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Selenate Reduction by Bacteria from a Selenium-Rich Environment

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Samples collected from Kesterson Reservoir were screened for bacterial presence and selenate reduction capability. Selenate concentrations of 100 mg/liter were not toxic to indigenous bacteria. Of the 44 samples collected, 20 possessed microbial populations capable of reducing selenate. Reduction was observed in 4% of the water samples, 92% of the sediment samples, and 100% of the soil samples. Microbial reduction of 100 mg of selenate per liter was complete within 1 week of incubation. Up to 75 mg of selenate per liter was reduced beyond selenite to an insoluble red precipitate. Data collected indicate that indigenous bacteria have a significant role in the biogeochemical cycling of selenium.

Selenium is a naturally occurring element of considerable biological interest because of the small difference between concentrations which are physiologically essential and concentrations which are toxic to organisms (6). Groundwater contamination by selenium occurs and is primarily a result of its solubilization and migration from soil due to chemical, physical, and biological processes. Selenium is found commonly in four inorganic oxidation states (6). Selenate (+VI valence state), the most oxidized form of selenium, and selenite (+IV valence state) are highly water soluble and are known to be toxic to biological systems at relatively low concentrations (parts per million). Elemental selenium (+0 valence state) is highly insoluble in water and therefore has minimum toxicity. The most reduced form of selenium is selenide (−II valence state), which may be a highly toxic gas but is seldom a biological threat because it is readily oxidized to insoluble elemental selenium in the presence of air.

Microorganisms possess the capacity to oxidize or reduce a wide variety of selenium-containing compounds (12). Microbial genera observed to have this ability include *Acinetobacter* (4), *Aeromonas* (4, 5), *Arthrobacter* (4), *Bacillus* (4, 17), *Candida* (9, 15), *Cephalosporium* (2), *Citrobacter* (4), *Corynebacterium* (4, 8), *Flavobacterium* (4, 5), *Fusarium* (2), *Micrococcus* (19), *Neurospora* (20), *Penicillium* (2, 3), *Pseudomonas* (4, 5), *Salmonella* (14), *Scopulariopsis* (2), and *Selenomonas* (11). While microbial reduction of selenite to insoluble elemental selenium and selenide has been widely reported (2–5, 7–11, 14, 17, 19, 20), reports of selenate being reduced to selenite, elemental selenium, or selenide (2–4, 7, 8) are less numerous. The reported extent of reduction is lower for selenate than for selenite. Microbial reduction of elemental selenium to selenide (7, 8, 19) and oxidation of elemental selenium to selenite and selenate have also been reported (17).

Although bacteria actively transform compounds containing selenium, the extent of microbial involvement in the natural biogeochemical cycling of selenium is unknown. This study was performed to determine the relative tolerance of microbial populations from selenate-rich environments to selenate and whether their ability to transform inorganic selenite and selenate might have an impact on the natural cycling of the element.

Sample collection and screening. In May of 1985, grab samples were collected from soil, water, and sediment near Kesterson Reservoir in California, an area known to have elevated levels of soluble selenium (18). Upon collection, each of the 44 samples was aseptically inoculated into sterile basal salts media containing 10 mg of selenate per liter as Na_2SeO_4 and lactate, glycerol, or glucose as a carbon source. The basal salts media contained (in grams per liter) ammonium sulfate, 0.3; calcium chloride dihydrate, 0.2; magnesium sulfate, 0.07; sodium chloride, 5.85; and potassium phosphate, 0.1; and (in milligrams per liter) boric acid, 0.6; cobaltous sulfate, 0.11; cupric sulfate, 0.08; manganous chloride, 0.63; zinc chloride, 0.22; and (in grams per liter) a single carbon source (glucose, glycerin or lactate as sodium lactate), 2.00. The pH was adjusted to 7.5 with 1 N NaOH. All media were steam sterilized at 121°C for 18 min. Inoculated media were incubated statically at 25°C for a period of 4 weeks. A noticeable increase in turbidity was observed for 42 of the 44 samples (95%) in at least one of the three carbon sources during the incubation period. Formation of a red precipitate was observed in 45% (20 of 44) of the samples and assumed to indicate the presence of insoluble forms of selenium such as the elemental form (12). Sample type (soil, sediment, or water) and carbon source (glycerin, glucose, or sodium lactate) were apparently related to the ability of the microbes to reduce selenate (Table 1). All of the soil samples (7 of 7), 12 (92%) of 13 sediment samples, and only 1 (4%) of 24 water samples exhibited selenate reduction in media initially containing 10 mg of selenate per liter. Microbial growth and reduction of selenate were observed more frequently in media containing sodium lactate or glycerin than in media containing glucose. Sodium lactate was generally associated with a more rapid formation of red precipitate than occurred with glycerin and was therefore chosen as the carbon source for further testing.

Total soluble selenium (Se +IV plus Se +VI) was determined by sample centrifugation (13,000 × *g*) and analysis of the supernatant by atomic absorption flame spectrophotometry (196.0 nm). Soluble selenite was quantified colorimetrically by the following procedure. To 500 μl of extracted supernatant, 40 ml of deionized H_2O (pH 1.70) and 1 ml each of EDTA-sulfate reagent and diaminobenzidine solution (1) were added. The tube containing this solution was placed in a boiling water bath for 5 min and then removed from the bath and cooled to ambient temperature. Ammonium hydroxide (5 N) was added until the pH was 8.5 ± 0.5. The solution was poured into a 50-ml graduated cylinder, and the volume was adjusted to 50 ml with rinsings of water (pH 8.5) from the empty tube. The solution was then poured into a

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TABLE 1. Relationship of sample type and carbon source to microbial growth and selenate reduction capability in a medium initially containing 10 mg of selenate per liter

Sample type (no. collected)	No. growing/no. reduced on carbon source:			Total no. growing/no. reduced
	Lactate	Glycerin	Glucose	
Soil (7)	7/5	7/7	5/0	7/7
Sediment (13)	12/10	11/10	11/8	12/12
Water (24)	22/1	20/0	14/0	23/1

250-ml separatory funnel with 10 ml of toluene and shaken for 30 s. The aqueous layer was discarded, and the organic layer was collected and analyzed for absorbance with a Spectronic 2000 UV-VIS spectrophotometer (Bausch & Lomb, Inc., Rochester, N.Y.) at a wavelength of 420 nm. Analytical standards containing (per liter) 100 mg of selenite, 75 mg of selenite plus 25 mg of selenate, 50 mg each of selenite and selenate, or 25 mg of selenite plus 75 mg of selenate were used for calibration. Selenate concentrations were calculated as the difference between the selenite and total soluble-selenium concentrations. Analysis of the red precipitate by an X-ray photoelectron spectrophotometer (Perkin-Elmer Physical Electronics, Norwalk, Conn.) with platinum standardization indicated that although the selenium present was not at a valence state of 0 (elemental), it was present in a more reduced form than selenite (presumably as an organoselenium compound). A nitric acid digestion of the red precipitate and subsequent analysis by atomic absorption flame spectrophotometry with a 5100 spectrophotometer (Perkin-Elmer) determined the quantity of precipitated selenium present.

Cultures possessing selenate reduction capability were streaked for isolation, and each was found to contain several morphologically distinct bacterial colonies. All isolates were gram-negative, facultatively aerobic, catalase- and oxidase-positive rods. Although each isolate was found to have the ability to reduce and precipitate ~80% of the selenite from a solution initially containing 100 mg of selenite per liter, only one isolate was capable of reducing selenate in pure culture. Strains which were incapable of reducing selenate in pure culture exhibited selenate reduction only when incubated in mixed culture. This observation indicates the possible existence of synergic relationships between these bacteria, which result in selenate reduction.

Selenate reduction trend. Inocula from the eight cultures which grew readily in a medium containing a selenate concentration of 10 mg/liter were transferred to basal salts media containing 100 mg of selenate and 2 g of lactate per liter. During culture growth, microbial quantification was estimated by direct cell counts with a Petroff-Hausser counting chamber, and concentrations of various oxidation states of selenium were assessed. Analyses revealed that all the selenate had been reduced to other forms of selenium after 3 weeks. Selenite was present in concentrations ranging from 40 to 80 mg/liter, indicating that 20 to 60 mg of selenium per liter had been reduced to insoluble selenium, a volatile selenide form, or both. The uninoculated control medium was found to contain the initial selenate concentration.

Five repetitions of this reduction experiment were conducted with the culture which exhibited the most extensive ability to reduce selenate and selenite. Samples were withdrawn periodically over an 18-day period and analyzed for selenium concentrations and microbial yield (Fig. 1). Typically, there was a rapid initial reduction of selenate to selenite which was complete within 4 to 11 days after inoculation. When ~90 to 95% of the selenate was reduced to selenite, the reduction of the selenite and precipitation of the resulting selenium compound began. The extent of this reduction stage was variable, with 20 to 80% of the selenium being removed from solution. Although measurements to verify the formation of selenide during the reduction processes were not made, such formation was presumed to occur, since 10 to 18% of the original 100 mg of selenium per liter was not detected in a soluble or insoluble form by the analytical methods described above.

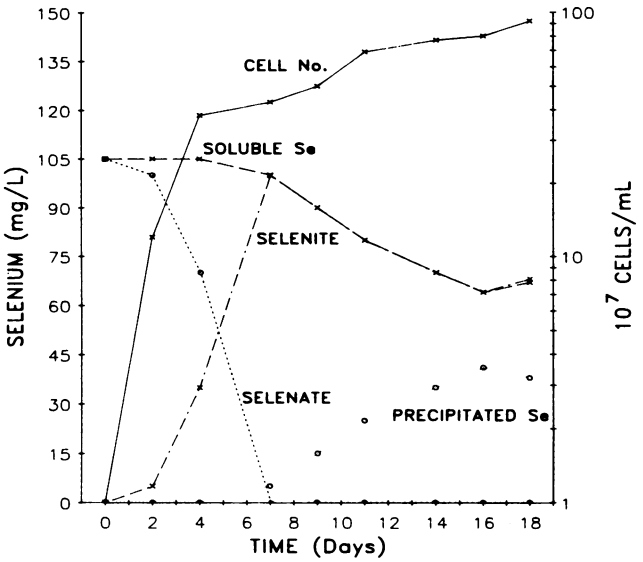

FIG. 1. Effect of time on microbial growth and reduction of selenium in media initially containing 100 mg of selenate and 2 g of lactate per liter. Symbols: ×, determined by measurement; ○, determined by calculation.

Effect of initial lactate and selenate concentration on selenate reduction ability. Tests were performed to determine the effects of initial carbon and selenate concentrations on microbial selenate reduction. An increased extent of selenate reduction occurred with increasing initial lactate concentrations over the range of 0.5 to 5 g/liter and was similar at 5 and 10 g/liter (Fig. 2). The rate of reduction increased with increasing carbon concentrations (from 0.5 to 10 g/liter). Further reduction of the remaining selenite appeared to be inhibited when >50 mg of selenium per liter had been reduced to below a +IV oxidation state. This effect was assumed to be the result of end product inhibition or a limiting substrate other than carbon. Results also indicated that for initial carbon concentrations of ≤1 g/liter, increasing the initial selenate concentration above 10 mg/liter resulted in a decreased efficiency of reduction. When the initial carbon concentration was 5 or 10 g/liter, a decrease in reduction efficiency (i.e., a deviation from the theoretical maximum) was observed at or above an initial selenate concentration of 47 mg/liter (Fig. 3).

Results of this research indicate that organisms resistant to elevated levels of soluble selenium exist and are capable of reducing selenate in concentrations of up to 100 mg/liter to less oxidized forms. Although some physical-chemical techniques for the removal of selenate are available, they are inefficient at present (13, 16). Efficient treatment methods currently can remove selenium from water only if the

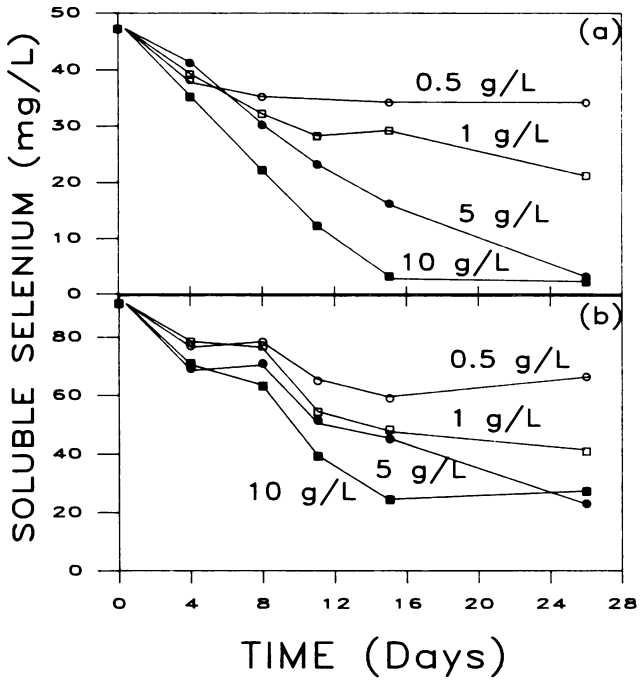

FIG. 2. Effect of initial lactate concentration on the ability of microbes to reduce soluble selenium to an insoluble precipitate from a solution initially containing 47 (a) or 91 (b) mg of selenium per liter as selenate.

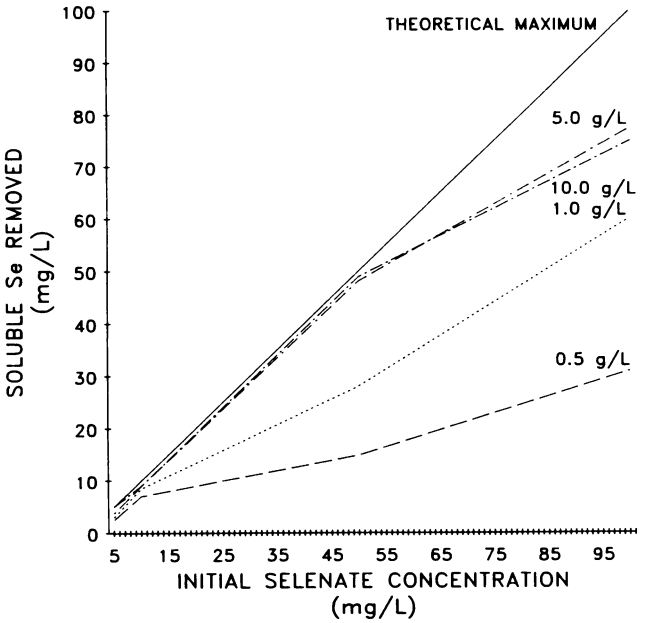

FIG. 3. Effect of initial selenate concentration on the extent of the microbial reduction ability, with various initial concentrations of lactate.

selenium occurs as selenite. The potential exists to use adapted indigenous bacteria in a treatment system that removes soluble and toxic forms of selenium from water. This may be accomplished by allowing microorganisms to reduce selenate to selenite and precipitated selenium, using chemical treatments to precipitate any remaining selenite, and physically filtering the precipitate from the water.

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