farm located in the San Joaquin Valley west of Fresno, Calif. This site was chosen because the high levels of selenate present greatly exceeded detection limits. Processing took place within 12 h of collection,

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† This paper is dedicated to the memory of Ivan Barnes.

during which time the core was stored at 6°C. The core was vertically extruded in 4-cm segments. Segments were placed in an N_2-pressured squeezer which expressed the pore waters (pH 9.5) through a 0.45-μm-pore-size sterile filter directly into a collection syringe. Pore waters were analyzed for sulfate and chloride with a Waters liquid chromatograph equipped with an IC-PAK A 10 polymethacrylate column. Selenium oxyanions were determined by flow through hydride generation atomic absorption spectrometry employing a closed-system HCl reduction for Se(IV) plus Se(VI) and no pretreatment for Se(IV). Selenate was arrived at by difference. Detection limit for Se(IV) plus Se(VI) was 30 nM; detection limit for Se(IV) was 2.5 nM (6, 35, 45).

Experiments with sediment slurries. Intertidal San Francisco Bay sediments were homogenized under N_2 with an equal volume of artificial bay water (ABW): (31) with or without 21 mM sulfate (pH of the ABW was 7.3). Sulfate-free slurries were prepared by homogenization in sulfate-free ABW, followed by centrifugation, rinsing, and resuspension of the compacted sediment in sulfate-free ABW. The homogenate was dispensed (15 ml) into serum bottles (122 ml) containing an additional 50 ml of ABW. Soluble substrates (glucose, sodium acetate, sodium lactate, sodium succinate), electron acceptors (Na_2SeO_4, $NaNO_3$, Na_2SO_4, FeOOH, MnO_2), or group VI analogs (Na_2MoO_4, Na_2WO_4, Na_2CrO_4) were added to the slurries at the final concentrations indicated in the text. Additional controls consisted of autoclaved sediment (250 kPa and 121°C for 30 min). MnO_2 and FeOOH suspensions were prepared as outlined elsewhere (20). Hydrogen was also employed as an electron donor by replacing the N_2 gas phase. Consumption of H_2 during the incubations was monitored by uptake from glass syringes (27). The slurries were crimp sealed under H_2 or N_2 and incubated in the dark at 20°C with rotary shaking. Subsamples were periodically withdrawn by syringe, centrifuged, filtered (0.45-μm pore size), and stored at 4°C until analyzed for selenium oxyanions as outlined above. Methane in the gas phase of the slurries was determined by flame ionization gas chromatography (27).

Respiration of [^{14}C] acetate by sediment slurries. Anaerobic (N_2 gas phase) sediment slurries were prepared (and incubated) as indicated above and dispensed into serum bottles (bottle volume, 34 ml; slurry volume, 16 ml). The ABW contained sulfate (10 mM) and acetate (5 mM), and in addition, selected samples received either Na_2MoO_4 or Na_2SeO_4 (1, 10, or 20 mM). All samples were injected with 5 μCi (200 μl) of [2-^{14}C]sodium acetate (specific activity, 50 mCi/mmol; ICN Pharmaceuticals Inc., Irvine, Calif.). The quantity of $^{14}CH_4$ and $^{14}CO_2$ in the headspace was monitored during the incubation by radio-gas chromatography (10). After 12 days of incubation, the total amount of $^{14}CO_2$ formed in the samples was measured by injection of 1.5 ml of 6 N HCl, followed by shaking for 1 h (200 rpm) before analysis by radio-gas chromatography.

Slurry experiments with ^{75}Se. Sediment slurries for radioisotope experiments were prepared as outlined above. Slurries contained 0.5 mM Na_2SeO_4 and received 0.2 μCi of carrier-free [^{75}Se]selenate (ICN Pharmaceuticals), and subsamples (2 ml) were periodically withdrawn and microcentrifuged. The radioactivities in the supernatant and the ABW-rinsed pellet (contained in the disposable plastic centrifuge tube) were individually counted on a Beckman model 8000 gamma counter (sample background, ~95 cpm). Subsamples of the gas phase (1 ml) were also periodically withdrawn, and their radioactivities were counted to determine the presence of alkylated selenium gases (e.g., [^{75}Se]dimethylselenide). To extract for [^{75}Se]selenide, pellet samples were acidified with 1 N H_2SO_4 and placed in an N_2 flushing-trapping train, and their radioactivities were counted (51). Experimental controls consisted of samples with air headspaces (O_2 consumed was replaced by syringe) and autoclaved samples under N_2, H_2, or H_2 plus 2 mM $Na_2 \cdot 9H_2O$.

To determine whether elemental selenium was the precipitation product of the experiments described above, we extracted ^{75}Se in sediments with solvents. Ten sediment slurry samples were prepared as described above, injected with [^{75}Se]selenate (0.1 μCi), and allowed to incubate under H_2. After 4 days, no significant counts remained in solution. Because red, amorphous Se^0(s) is soluble in CS_2 (48), this solvent was employed for extraction purposes. However, it was first necessary to wash the sediment pellets sequentially with solvents of decreasing polarity. The procedure was to centrifuge 1.5 ml of slurry, count the radioactivity in the supernatant, and suspend the pellet in 1.2 ml of deionized water. The slurry was centrifuged again, the radioactivity in the supernatant was counted, and the pellet was suspended in 1.2 ml of 95% ethanol. This procedure was repeated next with ethyl acetate. Finally, the pellet was suspended in an equal volume of CS_2, followed by an extraction interval (40 h at 4°C followed by 6 h at 20°C). The sample was then centrifuged, and the radioactivity in the supernatant was counted while the pellet was vortexed in new CS_2, centrifuged, decanted, and added to the previous CS_2 volume for counting. Pellet counts were also resolubilized by treatment with Na_2SO_3 (D. J. Velinsky, Ph.D. dissertation, Old Dominion University, Norfolk, Va., 1987). Pellet samples (1 ml) were put into tubes containing 4 ml of 1.25 M Na_2SO_3 plus 1.25 N H_2SO_4, sealed under N_2, and extracted for 48 h at 20°C, and centrifuged, followed by separate counting of the radioactivities in the supernatant and pellet.

An experiment was conducted to examine the oxidative resolubilization of ^{75}Se-precipitated counts associated with the sediment phase of slurries. Slurries were prepared as above and dispensed (25 ml/160-ml bottle sealed under H_2 with 1 μCi of [^{75}Se]selenate). After 9 days, most of the added counts were associated with the sediment pellet. Additions (~10 mmol/liter) of $NaNO_3$, MnO_2, FeOOH, or water (all from O_2-free stocks) were then made to the samples. The radioactivities in the supernatant and pellet fractions of centrifuged samples were counted over the course of a 22-day incubation by the procedures outlined above.

Isolation and physiological experiments with bacterial cultures capable of selenate respiration. For enrichments and isolations, the following medium was employed (grams per liter of deionized water): NaCl, 20.5; $MgCl_2 \cdot 6H_2O$, 0.5; $NaHCO_3$, 1.0; Na_2SeO_4, 3.8; K_2HPO_4, 0.225; KH_2PO_4, 0.225; $CaCl_2 \cdot 2H_2O$, 0.04; $(NH_4)_2SO_4$, 0.225; $MgSO_4 \cdot 7H_2O$, 0.09; sodium acetate, 2.72; cysteine hydrochloride, 0.06; $Na_2S \cdot 9H_2O$, 0.06; and the solutions of trace elements (9 ml) and vitamins (10 ml) of Wolin et al. (49). The final pH was 7.3. Liquid medium was dispensed (10 ml) under N_2-CO_2 (4:1) into Balch anaerobic culture tubes, crimp sealed, and autoclaved. Enrichments were established by inoculating tubes with sediment (0.2 ml) from the slurry samples which were exposed to acetate and selenate. After ~2 weeks of static incubation at 20°C, the inoculated tubes produced abundant red precipitate later identified as elemental selenium (see text). Enrichments were maintained with biweekly transfers. The enrichment was streaked onto roll tubes, and isolated red colonies were evident after 1 to 2 weeks of

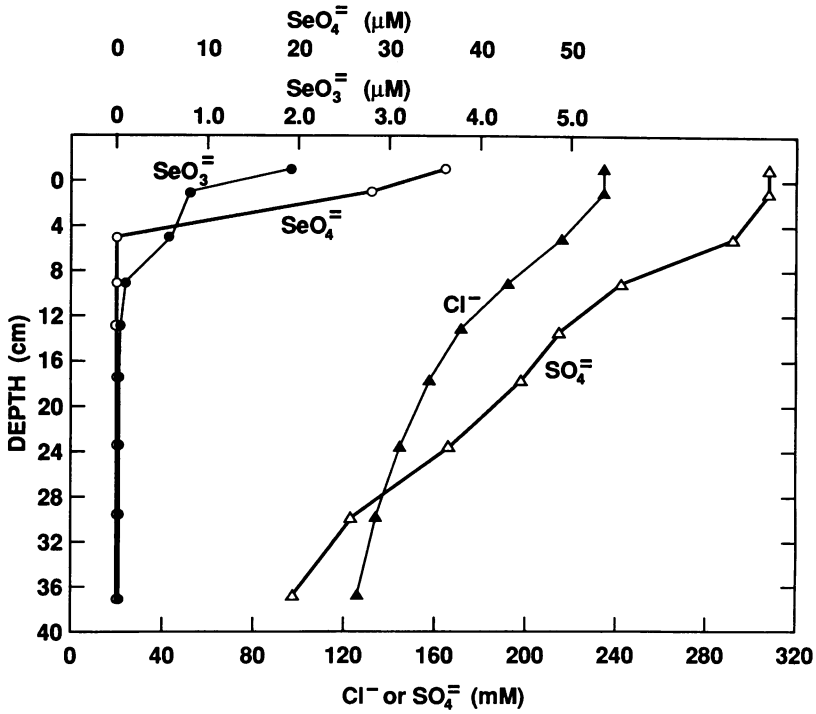

FIG. 1. Vertical profiles of dissolved selenate, selenite, sulfate, and chloride in a core taken from a San Joaquin Valley agricultural wastewater pond on 8 July 1988.

incubation. These colonies were picked and successfully transferred back to the liquid medium.

Growth of the isolate was determined by performing cell counts with acridine orange (15, 30). An experiment was conducted to demonstrate selenate-linked respiration of acetate. Culture tubes containing the above-described medium but with various concentrations of selenate (0, 0.5, 1.0, 2.0, 10.0, and 20.0 mM) and 2.5 μCi of [2-^{14}C]acetate were inoculated with 0.2 ml of a growing culture. After 28 days of incubation, tubes were injected with 1.5 ml of 6 N HCl, vigorously shaken, and, after 2 h, analyzed for $^{14}CO_2$ by radio-gas chromatography (see above). Similar growth and respiration experiments were also conducted on the initial enrichment culture with electron acceptors other than selenate (nitrate, trimethylamine oxide [TMAO], FeOOH, MnO_2, O_2). In addition, the effect of alternative electron acceptors (air, nitrate, TMAO, MnO_2, FeOOH) on the ability of enrichment cultures to remove [^{75}Se]selenate from solution was also examined. To determine whether the isolate was capable of denitrification, the acetylene-block assay was performed (3). Two culture tubes having medium lacking selenate but containing 10 mM nitrate were inoculated with 0.3 ml of a growing culture. One tube was incubated under N_2-CO_2, while the other had this atmosphere plus 2.6 ml of C_2H_2 (15% of the gas phase). After 48 h of incubation at 21°C, the quantity of N_2O in the headspaces of the tubes was determined by electron capture gas chromatography (32).

Scanning electron micrographs and X-ray diffraction. The procedures for the preparation of scanning electron micrograph-X-ray fluorescence micrographs of cultures have been described elsewhere (39a). X-ray diffraction analysis of dried precipitate from cultures was performed on a Nicolet X-ray diffraction unit model I-2.

RESULTS

Porewater profiles. Profiles of selenate, selenite, sulfate, and chloride in interstitial waters of a pond receiving agricultural wastewater are shown in Fig. 1. Selenate was abundant (36 μM) in the surface waters but was undetectable at depths below 2 cm. Selenite was about 20-fold less abundant than selenate and disappeared below 10 cm. In contrast, sulfate and chloride concentrations were reduced by only 68 and 54%, respectively, at the bottom of the core. Ratios of $(SeO_4^{2-} + SeO_3^{2-})/Cl^-$ were 1.65×10^{-3} in the surface water but rapidly decreased to 0.027×10^{-3} at the 4- to 6-cm depth interval. Ratios of SO_4^{2-}/Cl^- were constant (1.33 to 1.35) in the surface waters and upper 6 cm of sediment but progressively decreased with depth, reaching 0.79 at the bottom of the core (depth interval, 35 to 39 cm).

Sediment slurry experiments. In an initial experiment, selenate removal from slurries containing 21 mM sulfate proceeded more rapidly with H_2 than with N_2; however, after 7 days of incubation, both H_2 and N_2 slurries had removed >99.7% of the added selenate, while a more modest loss (~33%) was associated with an autoclaved control (Fig. 2). Selenite accumulated as a transient intermediate present at concentrations well below those of the initial selenate levels (<8 μM; data not shown). These results suggested that a bacterially mediated reduction of Se(VI) to a more reduced state than Se(IV) had occurred.

In a second experiment, the effect of several soluble electron donors (lactate, acetate, succinate, and glucose) on the removal of selenate (1.22 mM) from solution was examined (Table 1). After 5 days of incubation, unamended sediments removed 58% of the added selenate, while lactate- and acetate-amended slurries lost 100 and 95%, respectively. Succinate did not significantly speed the reaction, while

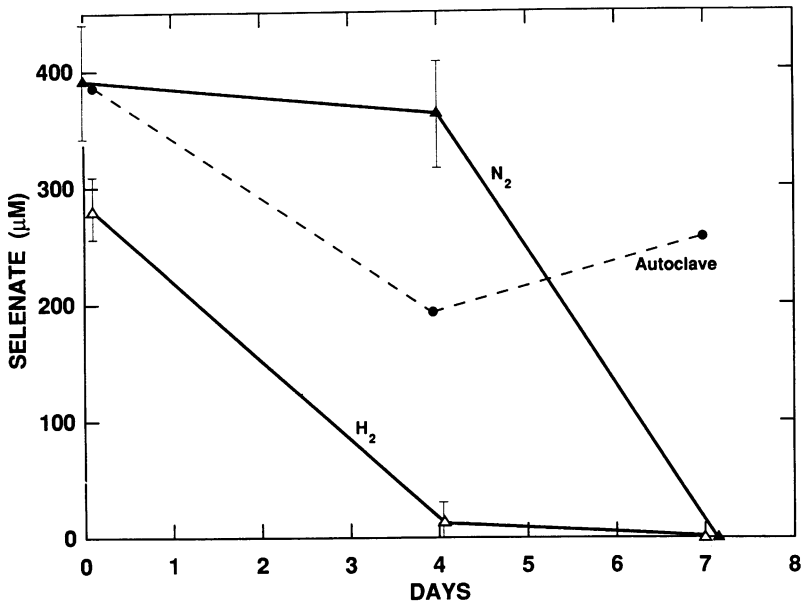

FIG. 2. Removal of added selenate from sediment slurries incubated under H_2 or N_2 or in an autoclaved control. Points represent the mean of three samples, and bars indicate 1 standard deviation (only one autoclaved control was run).

glucose appeared to have retarded selenate removal over that of the unsupplemented slurries. Significant quantities of selenite (0.160 to 0.272 mM) were present in the unamended, acetate, and succinate slurries at the end of the experiment.

The effects of several alternative electron acceptors on the removal of 5 mM selenate from sulfate-free slurries held under H_2 was investigated. Selenate removal was evident by day 4 in the unamended and FeOOH samples, and selenate disappeared completely by day 7 in these samples as well as those containing SO_4^{2-} (Fig. 3). Nitrate completely inhibited selenate removal during the incubation, and a partial inhibition (~72%) was achieved with MnO_2. A distinct color change to reddish brown was evident in all slurries except those amended with NO_3^- or MnO_2. This suggested that selenate was reduced to red elemental selenium, a fact we subsequently confirmed (see below). Significant levels of selenite were observed in some of the experimental flasks during the experiment. The highest values (0.76 to 1.3 mM) occurred by day 4 of the incubation in the unamended and the FeOOH-containing slurries and on day 7 in the sulfate-containing slurries (0.1 to 1.0 mM). No significant levels of selenite were observed in the slurries containing nitrate or MnO_2.

TABLE 1. Effect of various electron donors on selenate reduction in San Francisco Bay sediment slurries after 5 days of incubation[a]

Addition (10 mM)	SeO_4^{2-}[b] (mM)	SeO_3^{2-}[b] (mM)	SeO_4^{2-} lost (%)
None	0.510 (0.010)	0.160 (0.020)	58
Lactate	0.005 (0.001)	0.002 (0.000)	100
Acetate	0.058 (0.097)	0.272 (0.192)	95
Succinate	0.262 (0.380)	0.200 (0.143)	79
Glucose	0.817 (0.081)	0.001 (0.000)	33

[a] Initial levels of selenate and selenite were 1.22 and 0.001 mM, respectively.

[b] Results represent the mean of three samples, and values in parentheses indicate 1 standard deviation.

The effect of these various electron acceptors on the amount of selenate removed, methane produced, and H_2 consumed is shown in Table 2. In general, the quantity of H_2 consumed by the slurries was less than or equal to the total amount of electron-accepting equivalents present in the slurries. No significant levels of methane were produced in the slurries (with the exception of SO_4^{2-} plus WO_4^{2-}), indicating that the electron sinks for the H_2 oxidized were either the selenate or the alternative electron acceptors supplied. The only exception was the sulfate-amended slurries inhibited with tungstate. In this instance, selenate removal was totally inhibited and high levels of methane were present.

Sediment slurry experiments with [2-^{14}C]acetate. Slurries incubated with 10 mM sulfate formed $^{14}CH_4$ and $^{14}CO_2$ from [2-^{14}C]acetate (Fig. 4) and yielded very low $^{14}CH_4/^{14}CO_2$ ratios (0.0041). These values represented the metabolism of ~90% of the acetate. Incubation with 1 mM molybdate markedly inhibited $^{14}CO_2$ production (96%) but stimulated $^{14}CH_4$ formation 10-fold ($^{14}CH_4/^{14}CO_2$ ratio = ~1). Higher levels of molybdate had little further affect on $^{14}CO_2$ production but decreased $^{14}CH_4$ formation. The results for selenate contrasted with those for molybdate. Incubation with 1 mM selenate inhibited $^{14}CO_2$ production, although not to the same extent as that achieved with molybdate. However, 10 and 20 mM selenate resulted in significantly increased production of $^{14}CO_2$. Selenate only enhanced $^{14}CH_4$ production by ~35%, and there was no statistically significant effect of increasing selenate concentrations. The final $^{14}CH_4/^{14}CO_2$ ratio achieved with 20 mM selenate was 0.0083, and the quantity of $^{14}CO_2$ formed was 75% of that achieved in the absence of either molybdate or selenate.

Sediment slurry experiments with [^{75}Se]selenate. To identify the product(s) of selenate reduction in slurries, we conducted tracer experiments with $^{75}SeO_4^{2-}$. Loss of ^{75}Se from solution was associated with comparably increased levels of ^{75}Se in the sediments, and removal accounted for ≥95% of the added $^{75}SeO_4^{2-}$ (Fig. 5A). Similar results were achieved under an H_2 atmosphere, although the kinetics appeared to be slightly faster (Fig. 5B). No ^{75}Se was precip-

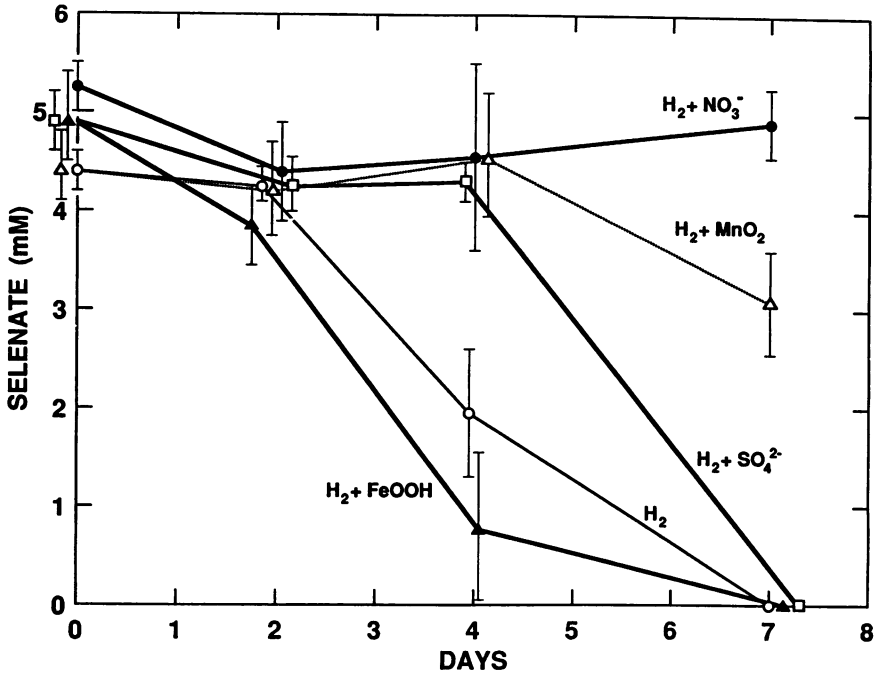

FIG. 3. Removal of added selenate from sulfate-free sediment slurries incubated under H_2 in the presence of various competitive electron acceptors. Points represent the mean of three samples, and bars indicate 1 standard deviation.

itated from solution in autoclaved (under H_2 or N_2) controls or in live slurries incubated under air (Fig. 5C). In addition, no precipitation occurred in autoclaved controls under air or in autoclaved controls under H_2 supplemented with 2 mM sodium sulfide (data not shown). No significant levels of ^{75}Se were detected in the gas phases of any of the slurries (Fig. 5A, B, and C), and in addition, ^{75}Se incorporated into the sediments in the "live" slurries was not acid volatile (Fig. 5A and B).

Removal of [^{75}Se]selenate from solution was inhibited by some but not all group VI oxyanions (Table 3). Chromate and tungstate effectively inhibited selenate removal. However, no inhibition occurred with molybdate.

Counts of ^{75}Se incorporated into the sediments could be extracted by the CS_2 extraction procedure (Table 4). Most of the counts (55%) were recovered in the CS_2, but significant quantities appeared in the ethyl acetate (15%) fraction and as a residual in the sediments (15%). Recovery was 92% for this procedure. Better recovery of $^{75}Se^0(s)$ was achieved by the sulfite oxidation method (Velinsky, Ph.D. dissertation). Re-

TABLE 2. Effect of alternative electron acceptors on selenate reduction, methane production, and hydrogen uptake by San Francisco Bay sediment slurries[a]

Addition (10 mmol/liter)	SeO_4^{2-} removed (mmol/liter)	CH_4 (mmol/liter)	H_2 (mmol/liter)
None	4.4	0.079	6.5
NO_3^-	0.4	0.0001	17.0
FeOOH	5.0	0.010	10.3
MnO_2	1.3	0.0004	21.0
SO_4^{2-}	4.9	0.115	7.2
SO_4^{2-} + WO_4^{2-}[b]	−0.7[c]	4.946	15.5

[a] Data represent the mean of three samples.
[b] Represents one sample only.
[c] Represents a nonsignificant increase of selenate.

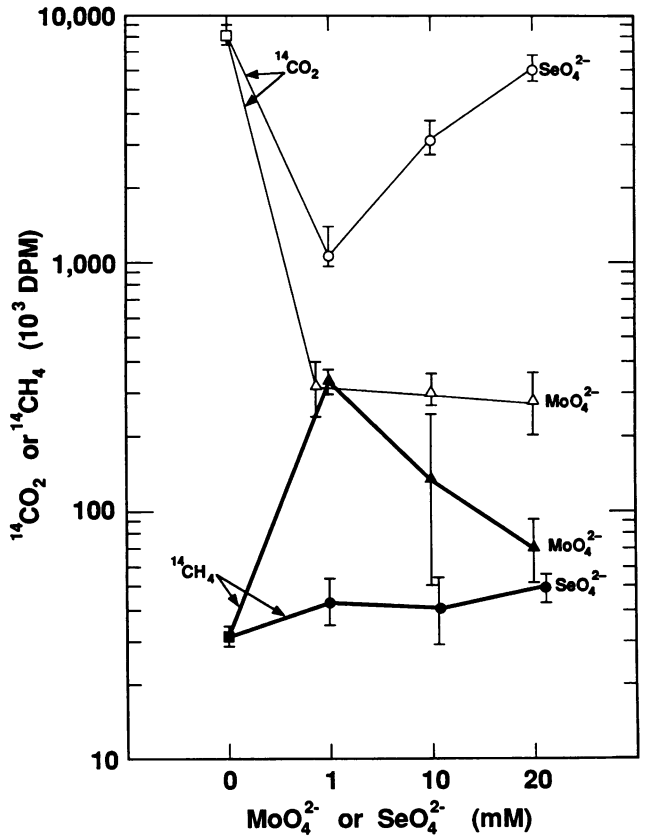

FIG. 4. Respiration of [2-^{14}C]acetate by sediment slurries containing 0, 1.0, 10.0, or 20.0 mM molybdate or selenate. Points represent the mean of three samples, and bars indicate 1 standard deviation.

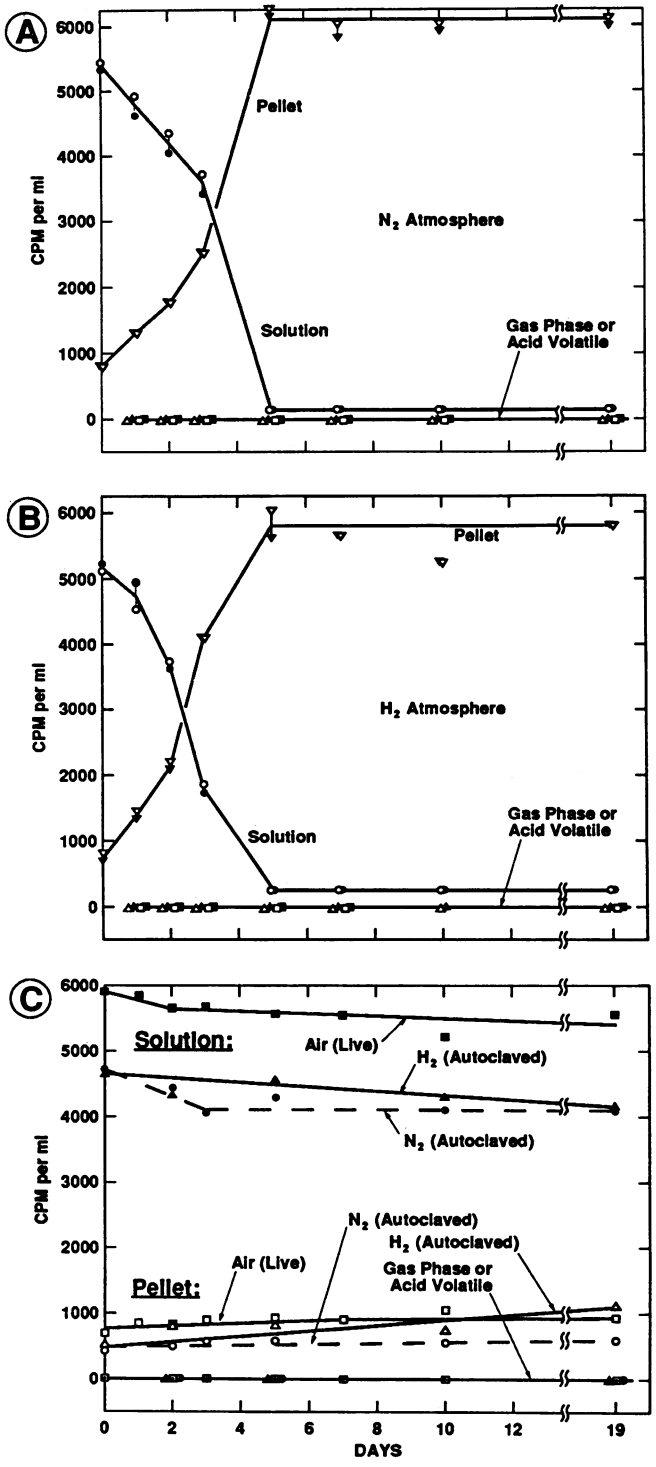

FIG. 5. Loss of ^{75}Se from solution and incorporation into sediments in slurries incubated under N_2 (A); or H_2 (B) or in autoclaved controls under N_2 or H_2 or live controls under air (C). All experimental conditions were run in duplicate; however, duplicates in panel C are not shown because of space limitations. All slurries initially contained 0.5 mM Na_2SeO_4.

TABLE 3. Inhibition of [^{75}Se]selenate reduction by group VI oxyanions[a]

Addition[b]	Counts in supernatant (dpm/ml)[c]
Na_2CrO_4	1,555
Na_2CrO_4	1,499
Na_2WO_4	1,364
Na_2WO_4	1,383
Na_2MoO_4	281
Na_2MoO_4	401

[a] Slurries were incubated in ABW plus sulfate (21 mM) under H_2 with 0.5 mM selenate.
[b] Concentration of inhibitors, 20 mM.
[c] After 12 days of incubation; total slurry mean disintegrations per minute per milliliter at start of experiment were 1,565 ± 58 (n = 6).

covery of Se^0(s) by oxidative solubilization was 74%, with 24% remaining in the sediments, which yielded a total recovery of 98% (Table 4).

The ability of various compounds to return the precipitated ^{75}Se into solution from the sediments is given in Table 5. In comparison with water controls, higher counts in solution occurred in the presence of FeOOH (about 3-fold) and nitrate (about 3.5-fold). However, incubation with MnO_2 produced only ~10% of the counts observed in the controls. Oxidative dissolution of ^{75}Se was small (~7%) when compared with the counts residual in the sediments.

Isolation of bacterial cultures and physiological experiments. Slow-growing enrichment cultures were obtained from inoculation of selenate-acetate medium with sediment from the slurry experiments. During growth, the enrichments produced either a red or black precipitate, the abundance of which increased with time. The more commonly observed red precipitate was soluble in CS_2 and could be solubilized by sulfite oxidation, as determined with ^{75}Se tracer (data not shown). The black precipitate was not soluble in CS_2 and was only slowly oxidized by sulfite treatment. These responses were consistent with that expected for red amorphous and black crystalline Se^0(s) (48). Analysis of both types of precipitates by scanning electron microscopy-X-ray fluorescence revealed amorphous (red) or crystalline (black) particles in which only Se was detected.

TABLE 4. Extraction of precipitated ^{75}Se from sediments with organic solvents or by sulfite oxidation[a]

Conditions	dpm/ml[b]	Recovery (%)
Total counts in slurry (before treatment)	1,507 (25)	
CS_2 extraction		
Distilled water	36 (4)	2
Ethanol	74 (8)	5
Ethyl acetate	228 (10)	15
Carbon disulfide	829 (40)	55
Counts remaining in pellet	232 (7)	15
Total recovered		93
Sulfite oxidation		
Supernatant	1,109 (45)	74
Pellet	369 (41)	24
Total recovered		98

[a] Extraction was performed after 4 days of incubation under H_2 when no significant counts (~9.7 ± 1.5 dpm) remained in solution.
[b] Values in parentheses indicate standard error of 10 samples.

TABLE 5. Dissolution of precipitated ^{75}Se in sediment slurries amended with various oxidants[a]

Addition (10 mmol/liter)	dpm/ml[b]	
	Supernatant	Total in slurry
Water	902	36,573
Water	1,035	36,194
Nitrate	3,666 (1,485)	37,154 (1,444)
MnO_2	112 (18)	35,445 (4,669)
FeOOH	3,686 (1,144)	40,474 (4,507)

[a] After 18 days of incubation.
[b] For duplicate or triplicate samples; parentheses indicate 1 standard deviation.

Because of the absence of significant quantities of other elements, the Se was presumed to be in the elemental state. X-ray diffraction analysis of the black precipitate revealed it to be composed of hexagonal Se^0(s). In some cultures, the initial red precipitate was observed to change into the black form over time (~2 weeks). No precipitate was formed in uninoculated media.

Incubation of the enrichment culture with [2-^{14}C]acetate (2.5 μCi per tube) for 28 days resulted in the formation of $^{14}CO_2$ for the following electron acceptors ($^{14}CO_2$ dpm per tube): none (0), 20 mM selenate (388,000), 10 mM nitrate (254,000), 10 mM TMAO (140,000), 20 mmol of MnO_2 per liter (10,300), 20 mmol of FeOOH per liter (131,000), and air (582,000). In another experiment, the enrichment culture was grown in medium containing 20 mM selenate with or without additional electron acceptors. Reduction of selenate was monitored as loss of [^{75}Se]selenate from solution. After 13 days of incubation, the percent selenate removed was as follows: regular selenate medium, 86%; with air, 8%; with 20 mM nitrate, 5%; with 40 mM nitrate, 3%; with 20 mM sulfate, 95%; with 40 mM sulfate, 83%; with 20 mM TMAO, 0%; with 40 mM TMAO, −6%; with 20 mmol of FeOOH per liter, 73%; and with 5 mmol of MnO_2 per liter, 17%.

An isolated red colony was picked from enrichment-streaked roll tubes and successfully transferred back to acetate-selenate medium. Cells consisted of gram-negative cocci (diameter, ~0.6 μm). Growth of the isolate (designated strain SeS) was rapid with nitrate or TMAO but considerably slower with selenate and only marginal under air (Table 6). After 6 weeks of incubation, however, selenate-grown cultures achieved densities comparable (~4 × 10^8 cells per ml) to those of nitrate- or TMAO-grown cultures.

A rough balance was achieved between the amount of selenate removed from culture fluid after growth, the final cell density, and the dry weight of the Se^0(s) precipitate. After 9 weeks of incubation, the dry weight of the washed red precipitate from a tube was 9.0 mg, while the residual selenate was 10.1 mM, indicating the removal of 9.9 mM selenate (~7.82 mg of Se per tube). With a final a cell density of 5 × 10^9 cells per 10 ml of medium and a cell volume of 0.113 μm^3 for spherical cells with a 0.6-μm diameter, the total cellular volume would be ~0.57 × 10^9 μm^3. Using a conversion factor of 5.6 × 10^{-13} g of C/μm^3 and assuming that C equals one-half of the cellular dry weight (5), this gives a total bacterial dry weight of 0.63 mg. Thus, the net Se weight of the precipitate (8.37 mg) divided by the expected weight (7.82 mg) yields a recovery of 107%.

TABLE 6. Growth of strain SeS with various electron acceptors

Addition[a]	10^6 Cells per ml at day:						
	0	2	3	4	5	6	11
None[b]	0.39				10		18
TMAO	0.44	15	79	105		580	
Nitrate	0.44	21	79	101		880	
Selenate	0.42				10		41
Air	0.27						3.9[c]

[a] TMAO, nitrate, and selenate were all added at 20 mM.
[b] Carry-over of selenate = ~0.4 mM.
[c] Taken after 14 days.

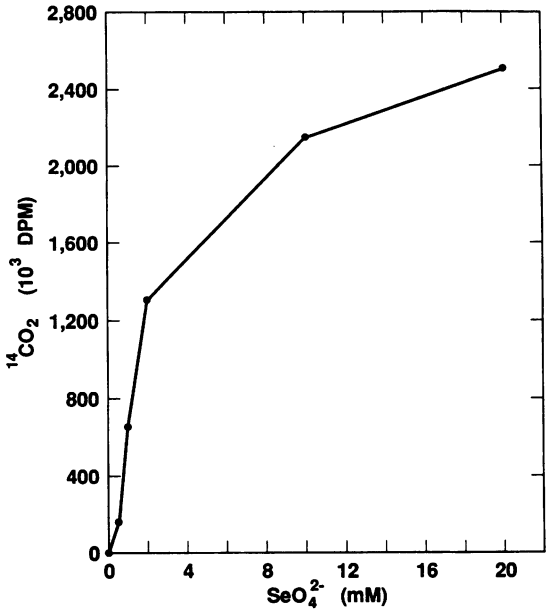

FIG. 6. Selenate-linked respiration of [2-^{14}C]acetate by strain SeS after 28 days of incubation in medium initially containing 20 mM selenate and 20 mM acetate.

Strain SeS respired selenate while growing on acetate. After 28 days of incubation at various selenate concentrations, progressively more red Se^0(s) was evident in tubes containing higher initial levels of selenate. Analysis for $^{14}CO_2$ indicated that more of this gaseous product was formed with increasing selenate concentrations (Fig. 6). The amount of $^{14}CO_2$ formed in the 20 mM selenate tube represented oxidation of 45% (9 mM) of the available acetate. When strain SeS was grown in nitrate medium, about 25-fold more N_2O was formed in the presence of C_2H_2 than in its absence (without acetylene, the isolate produced 0.1 and 3.5 nmol of N_2O at 0 and 48 h, respectively. With acetylene, the isolate produced 0.2 and 86.8 nmol of N_2O at 0 and 48 h, respectively.)

DISCUSSION

The interstitial profiles of selenate and selenite indicated that these oxyanions were removed by a process occurring near the sediment surface (Fig. 1). By contrast, removal of sulfate by sulfate reduction occurred at far greater depths. This pronounced spatial separation clearly indicated that two separate removal processes were operative in these sediments. Furthermore, the great abundance of sulfate ions (240 to 310 mM) in the upper core layers revealed that removal of selenium oxyanions was independent of sulfate, a point which contrasts with previous concepts with regard to

the biogeochemistry and microbiology of selenium which implied the involvement of sulfur-cycle organisms (12). The surface waters of these evaporation ponds contained abundant levels of nitrate (~2 mM) which, like the selenium oxyanions, also decreased rapidly with depth (R. S. Oremland, A. S. Maest, L. G. Miller, and J. T. Hollibaugh, manuscript in preparation).

To better understand the process(es) responsible for the selenate-selenite removal, we conducted slurry experiments with well-studied San Francisco Bay mud (10, 27, 32). This material was chosen for sampling convenience and is thought to be representative of processes occurring in anoxic, saline sediments. However, we were also able to repeat our results with sediments from the evaporation ponds, as well as with materials from selenium-impacted subsurface soils (N. Dubrovsky, J. Neil, J. T. Hollibaugh, and R. S. Oremland; and R. S. Oremland, A. S. Maest, L. G. Miller, and J. T. Hollibaugh, manuscripts in preparation).

Selenate removal in slurries was accelerated with H_2, lactate, and acetate and was inhibited by autoclaving (Fig. 2; Table 1). In addition, no significant acceleration occurred with succinate, and some retardation occurred with glucose. These results indicate that a microbial process was involved in selenate removal and that a measure of substrate specificity existed with regard to this process. The partial removal of traces (~1 μM) of selenate by biological activity in anoxic lake sediments has been observed by other workers (18). It was significant that H_2 and acetate were identified as potential substrates in our experiments because they are well recognized as key electron donors fueling terminal anaerobic processes like iron reduction, sulfate reduction, methanogenesis, etc. (19, 28, 47). Their addition to sediments results in the stimulation of these terminal processes (27, 31), as was observed for H_2 with regard to selenate (Fig. 2). Stimulation by lactate probably was a consequence of its ability to be quickly converted into acetate and H_2 in anoxic sediments.

Removal of high levels (5 mM) of selenate from slurries was unaffected by FeOOH or sulfate (Fig. 3). However, both nitrate and MnO_2 effectively inhibited selenate removal. Nitrate has been observed to interfere with selenate removal in soil column experiments (O. Weres, H. R. Bowman, A. Goldstein, E. C. Smith, and L. Tsao, Water Air Soil Pollut., in press). The removal process we observed was clearly reductive in nature because of the transient appearance of selenite in this and the preceding slurry experiment (Table 1). In addition, the marked appearance of a red coloration in the sediments implied the formation of amorphous, red Se^0(s). Because no significant selenite or red sediment coloration was present in nitrate- or MnO_2-containing samples, they served as controls for the observation of selenate reduction. All the hydrogen consumed in this experiment could be accounted for by the quantity of available electron acceptors, since no significant amounts of methane were formed (Table 2). The only exception to this was the case of the sulfate plus tungstate condition. However, because tungstate inhibits both sulfate reduction (29) and selenate reduction (Table 2), methanogenesis was stimulated by default (Fig. 7; see below). Two possibilities exist with regard to the mechanism(s) for inhibition of selenate reduction by nitrate and MnO_2: their action as competitive electron acceptors, or their ability to reoxidize reduced, precipitated Se from the sediments, or both. As we shall see below, experimental evidence clearly points to the former case.

Removal of selenate ions from solution resulted in their quantitative recovery as a precipitate in the sediments (Fig. 5). No significant quantities of gaseous Se or acid-volatile Se were detected when slurries were incubated under N_2 (Fig. 5A), H_2 (Fig. 5B), or in any of the controls (Fig. 5C). This indicated that reduced, alkylated gases like dimethylselenide (36) or selenide (e.g., H_2Se or FeSe) were not formed during the incubation. This latter point reinforces earlier observations made with these sediments in which only a tiny portion (≤~0.1%) of added [^{75}Se]selenate was converted to selenide in low-sulfate (<4 mM) slurries, even when they were amended with H_2 or lactate (51). The lack of selenate reduction in the controls (Fig. 5C) underscores that this was caused by an anaerobic, microbial process. This point was further stressed by the absence of any apparent selenate removal from autoclaved slurries incubated under highly reducing chemical conditions (H_2 atmosphere plus 2 mM sulfide). Thus, the observed selenate reduction was the result of direct biological activity and not due to the accumulation of reactive, bacterially formed reductants like sulfide.

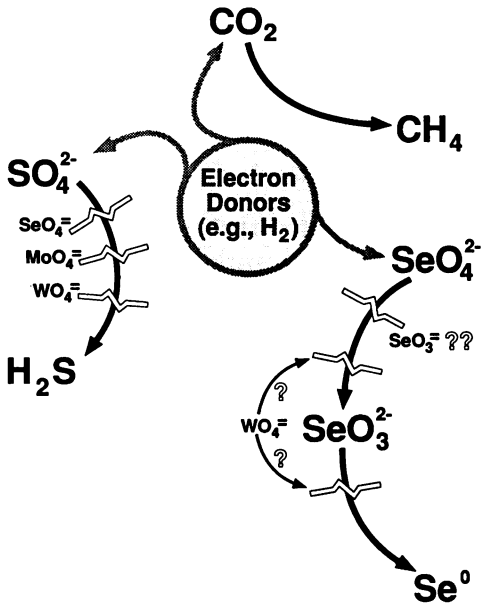

FIG. 7. Schematic representation of the possible modes of action of group VI inhibitors.

Since the precipitate was chemically more reduced than selenite and more oxidized than selenide, and because removal of high levels of selenate (5 mM) resulted in a red coloration in the slurries, elemental selenium was the logical product of the observed selenate reduction. This was confirmed in the extraction-oxidation experiments (Table 4). A significant quantity of counts remained in the sediment pellet in either the CS_2 extraction (15%) or the sulfite oxidation (24%). However, this can be explained by the presence of hexagonal, crystalline Se^0(s), a substance which is resistant to these extraction-oxidation procedures (48). The fact that this form of Se^0(s) was observed in some of the cultures (in addition to the red amorphous form) reinforces this explanation.

Sulfate did not inhibit selenate reduction in the slurry experiments (Fig. 3), in the enrichment cultures (see Results), or in [^{75}Se]selenate additions to slurries incubated with 0.1 to 10 mM sulfate and 0.1 to 10 mM selenate (data not shown). These results demonstrate that sulfate reduction was not the pathway for selenate reduction to Se^0(s). Thus,

the hypothesis generated from the selenate-sulfate core profiles (Fig. 1) has been confirmed.

The inhibitor results were, at first glance, contradictory but were nonetheless intriguing. Tungstate and chromate inhibited selenate reduction; however, no inhibition occurred with molybdate (Tables 2 and 3). Oxyanions of group VI elements inhibit sulfate-respiring bacteria (34), in part by destroying their intracellular ATP pools (43). However, a point often overlooked in inhibitor experiments with complex, mixed microbial populations is the unanticipated action of the inhibitor on nontarget organisms (29). Thus, although tungstate inhibits sulfate reduction, it also inhibits various molybdoenzymes which are not inhibited by molybdate. Relevant examples include the dimethyl sulfoxide reductase of *Escherichia coli* (4), which also reduces TMAO, nitrate, fumarate, hydroxylamine, and a variety of other compounds (46), as well as nitrate reductases (see review in reference 41). Thus, tungstate (and chromate) probably inhibited a similar enzyme involved in selenate reduction, while molybdate only interfered with sulfate reduction. These points are illustrated in Fig. 7. The fact that the isolated strain SeS grows on TMAO as well as nitrate (Table 6) suggests that some enzyme system similar to the dimethyl sulfoxide reductase or nitrate reductase is responsible for selenate reduction. However, from our current data we cannot predict the actual point of action of tungstate on selenate reduction. The compound may have inhibited the direct reduction of selenate or, alternatively, the subsequent reduction of selenite to Se^0(s), which could have resulted in the accumulation of toxic levels of selenite (Fig. 7). We observed a rapid accumulation of selenite (~0.022 μM) in the tungstate-inhibited slurry at the first sampling period (total incubation time, ~2 h), which at least suggests the latter case as a possible interpretation. However, other than implicating the involvement of a molybdoenzyme in the process of selenate reduction to Se^0(s), we cannot ascertain from our data the location of this enzyme in the overall pathway. This will be the subject for future investigations.

Clearly, selenate behaved differently from molybdate when these compounds were added to sediments to investigate acetate metabolism (Fig. 4). Molybdate lowered $^{14}CO_2$ production while stimulating that of $^{14}CH_4$, a result consistent with the stimulation of methanogenesis by inhibition of competitive sulfate reducers that has been reported by many investigators (29). However, the results for selenate, especially at the 10 and 20 mM levels, clearly indicated that it behaved as an oxidant for acetate metabolism to CO_2 rather than as an inhibitor of this process (although it certainly blocked the involvement of sulfate respirers). The fact that selenate did not significantly stimulate methanogenesis indicates that as for sulfate reduction or iron reduction (e.g., references 21 and 31), selenate-reducing bacteria have the potential to outcompete methanogens for common electron donors.

A small amount of precipitated ^{75}Se in sediments could be solubilized by incubation with nitrate or FeOOH (Table 5). It is possible that this occurred by an anaerobic oxidation of Se^0(s) analogous to that reported for sulfide by MnO_2 (2). However, only a small quantity of material (~7%) was "oxidized," and time course experiments indicated that these solubilized counts diminished rapidly by 3 weeks of incubation (data not shown). Thus, in terms of mass balances, reduction of selenate to Se^0(s) was the major reaction, while anaerobic reoxidation was only minor. Therefore, the inhibition of selenate reduction by nitrate and MnO_2 (Fig. 3) cannot be explained by rapid reoxidation. Furthermore, it is clear that, if anything, MnO_2 inhibited this oxidation (Table 5). Therefore, a more reasonable explanation is that nitrate and MnO_2 acted as preferred electron acceptors over selenate for bacterial respiration. Such phenomena have been well studied in sediments for a variety of processes like iron reduction versus methanogenesis or sulfate reduction (21) or sulfate reduction-denitrification versus methanogenesis (for reviews, see references 28 and 47). This interpretation is borne out by experiments with the enrichment culture in which selenate reduction (loss from solution) was inhibited by nitrate, MnO_2, and TMAO but not by sulfate or FeOOH, all of which sustained oxidation of acetate (see Results). Furthermore, the fact that strain SeS grew well on TMAO or nitrate (Table 6) and is a denitrifier (see Results) confirms the point that nitrate is preferred over selenate as an electron acceptor.

The fact that millimolar levels of selenate were reduced in slurry experiments (Fig. 3) and that these quantities of selenate could be linked to the metabolism of acetate in sediments (Fig. 4) indicated that isolation of a selenate-respiring culture was feasible. That strain SeS is capable of selenate-dependent growth was borne out in preliminary experiments (Table 6). The fact that a rough balance could be calculated between the amount of Se^0(s) formed and the quantity of selenate lost from solution indicated that a stoichiometric balance between reactant and product could be achieved. Finally, selenate-dependent acetate oxidation was demonstrated for the culture (Fig. 6), a fact which confirms that these organisms carry out a novel respiration. Although there were previous observations of what appeared to be Se^0(s) precipitation from selenate in various bacterial cultures (7, 9, 13, 17), these reports usually consisted of casual observations peripheral to the main thrusts of the investigations. Recently, Maiers et al. (24) reported Se^0(s) precipitation from selenate (maximum concentration, 1 mM) by enrichment cultures; however, it was not clear whether this occurred under aerobic or anaerobic conditions, and a respiratory linkage was not reported. However, a pseudomonad which respires selenate to selenite was recently isolated (J. Macy, personal communication). To our knowledge, that organism and strain SeS constitute the only bacteria identified thus far which are capable of performing this novel respiratory feat.

Although our physiological studies with SeS are only preliminary, a brief examination of the energy yields for anaerobic growth on selenate is instructive. We propose the following reaction using published values of ΔG_f^0 (44, 50):

$$4CH_3COO^- + 3SeO_4^{2-} \longrightarrow 3Se^0 + 8CO_2 + 4H_2O + 4H^+ \quad (1)$$

$$4(-369.3) + 3(-441.2) \longrightarrow 8(-394.4) + 4(-237.2)$$

Thus, $\Delta G^0 = -326$ kJ/mol of acetate. Because four protons are involved:

$$\Delta G^{0\prime} = \Delta G^0 + m\Delta G_f^*(H^+) = -1{,}463 \text{ kJ per reaction} \quad (2)$$

or −366 kJ/mol of acetate. Thus, the reduction of selenate to Se^0(s) with acetate is highly exergonic. However, because reduction of selenate to selenite with either H_2 or acetate is also highly exergonic (e.g., $G^0 = -162$ kJ/mol of H_2), growth with a selenite end product can also be achieved. This contrasts with an endergonic reaction associated with sulfate reduction to sulfite by H_2 ($G^0 = +20$ kJ/mol of H_2). At present, it is not clear whether strain SeS carries out a total reduction of selenate to Se^0(s) or a partial reduction to selenite followed by a later reduction of selenite to Se^0(s).

Other workers have observed that reduction of selenate to Se^0(s) is carried out sequentially by two organisms (J. Macy, personal communication). At present, we cannot confirm whether our strain SeS is pure, and these details will require future work. Bacterial Se^0(s) precipitation from selenite has been commonly reported for a variety of microbes including algae (16) and bacteria (25) and can be achieved chemically with strong reductants. However, the quantity of cysteine-sulfide in our medium (0.6 mM) was too little to precipitate selenite formed from the extent of selenate reduction to Se^0(s) which we observed (16 mM).

In summary, we discovered a novel bacterial respiratory process which profoundly influences the fate of selenium oxyanions. The dissimilatory reduction of selenate to Se^0(s) occurs independently of sulfate, and therefore the reductive Se cycle differs from that of sulfur. Indeed, that elemental Se is a product of the reductive Se cycle is in itself different from the S cycle because S^0(s) is neither a product nor intermediate in sulfate reduction but arises from partial oxidation of sulfide. It is significant, therefore, that Se^0(s) has been reported to be the major form of selenium in salt-marsh sediments (Velinsky, Ph.D. dissertation; Weres et al., in press). We observed the dissimilatory reduction of millimolar levels of added selenate (Fig. 5) and saw that this is quantitatively converted to Se^0(s) (Fig. 4; Table 4). In addition, we also performed experiments which indicated that selenate respiration occurs at nanomolar to micromolar selenate concentrations in sediments, and in situ assays have revealed that the upper few centimeters of the sediment column are the site for this activity (R. S. Oremland, A. S. Maest, L. G. Miller, and J. T. Hollibaugh, in preparation). These observations indicate that selenate respiration is the major sink for selenium removal in anoxic sediments and therefore leave upon the possibility of an agricultural waste treatment process based on sequestering Se^0(s) by bacterial respiration. In addition, the occurrence of relatively high levels of selenite in oxygenated waters of the oceans has been explained by oxidation of Se incorporated into organic matter by assimilatory reduction (11). We now propose an alternative hypothesis, namely, that dissimilatory reduction of selenate to selenite occurs within the reduced microzones of marine particles (1).

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LITERATURE CITED

1. **Alldredge, A. L., and Y. Cohen.** 1987. Can microscale chemical patches persist in the sea? Microelectrode study of marine snow, fecal pellets. Science **235**:689–691.
2. **Aller, R. C., and P. D. Rude.** 1988. Complete oxidation of solid phase sulfides by manganese and bacteria in anoxic marine sediments. Geochim. Cosmochim. Acta **52**:751–765.
3. **Balderston, W. L., B. Sherr, and W. J. Payne.** 1976. Blockage by acetylene of nitrous oxide reduction in *Pseudomonas perfectomarinus*. Appl. Environ. Microbiol. **31**:504–508.
4. **Bilous, P. T., and J. H. Weiner.** 1985. Dimethylsulfoxide reductase activity by anaerobically grown *Escherichia coli* HB101. J. Bacteriol. **163**:1151–1155.
5. **Bratback, G.** 1985. Bacterial biovolume and biomass estimations. Appl. Environ. Microbiol. **49**:1488–1493.
6. **Brimmer, S. P., W. R. Fawcett, and K. A. Kulhavy.** 1987. Quantitative reduction of selenate ion to selenite in aqueous samples. Anal. Chem. **59**:1470–1471.
7. **Brown, T. A., and A. Shrift.** 1980. Assimilation of selenate and selenite by *Salmonella typhimurium*. Can. J. Microbiol. **26**: 671–675.
8. **Bryant, R. D., and E. J. Laishley.** 1988. Evidence for two transporters of sulfur and selenium oxyanions in *Clostridium pasteurianum*. Can. J. Microbiol. **34**:700–703.
9. **Burton, J. A., Jr., T. H. Giddings, P. DeBrine, and R. Fall.** 1987. High incidence of selenite-resistant bacteria from a site polluted with selenium. Appl. Environ. Microbiol. **53**:185–188.
10. **Culbertson, C. W., A. J. B. Zehnder, and R. S. Oremland.** 1981. Anaerobic oxidation of acetylene by estuarine sediments and enrichment cultures. Appl. Environ. Microbiol. **41**:396–403.
11. **Cutter, G. A., and K. W. Bruland.** 1984. The marine biogeochemistry of selenium: a re-evaluation. Limnol. Oceanogr. **29**:1179–1192.
12. **Doran, J. W.** 1982. Microorganisms and the biological cycling of selenium. Adv. Microb. Ecol. **6**:17–32.
13. **Doran, J. W., and M. Alexander.** 1977. Microbial transformation of selenium. Appl. Environ. Microbiol. **33**:31–37.
14. **Ehrlich, H. L.** 1987. Manganese oxide reduction as a form of anaerobic respiration. Geomicrobiol. J. **5**:423–432.
15. **Hobbie, J. E., R. J. Daley, and S. Jaspar.** 1977. Use of Nuclepore filters for counting bacteria by fluorescence microscopy. Appl. Environ. Microbiol. **33**:1225–1228.
16. **Kumar, H. D., and G. Prakash.** 1971. Toxicity of selenium to the blue-green algae, *Anacyctis nidulans* and *Anabaena variabilis*. Ann. Bot. **35**:697–705.
17. **Lindblow-Kull, C., A. Shrift, and R. L. Gherna.** 1982. Aerobic, selenium-utilizing bacillus isolated from seeds of *Astragalus crotalariae*. Appl. Environ. Microbiol. **44**:737–743.
18. **Lipinski, N. G., P. M. Huang, U. T. Hammer, and W. K. Liaw.** 1987. The interaction of selenate and selenite with selected freshwater sediments. Int. Rev. Gesamten Hydrobiol. **72**:107–114.
19. **Lovley, D. R.** 1987. Organic matter mineralization with reduction of ferric iron: a review. Geomicrobiol. J. **5**:375–400.
20. **Lovley, D. R., and E. J. Phillips.** 1986. Organic matter mineralization with the reduction of ferric iron in anaerobic sediments. Appl. Environ. Microbiol. **51**:683–689.
21. **Lovley, D. R., and E. J. Phillips.** 1987. Competitive mechanisms for inhibition of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Appl. Environ. Microbiol. **53**:2636–2641.
22. **Lovley, D. R., and E. J. P. Phillips.** 1988. Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. Appl. Environ. Microbiol. **54**:1472–1480.
23. **Lovley, D. R., J. F. Stoltz, G. L. Nord, Jr., and E. J. P. Phillips.** 1987. Anaerobic production of magnetite by a dissimilatory iron-reducing microorganism. Nature (London) **330**:252–254.
24. **Maiers, D. T., P. L. Wichlacz, D. L. Thompson, and D. F. Bruhn.** 1988. Selenate reduction by bacteria from a selenium-rich environment. Appl. Environ. Microbiol. **54**:2591–2593.
25. **McCready, R. J. L., J. N. Campbell, and J. I. Payne.** 1966. Selenite reduction by *Salmonella heidelbergi*. Can. J. Microbiol. **12**:703–714.
26. **Myers, C. R., and K. H. Nealson.** 1988. Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science **240**:1319–1321.
27. **Oremland, R. S.** 1981. Microbial formation of ethane in anoxic, estuarine sediments. Appl. Environ. Microbiol. **42**:122–129.
28. **Oremland, R. S.** 1988. Biogeochemistry of methanogenic bacteria, p. 641–705. *In* A. J. B. Zehnder (ed.), Biology of anaerobic microorganisms. John Wiley & Sons, Inc., New York.
29. **Oremland, R. S., and D. G. Capone.** 1988. Use of "specific" inhibitors in biogeochemistry and microbial ecology. Adv. Microb. Ecol. **10**:285–383.
30. **Oremland, R. S., R. P. Kiene, I. Mathrani, M. J. Whiticar, and**

D. Boone. 1989. Description of an estuarine methylotrophic methanogen which grows on dimethylsulfide. Appl. Environ. Microbiol. **55**:994–1002.
31. **Oremland, R. S., and S. P. Polcin.** 1982. Methanogenesis and sulfate reduction: competitive and non-competitive substrates in estuarine sediments. Appl. Environ. Microbiol. **44**:1270–1276.
32. **Oremland, R. S., C. Umberger, C. W. Culbertson, and R. L. Smith.** 1984. Denitrification in San Francisco Bay intertidal sediments. Appl. Environ. Microbiol. **47**:1106–1112.
33. **Oremland, R. S., and J. P. Zehr.** 1986. Formation of methane and carbon dioxide from dimethylselenide in anoxic sediments and by a pure culture of an estuarine methanogen. Appl. Environ. Microbiol. **52**:1031–1036.
34. **Postgate, J. R.** 1952. Competitive and non-competitive inhibitors of bacterial sulfate-reduction. J. Gen. Microbiol. **6**:128–142.
35. **Presser, T. C., and I. Barnes.** 1984. Selenium concentrations in waters in the vicinity of Kesterson National Wildlife Refuge and the west grassland, Fresno and Merced counties, California. U.S. Geological Survey Water Resources Investigations report 85-4220. U.S. Geological Survey, Menlo Park, Calif.
36. **Reamer, D. C., and W. H. Zoller.** 1980. Selenium biomethylation products from soil and sewage sludge. Science **208**:500–502.
37. **Shrift, A.** 1954. Sulfur-selenium antagonism. I. Antimetabolite action of selenate on the growth of *Chlorella vulgaris*. Am. J. Bot. **41**:223–230.
38. **Shrift, A.** 1964. A selenium cycle in nature? Nature (London) **201**:1304–1305.
39. **Shrift, A., and E. Kelly.** 1962. Adaptation of *Escherichia coli* to selenate. Nature (London) **195**:732–733.
39a. **Smith, R. L., F. E. Strohmaier, and R. S. Oremland.** 1985. Isolation of anaerobic oxalate-degrading bacteria from freshwater lake sediments. Arch. Microbiol. **141**:870–879.
40. **Stadtman, T. C.** 1974. Selenium biochemistry. Science **183**:915–922.
41. **Stouthamer, A. H.** 1988. Dissimilatory reduction of oxidized nitrogen compounds, p. 245–303. *In* A. J. B. Zehnder (ed.), Biology of anaerobic microorganisms. John Wiley & Sons, Inc., New York.
42. **Sylvester, M. A., J. P. Deason, H. R. Feltz, and R. A. Engberg.** 1988. Preliminary results of the Department of the Interior's irrigation drainage studies, p. 665–677. *In* Proceedings on planning now for irrigation drainage studies. American Society of Civil Engineers, New York.
43. **Taylor, B. F., and R. S. Oremland.** 1979. Depletion of adenosine triphosphate in *Desulfovibrio* by oxyanions of group VI elements. Curr. Microbiol. **3**:101–103.
44. **Thauer, R. K., K. Jungermann, and K. Decker.** 1977. Energy conservation in chemotrophic anaerobic bacteria. Bacteriol. Rev. **41**:100–180.
45. **Vijan, P. N., and D. Leung.** 1980. Reduction of chemical interference and speciation studies in the hydride generation-atomic absorption method for selenium. Anal. Chim. Acta **120**:141–146.
46. **Weiner, J. H., D. P. MacIssac, R. E. Bishop, and P. T. Bilous.** 1988. Purification and properties of *Escherichia coli* dimethyl sulfoxide reductase, an iron-sulfur molybdoenzyme with broad substrate specificity. J. Bacteriol. **170**:1505–1510.
47. **Widdel, F.** 1988. Microbiology and ecology of sulfate- and sulfur-reducing bacteria, p. 373–416. *In* A. J. B. Zehnder (ed.), Biology of anaerobic microorganisms. John Wiley & Sons, Inc., New York.
48. **Windholz, M. (ed.).** 1976. The Merck index, 9th ed. Merck & Co., Inc., Rahway, N.J.
49. **Wolin, E. A., M. J. Wolin, and R. S. Wolfe.** 1963. Formation of methane by bacterial extracts. J. Biol. Chem. **121**:184–191.
50. **Woods, T. L., and R. M. Garrels.** 1987. Thermodynamic values at low temperature for natural inorganic materials. Oxford University Press, New York.
51. **Zehr, J., and R. S. Oremland.** 1987. Reduction of selenate to selenide by sulfate-respiring bacteria: experiments with cell suspensions and estuarine sediments. Appl. Environ. Microbiol. **53**:1365–1369.