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Dissimilatory Selenate Reduction Potentials in a Diversity of Sediment Types

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We measured potential rates of bacterial dissimilatory reduction of $^{75}SeO_4^{2-}$ to $^{75}Se^0$ in a diversity of sediment types, with salinities ranging from freshwater (salinity = 1 g/liter) to hypersaline (salinity = 320 g/liter and with pH values ranging from 7.1 to 9.8. Significant biological selenate reduction occurred in all samples with salinities from 1 to 250 g/liter but not in samples with a salinity of 320 g/liter. Potential selenate reduction rates (25 nmol of SeO_4^{2-} per ml of sediment added with isotope) ranged from 0.07 to 22 μmol of SeO_4^{2-} reduced liter^{-1} h^{-1}. Activity followed Michaelis-Menten kinetics in relation to SeO_4^{2-} concentration (K_m of selenate = 7.9 to 720 μM). There was no linear correlation between potential rates of SeO_4^{2-} reduction and salinity, pH, concentrations of total Se, porosity, or organic carbon in the sediments. However, potential selenate reduction was correlated with apparent K_m for selenate and with potential rates of denitrification (r = 0.92 and 0.81, respectively). NO_3^-, NO_2^-, MoO_4^{2-}, and WO_4^{2-} inhibited selenate reduction activity to different extents in sediments from both Hunter Drain and Massie Slough, Nev. Sulfate partially inhibited activity in sediment from freshwater (salinity = 1 g/liter) Massie Slough samples but not from the saline (salinity = 60 g/liter) Hunter Drain samples. We conclude that dissimilatory selenate reduction in sediments is widespread in nature. In addition, in situ selenate reduction is a first-order reaction, because the ambient concentrations of selenium oxyanions in the sediments were orders of magnitude less than their K_ms.

The presence of toxic selenium oxyanions in agricultural wastewaters which drain from seleniferous soils is widespread in the western United States and poses serious environmental problems (23). Lethal and teratogenic effects in waterfowl have been caused by these oxyanions (9, 15), possibly through biomagnification of environmental sources of selenium (13).

Dissimilatory reduction of selenate (DSeR), primarily to elemental selenium, occurs in anaerobic sediments (17, 18a); cultures of DSeR bacteria have been isolated from estuarine sediments (17) and from bioreactors (14). Although in situ rates of DSeR were measured in a selenium-impacted evaporation pond (18a), little is known about the general occurrence of this activity in nature. Herein we report that DSeR is a widespread phenomenon in sediments. We achieved this by making measurements of potential DSeR in which we injected a known quantity of unlabeled selenate along with radioisotopically labeled $^{75}SeO_4^{2-}$. This allowed us to make direct comparisons among sediment types without having to perform the tedious analyses for ambient concentrations of SeO^{42-} and SeO_3^{2-}. Measurement of potential DSeR is therefore analogous to denitrification potential, in which NO_3^- is added to samples to elicit N_2O production in the C_2H_2-block assay (for example, see reference 11).

Although selenium and sulfur are proximate group VIA elements, reduction of selenate and sulfate proceed by different biochemical pathways and are spatially segregated in nature: selenate reduction occurs in surficial sediments, while dissimilatory sulfate reduction occurs at greater depths (17, 18a, 27). On the other hand, denitrification is also localized in surficial sediments and is often nitrate limited (19). Nitrate, a common solute in agricultural waters, inhibits its respiratory selenate reduction in sediments (17), which implies that in nature denitrification and selenate reduction may proceed by similar mechanisms, with the former inhibiting the latter.

Here we report rates of potential DSeR in several environments, widely ranging in salinity and pH, including several which do not receive selenium-rich agricultural drainage. Our results indicate that a capacity for microbial selenate reduction is a common feature of a diversity of surficial sediment types in nature. Potential DSeR did not correlate with a variety of chemical factors (salinity, pH, or organic carbon) but was related to bacterial activity expressed as potential denitrification. Furthermore, the expression of potential selenate reduction activity in sediments appears to be limited by the concentration of selenate in a manner that displays Michaelis-Menten kinetics. The extent to which the presence of other group VI oxyanions or nitrate may inhibit selenate reduction appears to vary in different milieus.

MATERIALS AND METHODS

Sites, sampling, and chemical determinations. Surficial sediments (upper 5 to 10 cm) were collected from the following sites near Stillwater and Fallon, Nev. (10, 23), from 8 to 10 August 1989: Hunter Drain and Massie Slough, both agricultural drains; the littoral and pelagic zones of Big Soda Lake (5); the littoral zone of Lead Lake; a pond next to U.S. Route 50 near Fallon, which characteristically contains brine, and which we have designated roadside salina. Sediments were obtained from 11 to 13 August 1989 from two lakes in eastern California, i.e., Mono Lake (pelagic [18]) and June Lake (littoral). Surficial sediments were also taken 27 September 1989 from Searsville Lake (Stanford, Calif. [21]) and from two locations of San Francisco Bay, i.e., an intertidal mudflat (6) and a saltern of the Leslie Salt Co. (Redwood City, Calif.). Mason jars were filled to capacity with the sediments by direct scooping or by immediate

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transfer of material obtained with a mechanical sediment grabber (June Lake and pelagic sites). Potential DSeR experiments were initiated 2 to 18 h after on-site collection, and sediments were maintained in the shade at ambient air temperature until use. Sediment material used in the determination of apparent K_ms for selenate (see below) was stored in mason jars at 6°C for up to 9 weeks before use.

The salinities (range = 1 to 320 g/liter) and pH (range = 7.1 to 9.7) of the overlying waters were measured at each site. The total selenium content of the sediments was determined by hydride generation atomic absorption spectrometry after wet acid digestion of samples (7). This method quantifies the total atomic selenium without distinguishing between species [i.e., SeO_4^{2-}, SeO_3^{2-}, Se^0(s), S^{2-}, and organoselenium compounds].

Selenium oxyanion concentrations in interstitial waters were determined by flow-through hydride generation atomic absorption spectrometry (3, 20, 24). Interstitial waters were extracted from sediment cores by use of an N_2-pressurized squeezer as previously described (17). No pretreatment was used for Se(IV), but samples were required to be diluted at least 12-fold to provide sufficient volume for analysis. A closed-system HCl reduction of selenate to selenite was employed for determinations of Se(IV) plus Se(VI). Selenate concentrations were derived by the difference in selenite concentrations of the HCl-treated and untreated samples. Detection limits for Se(IV) or Se(IV) plus Se(VI) were 2.5 and 30 nM, respectively.

The water content (porosity) and organic carbon content of the sediments were determined by the method of Hedges and Stern (8). Dessicated sediment samples were acidified to remove inorganic carbon, and after being dried 50-mg portions were combusted with a Leco WR12 carbon determinator (Leco Corp., St. Joseph, Mich.). Carbon-analyzed steel standards were also combusted. Any volatile organic compounds which may have been lost from the sediments during processing were not included in calculations of organic carbon content.

Measurement of selenate reduction potential. Prior to the assay, the sediments were gently stirred with a spatula to achieve homogeneity (18a). Sediments were drawn into 5-ml syringes (hub ends removed; sediment volume = 3 ml), and the subcores were immediately sealed with latex rubber serum stoppers.

Selenate reduction potential was assayed by injecting $Na_2{}^{75}SeO_4$ (0.03 μCi/100 μl of total injectate; specific activity = 10,981 mCi/mmol; E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.) in isotonic solutions of NaCl, containing 5 mM $NaHCO_3$ (adjusted to ambient pH, deaerated with N_2) at several locations along the length of the sediment subcores, as the needle of the delivering microsyringe was withdrawn. The radiolabeled selenate was diluted with unlabeled Na_2SeO_4 (0.75 mM) to achieve a final concentration of 25 nmol/ml of sediment. During incubations, triplicate samples were periodically extruded and vortexed in disposable 15-ml centrifuge tubes containing 7 ml of the isotonic saline solution and 10 mM Na_2SeO_4 (chase solution) to dilute the radiolabeled substrate such that further accumulation of label in the sediments ceased. In the case of field samples, the centrifuge tubes were quick-frozen in a dry ice-propanol bath and stored at −75°C for later processing and gamma counting. Sediment samples taken from locations near the laboratory were processed immediately after assay in the same manner as field samples, except that the freezing and later thawing were omitted. Sediment suspensions were vortexed, and 1 ml of slurry was removed to determine the total counts added. The remaining slurry was centrifuged (5 min, 4,000 × *g*), the supernatant was discarded, and the pellet was rinsed twice with about 2 ml of chase solution before being suspended in 5 ml of chase solution and centrifuged again. Subsequently, the supernatant was discarded and the lower section of the tube containing the pellet was placed in a 20-ml polyethylene scintillation vial. The upper section of the tube was severed, and gamma activity in the vial was counted for 5 min with a Beckman Gamma 8000 gamma counter (Beckman Instruments, Inc., Irvine, Calif.). Assays were done at 25 ± 3°C unless stated otherwise. Rates of accumulation of elemental selenium (Se^0[s]) in the sediments, representing DSeR, were calculated by using first-order linear regressions ($r \geq 0.94$). Controls consisted of autoclaved sediments (200 kPa at 121°C for 1 h) which were processed (see above) upon cooling.

Measurement of denitrification potential. Sediment denitrification potential activity and selenate reduction potentials in each of several sediment types were studied simultaneously. The acetylene-block technique (1, 4) was used to measure denitrification in the presence of added nitrate. Sediment samples (3 ml) were suspended in 12 ml of homologous overlying water (filter sterilized), supplemented with $NaNO_3$ (final concentration = 1 mM), and sealed in Erlenmeyer flasks (headspace = 41.2 ml). After flushing with N_2 (10 min), C_2H_2 was added to a final partial pressure of 15 kPa and incubated at 25°C for 75 min with rotary shaking (100 rpm). Production of N_2O was measured by ^{63}Ni electron-capture gas chromatography (19).

Determination of apparent K_m for selenate. Several sediment types were examined for their apparent K_ms, a kinetic parameter reflecting enzymatic affinities of resident microorganisms for selenate. To document saturation kinetics for selenate, assays were conducted over a range of final selenate concentrations (from 0.9 pmol to 2 μmol per ml of sediment). Double-reciprocal plots were used to derive apparent K_m values, which were calculated on the basis of final selenate concentrations reflecting the water content (Table 1, porosity) of each sediment type.

Inhibition of selenate reduction. Inhibition of selenate reduction by nitrate, nitrite, and a number of group VI oxyanions was examined. Sediments were subcored and preincubated for 18 h with each inhibitor (100-μl injection of deaerated sodium salt solution; final concentration of inhibitors = 20 μmol/ml of sediment) to allow dispersion. Controls consisted of preincubation with amounts of NaCl yielding salinities equivalent to or greater than the salinity of the inhibitors. After preincubation, the subcores were incubated at 15°C for selenate reduction potential as described above. Duplicate subcores were sacrificed at time zero, after 21 h for Hunter Drain sediment, or after 0.5 h for Massie Slough sediment; these final time points were chosen because they were within the respective periods of linear selenate reduction activity.

Extractions. To verify that elemental selenium was produced by selenate reduction, $^{75}Se^0$(s) was extracted into CS_2 by using a sequence of decreasingly polar solvents (17). Selenate reduction assays were conducted with sediments from Hunter Drain, Big Soda Lake, and Massie Slough as described above but at 15°C. The chase solution from the final washings was discarded, and the sediment pellets were extracted immediately. The activities in the supernatants at each extraction step and in the final pellets were counted. Extraction patterns for selenate (initial time points from assay) and Se^0(s) (assay endpoints) were compared. Abiotic incorporation of ^{75}Se radiolabel in the form of adsorbed

TABLE 1. Chemical and physical properties of sampled sediments

Site	pH	Salinity (g/liter)	Porosity[a]	Organic carbon (% [dry wt])[b]
Massie Slough	7.3	1	0.53	2.1 ± 0.3
Big Soda Lake[c]	9.7[d]	27	0.25	0.3 ± 0.0
Lead Lake	7.8	8	0.32	0.8 ± 0.1
Searsville Lake	8.4	1	0.62	3.1 ± 0.3
Hunter Drain	7.6	60	0.38	1.1 ± 0.2
June Lake	7.1	2	0.72	4.1 ± 0.0
San Francisco Bay	8.0	27	0.62	1.1 ± 0.0
Big Soda Lake[e]	9.7[d]	89	0.89	10.0 ± 0.3
San Francisco salina	7.9	250	0.31	1.2 ± 0.1
Mono Lake[e]	9.8[f]	84	0.84	8.9 ± 0.1
Roadside salina	9.6	320	0.46	3.2 ± 0.1

[a] Grams of water per gram of wet sediment.
[b] Percentage (weight/weight) of dry sediment corrected for precipitated salt and hygroscopic adsorption ± standard error of $n = 3$ determinations.
[c] Littoral sediments.
[d] Reference 12.
[e] Pelagic sediments.
[f] Reference 26.

TABLE 2. Potential rates of selenate reduction in surficial sediments at 25°C and apparent K_ms for selenate

Site	Total selenium (μmol kg^{-1})	Selenate reduction (μmol l^{-1} h^{-1})	K_m (μM SeO_4^{2-})
Massie Slough	48 ± 0	22.07 (expt 1)	62[a]
		10.65[b] (expt 2)	720[b]
Big Soda Lake[c]	19 ± 0	3.57	34
Lead Lake	1 ± 0	3.01	16
Searsville Lake	19	1.91	20
Hunter Drain	14 ± 1	0.74	7.9
June Lake	5 ± 0	0.51	ND[d]
San Francisco Bay	4	0.41	22
Big Soda Lake[e]	140 ± 0	0.21	ND
San Francisco salina	2	0.12	ND
Mono Lake[e]	9 ± 0	0.07	ND
Roadside salina	8 ± 0	<0.01	ND

[a] Determined from a curve which did not reach saturation.
[b] Value determined at 15°C.
[c] Littoral sediments.
[d] ND, Not determined.
[e] Pelagic sediments.

selenite may occur over time in some circumneutral sediments (e.g., Hunter Drain) but not in all sediment types (e.g., alkaline Big Soda Lake) (18a). To distinguish between formation of $^{75}Se^0$(s) and adsorbed $^{75}SeO_3^{2-}$, subcores of autoclaved sediment were injected with $H_2{}^{75}SeO_3$ (0.01 μCi/100 μl of injectate; specific activity = 7,384 mCi/mmol; E. I. du Pont de Nemours and Co.) and extracted in the same manner.

Acid extraction of sediments was done to determine whether hydrogen selenide was a significant product of selenate reduction (27). Massie Slough, Hunter Drain, and Lead Lake sediment subcores were injected with $Na^{75}SeO_4$ (0.06 μCi/100-μl injection) and incubated at 25°C. After sufficient time for reduction of all added $^{75}SeO_4^{2-}$ (4 h), the subcores were extruded into serum vials and crimp sealed with butyl rubber stoppers (total sealed volume = 70.7 ml). The sediment was suspended in 10% (vol/vol) HCl (3 ml), and after 5 min a 10-ml sample of the gas volume was withdrawn with a Glaspak syringe and injected into another crimp-sealed serum vial (total sealed volume = 13.6 ml), displacing an equal volume of water from the vial. Gamma radiation in the smaller vial was quantified as described above.

RESULTS

Sediment characteristics. Chemical and physical characteristics of sediments from 11 chemically diverse sites are given in Table 1. Salinities ranged from 1 to 320 g/liter, and pH ranged from 7.1 to 9.8. The organic carbon content of the sediments ranged from 0.3 to 10.0% (dry weight), and porosities were from 25 to 89%. The total selenium content varied from 1 to 140 μmol of selenium per kg (dry sediment) (Table 2). None of the sites had detectable concentrations of selenate in the overlying water or interstitial water at the time of sampling, but selenite was present in interstitial waters of the upper 4 cm of Massie Slough (11 nM), Hunter Drain (144 nM), and Lead Lake (55 nM) sediments.

Rates of selenate reduction potential. Potential DSeRs occurred without any obvious time lags (Fig. 1). Rates of selenate reduction potential, as represented by the accumulation of $^{75}Se^0$(s) in the sediments, were significant and ranged from 0.07 to 22 μmol of SeO_4^{2-} liter^{-1} h^{-1} (Fig. 1 and Table 2). No activity was evident in autoclaved sediments from 10 of the 11 sites. Only in the case of the roadside salina was incorporation of radiolabel in autoclaved controls the same as in experimental subcores (Fig. 1). This was probably a result of precipitation of Na_2SeO_4 in this saturated brine. Massie Slough sediments were the most active, being 6.2-fold faster than sediments from the shoreline of Big Soda Lake, 7.3-fold faster than Lead Lake sediments, 30-fold faster than Hunter Drain sediments, and 315-fold faster than pelagic sediments from Mono Lake. There was no evident correlation between selenate reduction potential and any chemical or physical properties of the sediments. Correlation coefficients (r) for selenate reduction potential versus [H^+], salinity, total selenium content, organic carbon content, or porosity were, in order, 0.39, 0.33, 0.16, 0.21, or 0.14. However, the four lowest rates were all in hypersaline sediments (84 g/liter or more). A correlation was apparent ($r = 0.81$) between selenate reduction potential and denitrification potential when these two properties were studied in parallel experiments for eight of the sediment types (Fig. 2).

Determination of apparent K_m. The apparent affinities of selected sediment samples for selenate were determined. A representative selenate saturation curve (for Massie Slough sediment) is shown with the double-reciprocal plot (Fig. 3 [inset]). Rates of selenate reduction potential followed Michaelis-Menten kinetics in response to selenate concentration. There was no obvious correlation between K_m values (Table 2) and any other factor (e.g., [H^+], salinity, etc.), except potential reduction rates with selenate at 25 nmol/ml of sediment (assays at 25°C; $r = 0.92$).

The apparent K_m for selenate differed by 10-fold in two sediment samples taken from Massie Slough on separate occasions. The earlier sample (August 1989) had an apparent K_m of 62 μM (Table 2), whereas the sample taken in December 1989 had a value of 720 μM (Table 2 and Fig. 3). The highest rate of selenate reduction potential measured in any sediment type (135 μmol of SeO_4^{2-} liter^{-1} h^{-1}) was achieved in the second Massie Slough sample when the final selenate concentration was elevated to 2 μmol/ml of sediment (3.8 mM).

Inhibition of selenate reduction. The inhibitory effects of nitrate, nitrite, and various group VI oxyanions on selenate

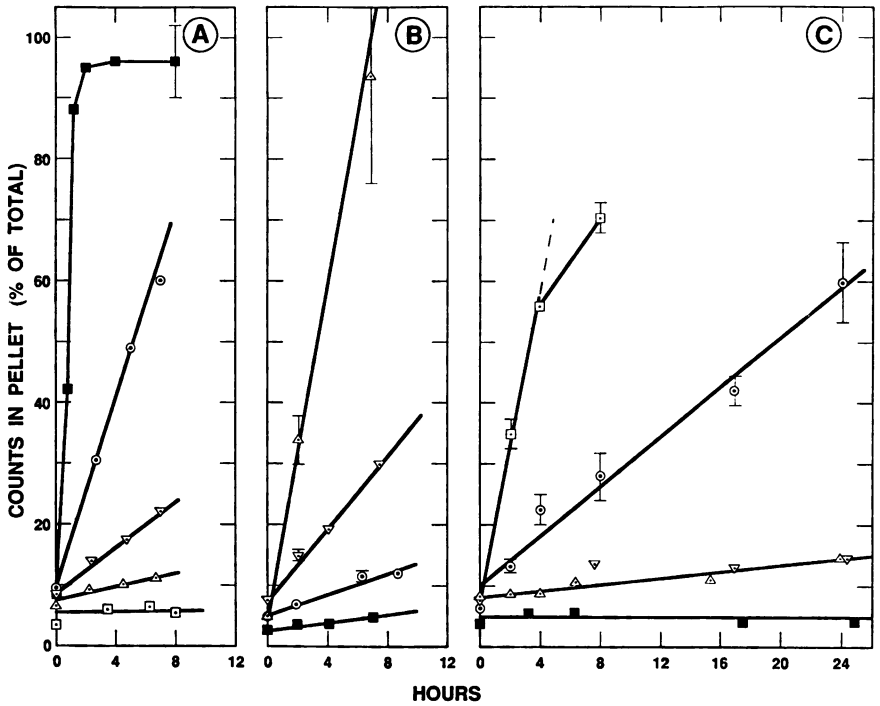

FIG. 1. $^{75}SeO_4^{2-}$ reduction in subcores containing 25 nmol of Na_2SeO_4 per ml of sediment at 25°C. Values are means ± SE of triplicate samples. (A) Massie Slough live (■), or killed (⊡); Searsville Lake (⊙); San Francisco Bay (▽); San Francisco salina (△). (B) Big Soda Lake, littoral (△) or pelagic (⊙); Hunter Drain (▽); Mono Lake (■). (C) Lead Lake (⊡); June Lake, live (⊙) or killed (■); Roadside salina, live (△) or, killed (▽).

reduction potential in sediments from Hunter Drain and Massie Slough were compared (Table 3). Freshwater Massie Slough sediment was strongly inhibited by 20 μmol of nitrate, nitrite, or tungstate per ml of sediment (inhibition, >84%), and to a lesser extent by molybdate and sulfate (inhibition, 70 and 47%, respectively). In Hunter Drain sediments, sulfate only minimally inhibited activity, but nitrate and nitrite each inhibited activity by about 71%. Tungstate and molybdate inhibited by 39 and 53%, respectively.

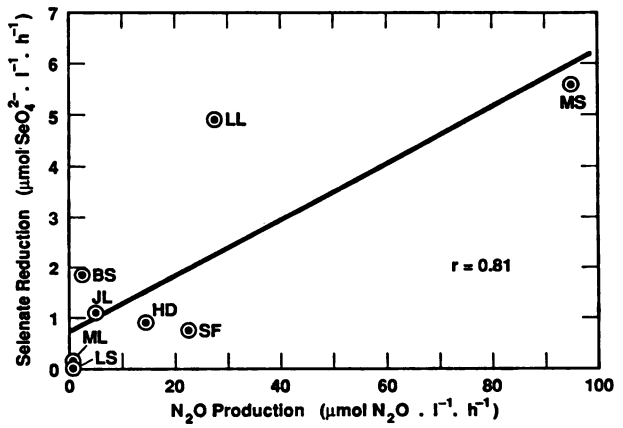

FIG. 2. DSeR potential versus denitrification potential for sediments assayed concurrently on 10 May 1990. Sediment types and the dates of their collection were: Massie Slough (MS; 20 March 1990); Big Soda Lake, littoral (BS; 18 December 1989); Lead Lake (LL; 21 March 1990); Hunter Drain (HD; 20 March 1990); June Lake (JL; 11 August 1989); San Francisco Bay (SF; 9 May 1990); Mono Lake (ML; 14 August 1989); San Francisco Bay saltern (LS; 9 May 1990). A first-order regression line of the data (r = 0.81) is drawn.

Extractions. Radiolabel was not detected in the gas phases of acidified subcores after selenate reduction occurred in Massie Slough, Big Soda Lake (littoral), Hunter Drain, and Lead Lake sediments, indicating that reduction to H_2Se did not occur. However, $^{75}Se^0$(s) was extracted into CS_2 from sediment pellets of Massie Slough, Big Soda Lake (littoral), and Hunter Drain (Fig. 4). At the final time points, incorporation of ^{75}Se into all the sediment pellets had reached a maximum, increasing in the washed pellets from approximately 5,200 dpm at the start of the experiment to about 52,000 dpm at its end (e.g., see Fig. 5A). All of the time zero extractions had a large proportion (25 to 38%) of water-soluble counts (Fig. 4). After selenate reduction, only 1 to 3% of total counts was extracted by water, while counts in the CS_2 fractions doubled (to 42% from 21% and to 36% from 19% for Massie Slough and Hunter Drain sediments, respectively). Big Soda Lake sediment had a large fraction (75%) residual in CS_2-extracted pellets (Fig. 4B), possibly indicating the formation of crystalline Se^0(s) (17). Rapid transition from time zero (SeO_4^{2-}) to final (Se^0[s]) extraction patterns for Massie Slough sediment (Fig. 5B) coincided with the period of most vigorous selenate reduction (Fig. 5A). This was also true for Hunter Drain and Big Soda Lake sediments (data not shown). The extraction patterns for $^{75}SeO_3^{2-}$ from all of the autoclaved (inactive) sediments differed from the Se^0(s) patterns in that 35 to 55% of the label was water soluble and approximately 16% was CS_2 soluble, except in Hunter Drain sediment (35% of counts in CS_2; Fig. 4).

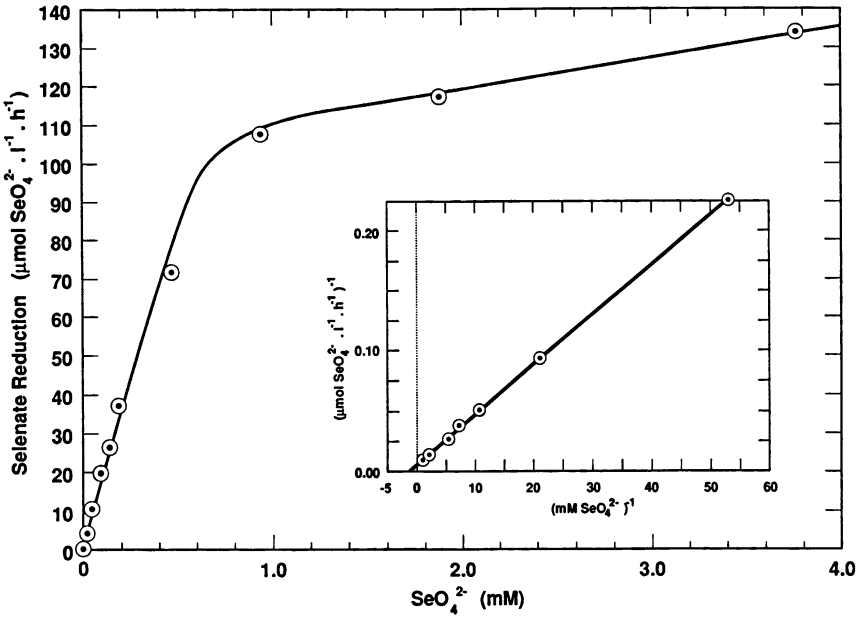

FIG. 3. SeO_4^{2-} reduction rates in sediment subcores from Massie Slough (December 1989) containing a series of Na_2SeO_4 concentrations at 15°C. Values are means of duplicates. Inset shows double-reciprocal plot of the data used to calculate the apparent K_m for selenate.

DISCUSSION

Sediments from 10 diverse environments showed a measurable capacity for selenate reduction in the presence of selenate at 25 nmol/ml of sediment. Several of these active sites do not receive agricultural drainage, including Searsville Lake, June Lake, Mono Lake, and the San Francisco Bay. None of the sites had detectable concentrations of selenate in the overlying or pore waters at the time of sampling in August 1989. Therefore, determinations of total selenium represent primarily Se^0(s) plus the small amount of selenite present in the Massie Slough, Hunter Drain, and Lead Lake sites. (Organoselenium compounds are generally only a minor component of porewaters from those three sites [T. Presser, personal communication].)

Selenate reduction in the sediments displayed Michaelis-Menten saturation kinetics (Fig. 3; Table 2), and autoclaved sediments were inactive (Fig. 1). These results are consistent with a microbiological origin for selenate reduction potential. DSeR to Se^0(s) through a selenite intermediate is performed by certain anaerobic bacteria (14, 17). The results of the acid and CS_2 pellet extraction experiments confirmed earlier evidence (18a) that the product of selenate reduction in sediments was Se^0(s) rather than H_2Se (Fig. 4 and 5). Either the red amorphous (CS_2-soluble) form or the black crystalline (CS_2-insoluble) form of Se^0(s) may be produced by DSeR (17), and the latter would account for the results observed with Big Soda Lake sediment (Fig. 4B).

TABLE 3. Inhibition of DSeR in Hunter Drain and Massie Slough sediments by nitrate, nitrite, and group VI oxyanions at final concentrations of 20 μmol/ml of sediment[a]

Inhibitor	SeO_4^{2-} (μmol liter^{-1} h^{-1})	
	Hunter Drain	Massie Slough
No addition	0.199	11.72
SO_4^{2-}	0.184 (7.5)	6.21 (47.0)
NO_3^-	0.058 (70.9)	0.36 (96.9)
NO_2^-	0.057 (71.4)	0.15 (98.7)
MoO_4^{2-}	0.094 (52.8)	3.52 (70.0)
WO_4^{2-}	0.122 (38.7)	1.86 (84.1)

[a] Values are mean rates (n = 2) of selenate reduction; standard errors were 1% or less. Parenthetic values are percent of inhibition relative to subcores receiving no addition. Preincubation and assays were done at 15°C.

It is possible that the selenate-reducing capacities of the various sediment types resulted from the combined activities of a number of distinct microorganisms, acting in parallel or sequentially (i.e., consortia of selenate and selenite reducers) (14). There was a correlation between denitrification potential and potential DSeR (Fig. 2). This, together with reports of denitrification by selenate reducing isolates (14, 17) and the inhibitory effect of 20 μmol of nitrate or nitrite per ml of sediment on selenate reduction (see below), may mean that denitrifiers were responsible for DSeR activity in the sediments. Dissolved nitrate concentrations in interstitial waters from Massie Slough, Lead Lake, and Hunter Drain ranged from 14 to 600 μM (unpublished data), and ambient nitrate in San Francisco Bay waters has been reported to be 4.6 to 89 μM (19). Since a time lag was not required for induction of potential DSeR, ambient nitrate concentrations did not preclude DSeR activity. Co-occurrence of DSeR with denitrification at nitrate-plus-nitrite concentrations of 0.4 mM has been observed in evaporation pond sediments with concentrations of selenium oxyanions of 0.6 μM (18a).

The apparent K_m values for selenate (Table 2), determined for six of the sites, were orders of magnitude higher than ambient concentrations of selenium oxyanions. Measured concentrations of selenium oxyanions in the surface waters and interstitial waters of the upper 5 cm of sediments from sites in the Stillwater area have ranged from beneath detectability (<13 nM [10]) to as high as 500 nM (e.g., Hunter Drain in March 1990; unpublished data), while apparent K_ms were 7.9 to 720 μM (Table 2). These values of apparent K_m are meant to be crude estimates because of possible constraints on selenate diffusion in the subcores during assay. Nevertheless, they are sufficient to illustrate that DSeR

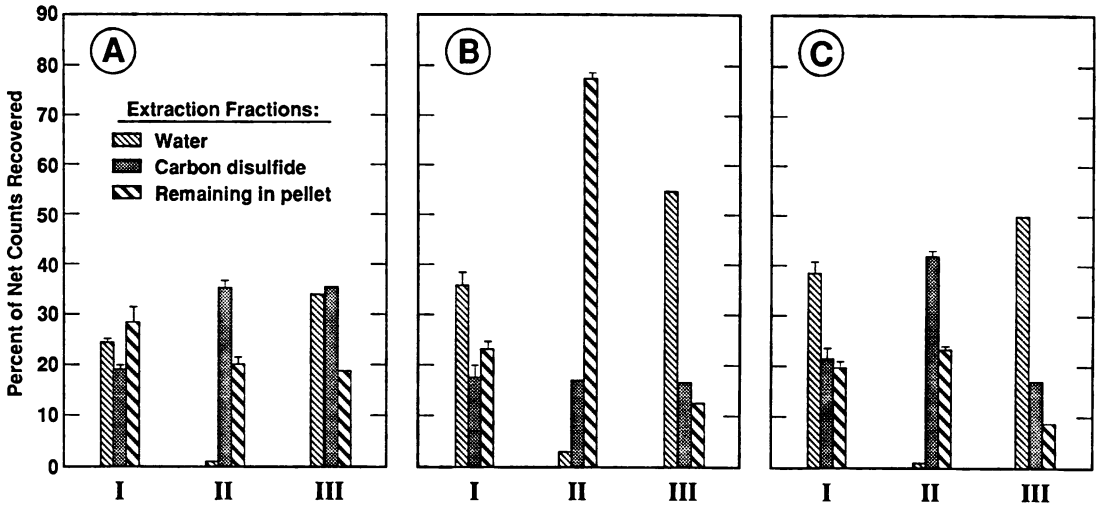

FIG. 4. Fractionation of extracted ^{75}Se counts from sediment pellets of Hunter Drain (A), Big Soda Lake, littoral (B), and Massie Slough (C). Roman numerals indicate: I, time zero immediately after injection of $^{75}SeO_4^{2-}$; II, final point, which was 96 h after injection for Hunter Drain and Big Soda Lake and 26 h for Massie Slough; III, extraction patterns for $^{75}SeO_3^{2-}$ from autoclaved material for each site. Values are means + standard errors of triplicate samples except for the bars designated III, which are from single samples. In panel B, bars designated II, the water- and ethanol-extractable counts were inadvertently combined.

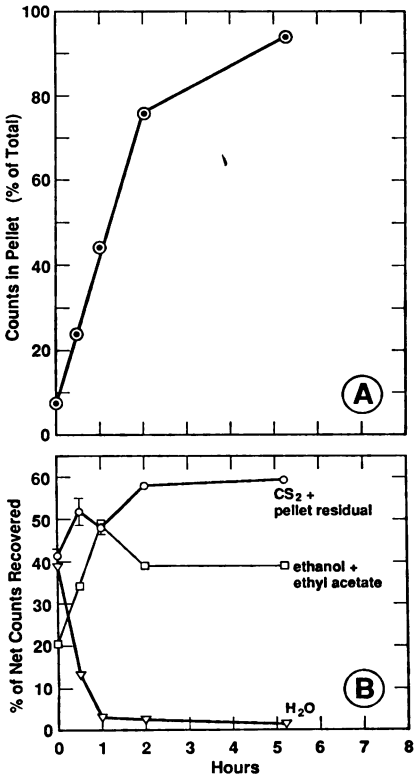

FIG. 5. Selenate reduction time course at 15°C for Massie Slough sediment subcores (A) and fractionation of ^{75}Se counts extracted from the pellet (B). Counts extracted in ethanol and ethyl acetate fractions are combined here for simplicity. Also combined are those counts in the carbon disulfide fraction with those remaining in the pellet after extraction. The final time point (26 h) is not shown here, but is included in Fig. 4.

follows Michaelis-Menten kinetics and that the apparent K_ms greatly exceed ambient concentrations of dissolved selenium oxyanions.

The apparent K_m values for selenate were a reflection of the composite activity of the entire selenate-reducing population in each sediment sample. The differences in apparent K_m for selenate between sediment types and the variability in K_m in different samples from the same site (e.g., Massie Slough) may have been engendered by a combination of factors, such as variable microbial population densities or perhaps different enzyme systems involved in the reduction of selenate.

It was thought that the use of inhibitors would yield evidence about the nature of the metabolic pathway used in DSeR. Inhibition of selenate immobilization by nitrate, in sediments from Hunter Drain and Massie Slough (Table 3), is consistent with earlier studies of San Francisco Bay sediment slurries (17). Whether nitrate acts to inhibit DSeR or competes with selenate for catalytic sites or electrons is unclear.

Inhibition of DSeR by molybdate in sediments from both Hunter Drain and Massie Slough was in contrast to the results reported for San Francisco Bay sediment slurries, but significant inhibition by tungstate was consistent with earlier work (17). The group VI oxyanions, molybdate and tungstate, inhibit sulfate respiration but not exclusively (16). Although tungstate interferes with the activity of various molybdoenzymes, including dimethyl sulfoxide reductase of *Escherichia coli* (2) and nitrate reductases (22), the fact that molybdate also significantly inhibited selenate reduction implies that DSeR was not catalyzed by a molybdoenzyme.

Evidence for the role of divergent metabolic pathways in selenate immobilization comes from the inhibition results. Selenate reduction in sediment slurries from San Francisco Bay was independent of sulfate reduction pathways (17). Similarly, there was little inhibition by sulfate of selenate reduction in Hunter Drain sediment. However, sulfate inhibited activity by 47% in Massie Slough sediment. This may indicate a significant contribution by dissimilatory sulfate-

reducing bacteria to total selenate reduction potential, or it could just be the result of a lag as was observed by Oremland et al. (17). *Desulfovibrio desulfuricans* reduced nanomolar concentrations of selenate to selenide when the concentration of sulfate was below 50 mM (27). However, 1 mM selenate irreversibly inhibited both selenate and sulfate dissimilatory reductions by this microorganism (27). In contrast, Massie Slough sediment showed vigorous reduction of 2 mM selenate (Fig. 3) and no production of $H_2{}^{75}Se$, making the involvement of sulfate-reducing bacteria unlikely.

A capacity for DSeR in sediments appears to be a widespread phenomenon, and the process is essentially consistent with previously detailed observations of San Francisco Bay sediment (17, 18a). The apparent K_ms for selenate, which were observed in several sediment types, indicate that DSeR is first order with respect to ambient selenium oxyanion concentrations and that the potential for DSeR is unlikely to be overwhelmed by ambient concentrations. We have observed in situ selenate turnover times as rapid as 0.55 h (Massie Slough) (R. S. Oremland, N. A. Steinberg, T. S. Presser, and L. G. Miller, unpublished data).

We can only speculate as to the function of DSeR in such a diversity of environments, including those where selenium oxyanions are not normally present. Although the reduction of selenate to Se^0(s) coupled with the oxidation of organic carbon is highly exergonic (17), the maintenance of constitutive DSeR capacity for the generation of metabolic energy would not seem to be advantageous in environments generally unaffected by selenium oxyanions. However, as a detoxification mechanism against transient low-level selenate concentrations, DSeR may be useful. Alternatively, DSeR potential could conceivably be the result of microbial enzyme systems with other functions but with broad substrate specificity, much like dimethyl sulfoxide reductase in *E. coli* (25).

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