ENVIRONMENTAL FACTORS AFFECTING SELENITE REDUCTION BY A MIXED CULTURE

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ABSTRACT: This study presents the results of batch experiments investigating selenite reduction by an enriched anaerobic mixed culture as a function of several environmental factors, including pH, temperature, different electron donors and acceptors, as well as initial selenite concentrations. The initial selenite reduction was a zero-order reaction and was inhibited at higher selenite concentrations (>33 mg Se/L). The optimal temperature/pH for microbial reduction occurred at 30°C and pH 7.2. Selenite reduction was affected in the presence of high concentrations of sulfate [45 times the Se(IV) molar concentration], significantly affected by nitrate [105 times the Se(IV) molar concentration], and completely inhibited by chromate and oxygen. Ethanol was the preferred carbon source for selenite removal, followed by acetate, citrate, lactate, and glucose. The selenite-acclimated culture also reduces selenate without any lag period.

INTRODUCTION

Selenium usually exists in environments at low concentrations, but due to human activities, it may be concentrated in specific areas and cause significant environmental concern, exemplified by drainage problems at San Joaquin Valley (Macy et al. 1993) and by the discharge of industrial wastes including processing wastes from the power industry. In natural environments, selenium can be present in different redox states, [e.g., Se(−II), selenide; Se(O), elemental selenium; Se(IV), selenite; or Se(VI), selenate]. The oxidized Se oxyanions may exist in different species [e.g., Se(IV) as H_2SeO_3, $HSeO_3^-$, or SeO_3^{2-}]. The concentration and speciation of Se in a given environment depend on the pH, redox conditions, solubility of its salts, complexing ability of ligands, and biological interactions, among others. Both selenate and selenite are toxic to living organisms.

Traditionally, chemical methods have been used for the removal of selenate and/or selenite (e.g., chemical coprecipitation, reduction, ion exchange, adsorption, photocatalytic reduction, and electrodialysis) (Sorg and Logson 1978; Merrill et al. 1986; Zingaro et al. 1997; Sanuki et al. 1999). Recently, microbial transformations of many metalloid oxyanions such as $As_2O_4^-$, CrO_4^{2-}, and MoO_4^{2-} to less toxic forms have been demonstrated in natural environments and in laboratories [e.g., Lovley (1993)]. The biological processes may offer a cost-effective means for metalloid oxyanion removal.

Microbial reduction of selenite to elemental Se was reported as early as the 1920s (Levine 1924). Since then, reduction of selenium oxyanions has been demonstrated in sediments [e.g., Oremland et al. (1989, 1990)] and in the aqueous phase (Tokunaga et al. 1996). Many microorganisms capable of reducing selenite/selenate have been isolated, the enzymes responsible for Se(IV)/Se(VI) reduction purified, the location of elemental selenium identified, and the final product of elemental Se(O) confirmed. The capability of many fungi, algae, and yeast cells in reducing Se(VI)/Se(IV) to volatile selenide is also well documented [e.g., Thompson-Eagle et al. 1989; Gharieb et al. 1995; Fan et al. 1998). The selenium oxyanion reducing bacteria are extremely diverse with respect to Gram stain, physiological properties, their ability/inability to use Se oxyanions for respiratory growth, and their ability/inability to utilize other electron acceptors of oxygen, nitrate, or sulfate.

Almost all of the studies of microbial reduction of selenium oxyanions have been conducted in pure cultures. Only a few studies were performed with mixed cultures (e.g., by a denitrifying consortium) (Rege et al. 1999). For practical application, the use of a pure culture may not be feasible due to carbon selectivity, potential contamination problems, and its survival in natural environments. Furthermore, electrons from organic compounds and H_2 generated by mixed cultures under fermentative conditions may facilitate the reduction of oxidized forms of the metals (Lovley 1993). Consequently, an investigation of selenite reduction by acclimated anaerobic sludge as a function of environmental factors was undertaken in the present study. Factors evaluated include the initial selenite concentration, pH, temperature, addition of different electron acceptors (e.g., sulfate, nitrate, and chromate), and different carbon sources.

MATERIALS AND METHODS

Enrichment of Selenite-Reducing Culture in Chemostat

The seeding sludge used for Se(IV) reduction was collected from an anaerobic digester in a local wastewater treatment plant. The synthetic wastewater, modified from Tomei et al. (1992) without addition of vitamin mixture, was used. The basal medium consists of the following in mg/L: CH_3COONa, 2,000; K_2HPO_4/KH_2PO_4, 800/675; NH_4Cl, 240; $CaCl_2 \cdot H_2O$, 110; $MgCl_2 \cdot H_2O$, 101; $FeCl_3$, 2; Na_2EDTA, 1.5; $MnCl_2 \cdot 4H_2O$, 0.5; $ZnCl_2$, 0.05; H_3BO_3, 0.05; $NH_4MnO_7 \cdot 4H_2O$, 0.05; $CoCl_3 \cdot 6H_2O$, 0.05; $NiSO_4$, 0.05; and $CuSO_4$, 0.03. $Na_2SeO_3 \cdot 5H_2O$ was used for Se(IV) input.

Two liters of sludge and 2 L of synthetic wastewater were initially mixed in an anaerobic reactor (4 L) and batch-fed for several days to acclimate microbial population to high Se(IV) concentration (55 mg/L as Se). Fresh synthetic wastewater was then pumped into the reactor to maintain a dilution rate D of 0.4 day^{-1} at $T = 25 \pm 2$°C. After 2 weeks of operation, a slightly reddish color (the color of elemental selenium) appeared, indicating that microbial reduction of selenite had begun. Other investigators (Tomei et al. 1995; Tucker et al. 1998) reported the appearance of red color in similar media, due to the cell lysis and release of internal selenium granules.

Batch Studies

The culture used for all batch studies was taken directly from the anaerobic chemostat reactor operated at $D = 0.4$

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day^{-1}. Approximately 2,000 mg/L of sodium acetate and appropriate amounts of selenite were externally added to the batch cultures. The culture was then evenly distributed (200 mL) into separate reactors, sealed with covers (rubber stoppers), and placed on a shaker. The batch experiments were performed to evaluate the effects of the initial Se(IV) concentrations (13–70 mg Se/L), pH (5.2–10), temperature (10–50°C), carbon source (ethanol, acetate, citrate, lactate, and glucose; each at 200 mg C/L), and electron source (oxygen, nitrate, sulfate, and chromate). The volatile suspended solids (VSS) was measured for each batch study. Samples were periodically withdrawn and filtered with Whatman GF/C (Maidstone, U.K.) and 0.45-μm filters; the diluted acidified filtrate was analyzed for soluble Se.

To ensure that the removal of selenite was solely biological, a control test was performed with the addition of mercuric chloride. Also, to check the role of the reducing power in Se(IV) reduction, the system without the addition of a carbon source was also conducted. To determine if the same culture had the capacity to reduce selenate, the sodium salt of selenate was added to the culture.

Analytical Methods

The pH and oxidation/reduction potential (ORP) were measured by directly inserting probes (Ingold 465-35-90-K9 and Ingold PT4865-35-90-K9, Switzerland) into the reactor. The measurement of soluble selenium was performed in an atomic absorption spectrophotometer (Perkin Elmer 5100 ZL, Norwalk, Conn.), following the procedures in Standard Methods [American Public Health Association (APHA) 1985]. Since the atomic absorption measures total soluble selenium, the selenium data from Se(VI) experiments contain Se(VI) and/or Se(IV) ions.

RESULTS AND DISCUSSIONS

Enriched Culture

At least three types of microorganism were observed in the chemostat reactor. No attempts were made to isolate the bacteria responsible for Se(IV) reduction. The results of the batch studies and the control reactor clearly indicated that Se(IV) reduction was a microbially mediated reaction (Fig. 1). Also, reducing power alone via endogenous respiration is inadequate for Se(IV) reduction. There were no replicate tests performed; the analytical error in these experiments was estimated to be <10%.

The selenite-reducing culture also reduced selenate at the initial concentration of 11 mg Se/L without any lag period (Fig. 2). Since the AA measures the total selenium concentration, the extent of Se(VI) reduction to Se(IV) or Se(0), or the distribution of the remaining Se between Se(VI) and Se(IV), is unclear. Nevertheless, the reduction of Se(VI) directly to elemental Se(0) has been reported (Oremland et al. 1989; Fujita et al. 1997). Clearly, the enzymes responsible for selenate reduction were present in the acclimated selenite-reducing culture. *Wolinella succinogenes* (Tomei et al. 1992), *Pseudomonas stutzeri* (Lortie et al. 1992), and *Thaueria selenatis* (Lawson and Macy 1995) have also been found to reduce both selenate and selenite. However, one particular anaerobic strain (Macy et al. 1989) could not reduce selenate, even though it could reduce selenite. Burton et al. (1987) reported that only 3 of 30 Se(IV)-reducing isolates from sediments could reduce Se(VI).

Batch Selenite Reduction

The Se concentration profile with time, along with pH and ORP data, is shown in Fig. 3. Similar to microbial reduction

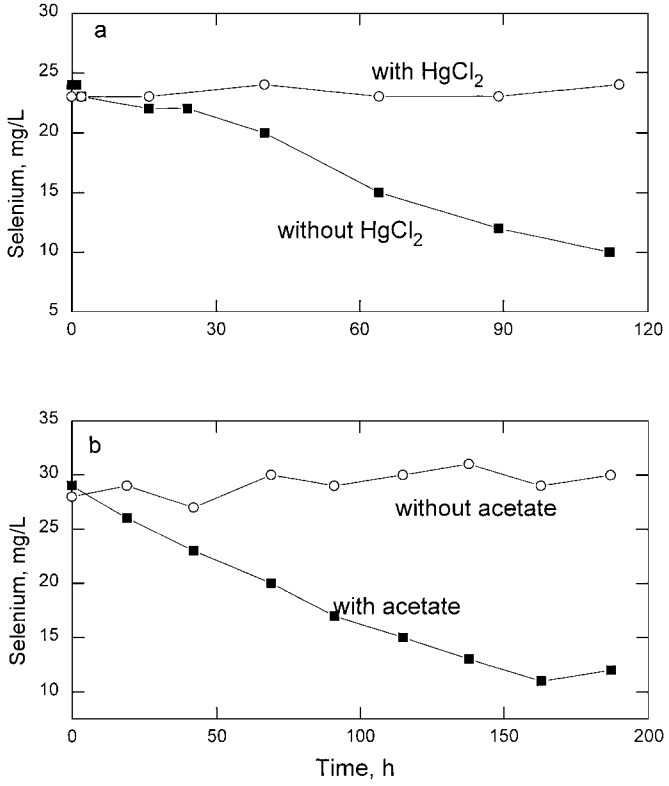

FIG. 1. Microbial Selenite Reduction in Se(IV) Acclimated Mixed Culture with or without: (a) Addition of $HgCl_2$ in Presence of Acetate as Electron Donor; (b) Addition of Acetate

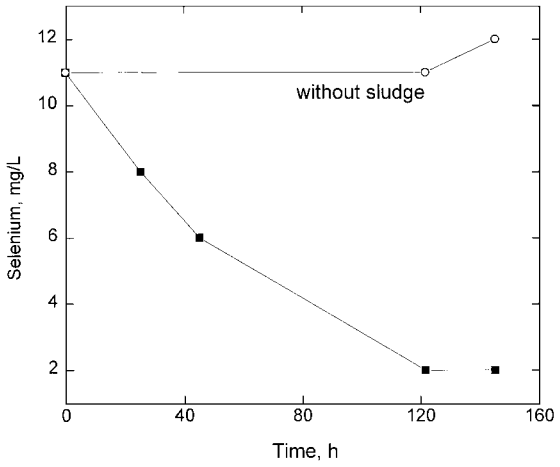

FIG. 2. Selenium Removal in Selenite-Acclimated Culture Fed with Selenate

of sulfate, nitrate, and chromate, an increase in pH was noticed and was attributed to the microbial reduction pathway. Macy et al. (1989, 1993) also reported a pH increase during the microbial SeO_4^{2-} reduction process. The ORP decreased from the initial value of −65 to −320 mV. The zero-order removal rate in Fig. 3 was approximately 0.15 mg Se/L-h (0.59 mg Se/g VSS-h). For comparison, the specific Se(IV) reduction rate for an acclimated denitrifying culture was one order of magnitude higher (Rege et al. 1999), and the Se(IV) reduction rate for the pure culture of *Pseudomonas stutzeri* was two orders of magnitude higher under aerobic conditions (Lortie et al. 1992).

Fig. 4 shows the effect of initial Se(IV) concentrations on microbial selenite reduction at a VSS concentration of 73 mg/L. The initial reduction rates (data within the first 50 h) increased as selenite concentration increased from 13 to 33 mg Se/L; thereafter, the rate decreased as the Se(IV) concentra-

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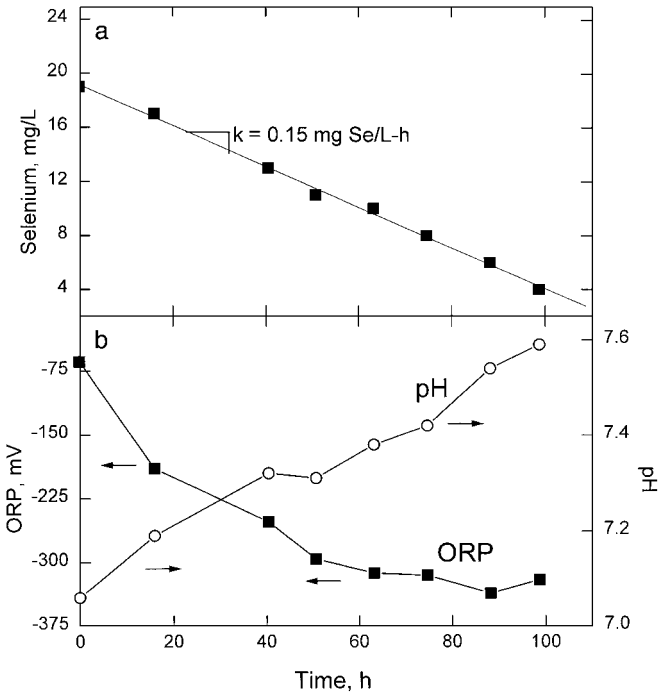

FIG. 3. Selenite Reduction and Corresponding pH and ORP Profiles

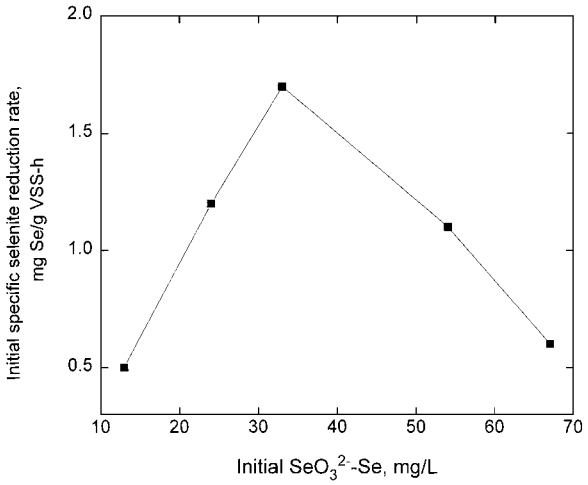

FIG. 4. Influence of Initial Selenite Concentration on Selenite Reduction

tions further increased. The toxicity of the selenite ion at higher concentrations may be responsible for the decreased selenite reduction rates. This same phenomenon of microbial inhibition at higher concentrations of Se(IV) was also reported by others with the pure culture of *P. stutzeri* (Lortie et al. 1992) and *D. desulfuricans* (Tomei et al. 1995).

The specific reduction rate and selenium removal efficiency [1 − (Se_t/$Se_{initial}$)] increased with the elevation of pH. The results (not shown) showed that the reduction efficiency after 90 h for low pH values (pH 5.2 and 6.1) was only 5%, for neutral pH (7.2 and 8) was 40–50%, and for high pH (9.2 and 10) was about 80%. Although several researchers have indicated that a neutral pH is optimal for oxyselenium ion reduction [e.g., Fujita et al. (1997)], others reported relatively high optimal pH ranges, [e.g., pH 7–9 (Lortie et al. 1992) and 9.7 (Oremland et al. (1990)]. However, in the current study, the high removal efficiency at higher pH values was attributed to chemical reactions (e.g,. coprecipitation) between selenite and other precipitates [e.g., $CaCO_3$ and $Mg(OH)_2$] in the culture medium. The content in two reactors (one with acetate and the

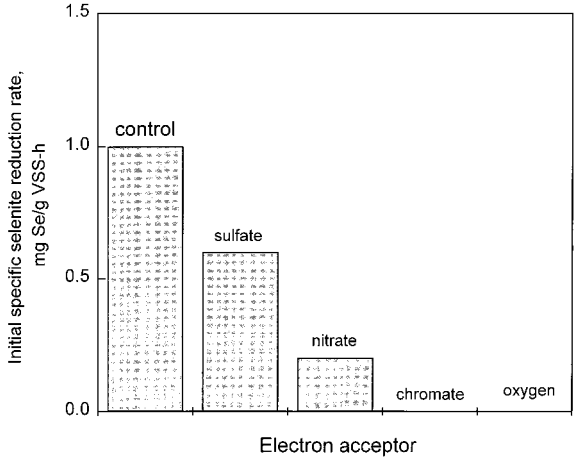

FIG. 5. Influence of Electron Acceptors on Selenite Reduction

other without acetate) was adjusted to pH 10 to confirm the potential chemical reaction at higher pH values. After 10 days of operation, both of these two reactors had a similar Se removal efficiency. Since the system without an exogenous electron donor was unable to reduce Se(IV) as shown in Fig. 1, it indicates potential chemical interactions at higher pH values. Chemical reduction of selenate/selenite with ferrous hydroxide under alkaline conditions has been reported (Murry 1988; Zingaro et al. 1997).

Temperature effects on selenite reduction were evaluated in the range between 10 and 50°C. The initial Se(IV) concentration was 25 mg/L. With the increasing temperature, the reduction rate increased and reached a maximum value at 30°C (not shown) and subsequently decreased with further increases in temperature. The same optimum temperature for selenium anion removal with pure cultures was also reported by Fujita et al. (1997).

The effects of electron acceptors (sulfate, nitrate, chromate, and oxygen) on Se(IV) reduction are shown in Fig. 5. Sulfate (500 mg/L as S) inhibited Se(IV) reduction, and nitrate (500 mg/L as N) significantly affected Se(IV) reduction. Under aerobic conditions or with the addition of chromate (50 mg/L as Cr), selenite removal did not occur. Since the enriched culture was obtained from the anaerobic digester, aerobic respiration may not be possible, as also reported by Oremland et al. (1989) with isolates from sediments. Chromate is known to be toxic to various bacteria. Since many strains of Se(VI)/Se(IV)-reducing bacteria are able to use sulfate as an electron acceptor (Oremland et al. 1989; Lortie et al. 1992), only extremely high levels of sulfate are thought to be inhibitory for Se(IV)/Se(VI) reduction. Losi and Frankenberger (1997) observed sulfate inhibition on Se(VI) reduction when the sulfate molar concentration was 230 times higher than selenate. As for nitrate, several studies have reported simultaneous denitrification and Se(IV)/Se(VI) reduction, although it will inhibit Se oxyanion reduction at much higher concentrations (Fujita et al. 1997; Losi and Frankenberger 1997).

Among the compounds tested (each at 200 mg/L as C), ethanol was the most effective in enhancing selenite removal (97%) after 10 days. Se(IV) removal efficiencies with acetate, citrate, and lactate were similar (62–67%), with glucose the least (41%). Oremland et al. (1989) also showed that glucose retarded the Se(VI)/Se(IV) reduction in sediments.

CONCLUSIONS

In this study, it was found that anaerobic sludge was easily acclimated to selenite, suggesting the mixed culture can be adapted for the remediation of selenium pollution. The extent of Se(IV) reduction and the factors affecting its reduction were

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investigated. The maximum initial specific zero-order reduction rate was approximately 2.8 mg Se/g VSS-h at pH 7.2 and 30°C. Selenite reduction was inhibited at high selenite concentrations. The inhibition of nitrate and sulfate at high concentrations on Se(IV) reduction is noted. Ethanol was the preferred carbon source for selenite removal, followed by acetate, citrate, lactate, and glucose. Microbial reduction of selenate by the selenite-acclimated cultures also occurred without any lag period.

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