Factors Affecting Reduction of Selenate to Elemental Selenium in Agricultural Drainage Water by *Enterobacter taylorae*

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Microbial reduction of selenate [Se(VI)] to elemental selenium [Se(0)] is a useful technique for removing Se from agricultural drainage water. A series of batch experiments were conducted in the laboratory to determine the effects of yeast extract (50−1000 mg/L), salinity (EC, 5−75 dS/m), and NO_3^- (5−100 mg/L) on the removal of Se(VI) (2000 μg/L) from drainage water by *Enterobacter taylorae*. Results showed that relatively high amounts of yeast extract (500 mg/L) were needed for *E. taylorae* to effectively reduce Se(VI) to Se(0). During a 7-day experiment, ∼95% of added Se(VI) was reduced to Se(0) in the low-salinity drainage water (5 dS/m) with NO_3^- values of 5−50 mg/L. In the high-salinity drainage water (50−75 dS/m), reduction of Se(VI) to Se(0) was limited. *E. taylorae* was also capable of reducing Se(VI) to Se(0) in the San Joaquin Valley drainage water, with a reduction of the added Se(VI) to Se(0) (73.8%) and Se(-II) (20%). This study suggests that *E. taylorae* may be used to treat Se(VI)-contaminated drainage water in the field.

KEYWORDS: Selenium speciation; selenate reduction; nitrate; salinity; yeast extract; *Enterobacter taylorae*

INTRODUCTION

Selenium (Se) contamination in the San Joaquin Valley wetlands of California is mainly associated with agricultural drainage water containing Se [mostly as selenate [Se(VI)]] (*1−3*). Bioaccumulation of Se in the Kesterson Reservoir in California has created a hazard to the waterfowl (*1, 2*). To protect wetland wildlife, Se in agricultural drainage water needs to be removed before its disposal to wetlands.

Bacterial reduction of Se(VI) to elemental Se [Se(0)] is a feasible technology in the removal of Se from agricultural drainage waters because of the insolubility of Se(0) (*3−8*). Many selenate-reducing bacteria isolated from different aquatic environments are capable of reducing Se(VI) to Se(0) (*3−9*), such as strain SES-3 isolated from an estuarine sediment (*10*), *Thauera selenatis* from Se(VI)-contaminated wastewater (*11*), *γ*-proteobacteria from solar evaporation pond salt (*12*), and *Enterobacter taylorae* from rice straw (*13*). In this study, we used *E. taylorae* to reduce Se(VI) to Se(0) in synthetic and natural drainage water and to determine the effects of yeast extract, salinity, and nitrate (NO_3^-) on the reduction of Se(VI) to Se(0) in a series of batch experiments.

MATERIALS AND METHODS

Materials. Natural drainage water was collected from the Westlands Water District, San Joaquin Valley, California. The water, with a pH of 7.9 and a salinity (electrical conductivity [EC]) of 7.4 dS/m contained 38.6 μg/L Se(VI), 4.29 μg/L selenite [Se(IV)], 0.567 μg/L organic Se, 3725 mg/L SO_4^{2-}, 509 mg/L Cl^-, 51.1 mg/L NO_3^-, 0.31 mg/L NH_4^+, and 0.125 mg/L PO_4^{3-}. The natural drainage water was passed through a 5 μm filter to remove detritus prior to use. Synthetic agricultural drainage water with an EC of 5.1 dS/m and a pH of 8 was prepared with the following constituents (in g/L): Na_2SO_4, 1.48; NaCl, 0.659; $NaHCO_3$, 0.275; $CaCl_2 \cdot 2H_2O$, 0.733; $MgSO_4$, 0.745; $(NH_4)_2SO_4$, 0.073; $Na_2B_4O_7 \cdot 4H_2O$, 0.176; KCl, 0.019; NaH_2PO_4, 0.044; $FeCl_2$, 0.0002; yeast extract, 0.5 (from 0.05 to 1 in the yeast extract effect experiment described below); glucose 0.5; and a trace element solution (*14*), 1 mL/L. $CaCl_2 \cdot 2H_2O$, $MgSO_4$, and other chemicals were separately dissolved in deionized water and autoclaved (18 psi at 121 °C) for 20 min before mixing on cooling. After mixing, a Se(VI) standard solution was added to the drainage water to a Se(VI) concentration of 2000 μg/L. In the salinity effect experiment described below, synthetic drainage water was autoclaved after salts (Na_2SO_4 and NaCl) were added to the drainage water. The Se(VI) and NO_3^- standard stock solution (10000 mg/L) was passed through a sterile 0.2 μm membrane filter prior to its addition to the drainage water.

E. taylorae was pregrown in a 1% tryptic soy broth (TSB) solution and incubated (30 °C) for 1 day. The solution was then centrifuged at 5000 rpm for 20 min. To remove the TSB residues, cells were washed three times by centrifugation (5000 rpm, 20 min) with 35 mL of the sterile synthetic drainage water described above. Washed cells were resuspended in the same solution to give an OD_{600} of 1.3.

Reduction of Se(VI) in Synthetic Drainage Water. A series of batch experiments were conducted in the laboratory to determine the effects of yeast extract, salinity, and NO_3^- on Se(VI) reduction to Se(0) in synthetic drainage water. The terms "yeast extract effect", "salinity effect", and "NO_3^- effect" represent three batches of experiments. In the yeast extract effect experiments, 200-mL portions of

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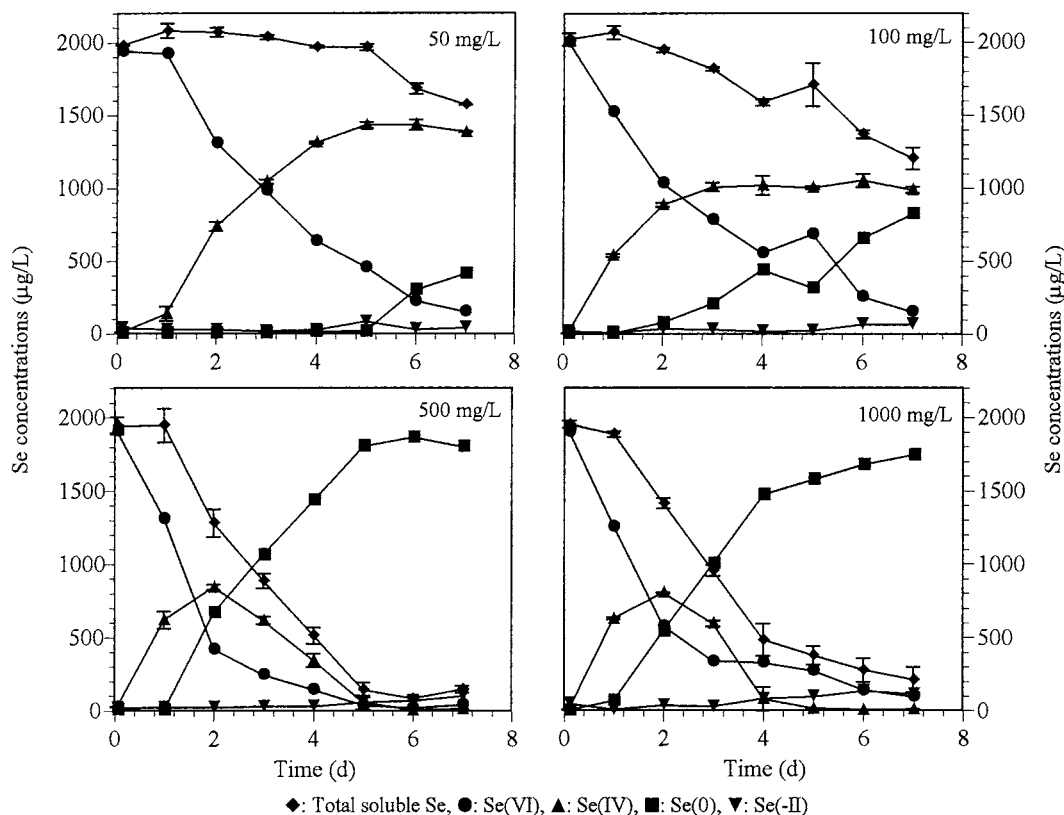† University of California.
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◆: Total soluble Se, ●: Se(VI), ▲: Se(IV), ■: Se(0), ▼: Se(-II)

Figure 1. Effect of yeast extract (50–1000 mg/L) on the changes of Se species in a synthetic drainage water upon inoculation with *E. taylorae*.

synthetic drainage water with yeast extract levels of 50, 100, 500, and 1000 mg/L were placed in each 250-mL flask, followed by 0.5 mL of washed cell suspension. In the salinity effect experiments, 200-mL portions of synthetic drainage water prior to autoclaving were placed in each 250-mL flask, followed by the addition of Na_2SO_4 and NaCl with a weight ratio of 3:1 to EC levels of 5, 25, 50, and 75 dS/m. The drainage water was autoclaved (18 psi at 121 °C) and cooled to room temperature, followed by the addition of 2000 μg/L Se(VI) and 0.5 mL of washed cell suspension. In the NO_3^- effect experiments, 200-mL portions of sterile synthetic drainage water were placed in each 250-mL flask, followed by spiking of NO_3^- to concentration levels of 5, 25, 50, and 100 mg/L and 0.5 mL of the washed cell suspension. All of the flasks were capped with sterile stoppers and incubated under a static condition at room temperature (21 °C). All three batches of the experiments were run in triplicate for 7 days. The samples were collected daily for analysis of Se species.

Reduction of Se(VI) in Natural Agricultural Drainage Water. To examine whether *E. taylorae* can survive in natural drainage water and reduce Se(VI) to Se(0), 200 mL of nonsterile natural drainage water spiked with a Se(VI) concentration of 2000 μg/L and 0.05% of yeast extract was added to each 250-mL flask, followed by the addition of 0.5 mL of the washed cell suspension. The drainage water without added washed cell suspension served as a control. Glucose was not added in this experiment because we found in a previous test that the addition of yeast extract alone to drainage water could also reduce Se(VI). All flasks were capped with sterile stoppers and incubated at room temperature (21 °C). The experiment was run in triplicate for 7 days. The water samples were collected daily for analysis of Se species.

Analysis. Selenium species in the drainage water were determined using a method developed by Zhang and Frankenberger (*15, 16*) after Se(0) was removed from solution by centrifugation at 12000 for 10 min. In brief, Se speciation was carried out as follows: Se(IV) in the water samples was determined in a pH 7 buffer solution. The sum of Se(IV) and selenide [Se(−II)] [organic Se and inorganic Se(-II)] was determined when the Se(-II) in the water samples was oxidized to Se(IV) by $Na_2S_2O_8$, which was indicated by precipitation of manganese oxides formed from the oxidation of added Mn^{2+}. The Se(-II) concentration was calculated as the difference between Se in this water

sample and the Se(IV) concentration determined in another subsample. Total soluble Se in the water samples was determined by oxidizing all Se to Se(VI) by $Na_2S_2O_8$, followed by reduction to Se(IV) in 6 N HCl. Se(VI) concentration was calculated as the difference between total soluble Se concentration and the sum of Se(IV) and Se(-II) concentration determined in another subsample. Se(0) was determined as the difference between added Se(VI) and total soluble Se. Se concentrations in all prepared solutions were analyzed by hydride generation atomic absorption spectrometry (HGAAS) (*15, 17*). The detection limit in the prepared solution was 0.5 μg/L.

RESULTS

Effect of Yeast Extract on the Se(VI) Reduction. The efficiency of the Se(VI) reduction is related to the amount of yeast extract added to the drainage water (**Figure 1**). In the high-yeast extract (500−1000 mg/L) drainage water, Se(VI) dropped rapidly during a 7-day experiment, from 1950 to 38.6− 93.2 μg/L. Se(IV) formed from Se(VI) reduction increased to 802−841 μg/L on day 2. Then, Se(IV) decreased with time to ∼5 μg/L. Se(0) and Se(-II) also increased with time. On the final day of the experiment, Se(0) was 1742−1800 μg/L and Se(−II) was 97.2−110 μg/L in the high-yeast extract drainage water.

In the low-yeast extract (50−100 mg/L) drainage water, Se(VI) also dropped rapidly during the 7-day experiment, from 2000 to 149−152 μg/L. Se(IV) formed from Se(VI) reduction increased to 1006 μg/L at day 3 in the 100 mg/L yeast extract drainage water and to 1310 μg/L at day 4 in the 50 mg/L yeast extract drainage water. Se(IV) was maintained at these levels during the rest of the experiment. Se(0) formed from Se(IV) reduction increased slowly with time. On the final day of the experiment, Se(0) and Se(-II) concentrations were 408 and 37.5 μg/L, respectively, in the 50 mg/L yeast extract drainage water. In the 100 mg/L yeast extract drainage water, Se(0) and Se(-II) concentrations were 820 and 64.4 μg/L, respectively.

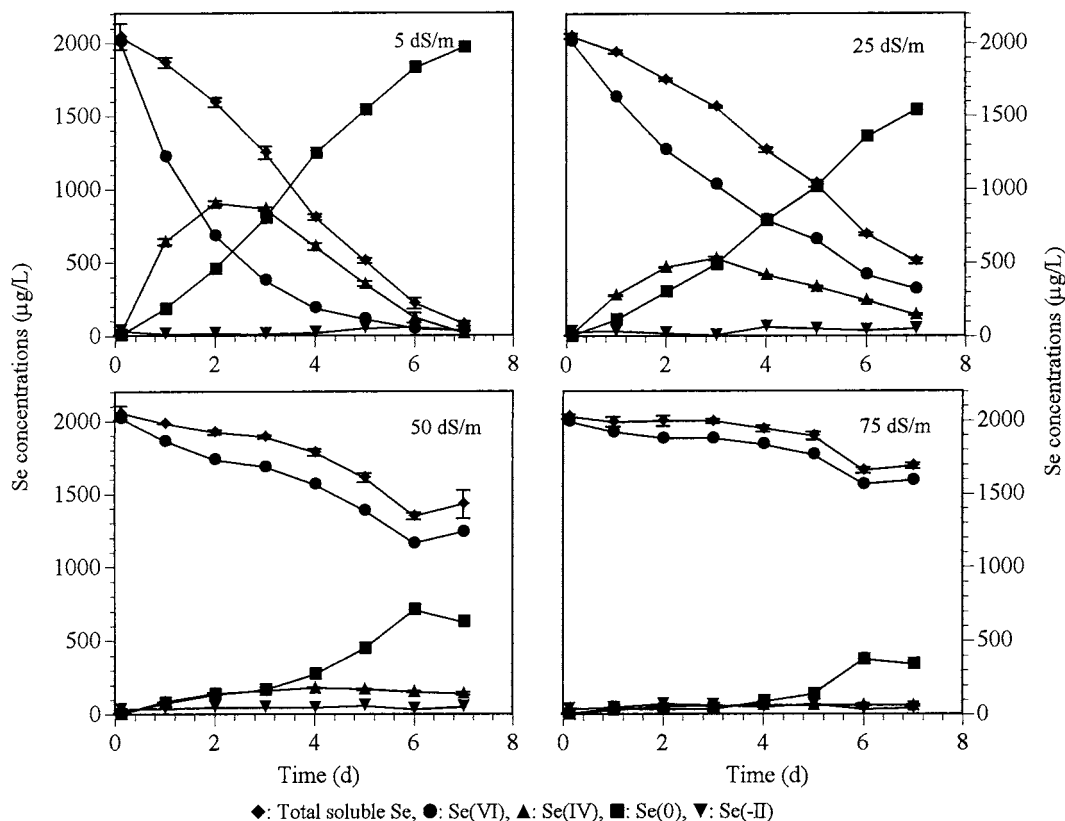**Figure 2.** Effect of salinity (5–75 dS/m) on the changes of Se species in a synthetic drainage water upon inoculation with *E. taylorae.*

◆: Total soluble Se, ●: Se(VI), ▲: Se(IV), ■: Se(0), ▼: Se(-II)

Effect of Salinity on Se(VI) Reduction. The relationship between the Se(VI) reduction and salinity (EC = 5, 25, 50, and 75 dS/m) in the synthetic drainage water is shown in **Figure 2**. In the high-salinity (EC = 50 and 75 dS/m) drainage water, Se(VI) decreased slowly during the 7-day experiment, from the 2039 to 1237−1588 μg/L. With the decrease of Se(VI) in the drainage water, Se(IV) formed from Se(VI) reduction increased slowly to a range of 60−61.6 μg/L in the 75 dS/m drainage water and to 170−178 μg/L in the 50 dS/m drainage water at days 4−5 and then slightly decreased with time. During the experiment, Se(0) increased to 339 μg/L in the 75 dS/m drainage water and to 624 μg/L in the 50 dS/m drainage water. Se(-II) was relatively stable in the drainage water, with a concentration range of 31−67.6 μg/L.

In the relatively low-salinity (EC = 5−25 dS/m) drainage water, Se(VI) dropped rapidly during the 7-day experiment, from 2043 to 31.5−318 μg/L. Se(IV) formed from Se(VI) reduction increased to 900 μg/L at day 2 in the 5 dS/m drainage water and to 528 μg/L at day 3 in the 25 dS/m drainage water. Then, Se(IV) decreased to 14.1 and 145 μg/L on the final day of the experiment in the 5 and 25 dS/m drainage water, respectively. Se(0) formed from Se(IV) reduction increased rapidly to 1964 and 1536 μg/L in the 5 and 25 dS/m drainage water, respectively. Se(-II) was low during the experiment, with a concentration range of 7.82−60.6 μg/L.

Effect of Nitrate on Se(VI) Reduction. The effect of NO_3^- on Se(VI) reduction in synthetic drainage water with NO_3^- levels of 5, 25, 50, and 100 mg/L is presented in **Figure 3**. In the relatively low NO_3^- (5−50 mg/L) drainage water, Se(VI) dropped rapidly from 1960 to 26.8−58.6 μg/L during the 7-day experiment. With the decrease of Se(VI) in the drainage water, Se(IV) formed from Se(VI) reduction increased with the highest detection on day 2 (540−703 μg/L). Then, Se(IV) decreased with time to ∼5 μg/L. Se(0) formed from Se(IV) reduction increased rapidly to 1839−1878 μg/L on the last day of the

experiment. Se(-II) was relatively stable during the experiment, with a concentration range of 9.11−68.1 μg/L.

In the relatively high NO_3^- (100 mg/L) drainage water, Se(VI) decreased slowly during the 7-day experiment, from 1979 to 506 μg/L. However, Se(IV) formed from Se(VI) reduction was maintained at a very low level of 10−20 μg/L during this experiment, with increases of Se(0) and Se(-II). On the final day of the experiment, Se(0) and Se(-II) concentrations were 1352 and 111 μg/L, respectively.

Se(VI) Reduction in Natural Drainage Water. Reduction of Se(VI) in natural drainage water with and without inoculation of *E. taylorae* is presented in **Figure 4**. In the drainage water with inoculation of *E. taylorae*, Se(VI) decreased rapidly during the 7-day experiment, from 2030 μg/L at the beginning of the experiment to 95.3 μg/L at day 7. Se(IV) formed from Se(VI) reduction increased to 469 μg/L on day 2. On the final day of the experiment, Se(IV) was 32.7 μg/L. Se(0) and Se(-II) increased with time to 1490 and 402 μg/L, respectively. In contrast, Se(VI) decreased slowly with time in the control to 1187 μg/L, with a low Se(IV) range of 0−32.5 μg/L during the 7-day experiment in the noninoculated drainage water. With the decrease of Se(VI) in the natural drainage water, Se(0) and Se(-II) increased to 500 and 316 μg/L, respectively.

DISCUSSION

Bacterial reduction of soluble Se(VI) to insoluble Se(0) has been proposed as a remedial technology in treating Se-contaminated drainage water in San Joaquin Valley, California (*3, 18*). Recently, Zahir et al. (*13*) isolated a Se(VI)-reducing bacterium, *E. taylorae*, from rice straw and used it to successfully reduce Se(VI) to Se(0) in an artificial high-salinity (15.5 dS/m) drainage water. We characterized the pathway of Se(VI) reduction in the drainage water as Se(VI) → Se(IV) → Se(0) → Se(-II). This study showed that the efficiency of Se(VI)

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◆: Total soluble Se, ●: Se(VI), ▲: Se(IV), ■: Se(0), ▼: Se(-II)

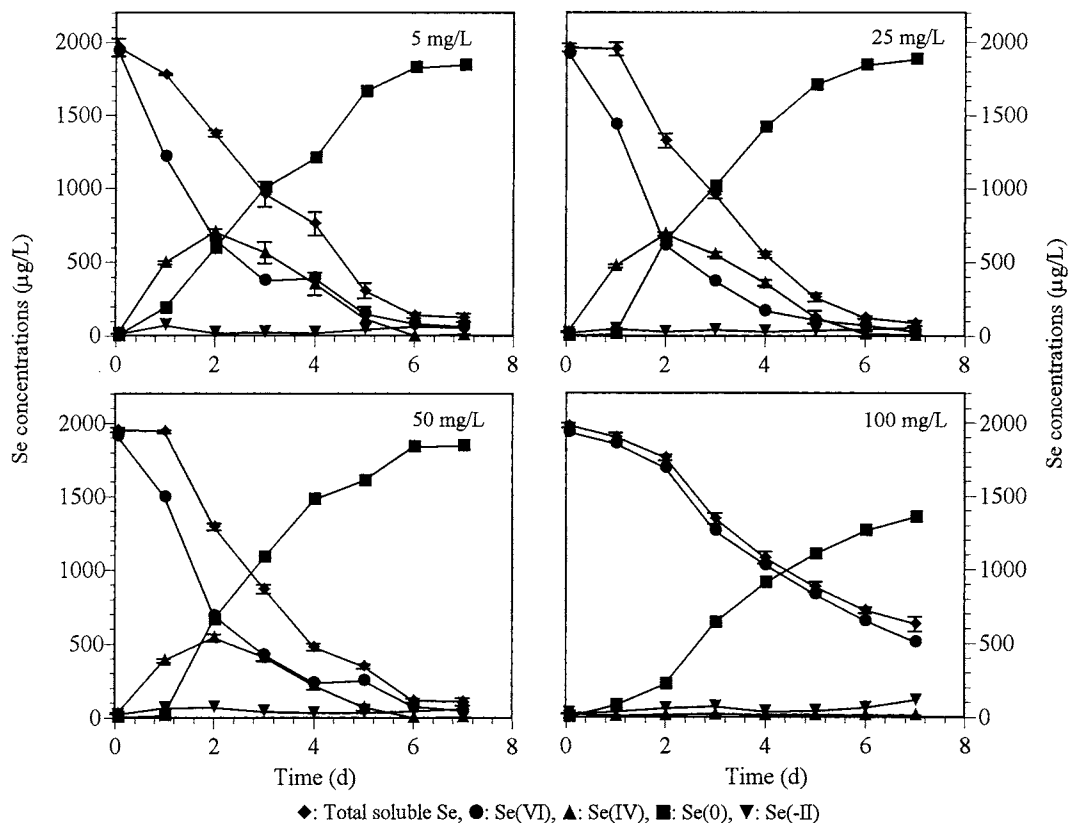**Figure 3.** Effect of NO_3^- (5–100 mg/L) on the changes of Se species in a synthetic drainage water upon inoculation with *E. taylorae.*

◆: Total soluble Se, ●: Se(VI), ▲: Se(IV), ■: Se(0), ▼: Se(-II)

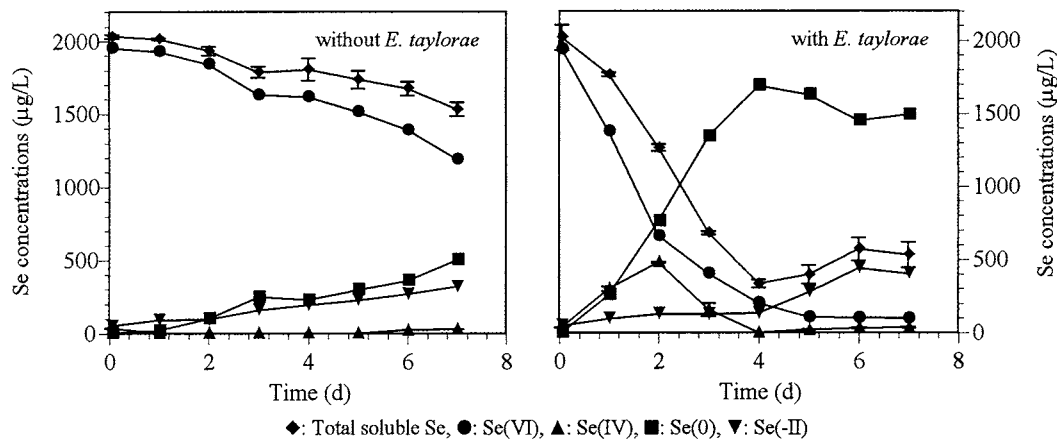**Figure 4.** Reduction of Se(VI) in natural drainage water spiked with 2000 μg/L of Se(VI), with and without inoculation with *E. taylorae.*

reduction by *E. taylorae* was related to the amount of yeast extract added, salinity, and NO_3^- levels in the drainage water.

Yeast extract is commonly used in culture media for microbial reduction of Se(VI) to Se(0) (*7, 10, 12, 19−21*). Fujita et al. (*19*) reported that yeast extract, as a growth factor, was required for *Bacillus* sp. SF-1 to reduce Se(VI) to Se(0), and casamino acids, a DNA/RNA mixture, or a vitamin mixture cannot replace the yeast extract. In this study, yeast extract was also shown to promote Se(VI) reduction to Se(0) by *E. taylorae.* In the drainage water with a relatively high level of yeast extract (500−1000 mg/L), ~89−93% of the added Se(VI) was reduced to Se(0) and ~5% to Se(-II). In the drainage water with low levels of yeast extract (50−100 mg/L), only 20−40% of the added Se(VI) was reduced to Se(0), although the amount of glucose added in the drainage water was the same in the experiments. The amount of yeast extract in the drainage water also affected the reduction process. In the high-yeast extract drainage water, Se(IV) was rapidly reduced to Se(0) when it was formed from

rapid reduction of Se(VI). In contrast, in the low-yeast extract drainage water, reduction of Se(IV) to Se(0) was limited, although Se(VI) reduction to Se(IV) proceeded rapidly. During the last 3−4 days of the experiment, a decrease in Se(VI) concentration, a slight change in the concentration of Se(IV), and an increase in Se(0) concentration in the low-yeast extract drainage water suggested that low amounts of yeast extract in the drainage water do not provide enough essential growth factors to support rapid bacterial reduction of Se(IV) to Se(0), which occurred in the high-yeast extract drainage water.

High-salinity drainage water often is the byproduct of agricultural irrigation in farmlands. The salinity in drainage water affected the efficiency of Se(VI) reduction to Se(0). In a study on the effect of sulfate, a major anion in agricultural drainage water, on the rate of Se(VI) reduction with washed cell suspension of *Desulfovibrio desulfuricans*, Zehr and Oremland (*22*) reported that the rate of Se(VI) reduction was negatively related to the amounts of SO_4^{2-} with a range of

0.2−50 mM. In this study, the reduction efficiency of Se(VI) to Se(0) by *E. taylorae* was also negatively associated with the salinity in the drainage water, with the major anions being SO_4^{2-} and Cl^-. During this 7-day experiment, the percentage of Se(VI) reduction to Se(0) followed a reverse trend with salinity levels in the drainage water: 95% (EC = 5 dS/m) > 81% (EC = 25 dS/m) > 30.5% (EC = 50 dS/m) > 16.7% (EC = 75 dS/m).

Nitrate is one of the most common anions found in agricultural drainage water, due to the application of fertilizers. NO_3^- is a competitive electron acceptor to Se(VI) in Se(VI) reduction to Se(0) in aquatic system (*23*, *24*). In a study on the microbial reduction of NO_3^- and Se(VI), Steinberg et al. (*21*) reported that NO_3^- reduction by an anaerobic, freshwater enrichment preceded that of Se(VI) reduction in an anaerobic medium with equal amounts of Se(VI) and NO_3^- of 20 mM. Fujita et al. (*19*) reported that Se(VI) reduction by *Bacillus* sp. SF-1 in a basal medium with 1 mM of Se(VI) was completely inhibited when 20 mM NO_3^- was added to the medium. NO_3^- and Se(VI) can also be simultaneously reduced in the medium with washed-cell suspensions of *Sulfurospirillum barnesii* and lactate as the electron donor (*8*). In this study, reduction of Se(VI) to Se(0) was related to the amounts of NO_3^- in the drainage water. A NO_3^- range of 5−50 mg/L did not affect Se(VI) reduction to Se(0). About 95% of added Se(VI) was reduced to Se(0) in the drainage water during our 7-day experiment. Se(VI) reduction was slightly slowed when NO_3^- was increased to 100 mg/L in the drainage water. Similar results can be also found in a recent study on bacterial Se(VI) reduction using rice straw, when Zhang and Frankenberger (*25*) reported that the presence of NO_3^- in a rice straw solution retarded Se(VI) reduction to Se(0). During a 14-day study, ∼93% of the added Se(VI) (1000 μg/L) was reduced to Se(0) during a period of 7 days in the rice straw solution with the 100 mg/L NO_3^-. It took 12 days for 93% of the added Se(VI) to be reduced to Se(0) in the rice straw solution with 250 mg/L NO_3^-. In the rice straw solution with 500 mg/L NO_3^-, Se(VI) was not reduced until day 14, when almost all of the NO_3^- was removed from solution via denitrification.

This study showed that *E. taylorae* can survive in natural drainage water and also effectively reduce Se(VI) to Se(0). In the absence of *E. taylorae*, only 24.6 and 15.6% of the added Se(VI) was reduced to Se(0) and Se(-II), respectively. Upon the addition of *E. taylorae* to the drainage water, 73.8 and 20% of the added Se(VI) was reduced to Se(0) and Se(-II), respectively. In the San Joaquin Valley, California, the values of EC in drainage water, with the major anions of SO_4^{2-} and Cl^-, range from 2.25 to 21.5 dS/m (*26*). NO_3^- typically ranges from 3 to 234 mg/L (*26*). Therefore, *E. taylorae* may be a potential Se(VI) reducer to remediate Se-contaminated drainage water with relatively low NO_3^- levels in the San Joaquin Valley, California

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