

Removal of Selenate in River and Drainage Waters by *Citrobacter braakii* Enhanced with Zero-Valent Iron

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A cost-effective remediation method is needed to remove selenium (Se) from Se-contaminated water. In this study, a selenate [Se(VI)]-reducing bacterium, *Citrobacter braakii*, that is capable of using molasses as a carbon source to reduce Se(VI) from natural river and drainage waters was isolated. During an 8-day experiment, 87–97% of the added Se(VI) in New River water and White River water, California, was reduced to elemental Se [Se(0)] or transformed to organic Se. In highly saline drainage water, removal of Se(VI) by *C. braakii* was limited, with 20% Se(VI) removal in a 7-day experiment. Addition of zero-valent iron (ZVI) into these waters along with *C. braakii* inoculation significantly enhanced the removal of Se(VI) and reduced the formation of organic Se. This study suggests that the combination of a bacterial treatment using inexpensive molasses and ZVI can effectively remove Se from natural river water and agricultural drainage waters.

KEYWORDS: Selenium speciation; selenate reduction; zero-valent iron; molasses; *Citrobacter braakii*; selenium-contaminated water

INTRODUCTION

Removal of selenium (Se) from Se-contaminated agricultural drainage water has been studied for the past 20 years since the discovery of irrigation-induced Se poisoning of waterfowl (1). These studies mainly include physical–chemical removal of Se by zero-valent iron filings, ferrous hydroxides, ion exchange, reverse osmosis (1), and biological removal of Se by volatilization to the atmosphere (2–6) and by microbial reduction of soluble selenate [Se(VI)] to insoluble elemental Se [Se(0)] (7–11). Although some of these technologies have been tested in the field, the goal of finding a practical, cost-effective technology for treating Se-contaminated water has not yet been accomplished.

Many bacteria are capable of reducing soluble Se(VI) to insoluble Se(0), including *Sulfurospirillum barnesii*, *Enterobacter cloacae*, *Thauera selenatis*, *Enterobacter taylorae*, and *Citrobacter freundii* (7–11). For efficient reduction of Se(VI) to Se(0), Se(VI)-reducing bacteria use electron donors such as acetate, lactate, and glucose as sources of carbon/energy/electrons (7–11). The high cost of these chemicals makes them less practical for remediating Se-contaminated water under field conditions (7). There is a need to search for an effective and inexpensive organic carbon source that bacteria can use to reduce soluble Se(VI) to insoluble Se(0).

Molasses has been used as a cost-effective organic carbon source to reduce Cr(VI) to Cr(III) and NO_3^- to NH_4^+ (12, 13). Molasses can be obtained in the San Joaquin Valley, California,

at a wholesale price of \$60–90/ton (1), which is much cheaper than the chemicals mentioned above. Therefore, molasses could be a cost-effective organic carbon source that can be used by Se(VI)-reducing bacteria to reduce Se(VI) to Se(0).

Zero-valent iron (ZVI) is an inexpensive and moderately strong reducing agent. It has been used to remove many common environmental contaminants, such as As, Cr(VI), U(VI), NO_3^- , and Se(VI) (14–18). ZVI has also been used in bacteria treatment systems to enhance the degradation of Cr(VI), NO_3^- , TCE, PCE, and RDX (19–23). The addition of ZVI to a biotreatment system in the removal of Se from Se-contaminated water has not yet been tested.

In this study, we have isolated a Se(VI)-reducing bacterium from a field rice straw treatment system (communications with Carla Scheidlinger, Broadview Water District, California) used to remove Se from agricultural drainage water. This bacterium utilizes molasses while reducing Se(VI) in natural river water and drainage water in a series of the batch experiments. The enhancement of the removal of Se(VI) by the addition of ZVI has also been studied.

MATERIALS AND METHODS

Materials. Natural water used in this study included both river water and agricultural drainage water collected from the New River, White River, and the western San Joaquin Valley, California. The concentrations of total Se, NO_3^- -N, and PO_4^{3-} -P, pH, Eh, and salinity (electrical conductivity) in these waters are presented in **Table 1**. All of the waters were passed through a 5 μm filter (VWR, Bristol, CT) to remove detritus prior to use. Na_2SeO_4 (Sigma, St. Louis, MO) standard solution [Se(VI), 10000 mg/L] was passed through a sterile 0.2 μm membrane filter prior to its addition to river water. ZVI (40–60 mesh) was obtained

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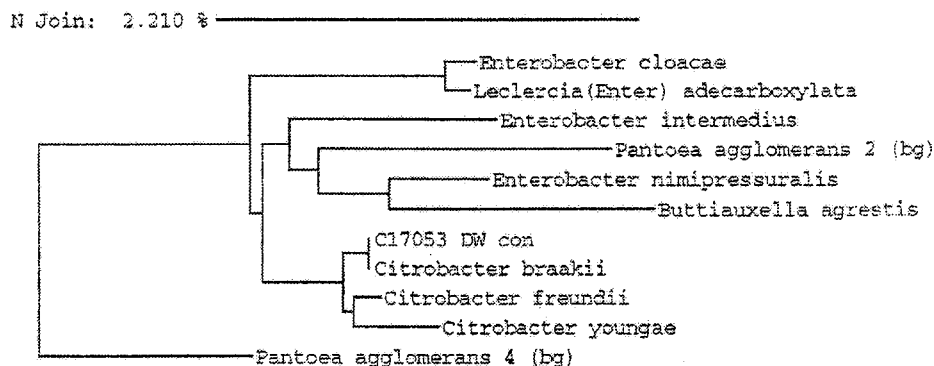


Figure 1. Neighbor-joining phylogenetic tree of *C. braakii* identified by 16S rDNA sequence analysis.

Table 1. Redox Potential (Eh), pH, Electrical Conductivity (EC), and Concentrations of Se, NO_3^- -N, and PO_4^{3-} -P in River and Agricultural Drainage Waters

water	Se ($\mu\text{g/L}$)	NO_3^- -N (mg/L)	PO_4^{3-} -P (mg/L)	pH	EC (dS/m)	Eh (V)
New River	3.2	7.4	0.43	7.88	2.28	0.438
White River	2.3	23.36	0.55	8.24	1.93	0.395
drainage	338 ^a	34.07	0.07	7.37	17.26	0.412

^a Only Se(VI) in drainage water.

from Peerless (Peerless Metal Powers and Abrasive, Detroit, MI) and was used as received. The surface area of the ZVI particles was 1.63 m^2/g , which was determined by a BET surface area analyzer (Micromeritics ASAP 2010).

Isolation of the Se(VI)-Reducing Bacterium. The water sample used to isolate the Se(VI)-reducing bacterium was collected from a field rice straw treatment system used to remove Se from agricultural drainage water in the San Joaquin Valley, California. An aliquot of the treated water was spread onto tryptic soy agar (TSA; Difco, Detroit, MI) plates containing 50 mg/L of Se(VI). Plates were incubated at 30 $^\circ\text{C}$ for 48 h when some colonies with red Se(0) precipitates were observed on the TSA plates. The red colonies were restreaked on TSA plates with and without Se(VI) to ensure that the red color of the colonies was not due to a bacterial pigment. One pure bacterial isolate was identified as *Citrobacter braakii* by analysis of the 16S rRNA gene sequence (MIDI Labs, Newark, DE) (Figure 1).

Removal of Se(VI) in Natural River Water. To examine whether the Se(VI)-reducing bacterium *C. braakii* could reduce Se(VI) in different natural waters supplemented with molasses and whether the addition of ZVI into these waters would enhance the removal of Se(VI) by *C. braakii*, a series of batch experiments were conducted. *C. braakii* was pregrown in a 1% tryptic soy broth (TSB; Difco) solution and incubated (30 $^\circ\text{C}$) overnight. The solution was then centrifuged at 5000 rpm for 20 min. To remove the TSB residues, *C. braakii* cells were washed three times with 30 mL of sterile natural water by centrifugation. Washed cells were resuspended in the same solution to give an OD_{600} range of 0.59–0.62 for the experiments described below.

In the experiment, 200 mL of nonsterile river water containing 0.1% of molasses was added to each 250 mL Erlenmeyer flask. The flasks were spiked with Se(VI) to provide a final concentration of 1000 $\mu\text{g/L}$. Four different variables were tested in the experiment including (i) a control without the addition of washed *C. braakii* cell suspension, (ii) the addition of 1 mL of washed *C. braakii* cell suspension, (iii) the addition of 1 g of 40–60 mesh ZVI, and (iv) the addition of 1 mL of washed *C. braakii* cell suspension and 1 g of 40–60 mesh ZVI. The flasks were capped with sterile stoppers and incubated under a static condition at room temperature (21 $^\circ\text{C}$). The experiment was run in triplicate for 8 days. The river water samples were collected at days 0, 1, 2, 3, 4, 6, and 8 for analysis of Se species. To examine whether elevated SO_4^{2-} and Cl^- , dominant anions in agricultural drainage water (24), affected the removal of Se(VI), a batch experiment was conducted with White River water fortified with 30 mM concentrations of SO_4^{2-} and Cl^- . The EC value of the White River water with the addition of

SO_4^{2-} and Cl^- was 8.33 dS/m. Other experimental conditions were the same as in the experiments using river water described above.

Removal of Se(VI) in Agricultural Drainage Water. In this experiment, nonsterile natural agricultural drainage water was treated with molasses to provide a final concentration of 0.1%. The Se concentration (338 $\mu\text{g/L}$ of Se) in the drainage water was determined before the experiment. Molasses-fortified drainage water (200 mL) without the addition of Se(VI) was added to each 250 mL Erlenmeyer flask. The four different variables used in this experiment were the same as in the experiments using river water described above. The flasks were capped with sterile stoppers and incubated under a static condition at room temperature (21 $^\circ\text{C}$). The experiment was run in triplicate for 7 days. The drainage water samples were collected at days 0, 1, 2, 3, 5, and 7 for analysis of Se species.

Analysis. Selenium species in the water samples were determined using a method developed by Zhang and Frankenberger (25). Directly measured Se species included total soluble Se, Se(IV), and Se(IV) plus organic Se [organic Se(-II) plus organically bound Se]. Se(VI), removed Se [Se(0) and/or Se adsorbed to ZVI and iron oxyhydroxides], and organic Se were determined by the difference method. Se concentrations in all of the prepared solutions were analyzed by hydride generation atomic absorption spectrometry (HGAAS) (26).

The rate constant for the removal of Se(VI) by *C. braakii* in the waters was calculated using a simple first-order kinetics equation: $d[\text{Se(VI)}]/dt = -k[\text{Se(VI)}]$, where k is the rate constant of Se(VI) removal. Se(VI) data that were close to zero were not used because these data were not in a linear range in the rate constant calculation.

RESULTS

Removal of Se(VI) from River Water. The removal of Se(VI) in river water during an 8 day experiment is illustrated in Figures 2 and 3. Se(VI) showed little change in the water without the addition of *C. braakii* and decreased rapidly from 1000 to 13.8 and 103 $\mu\text{g/L}$ in New River water and White River water inoculated with *C. braakii*, respectively. On the final day of the experiment, Se(IV), Se(0), and organic Se concentrations were 12.3, 874, and 99.8 $\mu\text{g/L}$ in New River water and 25.6, 700, and 171 $\mu\text{g/L}$ in White River water, respectively. Addition of ZVI into the water inoculated with *C. braakii* significantly increased the removal of Se(VI) from the water. Se(VI) dropped rapidly from 1000 to 6.1–8.4 $\mu\text{g/L}$ in both river waters during the first 3 days of the experiment. On the final day of the experiment, only organic Se existed in the water at a level of 21.2–23.8 $\mu\text{g/L}$. In contrast, the removal of Se(VI) slowed in the water supplemented with only ZVI. About 420 $\mu\text{g/L}$ of the added Se(VI) was removed in 8 days, with no soluble Se(IV) or organic Se in either river water.

The removal of Se(VI) in White River water to which 30 mM concentrations of SO_4^{2-} and Cl^- had been added is presented in Figure 4. During an 8 day experiment, Se(VI) showed little change in this water without the addition of *C. braakii*. In comparison to the same river water without the

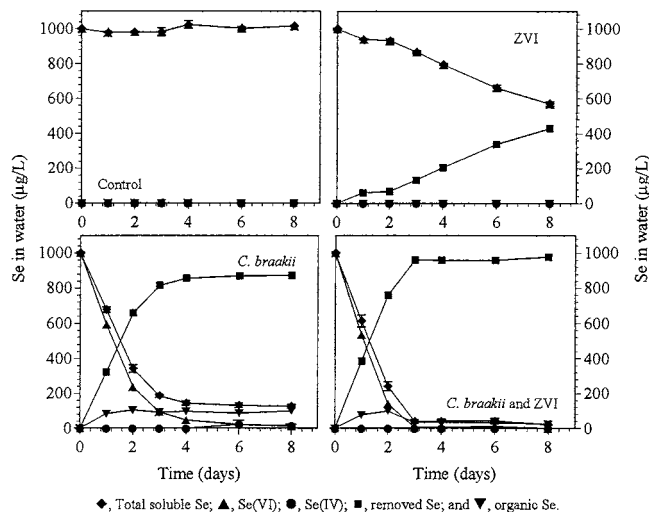


Figure 2. Removal of Se from New River water containing 0.1% of molasses under four conditions: (i) control (without the addition of washed *C. braakii* cell suspension); (ii) addition of 1 mL of washed *C. braakii* cell suspension; (iii) addition of 1 g of 40–60 mesh ZVI; (iv) addition of 1 mL of washed *C. braakii* cell suspension and 1 g of 40–60 mesh ZVI. Error bars indicate one standard deviation ($n = 3$).

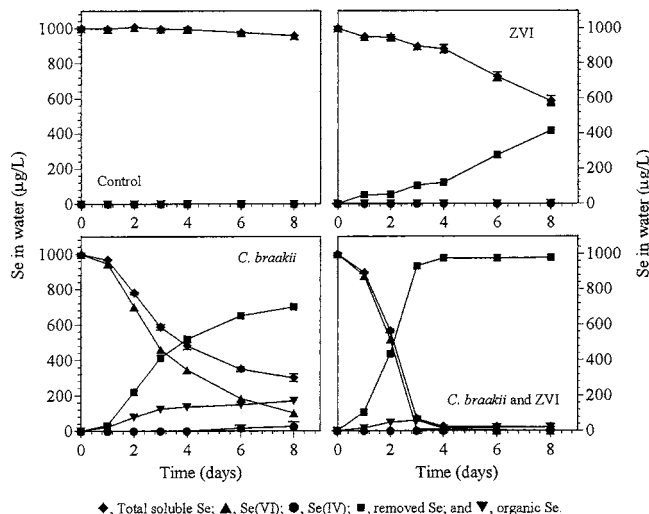


Figure 3. Removal of Se from White River water containing 0.1% of molasses under four conditions: (i) control (without the addition of washed *C. braakii* cell suspension); (ii) addition of 1 mL of washed *C. braakii* cell suspension; (iii) addition of 1 g of 40–60 mesh ZVI; (iv) addition of 1 mL of washed *C. braakii* cell suspension and 1 g of 40–60 mesh ZVI. Error bars indicate one standard deviation ($n = 3$).

addition of 30 mM SO_4^{2-} and Cl^- described above, removal of Se(VI) was slowed in this water inoculated with *C. braakii*, with a decrease from 1000 to 317 $\mu\text{g/L}$. On the final day of the experiment, Se(IV), Se(0), and organic Se concentrations were 25.6, 492, and 165 $\mu\text{g/L}$ in this river water. Addition of ZVI into the water inoculated with *C. braakii* significantly increased the removal of Se(VI) from the water. Se(VI) decreased rapidly from 1000 to 36.4 $\mu\text{g/L}$ in the water during the first 3 days of the experiment. On the final day of the experiment, only organic Se existed in the water, at a low level of 22.3 $\mu\text{g/L}$. In contrast, Se(VI) decreased from 1000 $\mu\text{g/L}$ to 706 $\mu\text{g/L}$ in the water amended with only ZVI during the 8 day experiment, with no soluble Se(IV) and organic Se in the water.

Removal of Se(VI) from Highly Saline Drainage Water. Removal of Se(VI) from a highly saline drainage water (EC,

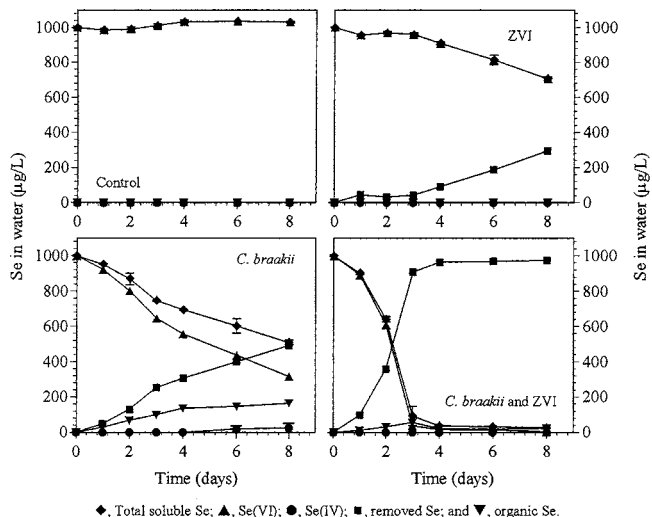


Figure 4. Removal of Se from White River water with the addition of 30 mM each SO_4^{2-} and Cl^- and 0.1% of molasses under four conditions: (i) control (without the addition of washed *C. braakii* cell suspension); (ii) addition of 1 mL of washed *C. braakii* cell suspension; (iii) addition of 1 g of 40–60 mesh ZVI; (iv) addition of 1 mL of washed *C. braakii* cell suspension and 1 g of 40–60 mesh ZVI. Error bars indicate one standard deviation ($n = 3$).

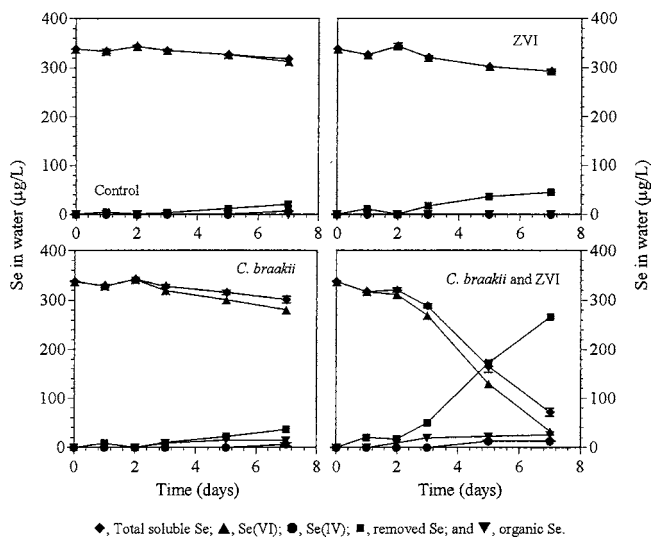


Figure 5. Removal of Se from highly saline agricultural drainage water containing 0.1% of molasses under four conditions: (i) control (without the addition of washed *C. braakii* cell suspension); (ii) addition of 1 mL of washed *C. braakii* cell suspension; (iii) addition of 1 g of 40–60 mesh ZVI; (iv) addition of 1 mL of washed *C. braakii* cell suspension and 1 g of 40–60 mesh ZVI. Error bars indicate one standard deviation ($n = 3$).

17.3 dS/m) containing 0.1% molasses is presented in **Figure 5**. During a 7 day experiment, Se(VI) changed little in the drainage water without addition of *C. braakii* and decreased slightly from 338 to 281 $\mu\text{g/L}$ in the drainage water to which *C. braakii* had been added, with a low level of Se(IV) (6.3 $\mu\text{g/L}$) and organic Se (14.3 $\mu\text{g/L}$) at the final day of the experiment. Addition of ZVI into the drainage water inoculated with *C. braakii* significantly enhanced the removal of Se(VI). Se(VI) dropped rapidly from 338 to 32.9 $\mu\text{g/L}$ in 7 days. In contrast, Se(VI) decreased from 338 $\mu\text{g/L}$ to 293 $\mu\text{g/L}$ in the drainage water to which only ZVI had been added, with no soluble Se(IV) and organic Se in the water.

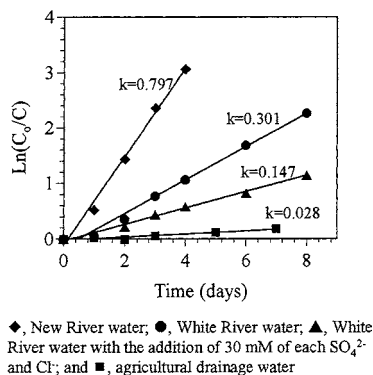


Figure 6. First-order kinetics of Se(VI) removal from river and drainage waters by *C. braakii*.

DISCUSSION

Bacterial reduction of Se(VI) to Se(0) is a useful remediation technique for removing Se from Se-contaminated water. In aquatic systems, bacteria often need specific organic materials as a carbon, energy, and electron source (7–11) to reduce Se(VI). In this study, *C. braakii* was capable of using inexpensive molasses to reduce Se(VI) to Se(0), with a rate constant of Se(VI) removal at 0.797 h^{-1} in New River water and 0.301 h^{-1} in White River water (Figure 6). At the end of the experiment, 98.6 and 89.7% of the added Se(VI) was removed from New River water and White River water, respectively. In contrast, Se(VI) was not removed in the river water without inoculation of the Se(VI)-reducing bacterium, *C. braakii*.

The relatively lower removal rate of Se(VI) in the White River water compared to that in the New River water might be attributed to the relatively greater NO_3^- content in White River water (Table 1). NO_3^- has been reported as a competitive electron acceptor during Se(VI) reduction to Se(0) in aquatic systems (27, 28). In a study on the bacterial reduction of NO_3^- and Se(VI), Steinberg et al. (27) reported that NO_3^- reduction by an anaerobic, freshwater enrichment preceded Se(VI) reduction in an anaerobic medium with equal amounts of Se(VI) and NO_3^- of 20 mM. Fujita et al. (28) reported that Se(VI) reduction by *Bacillus* sp. SF-1 in a basal medium with 1 mM Se(VI) was completely inhibited when 20 mM NO_3^- was added to the medium.

The increased salt contents in the White River water affected Se(VI) removal by *C. braakii*. The rate constant of Se(VI) removal decreased to 0.147 h^{-1} (Figure 6) in the White River water to which 30 mM concentrations of SO_4^{2-} and Cl^- (8.33 dS/m) had been added. In the highly saline drainage water (17.3 dS/m) collected from the western San Joaquin Valley, California, removal of Se(VI) by *C. braakii* was limited, with 17% Se(VI) removal in a 7 day experiment. The salt effect on Se(VI) reduction was also observed by Zehr and Oremland (29), who reported that the rate of Se(VI) reduction with washed cell suspension of *Desulfovibrio desulfuricans* was reduced with an increased SO_4^{2-} .

ZVI as an inexpensive and moderately strong reducing agent has been used to remove Se(VI) from water through reductive and adsorptive processes during its corrosion to iron oxyhydroxides (18, 30, 31). For example, green rust, one of the iron oxyhydroxides, can serve as a reducing agent to abiotically reduce Se(VI) to Se(IV) and Se(0) (32, 33). Ferrihydrite and goethite are strong adsorbents that can be used to effectively remove Se(IV) from water (34, 35). In the present study, addition of a small amount of ZVI removed Se from the river and drainage waters without the inoculation of *C. braakii*, with

Se removal percentages of 42.9, 41.6, 29.4, and 13.3 in the New River, White River, White River with the addition of 30 mM concentrations of SO_4^{2-} and Cl^- , and the saline drainage waters, respectively. Removal of Se(VI) appeared to be negatively related to the salt content in the water. In our previous study on the removal of Se(VI) in the presence of various concentrations of Cl^- , SO_4^{2-} , NO_3^- , HCO_3^- , and PO_4^{3-} , we have reported that the removal rate of Se(VI) declined with an increase of each anion concentration in solution (18).

Addition of ZVI into the river and drainage waters inoculated with *C. braakii* enhanced the removal of Se(VI). Combination of bacterial Se(VI) reduction with Se removal by ZVI almost completely removed all of Se(VI) in these waters during a period of 7–8 days. The benefits of adding ZVI into a biotreatment system might be that ZVI can rapidly remove dissolved O_2 ($2\text{Fe}^0 + 2\text{H}_2\text{O} + \text{O}_2 = 2\text{Fe}^{2+} + 4\text{OH}^-$). Rapid removal of dissolved O_2 supports an anaerobic environment, thus enhancing rapid reduction of Se(VI) to Se(IV) and then to Se(0) by *C. braakii*. Adsorption of Se(IV) to iron oxyhydroxides formed from ZVI corrosion also enhances the removal of Se. Some researchers have reported that H_2 released from ZVI corrosion ($\text{Fe}^0 + 2\text{H}_2\text{O} = \text{Fe}^{2+} + 2\text{OH}^- + \text{H}_2$) stimulates anaerobic bioremediation by serving as electron donor (19–23). In this study, it is not known whether *C. braakii* can use H_2 as electron donor to reduce Se(VI).

Formation of organic Se is a concern during the removal of Se(VI) by Se(VI)-reducing bacteria due to the much higher bioavailability of organic Se compared with inorganic Se(VI) and Se(IV) (36). In this study, 10–17.1% of the added Se(VI) in river waters and 4% in drainage water were transformed to organic Se by *C. braakii* during a period of 7–8 days. Addition of ZVI to the river waters inoculated with *C. braakii* not only enhanced Se(VI) removal but also significantly reduced the formation of organic Se to a low level of 2% of the added Se. However, organic Se was slightly high in drainage water augmented with both ZVI and *C. braakii* during a 7 day experiment, with 85% removal of the total Se.

Agriculture productivity in the San Joaquin Valley, California, generates high-Se drainage water, which has a concentration range of 140–1400 $\mu\text{g/L}$ in many areas of the valley (37). In the Salton Sea region, elevated Se is in the range of 3–300 $\mu\text{g/L}$ in the subsurface drainwater (38). This study suggests that a bacterial treatment system using both inexpensive molasses and ZVI can effectively remove Se from natural river water and agricultural drainage water. One major concern in the use of *C. braakii* for the removal of Se from Se-contaminated water is that *C. braakii* can transform inorganic Se to organic Se when molasses is added to water. A further study to screen Se(VI)-reducing bacteria that can use molasses as a carbon source to effectively reduce Se(VI) to Se(0), with little production of organic Se, is ongoing.

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