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Pilot-Scale Selenium Bioremediation of San Joaquin Drainage Water with *Thauera selenatis*

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This report describes a simple method for the bioremediation of selenium from agricultural drainage water. A medium-packed pilot-scale biological reactor system, inoculated with the selenate-respiring bacterium *Thauera selenatis*, was constructed at the Panoche Water District, San Joaquin Valley, Calif. The reactor was used to treat drainage water (7.6 liters/min) containing both selenium and nitrate. Acetate (5 mM) was the carbon source-electron donor reactor feed. Selenium oxyanion concentrations (selenate plus selenite) in the drainage water were reduced by 98%, to an average of 12 ± 9 μg/liter. Frequently (47% of the sampling days), reactor effluent concentrations of less than 5 μg/liter were achieved. Denitrification was also observed in this system; nitrate and nitrite concentrations in the drainage water were reduced to 0.1 and 0.01 mM, respectively (98% reduction). Analysis of the reactor effluent showed that 91 to 96% of the total selenium recovered was elemental selenium; 97.9% of this elemental selenium could be removed with Nalmet 8072, a new, commercially available precipitant-coagulant. Widespread use of this system (in the Grasslands Water District) could reduce the amount of selenium deposited in the San Joaquin River from 7,000 to 140 lb (ca. 3,000 to 60 kg)/year.

Irrigation of agricultural lands on the west side of the San Joaquin Valley, Calif., has resulted in the leaching of naturally occurring selenium into subsurface drainage water. This water typically contains selenium oxyanion concentrations (mostly selenate) of 230 to 640 μg/liter (2, 20; present study). Nitrate, present because of agricultural activities, is found in the drainage water at concentrations of 45 to 92 mg/liter (20; present study). Disposal of this drainage water has led to serious environmental problems. In 1983, high selenium concentrations in the drainage water transported via the San Luis Drain into the Kesterson National Wildlife Refuge were linked to reproductive problems, deaths, and deformities of waterfowl (19, 24). These findings led to the closure of the San Luis Drain and Kesterson Reservoir in 1985.

Since closure of the San Luis Drain, the drainage water has been disposed of by using evaporation ponds or by transporting the water to the San Joaquin River. Over the last 9 years, roughly 7,000 lb (ca. 3,000 kg) of selenium per year have been deposited in the river from the Grasslands Water District and adjacent Grasslands areas (9). By the end of 1996, the Grasslands Bypass Channel will be functional, and drainage water from the Grasslands Water District and adjacent Grasslands areas will be transported via the reopened San Luis Drain to Mud Slough and then into the San Joaquin River (22). Thereafter, the total amount of selenium that will be permitted to flow into the river may not exceed the levels of the last 9 years (9). For discharges to remain within these limits, it would be advantageous to remove as much selenium as possible from the drainage water before it flows into the river.

While removal of selenium can potentially be accomplished by physical-chemical methods such as reverse osmosis, chemical precipitation, or adsorption, these methods are not always effective and can be quite expensive (12).

Recent studies suggest that microbiological selenium detoxification may be the simplest and most economical approach (7, 13, 20). In previous experiments (13), a laboratory-scale biological reactor, inoculated with the selenate-respiring bacterium *Thauera selenatis*, was successfully used to reduce the concentration of selenium oxyanions in drainage water to levels lower than 5 μg/liter. The organism was maintained in the reactor by selective pressure as long as a carbon source-electron donor (acetate) was supplied and conditions that allowed growth in drainage water were provided. These results suggested that *T. selenatis* could be employed in a pilot-scale biological reactor system for selenium remediation.

Because of its unique metabolism, *T. selenatis* is particularly well suited for use in selenium bioremediation systems. The organism, a gram-positive motile rod, grows both aerobically and anaerobically. When grown with an appropriate carbon source-electron donor such as acetate, *T. selenatis* respires anaerobically, by using either selenate or nitrate as the terminal electron acceptor, reducing the selenate to selenite and nitrate to nitrite. The terminal reductases used to reduce selenate and nitrate are not the same, having different pH optima and different locations within the cell; the selenate reductase, which catalyzes the reduction of selenate to selenite, is located in the periplasm, whereas the nitrate reductase, which catalyzes the reduction of nitrate to nitrite, is located in the cytoplasmic membrane (18). When grown with nitrate (present in drainage water) and selenate, *T. selenatis* reduces both of these electron acceptors concomitantly (18). Apart from organism SES-3 (16, 17), which also has a specific enzyme for selenate reduction, all other organisms known to reduce selenate appear to do so with a nonspecific enzyme system (e.g., nitrate reductase). For instance, certain denitrifying bacteria are able to reduce selenate but only if any nitrate that is present has first been reduced (20, 21).

The nitrite reductase which is found in the periplasm of *T. selenatis* during denitrification (15) catalyzes the reduction of

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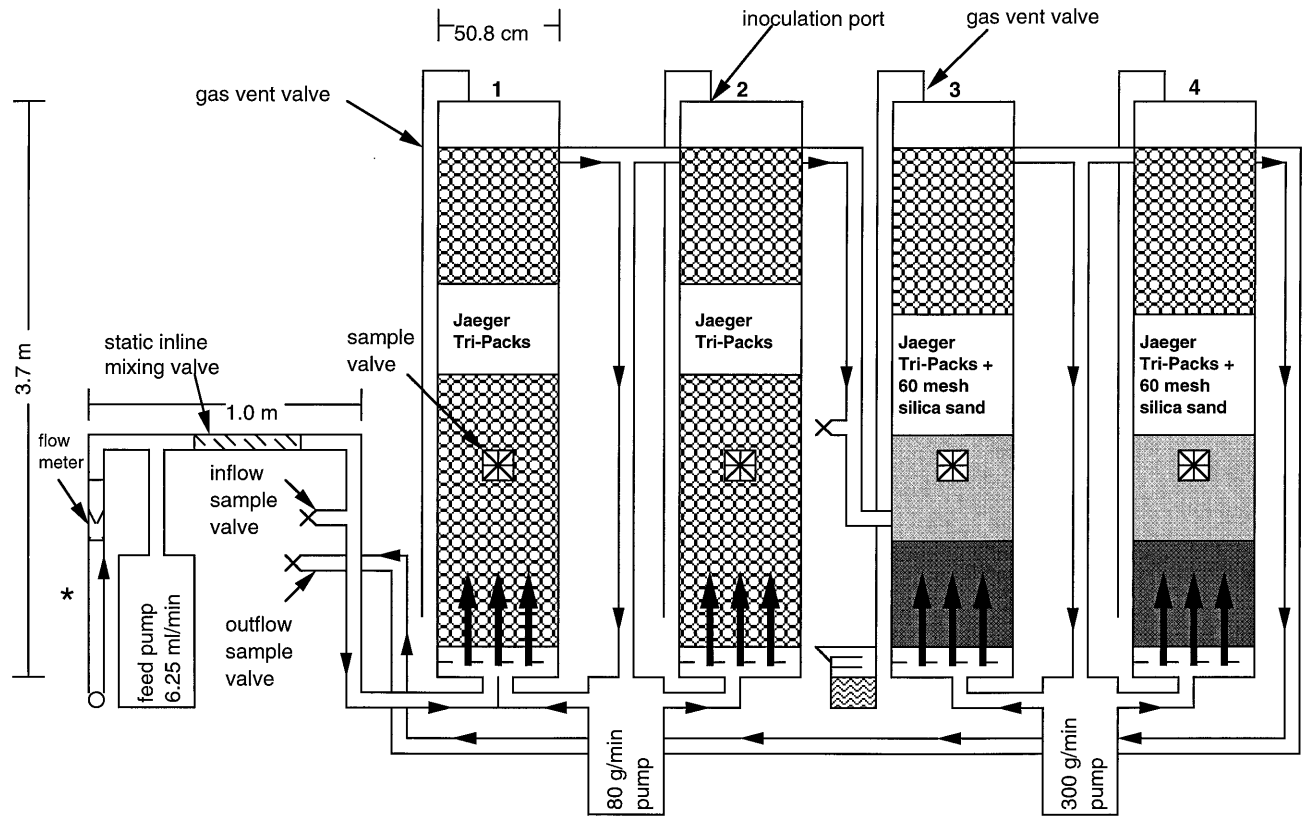

FIG. 1. Schematic representation of the pilot-scale biological reactor used for bioremediation of selenium oxyanions in agricultural drainage water. The gas vent valve represented on tank 3 is present on all tanks. The point at which methylene blue was added for determination of residence times is indicated by an asterisk.

selenite to elemental selenium in addition to reducing nitrite to nitric oxide (5). Active denitrification is, therefore, required for the complete reduction of selenate to elemental selenium by *T. selenatis* (5).

To determine whether the reactor design used in laboratory studies would be effective on a larger scale, a pilot-scale biological reactor system was constructed on a site in the Panoche Water District and was inoculated with *T. selenatis*. The present study describes the experiments that were carried out with this system in the treatment of selenate-containing drainage water from the Panoche Water District. Optimal conditions for the reduction of selenate to elemental selenium in the pilot-scale reactor were determined. The feasibility of using commercially available coagulants and flocculants to facilitate the removal of elemental selenium from the reactor effluent was also tested.

MATERIALS AND METHODS

Chemicals. Sodium hydroxide (50%) and high-performance liquid chromatography-grade carbon disulfide were obtained from Fisher Scientific. Standard Se reference solution (selenite) and bovine liver standard for the determination of total Se were from the same source, as noted previously (13). Glacial acetic acid was obtained from Chemical Central Brokers, Hayward, Calif. Anhydrous sodium acetate was obtained from Van Waters and Rogers, San Jose, Calif. All other chemicals were of the highest purity commercially available.

Biological reactor studies. The pilot-scale biological reactor constructed and used in these studies was housed in a shed located in the Panoche Water District near Firebaugh, Calif. The temperature in the shed was maintained at 22 to 28°C. The reactor (Fig. 1) consisted of four medium-packed tanks. The volume of each tank was approximately 0.8 m^3. These tanks were constructed of grade SDR 41 polyvinyl chloride pipe (Harvel Plastics, Easton, Pa.) and were approximately 3.8 m tall and 50.7 cm in diameter. A 50.7-cm-diameter disk was positioned 35.5 cm from the bottom of each tank. The disk was perforated with 0.64-cm-diameter holes distributed every 2.5 cm from the center. Each tank was sealed with two polyvinyl chloride caps (Harvel Plastics); those on the bottom were reinforced with fiberglass. Gas vent valves were installed at the top of each tank; the outlet, located at the bottom of the tank, was submerged in water to maintain anaerobicity (Fig. 1). Tanks 1 and 2 were filled with Jaeger Tri-Packs (2 in. diameter [1 in. = 2.54 cm]; Jaeger Products Inc., Houston, Tex.). Tanks 3 and 4 contained 136 kg each of 60-mesh silica sand. On day 108, to prevent clogging, approximately 90 kg of sand was removed from each tank and replaced with 0.27-m^3 Jaeger Tri-Packs. Some sand was left in the tanks to provide as much surface area as possible for the growth of bacteria. The remainder of the piping was composed of 2-in. (ca. 5-cm) and 3-in. (ca. 8-cm) schedule 40 and schedule 80 polyvinyl chloride. Flow through the system was maintained at 3.8 or 7.6 liters/min (1 or 2 gal/min) with a 0- to 15-gal/min (ca. 0- to 57-liters/min) centrifugal pump (model PE50E1; Peerless, Fresno, Calif.). The flow rate was monitored with an in-line flow meter (model 44750; White Industries, Westminster, Calif.); the flow meter was calibrated every week by determining the water outflow rate (actual rate was always within 5% of the flow meter reading). The source of drainage water for this pump was a 15,000-liter-constant head tank located outside the shed.

Concentrated acetic acid feed was introduced into the system with an electronic metering pump (model A7; Milton Roy, Acton, Mass.). Initially, the feed was prepared by mixing glacial acetic acid (17.4 M) and tap water. The pH of this solution was adjusted to 6.0 to 6.5 with concentrated (50%) sodium hydroxide, such that the pH of water in tank 1 was approximately 6.8 to 7.0. After it was shown to enhance the growth of *T. selenatis* in drainage water, 1.43 M K_2HPO_4 (pH 7.0) was added to a final concentration (after dilution) of 0.02 mM (day 109). The method of feed preparation was changed during the study (day 136) to bring the final pH of the feed close to that of the pK_a of acetic acid. Equal volumes of 5.5 M acetic acid and 5.5 M sodium acetate were mixed, resulting in a 5.5 M solution at pH 5.1. To ensure complete mixing of acetate feed and inflow water, a static in-line mixing valve was placed between the feed-inflow union and the first tank. Water was recirculated through the first two tanks at approximately 150 liters/min, with an 80-gal/min (ca. 300-liters/min) centrifugal pump (model 2ST1E100; G & LA Goulds, San Jose, Calif.); a flow meter was used to estimate the recirculation flow rate. Water was recirculated through the second two tanks at 500 liters/min with a 300-gal/min (ca. 1,000-liters/min) centrifugal pump (Circle E model RC300-300); the recirculation flow rate was an estimate based upon the control valve being open at 50% capacity.

Inoculation and sampling. The medium and methods used to cultivate *T. selenatis* were as described previously (14, 18). Harvested cells (approximately 6.5 g [wet weight] from a 10-liter culture) were resuspended in 100 ml of 25% glycerol–50 mM phosphate buffer (pH 7.0), frozen in liquid nitrogen, and stored at −70°C until they were used. To inoculate the reactor, the cells were thawed and divided into four equal portions that were poured into tanks 1 to 4 through inoculation ports located at the top of each tank (Fig. 1). Water samples from the reactor (inflow, outflow, and tanks 1 to 4) were taken from sampling ports shown in Fig. 1. The samples (1.8 to 4.5 ml) were immediately acidified with concentrated HCl (20 μl/ml of sample) and snap-frozen in liquid nitrogen. Samples were transported to the laboratory in liquid N_2 and stored at −20°C until analyses were performed.

Determination of residence time. On three different days when the drainage water flow rate was 7.6 liters/min, 1 liter of a 10% solution of methylene blue was added to the reactor at an inlet port located 1.5 m before the feed-inflow unit (Fig. 1). Samples of outflow water were taken at 10- to 15-min intervals over a period of 240 min. The samples were immediately frozen in liquid nitrogen and transported to the laboratory. After thawing, the A_{660} of the samples was measured. The concentration of methylene blue in the outflow was calculated from the absorbance measurements with an extinction coefficient of 12.08 $g \cdot liter^{-1} \cdot cm^{-1}$. Residence time in the reactor was determined as described previously (13).

Determination of optimal phosphate concentration for growth of microorganisms in drainage water. The minimum level of phosphate required for maximal growth of microorganisms in the reactor was determined in roll tubes with reactor-inflow drainage water, supplemented with acetate (10 mM), nitrate (10 mM), and phosphate (final concentrations, 0, 0.015, 0.15, and 1.5 mM). The tubes were inoculated with 0.1 ml of anaerobic outflow water containing microorganisms from the reactor and incubated overnight at 28°C. The minimum phosphate concentration that supported maximum growth was 0.015 mM (as estimated by measuring optical density at 600 nm); high amounts did not inhibit growth. A slightly higher concentration (0.02 mM final concentration) was added to the drainage water flowing into the reactor system.

Estimation of numbers of selenate- and nitrate-respiring bacteria. To estimate the numbers of selenate- and nitrate-respiring bacteria present, the Tri-Packs were removed from tanks 2 and 4, sealed in plastic bags, and transported on ice to the laboratory. The Tri-Packs from tank 2 were covered with reddish-pink microorganisms, while those from tank 4 were black. Material from each Tri-Pack (0.96 g, tank 2; 0.91 g, tank 4) was scraped into a Balch tube containing 2.0 ml of sterile anaerobic minimal medium and five to six glass beads. After mixing vigorously with a vortex mixer, the contents of the tubes were serially diluted (10^{-1} to 10^{-7}) into the same medium. These dilutions were transferred into a Coy anaerobic chamber, and 0.1 ml of each was spread onto minimal agar medium (1.5% Oxoid purified agar) containing acetate (10 mM) and vitamins plus nitrate (10 mM) and/or selenate (10 mM). The plates were incubated at 28°C in the anaerobic chamber. Colonies were counted after 10 days. Selenate- and nitrate-respiring colonies were identified as described previously (13). *T. selenatis* was identified as described previously (13).

Extraction of elemental selenium from outflow water. Elemental selenium was extracted from outflow water with carbon disulfide (6).

Coagulation and flocculation of elemental selenium. Samples were sent to the Wastewater Treatment Division at Nalco Chemical Company (Naperville, Ill.) for flocculation and coagulation treatment. The methods they used (described below) are standard and can be applied to large-scale operations. Two polymer coagulation methods were used to flocculate and coagulate the elemental selenium present in an outflow water sample when selenium oxyanion concentrations were less than 5 μg/liter. First, Nalco's Nalmet 8702 soluble heavy-metal precipitation-coagulation program was tested. Nalmet 8702 is an anionic liquid heavy-metal removal product. Samples (500 ml) of pilot reactor outflow water (pH unadjusted [approximately 7.5] and pH adjusted to 6.0) were treated with 100-, 200-, and 300-mg/liter doses. The samples were mixed at 100 rpm for 5 min and 20 rpm for 5 min and then allowed to settle for 10 min. The clear supernatant was then filtered through a 0.45-μm-pore-size filter and analyzed for total selenium. Experiments with cationic coagulants were also performed. Samples were treated with 150 mg of Nalco 7156 per liter, 120 mg of Nalco 8189 per liter, or 200 mg of Nalco 7157 per liter. In each sample, a light, fluffy floc that failed to settle after 10 min formed. An anionic flocculant was used to increase the rate of settling. The resulting clear supernatant was filtered through a 0.45-μm-pore-size filter and analyzed for total selenium. A control sample (untreated outflow water) was also filtered, and the total selenium content was determined.

Selenium, nitrate, nitrite, and acetate analyses. Selenate, selenite, and elemental selenium were analyzed by hydride generation-atomic absorption spectroscopy (HG-AAS; Perkin-Elmer model 3030B). The limit of detection was 5 μg/liter. Controls were performed to ensure that there was no interference from organic compounds with this method. Five inflow and five outflow samples, taken between days 63 and 74, were analyzed by HG-AAS and fluorometry (13, 23). When the methods were compared, the selenate-plus-selenite values obtained differed by no more than 5 μg/liter. In addition, there was no evidence of interference or poor recovery when samples to be analyzed with HG-AAS were spiked with Se standard (300 μg/liter). Nitrate, nitrite, and acetate were analyzed as described previously (13, 14).

RESULTS

Pilot-scale biological reactor studies. Drainage water was treated in the pilot-scale biological reactor for a total of 186 days, from June through November 1995. During this period, the concentration of selenate in the water entering the reactor ranged from 160 to 640 μg/liter (2.0 to 8.1 μM). The minimum value was observed in mid-July, and the maximum value was observed in early October. The concentration of selenite in the water was usually less than 5 μg/liter, with the exception of 14 different days when the selenite concentration was between 5 and 40 μg/liter. The nitrate concentration of the inflow water was 3.1 to 6.5 mM (43 to 92 mg of NO_3^--N per liter), while the concentration of nitrite was never more than 0.07 mM (<1 mg NO_2^--N/liter). For the first 3.5 months, water entered the reactor system at a rate of 3.8 liters/min; the flow rate was increased to 7.6 liters/min at day 108 and continued at that rate until the end of the experiment. The pH of the drainage water ranged from 6.8 to 7.0, and the temperature was between 19 and 25°C.

Because the optimum pH for growth of *T. selenatis* with selenate as the terminal electron acceptor was found to be 7.0 (although selenate reduction was still active at pH 8) (18), the inflow water pH was maintained as close to this value as possible during days 1 to 136. This was accomplished by adjusting the pH of the acetate feed (see Materials and Methods). To achieve effective nitrate and selenium oxyanion reduction, the amount of acetate fed into the reactor was varied from 1.0 to 17.0 mM. At none of these concentrations (during days 1 to 136) did complete nitrate reduction consistently occur, although on one occasion when the nitrate level in the water was low (3.1 mM), an acetate concentration of 1.5 mM was sufficient for nitrate reduction. Selenium oxyanion reduction during the same period was also poor (i.e., no greater than 20% reduction) (data not shown). During the first 136 days of the experiment, the bioreactor was populated mainly by white flocculent microorganisms that seemed to increase in number with increasing acetate concentration. Microscopic examination showed the flocs to consist primarily of long, thin, rod-shaped bacteria. The greatest density of these bacteria was found in the first tank; subsequent tanks had less biomass but contained some material that had the characteristic reddish color of elemental selenium.

Efficient selenium oxyanion reduction in the reactor system was achieved only when the pH of the feed was near the pK_a of acetate (4.76). The change in feed pH was made on day 136 and resulted in a drop in the pH of tank 1 from 7.0 to 6.5. The feed was pumped into the reactor inflow water at a rate of 6.25 ml/min, resulting in an acetate concentration in inflowing drainage water of 5 mM. Approximately 4 mM acetate was consumed.

Following this change in the acetate feed, effective selenium oxyanion reduction began to occur (Fig. 2). Levels lower than the detection limit of the selenium analysis were often achieved.

The selenium oxyanion concentration of the inflow water averaged 289 ± 90.4 μg/liter (average ± standard deviation) (Fig. 2). In the treated outflow water, the level of selenium oxyanions remaining was less than 5 μg/liter on 47% of the days sampled. If levels lower than the detection limit of the selenium assay are assumed to be 5 μg/liter, then for days 136 to the end of the experimental period (excluding days 147 to 149 and 155), the average level of selenium oxyanions remaining in drainage water after treatment was 12 ± 9 μg/liter. Since the concentration of selenium detected was less than 5 μg/liter much of the time, the average in the outflow water must have

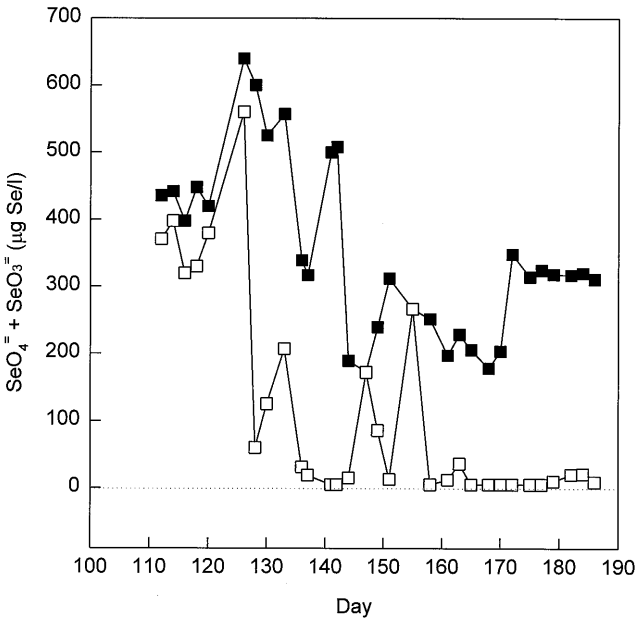

FIG. 2. Concentration of selenium oxyanions (SeO_4^{2-} plus SeO_3^{2-}) in drainage water flowing into and out of the pilot-scale biological reactor system. During the period shown, the flow rate through the reactor was 7.6 liters/min. The flow was interrupted on days 147 to 149 and on day 155. Symbols: ■, inflow; □, outflow.

been less than 12 μg/liter. During the same period, the average nitrate concentration of the inflow water was 4.6 ± 0.58 mM. This nitrate was effectively reduced to <0.1 mM nitrate and <0.01 mM nitrite (Fig. 3). The average temperature and pH values for water samples taken were as follows: inflow, 23.0°C, pH 6.2; tank 1, 23.6°C, pH 6.5; tank 2, 24.7°C, pH 7.0; tank 3, 26.3°C, pH 7.4; tank 4, 26.7°C, pH 7.5; outflow, 26.2°C, pH 7.5. The residence time at a flow rate of 7.6 liters/min ranged from 190 to 199 min.

Effective selenium oxyanion reduction from day 136 to day 186 was interrupted on two occasions. On days 147 to 149 and on day 155, the reactor system was not functioning properly because the water supply was cut off accidentally during unrelated on-site construction. Following restoration of the water supply, selenium oxyanion concentrations in the outflow water rapidly returned to the low levels achieved prior to each incident (Fig. 2).

Numbers of selenate- and nitrate-respiring microorganisms. On day 175, during the period of optimal selenium oxyanion reduction, the Tri-Packs were removed from tanks 2 and 4 and the numbers of nitrate- and selenate-respiring bacteria per gram of biomass were determined. The numbers of nitrate-respiring bacteria in tanks 2 and 4 were 2.6×10^8/g and 4.2×10^8/g, respectively. The number of selenate-respiring bacteria were approximately 200-fold lower, with 1.2×10^6/g in tank 2 and 1.8×10^6/g in tank 4. The selenate-respiring bacteria were found to be *T. selenatis*. Although the numbers of selenate-respiring *T. selenatis* and nitrate-reducing bacteria in tank 2 were somewhat lower than those in tank 4, tank 2 appeared to contain the greatest amount of biomass. Material in this tank and in tank 3 was pink-red. Tank 1 contained mostly white material, while that in tank 4 was red-brown to black.

Recovery of elemental selenium from outflow water. Elemental selenium was extracted from outflow water samples taken on the final two sampling days of the study, when selenium oxyanion concentrations were low. Elemental selenium

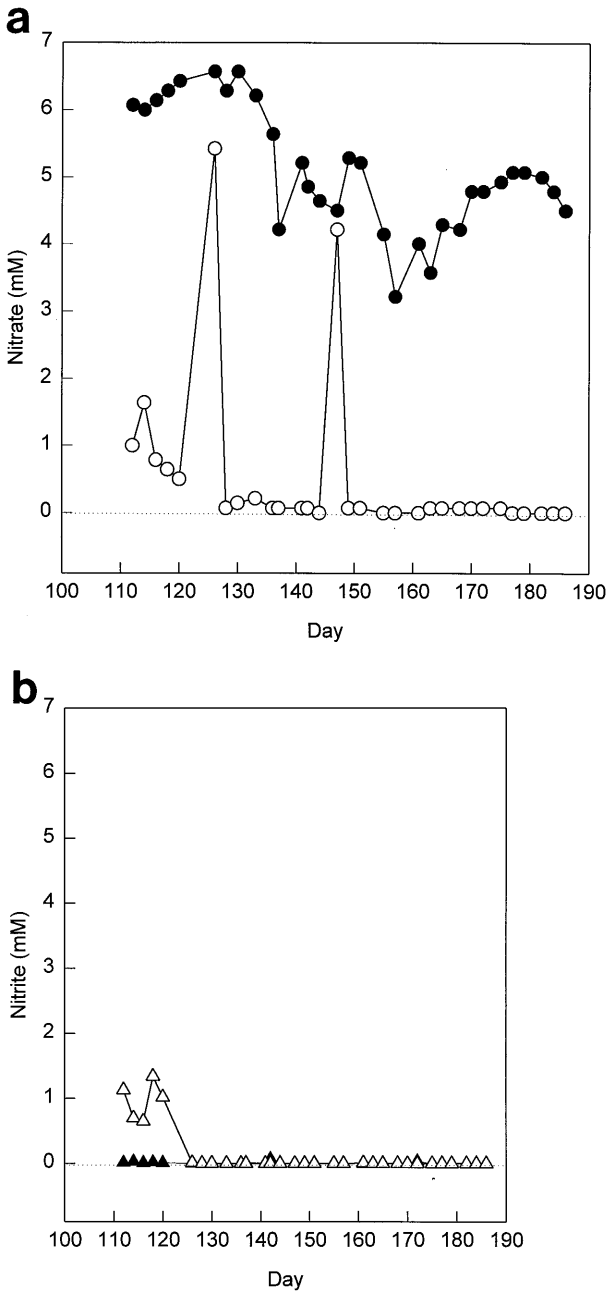

FIG. 3. Concentration of nitrate (a) and nitrite (b) in drainage water flowing into and out of the biological reactor. Drainage water flowed through the system at a rate of 7.6 liters/min. The flow was interrupted on days 147 to 149 and on day 155. Symbols: ●, inflow nitrate; ○, outflow nitrate; ▲, inflow nitrite; △, outflow nitrite.

was present as 91 and 96% of the total selenium recovered on days 184 and 186, respectively.

Removal of elemental selenium from outflow water. Removal of the elemental selenium from the outflow water by polymer coagulation proved to be highly successful. Water to be treated contained 237 μg of elemental selenium per liter; after treatment, the filtrate from all samples contained 12 μg/liter or less. The best results (97.9% removal; less than 5 μg of elemental selenium per liter) were achieved when outflow water (pH 6.0) was treated with 100 mg of Nalmet 8072 per liter.

DISCUSSION

The future of California agriculture depends on proper management and disposal of selenium-contaminated drainage water from the San Joaquin Valley. The value of crops produced during 1995 in this region was estimated to be 18 billion dollars (4). It is, therefore, critical that a simple and environmentally sound method for selenium removal from drainage water be developed. The present study describes such a method.

Panoche Water District drainage water was effectively treated in a pilot-scale biological reactor system inoculated with the selenate-respiring bacterium *T. selenatis*; the carbon source-electron donor reactor feed used was acetate (5 mM). *T. selenatis* was maintained in the nonsterile reactor by selective pressure (i.e., the presence of acetate and selenate). Selenium oxyanions, present primarily as selenate in the drainage water, were reduced consistently by 98% (frequently to levels below 5 μg/liter, the detection limit of the selenium assay).

In the pilot-scale reactor system, it was possible to treat 10,915 liters of drainage water per day (i.e., 7.6 liters/min; mean residence time, 195 min). In a previous study, drainage water was successfully treated in a laboratory-scale reactor (13). The pilot reactor described in this study represents a 1,000-fold scale-up of that system.

Concomitant with selenium oxyanion reduction, the nitrate present in drainage water was also reduced by 98%. Nitrite was not present in the treated water; therefore, complete denitrification had occurred. Such a biological system could, therefore, easily be used for removal of nitrate from drinking water.

Critical to effective treatment of drainage water is the physical removal of elemental selenium formed during reduction of selenium oxyanions. Results obtained in this study demonstrate that the elemental selenium can be coagulated and flocculated with the commercially available polymer Nalmet 8702 (manufactured by the Nalco Chemical Company [8]). The optimum conditions (i.e., optimum Nalmet 8702 concentration and optimum pH, etc.) for selenium coagulation and flocculation with Nalmet 8702 must still be determined. Following Nalmet 8702 treatment, the coagulated and flocculated material can be removed from water by one of several methods, including filtration (20) or flotation (6); the latter appears to be the preferred approach. Optimization of coagulation, flocculation, and selenium removal from treated drainage water must still be carried out on a pilot scale.

One of the major costs of operating a biological reactor system (20) is that of the carbon source-electron donor feed. On the basis of the results of the present study, where acetate was the feed, it was estimated that the cost of feed for a farm-scale reactor is $6.2/10,000 liters (i.e., $459/day, treating 740,000 liters/day). Use of acetate for selenium bioremediation is, therefore, expensive. In comparison, the cost of methanol as a feed source (taking into account the amount of methanol that would be needed to permit complete reduction of the nitrate in drainage water) would be $1/10,000 liters of drainage water (i.e., $76/day). Unfortunately, methanol cannot be used by *T. selenatis* as a substrate for growth. Its use in a pilot-scale biological reactor in the past has resulted in a system capable of denitrification only (10, 20). While selenium oxyanion reduction was observed in such systems, once nitrate was reduced (10, 20), the reduction was slow and the level to which selenium was reduced was not as low (16 to 50 μg/liter at best) as that achieved in *T. selenatis* biological reactor systems (13; present study). Slow reduction requires reactor systems that are much larger than that described in this study (10, 20).

To reduce the cost of feed for a *T. selenatis* bioremediation system, alternate feed sources have been sought. Experiments have been carried out to determine the optimum conditions for the fermentation of the abundant waste product whey to acetate, propionate, and butyrate. These substrates are utilized rapidly by *T. selenatis* for growth (15). The fermented whey has been used as a feed for selenium bioremediation in a laboratory-scale reactor treating Panoche drainage water, and levels of selenium oxyanions have been reduced by 98% (1). Use of fermented whey would reduce bioreactor feed costs to about $2/10,000 liters of drainage water (i.e., $148/day; 740,000 liters treated per day). This is significantly less than the cost of using acetate ($459/day). Clearly, the whey fermentation must be optimized (which will very likely result in a decrease in this cost) and tested on a pilot scale.

With regard to practical use of the *T. selenatis* system, it may be possible to construct individual bioremediation systems on farms where selenium-contaminated drainage water is a problem. Alternatively, the *T. selenatis* system could also be used in conjunction with agroforestry systems that have been shown to be an effective means of reducing the volume of drainage water (3). A hypothetical agroforestry system of 10 ha of salt-tolerant trees and 4 ha of halophytes is capable of treating the amount of drainage water generated by 650 ha of conventional crops. While the volume of water is reduced, the selenium remains in the water and the concentration is increased. Such salt-concentrated and selenium-containing wastewater could be treated in a *T. selenatis* reactor. In preliminary experiments with this concentrated saline water, selenium oxyanions were reduced by 95% in a lab-scale *T. selenatis* reactor system (11).

Another biological selenium removal system, the algal-bacterial selenium removal system, has been described recently (7). It was thought that the microalgae generated during the first steps of the process might be used as a source of fermentable carbon that could be an inexpensive feed for the *T. selenatis* reactor. Preliminary experiments were, however, not successful. The microalgae proved to be quite recalcitrant to anaerobic fermentation, even after heat treatment (1).

In conclusion, the present study demonstrates that a simple, effective, and environmentally sound method for selenium bioremediation now exists; although pilot-scale studies to optimize elemental selenium removal and to ferment whey to an inexpensive feed must still be done. If reactor systems were eventually used on the various farms in the Grasslands Water District, it should be possible to reduce the amount of selenium entering the San Joaquin River annually by 98% (i.e., from 7,000 lb [ca. 3,000 kg] to 140 lb [ca. 60 kg]/year).

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