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Application of Redox Mediator To Accelerate Selenate Reduction to Elemental Selenium by *Enterobacter taylorae*

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Acceleration of bacterial reduction of selenate [Se(VI)] to insoluble elemental Se [Se(0)] plays an important role in Se bioremediation. Anthraquinone-2,6-disulfonate (AQDS), a redox mediator, was assessed for its ability to enhance the reduction of Se(VI) (2000 μg/L) to Se(0) by *Enterobacter taylorae* in various media. The results showed that addition of AQDS did not increase Se(VI) reduction in the media containing 50 and 250 mg/L yeast extract, suggesting that *E. taylorae* cannot directly use anthrahydroquinone-2,6-disulfonate (AHQDS, a reduced form of AQDS) to respire Se(VI). An increase of yeast extract concentration from 50 to 250 mg/L in the medium dramatically enhanced the AQDS function for rapid reduction of selenite [Se(IV)] to Se(0). During an 8-day experiment, 85−91% of the added Se was reduced to Se(0) in the AQDS-amended medium in comparison to formation of 46% of Se(0) in the medium without AQDS. These results show that redox mediators have great application potential in bioremediation of Se in Se-contaminated water.

KEYWORDS: Anthraquinone-2,6-disulfonate; *Enterobacter taylorae*; redox mediator; selenium reduction; selenium speciation

INTRODUCTION

Selenium (Se) contamination of aquatic systems in California mainly results from agricultural drainage water (*1*, *2*). In some areas of the San Joaquin Valley in California, Se in agricultural drainage water is frequently found at elevated levels of 140−1400 μg/L (mostly as selenate [Se(VI)]) (*3*−*5*). The bioaccumulation of Se in aquatic systems creates serious hazards to fish and waterfowl (*6*−*8*). Consequently, it is very important for scientists and wetland managers to find ways to remove or reduce Se from agricultural drainage water before it is disposed into aquatic systems.

In the aquatic system, Se(VI) can be used in microbial respiration as a terminal electron acceptor for growth and metabolism. Several bacteria isolated from different environments are capable of reducing Se(VI) to elemental Se [Se(0)], for example, *Bacillus* sp. SF-1, *Bacillus* sp. RS1, *Citerobacter freundii*, *Citerobacter braakii*, *Enterobacter cloacae*, *Enterobacter taylorae*, *Sulfurospirillum barnesii*, and *Thauera selenatis* (*4*, *9*−*15*). Because of the insolubility of Se(0) in aquatic systems, reduction of Se(VI) to Se(0) is considered to be a useful technique for removing Se from agricultural drainage water. For example, in a pilot-scale Se bioremediation system using the Se(VI) reducer *T. selenatis* and acetate in the liquid phase as an electron donor, Cantafio et al. (*4*) reported that bacterial reduction of Se(VI) to Se(0) proceeded rapidly in a series of four columns filled with Jaeger Tri-packs and/or silica sand. About 98% of the Se(VI) and selenite [Se(IV)] in the agricultural drainage water was reduced.

Humic substances (HS) are widespread in terrestrial and aquatic environments. The redox-active quinone moieties of HS have been recently shown to play important roles as electron carriers to stimulate the reductive biotransformation of azo dyes and nitroaromatic and polyhalogenated contaminants by shunting electrons between an external electron donor and the contaminants (*16*). For example, anthraquinone-2,6-disulfonate (AQDS) can increase the reaction rate by acting as a redox mediator that shifts electrons between its oxidized, quinone from AQDS and its reduced, hydroquinone from AHQDS (anthrahydroquinone-2,6-disulfonate) (*17*). van der Zee et al. (*18*) reported that the first-order rate constant for the reduction of Reactive Red 2 (RR2) dye by methanogenic granular sludge could be increased 7-fold when AQDS was added into RR2-containing wastewater. Coates et al. (*19*) revealed that bacteria can directly use AHQDS as an electron donor to reduce NO_3^- to N_2 with acetate as a carbon source. The redox potential of NO_3^-/N_2 in an aquatic system is very similar to that of Se(VI)/Se(IV). Therefore, AHQDS might be used by Se(VI)-reducing bacteria as an electron mediator to accelerate Se(VI) reduction to Se(0) in aquatic systems.

The reduction of Se(VI) to Se(0) involves a two-step pathway [Se(VI) → Se(IV) → Se(0)]. For effective removal of Se from water, both Se(VI) and Se(IV) need to be rapidly reduced. In this study, we investigated the effect of AQDS on the reduction of Se(VI) and Se(IV) to Se(0) in a medium by *E. taylorae*. The

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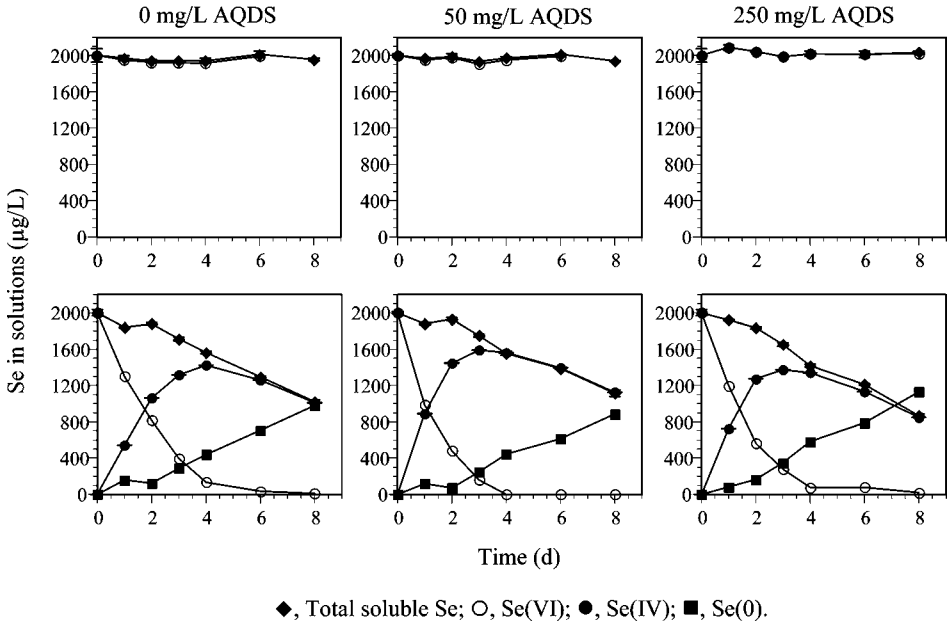

Figure 1. Effect of AQDS on the reduction of Se(VI) (2000 μg/L) to Se(0) in medium containing 50 mg/L yeast extract. Top row, control experiments without the addition of *E. taylorae*; bottom row, experiments with *E. taylorae*. Error bars indicate one standard deviation ($n = 3$).

enhancement of Se(VI) and Se(IV) reduction to Se(0) was characterized in a series of batch experiments.

MATERIALS AND METHODS

Materials. All experiments were conducted in a FTW culture medium (*13*). The medium was prepared with the following constituents (in grams per liter): K_2HPO_4, 0.225; KH_2PO_4, 0.225; $(NH_4)_2SO_4$, 0.225; NaCl, 0.46; $CaCl_2 \cdot 2H_2O$, 0.005; $MgSO_4$, 0.117; $FeCl_2$, 0.0005; glucose 0.5; yeast extract, 0.05 or 0.25, and trace element solution (*20*), 1 mL/L. After addition of AQDS ranging from 0, 25, and 250 mg/L, the medium was autoclaved (18 psi at 121 °C) for 20 min. The Se(VI) and Se(IV) standard stock solutions (10 000 mg/L) were passed through a sterile 0.2 μm membrane filter prior to their addition to the medium.

Effect of AQDS on Reduction of Se(VI) to Se(0). *E. taylorae* was isolated from rice straw in our previous study and was selected in this work because of its ability to reduce Se in drainage water (*14*). *E. taylorae* was initially pregrown in a 1% tryptic soy broth (TSB) solution and incubated at 30 °C overnight. The culture was then centrifuged at 5000 rpm for 20 min. To remove the TSB residues, the cells were washed four times with 30 mL of the sterile medium by centrifugation. Washed cells were resuspended in the sterile medium to give an OD_{600} range of 1.01−1.05 for the experiments described below.

The first experiment was conducted in the laboratory to determine the effect of the addition of AQDS on reduction of Se(VI) to Se(0) by *E. taylorae* in the FTW medium. *E. taylorae* is a facultative bacterium, and it is capable of reducing Se(VI) in the presence of air. Therefore, the medium was used without any treatments after sterilization. In the experiment, 38 mL of the medium containing 50 or 250 mg/L of AQDS was added to each 40-mL EPA glass vial, with white polypropylene open-top closures with TFE/silicone septa. The vials were spiked with Se(VI) to give a final concentration of 2000 μg/L and inoculated with 0.2 mL of *E. taylorae* cell suspension. The vials were capped and incubated under a static condition at room temperature (20 °C). Another batch experiment without the addition of AQDS was conducted for a comparison. The experiments were run in triplicate for 8 days. The samples of culture medium were collected in a clean laminar flow hood by use of a sterilized pipet tip at an interval of 1−2 days for analysis of Se species.

Effect of AQDS on Reduction of Se(IV) to Se(0). After obtaining the results from the first experiment described above, we found that the addition of 250 mg/L AQDS and 250 mg/L yeast extract in the medium significantly enhanced the formation of Se(0). Therefore, we next tested the effect of AQDS on the reduction of Se(IV) to Se(0) by *E. taylorae* in the medium. In this experiment, 38 mL of the medium containing 250 mg/L each AQDS and yeast extract was added to each 40-mL EPA glass vial. The vials were spiked with Se(IV) to give a final concentration of 2000 μg/L, followed by inoculation with 0.2 mL of *E. taylorae* cell suspension. The vials were capped and incubated under a static condition at room temperature (20 °C). Another experiment with the added yeast extract but without the addition of AQDS was conducted for a comparison. The experiments were run in triplicate for 8 days. The samples of culture medium were collected in a clean laminar flow hood by use of a sterilized pipet tip at an interval of 1−2 days for analysis of Se species.

Analysis. Selenium species in the medium samples were determined by a method developed by Zhang and Frankenberger (*21*) and Zhang et al. (*22*) after removal of Se(0) from the solution by centrifugation (Beckman microfuge 11) at 12 000 rpm for 14 min. Directly measured Se species included total soluble Se, Se(IV), and Se(IV) plus selenide [Se(−II)]. Se(VI), Se(0), and Se(−II) were determined by the difference method (*21, 22*). Se concentrations in all the solutions were analyzed by hydride generation atomic absorption spectrometry (HGAAS). The rate constant of total soluble Se [Se(VI) plus Se(IV)] removal by *E. taylorae* in the experiments was calculated from a simple first-order kinetics equation: $dSe/dt = -k[Se]$, where k is the rate constant for soluble Se removal and [Se] is the concentration of total soluble Se.

RESULTS

Effect of AQDS on Reduction of Se(VI) to Se(0). The reduction of Se(VI) to Se(0) in the medium is illustrated in Figures 1 and 2. During an 8-day experiment, there was little change in Se(VI) levels in the uninoculated control vials. In the presence of *E. taylorae*, 2000 μg/L Se(VI) was almost entirely reduced in the medium amended with different amounts of yeast extract and AQDS. The changes in Se(IV) concentration, an intermediate product of Se(VI) reduction to Se(0), varied. In the medium amended with 50 mg/L yeast extract, Se(IV) concentrations initially increased to a maximum of 1430, 1590, and 1370 μg/L at day 3−4 during the rapid reduction of Se(VI), and then decreased to 1010, 1110, and 850 μg/L at day 8 in the media amended with 0, 50, and 250 mg/L AQDS, respectively. Simultaneously, Se(0) increased to 980, 885, and 1130 μg/L, respectively.

In the medium amended with 250 mg/L yeast extract but no AQDS, the Se(IV) concentration also increased to 1470 μg/L

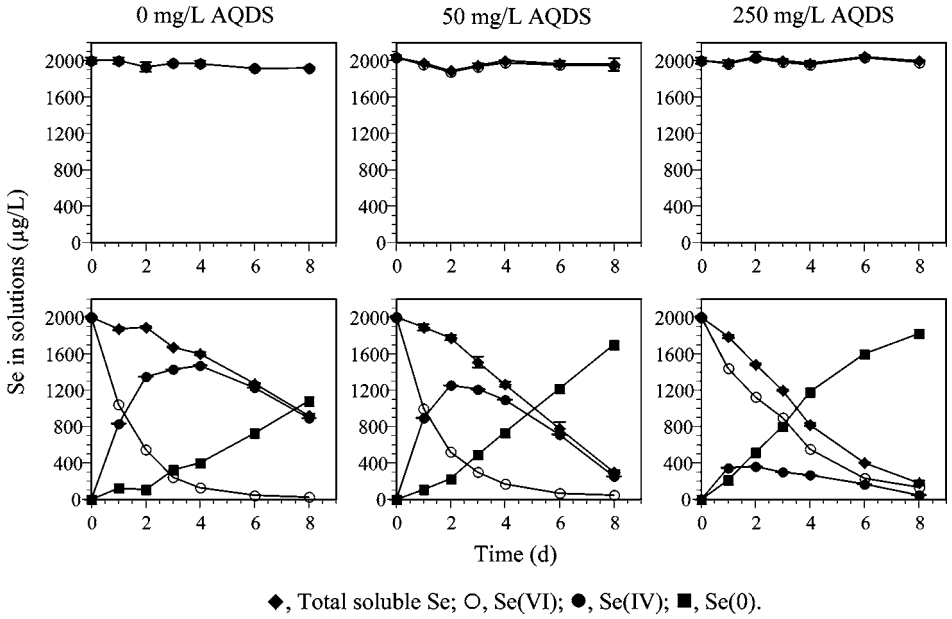

Figure 2. Effect of AQDS on the reduction of Se(VI) (2000 μg/L) to Se(0) in medium containing 250 mg/L yeast extract. Top row, control experiments without the addition of *E. taylorae*; bottom row, experiments with *E. taylorae*. Error bars indicate one standard deviation ($n = 3$).

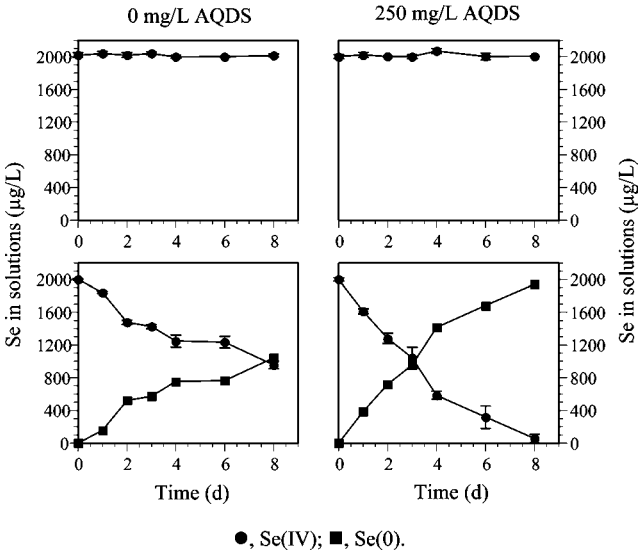

Figure 3. Effect of AQDS on the reduction of Se(IV) (2000 μg/L) to Se(0) in the medium containing 250 mg/L yeast extract. Top row, control experiments without the addition of *E. taylorae*; bottom row, experiments with *E. taylorae*. Error bars indicate one standard deviation ($n = 3$).

at day 4 and then decreased to 890 μg/L. Formation of 1080 μg/L Se(0) was observed under these conditions. Significantly greater activity was observed in the medium containing 250 mg/L yeast extract and a similar concentration of AQDS. In that instance, the Se(IV) concentration rose to 1250 and 360 μg/L at day 2 and then gradually dropped to 250 and 47 μg/L at day 8 with the 50 and 250 mg/L amendments with AQDS, respectively. Simultaneously, Se(0) increased to 1700 and 1820 μg/L, respectively.

Effect of AQDS on Reduction of Se(IV) to Se(0). Reduction of Se(IV) to Se(0) in the medium amended with 250 mg/L yeast extract is presented in Figure 3. During an 8-day experiment, the concentration of Se(IV) changed little in the uninoculated control medium. In the presence of *E. taylorae*, Se(IV) concentrations decreased with time from 2000 to 960 μg/L during a period of 8 days, with Se(0) formation of 1040 μg/L. When 250 mg/L AQDS was added to the medium, Se(IV) was rapidly reduced to 56 μg/L, resulting in formation of 1940 μg/L Se(0).

DISCUSSION

Bacterial reduction of soluble Se(VI) to insoluble Se(0) is a useful remedial technique for removing Se from water. The reduction of Se(VI) to Se(0) involves a two-step pathway with the first reduction of Se(VI) to Se(IV) and then Se(IV) to Se(0). For removal of Se from water, bacteria need specific carbon and electron sources to effectively reduce both Se(VI) and Se(IV) to Se(0). For example, Oremland et al. (*15*) reported that *S. barnesii* used lactate as an effective electron donor in Se(VI) reduction. *T. selenatis* metabolized acetate (*4*), while *E. cloacae* SLD1a-1 utilized glucose (*13*) in reduction of Se(VI) to Se(0). The present study shows that the efficiency of Se(VI) reduction to Se(0) by *E. taylorae* is related to the amounts of yeast extract and AQDS added to the medium.

Yeast extract is an important organic carbon source commonly used in the bacterial reduction of Se(VI) to Se(0). Zhang et al. (*11*) reported that yeast extract promoted Se(VI) reduction to Se(0) by *C. freundii*. During 8 days of incubation, about 89−96% of the added Se(VI) (500−4500 μg/L) was reduced to Se(0) in a medium amended with glucose and yeast extract. In this study, total soluble Se [Se(VI) plus Se(IV)] removal was relatively slow in the medium containing a low concentration of yeast extract (50 mg/L). Addition of AQDS did not significantly increase the removal of soluble Se, k values being 0.083, 0.073, and 0.11 day^{-1} in the medium containing 0, 50, and 250 mg/L AQDS, respectively (Table 1). On the final day of the experiment, Se(0) accounted for only 49%, 44%, and 57% of the added Se, respectively. However, an increase of yeast extract concentration from 50 to 250 mg/L dramatically enhanced the AQDS function for rapid removal of soluble Se. In comparison to a slight increase of k value from 0.083 to 0.095 day^{-1} in the medium without the addition of AQDS, k values were much higher in the medium containing 50 and 250 mg/L AQDS, being 0.23 and 0.31 day^{-1}, respectively. The resultant Se(0) amounted to 85−91% of the added Se(VI).

Rapid removal of soluble Se in the medium containing a high level of yeast extract (250 mg/L) can be attributed to rapid

Table 1. Rate Constant of Total Soluble Se [Se(VI) plus Se(IV)] Removal in the Experiments Amended with Different Amounts of Yeast Extract and AQDS

yeast extract (mg/L)/AQDS (mg/L)	rate constant (k, days^{-1}) total soluble Se
50/0	0.083
50/50	0.073
50/250	0.11
250/0	0.095
250/50	0.23
250/250	0.31

reduction of Se(IV) due to the addition of AQDS. In the Se(IV) reduction experiment, Se(IV) was reduced much more rapidly in the medium amended with AQDS than that without AQDS. Rapid reduction of Se(IV) was also observed in the Se(VI) reduction experiment, where only a small amount of Se(IV) was found in the medium amended with 250 mg/L yeast extract and AQDS. These results indicate that Se(IV) was rapidly reduced to Se(0) when it was formed by the reduction of Se(VI). Therefore, a relatively high amount of yeast extract is needed for *E. taylorae* to reduce AQDS to AHQDS while Se(VI) is being reduced, and then to use AHQDS as a electron donor to enhance Se(IV) reduction.

Addition of AQDS had little effect on Se(VI) reduction even at a high level of yeast extract (250 mg/L) in the medium when Se(IV) was rapidly reduced to Se(0). These findings indicate that *E. taylorae* employed AHQDS to reduce Se(IV) but not Se(VI).

The redox-active quinone groups play important roles as electron carriers in stimulating the reductive biotransformation of azo dyes, nitroaromatic and polyhalogenated contaminants, and NO_3^- by shunting electrons between an external electron donor and the contaminants. Results from this study showed that although *E. taylorae* was unable to use AHQDS as a redox mediator to enhance Se(VI) reduction, it is capable of using AHQDS as an electron donor in the reduction of Se(IV) and thus increasing the formation of insoluble Se(0). Rau et al. (*23*) reported different hydroquinone/quinone couples have a redox potential (E'_o) range from −350 to 280 mV. Therefore, selection of a suitable quinone, which not only serves Se reducers in acceleration of Se(VI) and Se(IV) reduction but also limits the formation of organic Se, would play an important role in Se bioremediation.

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