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Bioreduction of Selenate Using a Hydrogen-Based Membrane Biofilm Reactor

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A H_2-based, denitrifying and sulfate-reducing membrane-biofilm reactor (MBfR) was shown to be effective for removing selenate (Se(VI)) from water or wastewater by reducing it to insoluble Se^0. When Se(VI) was first added to the MBfR, Se(VI) reduction—first to selenite (Se(IV)) and then mostly to Se^0—took place immediately and then increased over three weeks, suggesting enrichment for dissimilatory selenium-reducing bacteria. Increasing the H_2 pressure improved the Se(VI) reduction rate and total-Se removal, and lowering the influent Se(VI) concentration from 1000 to 260 μg Se/L increased the average Se removal to 94%, which corresponded to an effluent Se concentration of less than 12 μg Se/L, a value well below the standard of 50 μg Se/L. The fact that the effluent suspended solids contained reduced Se suggests that Se^0 was retained in the biofilm, which detached to form the effluent suspended solids. A series of short-term experiments elaborated on how decreased influent selenate loading and increased H_2 pressure could systematically improve the reduction of Se-(VI) and removal of total Se. Short-term experiments also demonstrated that selenate reduction improved with lower influent nitrate concentration, suggesting that H_2 was more available for selenate reduction when the H_2 demand for denitrification was smaller. Complete sulfate reduction, which occurred in parallel to nitrate reduction, dominated the electron-equivalent flux. Like selenate reduction, but unlike nitrate reduction, sulfate reduction was sensitive to H_2 pressure and appeared to be inhibited by selenate. Finally, selenate reduction was relatively insensitive to pH in the range of 7.0 to 9.0. This research shows that the MBfR can be effective for removing Se(VI) in water or wastewater to below the 50 μg Se/L standard.

Introduction

Selenium is widely used in a variety of industries, including production of glass, pigments, pesticides, stainless steel, and photoelectric cells (*1*). Selenium contamination in the wetlands of the San Joaquin Valley, California, is mainly associated with agricultural drainage water containing Se, mostly as selenate (Se(VI)), along with high levels of nitrate and sulfate (*2*−*5*). Selenium is a naturally occurring element of considerable biological interest because of the small difference between concentrations that are physiologically essential and concentrations that are toxic to organisms (*6*). Technology to remove selenate from industrial wastewater, groundwater, and drinking water is an emerging need.

Inorganic selenium is found commonly in four oxidation states. Se(VI), the most oxidized form of selenium, and Se(IV) are highly water soluble and are toxic to biological systems at relatively low concentrations. Elemental selenium (Se^0) is highly insoluble in water and therefore has much lower toxicity. The most reduced form of selenium is selenide (Se(−II)), which may be a highly toxic gas, but is seldom a biological threat because it is readily oxidized to insoluble Se° in the presence of air. The maximum contaminant level (MCL) for drinking water is 50 μg-Se/L of total selenium (*7*).

Thus far, physicochemical methods such as chemical precipitation, catalytic reduction, reverse osmosis, and ion-exchange mainly have been used for removing soluble Se from industrial wastewater (*8*−*12*). While most of these methods are suitable for the removal of selenite, they are less effective in removing selenate (*13*). Furthermore, physicochemical methods are costly and often generate problematic residues.

Under appropriate environmental conditions, selenate can be bio-reduced into elemental selenium (Se^0) via selenite as an intermediate (*14*−*18*), and the reduction is dissimilatory (*19*−*20*). Se^0 is insoluble and can be removed by filtration or centrifugation. Since Se^0 has little or no toxicity (*14*, *16*) and can be easily removed from the aqueous phase (*21*), biological treatment can be an effective and economical process for removing soluble Se, especially selenate. Some biological treatment systems have already been reported, including an algal-bacterial Se-removal system (*22*), a sludge-blanket reactor system (*23*), and suspended-growth reactor systems bioaugmented with selenate-reducing bacteria (*4*, *24*). In all these systems, selenate and selenite are reduced using an organic carbon source as the electron donor. In drinking-water treatment, heterotrophic reduction has the potential for leaving a donor residual that requires significant post-treatment to produce safe and biologically stable water (*25*). On the other hand, autotrophic reduction using an inorganic electron donor, such as hydrogen or reduced sulfur (*26*), does not result in the donor-residual problem.

Autotrophic reduction with hydrogen (H_2) as the electron donor has at least two major advantages (*27*−*28*). First, H_2 has very low water solubility, which means that essentially no residual donor carries over in the treated water. Second, it is nontoxic to humans. Despite its advantages, H_2 has not been widely used, because sparging has been required to supply H_2 gas. Sparging creates the possibility for creating H_2 off gas and a combustible combination with oxygen in air (*29*). To solve this problem, the authors' group developed a hollow-fiber membrane biofilm reactor (MBfR) that delivers hydrogen gas directly to the biofilm by molecular diffusion through the biofilm's substratum, which is a bubbleless membrane. Further details on the MBfR and its operation are in several publications (*27*, *28*, *30*−*32*).

In screening studies for a wide range of oxidized contaminants, Nerenberg and Rittmann (*33*) showed immediate reduction of selenate in MBfRs in which nitrate or oxygen was the primary electron acceptor. In this research, we

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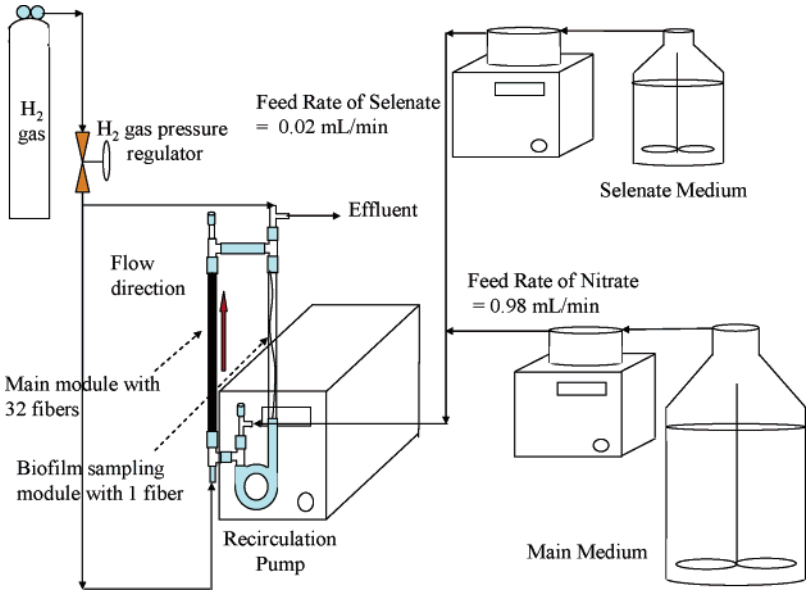

FIGURE 1. Schematic of the bench-scale MBfR used to investigate Se reduction.

TABLE 1. Physical Characteristics of the MBfR System

	units	main tube	coupon tube	reactor total
tube inside diameter	cm	0.6	0.5	
number of hollow fibers		32	1	33
cross-sectional area fibers	cm^2	0.0197	0.0006	0.0203
feed rate	mL/min			1
recirculation rate	mL/min			150
net cross sectional area	cm^2	0.26	0.20	
fiber surface area	cm^2	70.4	2.2	72.6
liquid velocity	cm/min	570.3	766.3	
MBfR system volume	mL			23.9
average hydraulic detention time	min			23.9

evaluate in detail the potential for using the H_2-based MBfR for reducing selenate to Se^0 and removing the solid-phase Se^0. In particular, we investigate (1) acclimation of a denitrifying and sulfate-reducing MBfR to Se(VI) addition; (2) the fate of Se^0 in the reactor; (3) the effects of selenate loading, H_2 pressure, nitrate loading, and pH on selenate reduction; and (4) competition between selenate reduction and sulfate reduction.

Materials and Methods

Experimental Setup. A schematic of the MBfR used in this study is shown in Figure 1, and the physical characteristics of the reactors are provided in Table 1. The MBfR system consisted of two glass tubes connected with Norprene tubing and plastic barbed fittings. One glass tube contained a main bundle of 32 hollow-fiber membranes (model MHF 200TL, a composite bubbleless gas-transfer membrane produced by Mitsubishi Rayon), each 25 cm long. Biofilm samples were collected by cutting short lengths of a separate "coupon" fiber, located in the second glass tube. This allowed sample collection without disturbing the main bundle of fibers and without causing a significant change in total biofilm surface area in the reactor. Results for biofilm sampling are not presented in this manuscript.

Operating Conditions. A single peristaltic manifold pump (Gilson Minipuls 3, Middleton, WI) was used with PVC tubing to give a nitrate-medium-feed rate of 0.98 mL/min. A second manifold pump supplied the selenate stock solution at a rate of 0.02 mL/min into the feed line from the nitrate medium, thereby giving a total feed flow rate of 1 mL/min. The recirculation rate was 150 mL/min, giving a recirculation ratio of 150 that promoted completely mixed conditions. Another advantage of a high recirculation rate was the high hydraulic shear on the fibers, providing a dense biofilm (*34*) that minimized the accumulation of excessive biofilm that might clog the reactor. The standard H_2 pressure for both reactors was 2.5 psi (0.17 atm), and the H_2 pressure was increased to 4 psi (0.27 atm) to evaluate the effect of H_2 availability.

Feed Medium, Stock Solutions, and Mixed Influent. The composition of the main feed medium was (g/L): KH_2PO_4 0.128, 100, Na_2HPO_4 0.434, $MgSO_4{\cdot}7H_2O$ 0.2, $NaNO_3$ as N 0.03, $CaCl_2{\cdot}2H_2O$ 0.001, $FeSO_4{\cdot}7H_2O$ 0.001, and 1 mL of trace mineral solution. The trace mineral solution (mg/L) consisted of $ZnSO_4{\cdot}7H_2O$ 100, $MnCl_2{\cdot}4H_2O$ 30, H_3BO_3 300, $CoCl_2{\cdot}6H_2O$ 200, $CuCl_2{\cdot}2H_2O$ 10, $NiCl_2{\cdot}6H_2O$ 10, $Na_2MoO_4{\cdot}2H_2O$ 30, and Na_2SeO_3 30. The main medium was prepared in an 8-L glass bottle (Pyrex) and filter sterilized into another sterile 8-L glass bottle using a capsule filter (Pall SuporCap 100, Pall Corporation, Ann Arbor, MI). The selenate feed medium contained deionized water with 50 mg Se/L (from Na_2SeO_3). When the main and selenate media were mixed, the nominal influent concentrations were 5 mg NO_3^- N/L, 78.5 mg SO_4^{2-}/L, and 1000 μg Se/L; these nitrate and sulfate concentrations are representative of selenate-contaminated groundwater in the San Joaquin Valley (*2*−*5*), and selenate was 20 times a typical concentration to accentuate our ability to study its fate and kinetics. Actual concentrations were measured daily and are reported. All feed media were purged with nitrogen gas to eliminate dissolved O_2 in the influent. To determine

TABLE 2. Variable System Conditions for the Short-Term Experiments[a]

H_2 pressure (psi) (1 psi = 0.068 atm)	influent concentration		influent pH
	selenate (μg Se/L)	nitrate (μg N/L)	
	series 1		
2.5	1000	5000	7.5
	500		
	250		
	100		
	series 2		
2.5	1000	5000	7.5
4.0			
5.5			
	series 3		
2.5	1000	10 000	7.5
		5000	
		2500	
		1000	
		0	
	series 4		
2.5	1000	5000	6.5
			7.1
			7.6
			8.2
			8.9

[a] The influent SO_4^{2-} concentration was 78.5 mg SO_4^{2-}/L in all experiments.

the effect of Se loading, the influent concentration of selenate was reduced to 250 μg Se/L in the presence of the same concentrations of nitrate and sulfate.

Inoculation and Startup. The inoculum was obtained from a pilot-scale MBfR operated at La Puente, CA (*32*). The pilot-scale reactor treated groundwater with approximately 5 mg N/L of nitrate and 60 μg/L of perchlorate. It had two modules in series; most of the nitrate was removed in the first module and most of the perchlorate was removed in the second. Biofilm samples were taken from the first and second modules and mixed together. This mixture was added to a sterile glycerol solution, with a final concentration of 25% glycerol, and stored at −80 °C. For inoculating the MBfR, biofilm samples were thawed, washed twice by centrifuging for 15 min at 5000*g*, and re-suspended in 10 mL of sterile minimal medium without electron donor. A 1.5-mL aliquot of the washed biofilm suspension was added into the reactor.

Following inoculation, H_2 gas was supplied to the lumen of the fibers at 2.5 psi (0.17 atm), and the reactor was operated in recirculation mode for 24 h to establish biofilm colonization. Then, feed medium without selenate was applied at a rate of 0.2 mL/min to the MBfR. This approach of initiating the biofilm in advance of adding the target contaminant followed our established experimental protocol (*33*, *38*) for testing a range of oxidized contaminants. The concentration of nitrate in the effluent reached steady-state after 3 days, and then the feed rate was increased to 1.0 mL/min. After nitrate was completely removed (ca. 20 days), selenate was added to the influent of MBfR at 1000 μg/L as Se.

Short-Term Experiments. Short-term experiments were conducted to investigate systematically how H_2 availability, selenate loading, nitrate loading, and pH affected selenate reduction. The experiments were organized into four series, listed in Table 2. Prior to each experiment, the reactor was returned to the steady-state condition with an influent of 1000 μg Se/L selenate, 5 mg N/L nitrate, and 78.5 mg SO_4^{2-}/L, and 2.5 psi H_2 pressure. For each short-term test, the change of system conditions lasted for 2 h before samples were taken. With a liquid retention time of 24 min in the MBfR, 2 h (more than 5 liquid retention times) was long enough for the system to reach a pseudo-steady-state, which is defined as a condition in which the liquid concentration reached a stable state, while the biofilm accumulation and biomass were not changed significantly from the true steady state.

Sampling and Analysis. The performance of the reactors was monitored by analyzing influent and effluent samples taken on a daily basis and immediately filtered through a 0.2-μm membrane filter (Pall Corp., Ann Arbor, MI). After sample centrifugation (15 000*g*, 10 min), total soluble selenium (Se(IV) + Se(VI)) was determined by ICP-MS (PQExCell, VG Elemental). To investigate the degree of reduction of oxidized contaminants, the Se(IV) concentration was analyzed by a fluorometric method (Standard method 3500-Se E) (*35*). The concentration of Se(VI) was calculated by subtracting Se(IV) from total Se in the effluent. This approach assumes that selenium reduced to Se^0 results in a solid removed by filtration plus centrifugation (*36*−*37*); thus, the concentration of selenium removed as Se^0 was computed as the difference between influent and effluent total Se. Analyses for nitrate and nitrite were carried out by ion chromatography using an AS-11 column, an AG-11 precolumn, and a 200-μg/L injection loop, as described by Nerenberg (*38*). The sulfate concentration was measured using a capillary ion analyzer (CIA) (Millipore Corp., Milford, MA), and the dissolved sulfide concentration in the aqueous phase was measured using a colorimetric method based on methylene blue formation (*39*).

To determine how much Se^0 was associated with the effluent suspended solids, hydrogen peroxide (H_2O_2) was added to unfiltered effluent samples, because it can oxidize Se^0 to soluble forms (*40*) analyzed by ICP/MS. The suspended solids (SS) concentration was measured following Standard Methods 2540 E (*35*).

A headspace-analysis method was used to determine dissolved H_2 concentrations (*41*). A 1-mL liquid sample was transferred from the reactor to a 160-mL serum vial previously outgassed with N_2. The vials were shaken vigorously by hand for 5 min to liberate the dissolved H_2. A gastight syringe was used to sample the headspace (1 mL) and test for H_2 by the reduction gas analysis (RGA3, Trace Analytical, Menlo Park, CA). In this method, H_2 is directed through a HgO bed and produces Hg gas, which is measured by an ultraviolet photometer. Once the H_2 concentration was known, Henry's law and mass balance were used to determine the dissolved H_2 concentration (*42*). The Henry's constant was 7.9×10^{-7} mol/cm^3 water/(mol/cm^3 gas) for the assay temperature of 25 °C.

Results and Discussion

Startup and Steady-State Experiments. Figure 2 presents the experimental results for the startup and steady-state phases. In the first few days of operation, nitrate was partially converted to nitrite, but the nitrate and nitrite concentrations in the effluent dropped to less than 15 μg N/L within 10 days. Sulfate reduction began within 3 days of operation and occurred over most of the experimental period. After selenite was reduced completely, sulfide as a byproduct of sulfate reduction was stably observed at 8 ± 1 mg/L. This result supports that sulfate concentration below 78 mg/L and sulfide up to 8 mg/L were not inhibitory for selenate and selenite reductions.

Se(VI) was reduced to Se(IV) within 1 day of Se(VI) addition (begun on day 25). Up to day 38, Se(VI) was reduced exclusively to Se(IV), which is completely soluble. On day 38 (or 13 days after Se(VI) feeding began), Se(IV) began to be removed through reduction to Se^0. The maximum removal (587 μg Se/L, or 62%) was observed on day 47 and remained steady to day 65. This steady increase in Se(VI) reduction suggests that dissimilatory selenate-reducing bacteria were

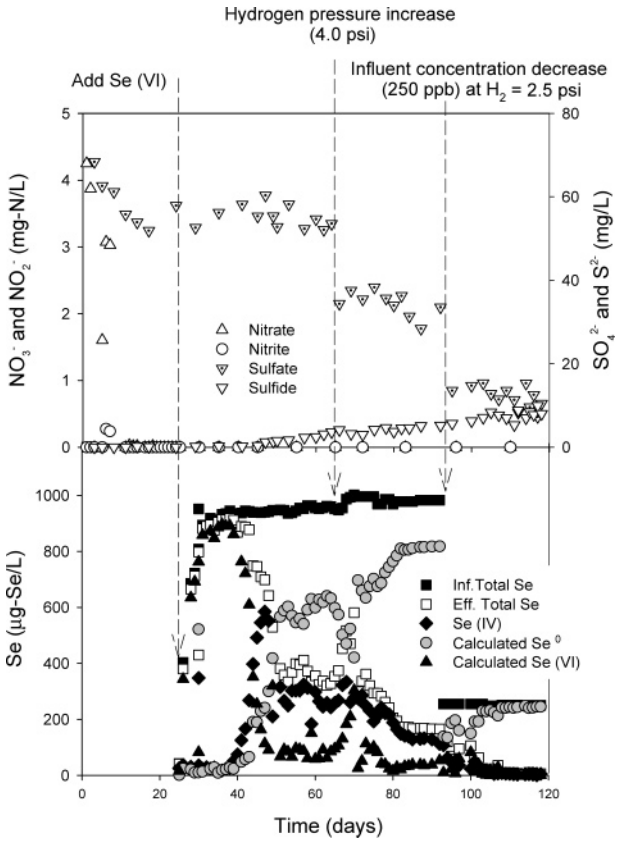

FIGURE 2. Effluent concentrations of nitrate, nitrite, total Se, Se(IV), and calculated Se(VI), along with influent Se(VI) and calculated Se^0 retained in the biofilm. Influent concentrations are 5 mg/L of NO_3^- N and 1000 μg/L of SeO_4^{2-} as Se. (upper left *Y* axis: NO_3^- and NO_2^- in mg N/L; upper right *Y* axis: SO_4^{2-} and S^{2-} in mg/L; lower left *Y* axis: Se species in μg Se/L).

present in the biofilm, but continuous exposure to Se(VI) provided a selective pressure that further enriched these bacteria.

The H_2 pressure was changed to 4.0 psi (0.27 atm) on day 65 to improve the extent of Se(VI) reduction, which increased continually up to day 81 after a transient decline. From day 82 to day 92, the average concentrations of Se(IV) and Se removed as Se^0 were 135 ± 8 and 810 ± 5 μg Se/L, respectively. The average removal of Se in this period was about 83%, which shows that H_2 availability controlled the reduction kinetics of Se(VI) to Se^0.

On day 93, the influent Se(VI) loading was lowered (new initial Se(VI) concentration of 260 μg Se/L on day 93) to evaluate the effect of Se(VI) loading on Se(VI) reduction. The H_2 pressure was returned to 2.5 psi (0.17 atm). Within 10 days, selenate was almost 100% reduced to solid Se^0 with the lower selenate loading.

Table 3 summarizes the Se(VI) and Se(IV) concentrations in the effluent, the calculated concentration of Se^0 removed, and the average selenium removal percentages and fluxes for the three steady states. The Se(VI) fluxes ranged from 0.034 to 0.126 g Se(VI)/m^2 of biofilm surface area/day for this set of experiments. The key conclusion from the steady-state experiment is that selenate flux and H_2 pressure to the hollow fibers controlled the effluent Se(VI) concentration and % selenium removal. A selenate flux of 0.126 g/m^2·d gave an effluent Se(VI) of 87 μg/L and 62% Se removal when the H_2 pressure to the fibers was 2.5 psi. Reducing the flux to 0.034 g/m^2·d increased total Se removal to 94%, while the effluent Se(VI) declined to 7.2 μg/L. Increasing H_2 pressure to 4 psi allowed a Se(VI) flux of 0.130 g Se(VI)/m^2·d, giving a higher percent Se removal (78%) and a lower Se(VI) concentration (55 μg Se/L) than 2.5 psi for the same Se(VI) influent concentration. This interplay of flux and H_2 pressure is explored in more detail with the short-term experiments. From a regulatory point of view, the effluent Se concentration (soluble Se(VI) + Se(IV)) was below the MCL (50 μg/L) for the lower Se(VI) loading.

Fate of Se^0 and Total Se. The selenium content of the SS was measured in the MBfR effluent on day 115. The SS concentration was 0.05 ± 0.01 mg SS/L, and total dissolved selenium was 6.8 μg Se/L. Five effluent samples were treated with 10% hydrogen peroxide, filtered by 0.2-μm membrane filter, and analyzed by ICP/MS. The dissolved concentration of selenium species was 39.5 ± 0.6 μg Se/L, making the Se^0 concentration present in the SS 32.7 μg Se/L. Thus, most of the effluent suspended solids were Se^0 (i.e., 32.7 μg Se/L/50 μg SS/L = 65%), while most of the total Se in the effluent was Se^0 in the SS (i.e., 32.7 μg Se/L/39.5 μg/L = 83%). Thus, good removal of total Se requires that the suspended solids be removed from the effluent. These results also imply that Se^0 was captured in the biofilm and subsequently removed from the MBfR system as detached biofilm.

Short-Term Experiments. In the first short-term series, the influent selenate concentration was set to 100, 250, 500, or 1000 μg Se/L, with applied H_2, influent nitrate, and influent sulfate at the steady-state values of 2.5 psi (0.17 atm), 5 mg N/L, and 78.5 mg SO_4^{2-}/L, respectively. Results for selenium are shown in Figure 3. Nitrate reduction (not shown) was >99.5% in all cases and not affected by the influent selenate load. Sulfate reduction and flux decreased, however, as the influent Se(VI) concentration increased. Selenite was detected for influent selenate concentrations of 500 and 1000 μg Se/L. Se reduction to Se^0 increased to 649 ± 23 μg Se/L as the influent Se(VI) concentration increased to 1000 μg Se/L, but the percentage removal declined to 65%. With a fixed H_2 availability (2.5 psi or 0.17 atm) and fixed biofilm accumulation, the Se(VI) concentration in the effluent from the Se-

TABLE 3. Summary of Steady-State Results for the Selenate-Reducing MBfR

	steady-state period (days)		
	51–64	**82–92**	**106–118**
H_2 pressure (psi) (1 psi = 0.068 atm)	2.5	4.0	2.5
average influent concentration of Se (μg Se/L)	950	1,000	260
Se(VI)	87 ± 25 μg Se/L	55 ± 4 μg Se/L	7.2 ± 6.1 μg Se/L
calculated Se(IV)	270 ± 40 μg Se/L	135 ± 8 μg Se/L	4.4 ± 1.9 μg Se/L
Se removed as Se^0	590 ± 30 μg Se/L	810 ± 5 μg Se/L	245 ± 6 μg Se/L
sulfate concentration in effluent	54 ± 2 mg SO_4^{2-}/L	35 ± 3 mg SO_4^{2-}/L	13 ± 3 mg SO_4^{2-}/L
average removal of nitrate	99%	100%	100%
average removal of total Se	62%	83%	94%
Se(VI) flux (g Se(VI)/m^2·d)	0.126	0.130	0.034
nitrate flux (g NO_3^-/m^2·d)	0.689	0.667	0.712
sulfate flux (g SO_4^{2-}/m^2·d)	3.95	6.34	8.42

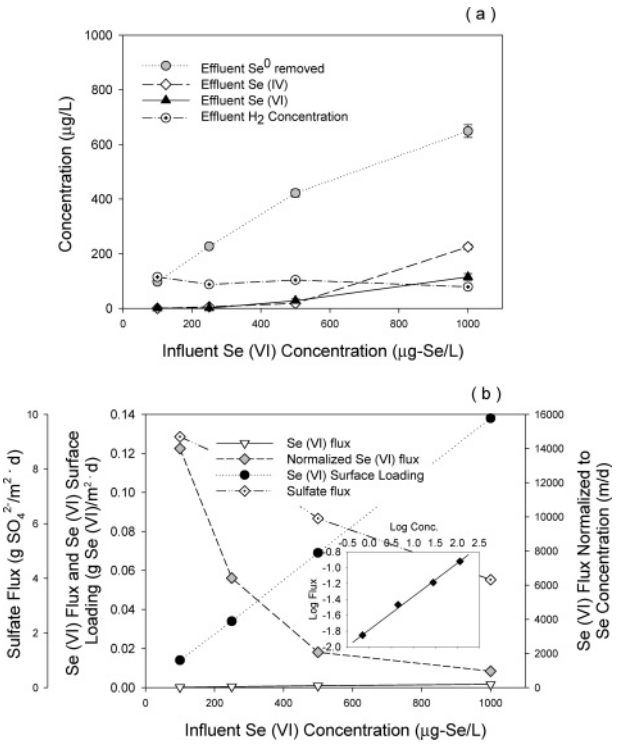

FIGURE 3. Results for the first short-term series (selenate load variable): (a) selenate, selenite, Se^0 removed, and H_2 concentration in the effluent; (b) Se(VI) flux, Se(VI) flux normalized to effluent Se(VI) concentration, Se(VI) surface loading, and sulfate flux; (inset) logarithm of Se(VI) flux plotted against the logarithm of the effluent Se(VI) concentration. (Se species concentration in μg Se/L, H_2 concentration in μg/L, Se(VI) flux and Se(VI) surface loading in g Se(VI)/m^2·d, Se(VI) flux normalized to Se(VI) concentration in m/d).

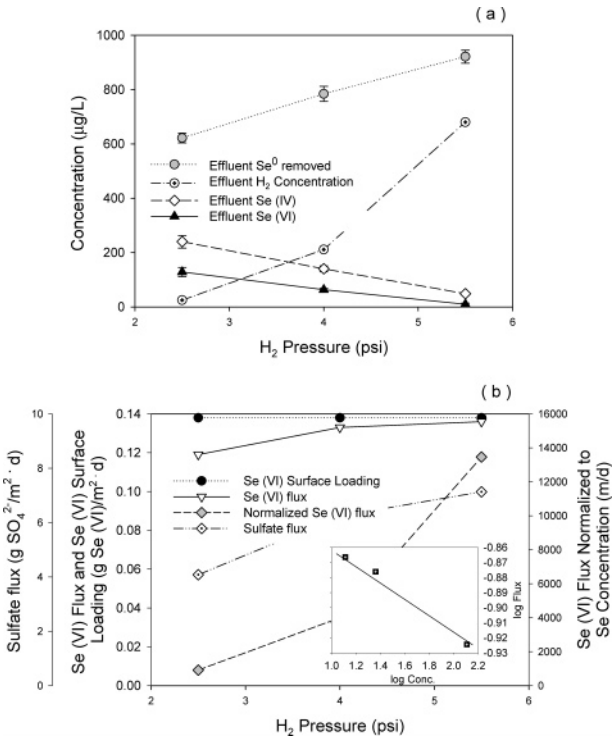

FIGURE 4. Results for the second short-term series (H_2 pressure variable): (a) selenate, selenite, Se^0 removed, and H_2 concentration in the effluent; (b) Se(VI) flux, Se(VI) flux normalized to effluent Se(VI) concentration, Se(VI) surface loading, and sulfate fux; (inset) logarithm of Se(VI) flux versus logarithm of effluent concentration of Se(VI). (Se species concentration in μg Se/L, H_2 concentration in μg/L, Se(VI) flux and Se(VI) surface loading in g Se(VI)/m^2·d, Se(VI) flux normalized to Se(VI) concentration in m/d).

reducing MBfR increased with loading up to 115 μg Se/L. Although the highest percentage Se(VI) removal was observed at the lowest influent loading (99.9% for 100 μg Se/L), over 95% removal was achieved with 500 μg Se/L in the influent. The effluent residual H_2 was relatively constant at 97 $\pm$ 17 μg/L for all experiments. This means that H_2 consumption was essentially constant within this series; the reason for this is explained later.

The Se(VI) fluxes (denoted J) into the biofilm ranged from 0.014 to 0.120 g Se(VI)/m^2·d and gradually increased when the influent Se(VI) loading increased from 0.02 to 0.2 g/m^2·d. Although Se(VI) flux was smallest for the lowest Se(VI) loading and concentration (denoted S) in the effluent from the MBfR, the Se(VI) flux normalized to effluent concentration (m/d), i.e., the pseudo-first-order rate coefficient for Se(VI), was dramatically higher for the lowest Se(VI) concentration. To estimate the reaction order, log J versus log S is plotted in the inset of Figure 3. The slope is 0.41, which is close to the well-known half-order kinetics was for deep biofilms in which the reaction is zero order in substrate concentration (*43*–*44*). The impact of a reaction order smaller than one is that increases in S give less than proportional increases in J, which means that percentage removal declines for higher loading. This is exactly the result shown in Figure 3 for Se(VI) concentration and loading. Thus, the results support that Se(VI) reduction was largely diffusion-limited in a deep biofilm, but the bacteria reducing selenate had high affinity for Se(VI).

In the second short-term experiment, the applied H_2 pressure was 2.5 psi (0.17 atm), 4.0 psi (0.27 atm), or 5.5 psi (0.37 atm), with a fixed selenate influent of 1000 μg Se/L. Influent nitrate and sulfate were the standard values of 5 mg NO_3^- N/L and 78.5 mg SO_4^{2-}/L, respectively. Denitrification was at least 99.5% (not shown) and not affected by H_2 pressure. On the other hand, sulfate reduction was significantly affected as H_2 pressure increased. As shown in Figure 4, increasing H_2 pressure caused a steady decrease in effluent selenate and selenite, and Se^0 removal continually increased, reaching 921 $\pm$ 24 μg Se/L (~92%) at 5.5 psi. At 2.5 psi applied pressure, the effluent residual H_2 was 23 μg/L. As the H_2 pressure increased to 4.0 psi, the reduction of Se(VI) increased by 9%, despite the Se(VI) concentration being lower. Increasing the H_2 pressure to 5.5 psi gave a further increase to Se reduction with still lower Se(VI), but the effluent H_2 concentration rose to $\geq$680 μg/L. Increasing the applied H_2 pressure to 5.5 psi at the given selenate, nitrate, and sulfate loading caused an increasing effluent H_2 concentration, which suggests that H_2 limitation in the biofilm was minimal for the highest H_2 pressure.

As H_2 pressure increased from 2.5 to 5.5 psi, the Se(VI) flux normalized by its effluent concentration increased from 912 to 13 500 m/d (Figure 4b). This demonstrates that selenate reduction strongly depended on H_2 availability. This is underscored by the negative reaction order (−0.05) shown in the inset to Figure 4b. The flux increased with decreasing Se(VI) concentration because of the increased H_2 availability, which increased the active depth of the biofilm for Se(VI) reduction.

In the third short-term experiment, the influent nitrate concentrations were varied from 0 to 10 mg N/L, with H_2 pressure and selenate and sulfate concentrations at the steady-state values of 2.5 psi, 1000 μg Se/L, and 78.5 mg SO_4^{2-}/L, respectively. As shown in Figure 5, effluent selenate and selenite concentrations modestly increased when effluent nitrate concentration increased, and the amount of Se^0 removed gradually decreased to 818$\pm$13 μg Se/L as influent

TABLE 4. Comparison of Fluxes for Selenate, Sulfate, and Nitrate from the Short-Term Experiments

influent NO_3^- concn. (mg·N/L)	influent Se(VI) concn. (μg/L)	H_2 pressure (psi)	electron-equivalent flux (eq/m²·d)			sum of the fluxes in electron equivs (eq/m² d)	distribution of fluxes (%)			sulfate flux normalized by sulfate effluent concn[b] (m/d)
			Se (VI)[a] (eq/m² d)	NO_3^-	SO_4^{2-}		Se (VI)	NO_3^-	SO_4^{2-}	
Se (VI) concentration loading										
5	1000	2.5	0.00168	0.0556	0.329	0.387	0.4	14.4	85.2	75.5
	500		0.00091	0.0560	0.516	0.573	0.2	9.8	90.0	172
	250		0.00048	0.0574	0.702	0.760	0.1	7.5	92.4	424
	100		0.00020	0.0550	0.765	0.820	0.03	6.7	93.3	640
H_2 pressure										
5	1000	2.5	0.00166	0.0569	0.340	0.399	0.4	14.3	85.3	79.4
		4.0	0.00186	0.0538	0.528	0.584	0.3	9.2	90.5	181
		5.5	0.00190	0.0549	0.653	0.709	0.3	7.7	92.0	324
NO_3 (mg/L)										
10	1000	2.5	0.00172	0.1107	0.289	0.402	0.4	27.6	72.0	62.2
5			0.00171	0.0554	0.322	0.379	0.5	14.6	84.9	72.9
2.5			0.00179	0.0277	0.327	0.356	0.5	4.8	91.7	74.6
1			0.00182	0.0111	0.345	0.358	0.5	3.1	96.4	81.3
0			0.00187		0.338	0.340	0.5		99.5	78.8

[a] Calculated by $\frac{J_{Se}}{EW_{SE}} = \frac{\text{Influent flow rate}(Q) \times \text{removed Se(VI)}(\Delta S)}{\text{Total biofilm surface(aV)} \times EW_{Se}}$, where Q is in m³/d, ΔS is in g-Se/m³, aV is in m², EW is in g-Se/e⁻ equivalent for reduction of Se(VI) to Se^0, and J is in g-Se/m²·d. [b] Calculated with $= \frac{J_{SO_4^{2-}}}{S} = \frac{g - SO_4^{2-}}{m^2 \times d} \Big/ \frac{g - SO_4^{2-}}{m^3} = \frac{m}{d}$

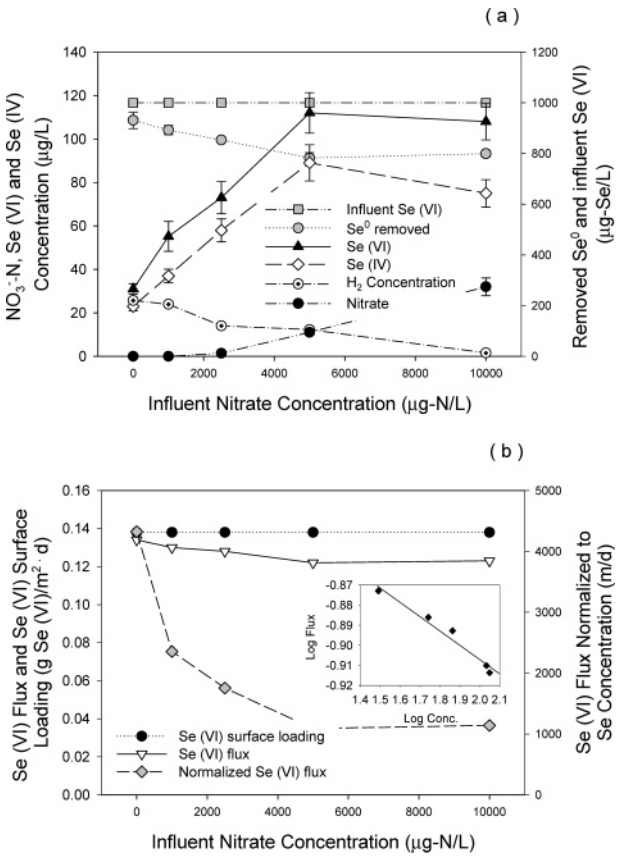

FIGURE 5. Results for the third set of short-term experiments (nitrate load variable): (a) nitrate, selenate, selenite, Se^0 removed, and H_2 concentration in the effluent; (b) Se(VI) flux and Se(VI) flux normalized to effluent Se(VI) concentration; (inset) logarithm of Se(VI) flux versus logarithm of effluent concentration of Se(VI). (Se species concentration in μg Se/L, H_2 concentration in μg/L, Nitrate concentration in μg N/L, Se(VI) flux and Se(VI) surface loading in Se(VI)/m²·d, Se(VI) flux normalized to Se(VI) concentration in m/d).

nitrate concentration increased. With zero nitrate in the influent, the percentage removal of selenate increased to 96%.

When the applied H_2 pressure was fixed (at 2.5 psi in Figure 5), the bulk-liquid H_2 concentration decreased as nitrate loading increased, due to greater demand for H_2. The lowering of H_2 concentration identifies increasing H_2 limitation for all reduction reactions. As nitrate surface loading increased up to 2.0 g NO_3^- N/m²·d (initial nitrate concentration: 10 mg N/L), the Se(VI) fluxes only declined a small amount. However, the Se(VI) flux normalized to Se(VI) effluent concentration sharply decreased as the nitrate surface load increased. Thus, nitrate reduction competed more strongly for H_2, and the H_2 concentration in the bulk liquid declined. The reaction order for Se(VI) reduction was again negative (−0.07) and reinforces the role of H_2 availability.

Biological reduction of oxidized contaminants is usually straightforward when (1) the contaminant is a dissimilatory acceptor, or provides energy for bacterial growth; (2) transfer of electrons from the donor to the acceptor is thermodynamically favorable ($\Delta G^\circ \ll 0$); and (3) the contaminant concentration is high enough to support a steady-state microbial population (*44*). Condition 2 is true for selenate, nitrate, and sulfate, as shown here:

$$SeO_4^{2-} + 3H_2 + 2H^+ \rightarrow Se^0 + 4H_2O \qquad \Delta G^\circ = -71 \text{ KJ/e}^- \quad (1)$$

$$NO_3^- + 2.5H_2 + H^+ \rightarrow 0.5N_2 + 3H_2O \qquad \Delta G^\circ = -112 \text{ KJ/e}^- \quad (2)$$

$$SO_4^{2-} + 4H_2 + 1.5H^+ \rightarrow 0.5H_2S + 0.5HS^- + 4H_2O \qquad \Delta G^\circ = -19 \text{ KJ/e}^- \quad (3)$$

The fact that reductions of each acceptor increased over time supports that conditions 1 and 3 also were true.

In the fourth short-term series, the influent pH values were set to 6.5, 7.1, 7.6, 8.2, or 8.9, with applied H_2 pressure, influent selenate concentration, influent sulfate, and influent nitrate concentration at their steady-state values of 2.5 psi, 1,000 μg Se/L, 78.5 mg/L, and 5 mg N/L, respectively. As shown in Figure 6, nitrate, sulfate, and selenate reductions were relatively insensitive to pH from 7.0 to 9.0, although a maximum selenate removal occurred at pH of around 7.5. Selenate reduction slowed significantly at pH = 6.5. Also, the

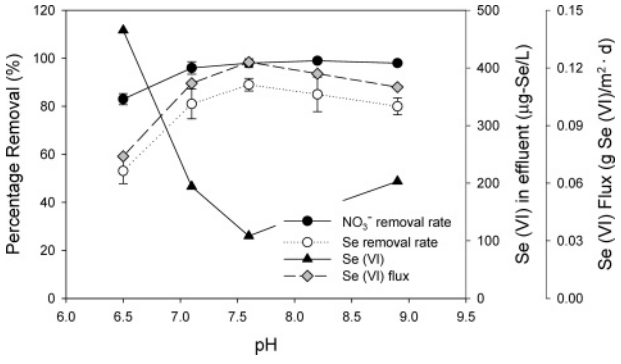

FIGURE 6. Results for the fourth set of short-term experiments (influent pH variable). Percentage removals, effluent concentration of Se(VI), and Se(VI) flux. (left *Y* axis: removal percentage in %, right *Y* axis: Se(VI) concentration in effluent (μg Se/L), and right off axis: Se(VI) flux in g Se(VI)/m²·d).

trend of Se(VI) flux depending on the change of pH was similar as that of Se removal rate, and nitrite was not an intermediate at any pH.

Relationship between Sulfate Reduction and Selenate Reduction. Table 4 shows the electron-equivalent fluxes of electron acceptors—Se(VI), NO_3^-, and SO_4^{2-}—and the percentage distribution of each flux. Sulfate reduction was the biggest consumer of electrons for all short-term experiments. Sulfate reduction was at least 72% of the electron flow, and it averaged 89%. On the other hand, selenate reduction was always a very small percentage, less than 0.5%. This means that the total demand for H_2 was largely controlled by sulfate reduction, not selenate reduction, and this explains why the selenate loading had no effect on H_2 concentration (first short-term experiment). Sulfate reduction was highly sensitive to H_2 pressure based on its normalized fluxes. Also, the sum of the fluxes in electron-equivalents increased gradually with the H_2 pressure, since sulfate reduction dominated the electron-equivalent flux.

Se(VI) seemed to inhibit sulfate reduction, because sulfate fluxes normalized by sulfate concentrations clearly increased when Se(VI) concentration in the effluent decreased. These results imply that Se(VI) interacted with the sulfate reductase, and we note inhibition of sulfate reduction at selenate/sulfate ratios as low as 0.001−.01 g Se(VI)/gSO_4^{2-}. Postgate (*45*) reported partial inhibition of sulfate reduction at selenate/sulfate ratios between 0.02 and 0.1, and Zehr and Oremland (*46*) demonstrated complete inhibition at ratios between 0.02 and 1.0.

Comparison of Fluxes between Nitrate/Perchlorate Reduction and Selenate Reduction. In the previous work of the authors (*38*), nitrate in the reactor slowed perchlorate reduction for a fixed H_2 pressure, which suggested that partial nitrate removal may not be feasible when perchlorate reduction is the treatment goal. For example, when nitrate increased from 0 to 10 mg N/L, perchlorate reduction decreased from 6.5 to 2.9 e^- meq/m²·d. On the other hand, the presence of perchlorate had no impact on denitrification. In the current study, selenate reduction was affected by the nitrate concentration in the reactor. Although the trend was similar to that for perchlorate, the effect was less dramatic in this case: The selenate flux decreased from 1.9 to 1.7 e^- meq/m²·d as the NO_3^- concentration increased from 0 to 10 mg N/L. In this study, H_2 appeared to be the factor controlling the selenate flux, not inhibition from nitrate, as was postulated, but not proven for perchlorate (*38*). In the earlier study (*38*), nitrate and perchlorate removals did not increase for the relatively higher H_2 pressure (5.5 psi), but selenate reduction increased by 13% as the H_2 pressure was increased in this study. Perhaps the high H_2 demand from sulfate reduction, which was not present in the earlier study (*38*), explains the greater sensitivity to H_2 pressure with selenate. Although selenate reduction was relatively insensitive to pH in this study, perchlorate reduction was more pH sensitive (*38*). Thus, the comparison of the results for selenate versus perchlorate shows generally similar trends, but differences in the quantitative details due to having different oxidized contaminants, different experimental conditions (particularly sulfate reduction), or both.

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