Selenium (Se) Removal from Copper Refinery Wastewater Using a Combination of Zero-Valent Iron (ZVI) and Se(VI)-Reducing Bacterium, *Thaurea selenatis*

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**Abstract**

The copper refinery process produces Se(VI)-bearing wastewater with a high content of Cl\(^-\) and SO\(_4^{2-}\) ions. To overcome the negative effect caused by Cl\(^-\) and SO\(_4^{2-}\) ions on Se(VI) reduction and its following removal, this study investigated the possible synergistic effect of the combination of Se(VI)-reducing bacterium, *Thaurea (T.) selenatis* and zero-valent iron (ZVI). In the presence of SO\(_4^{2-}\) (200 mM) and Cl\(^-\) (300 mM), the following was observed: (i) ZVI alone was unable to remove Se both under strictly aerobic and micro-aerobic conditions. (ii) Se(VI) reduction by *T. selenatis* alone was severely inhibited under anaerobic conditions (and thus no microbial growth was observed). (iii) On the other hand, *T. selenatis* was capable of growth and Se(VI) reduction under micro-aerobic conditions. (iv) Combining *T. selenatis* and ZVI under micro-aerobic conditions showed a synergistic effect on Se(VI) reduction, readily facilitating Se removal. This synergistic effect was optimized by adjusting the pH to near neutral (optimal for *T. selenatis* growth), but by adjusting the temperature to 35°C (sub-optimal for *T. selenatis* growth): Se removal of 55% by *T. selenatis* alone, was significantly improved to 98% by combining *T. selenatis* and ZVI. The proposed key process to display the synergistic effect on Se removal under micro-aerobic conditions is as follows: (i) Using the remaining T. *selenatis* anaerobic Se(VI) respiration by aerobic respiration, (ii) ZVI indirectly serves as a reducing agent to maintain low DO levels, consequently readily switching from aerobic to anaerobic Se(VI) respiration by *T. selenatis*. (iii) ZVI may also be acting directly for Se deposition by reducing microbially-produced intermediate Se(IV), which is more reactive than original Se(VI). The present findings could be used as a basis for developing an economically feasible and environmentally harmless bio-treatment technology for Se(VI) containing copper refinery wastewaters.

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influential level. Zhang et al.\textsuperscript{5} reported that reduction of Se(VI) by ZVI was negatively related to the salt concentration in the water: The Se(VI) removal rate by ZVI declined with an increase of concentrations of anions such as Cl\textsuperscript{−}, SO\textsubscript{4}\textsuperscript{2−}, NO\textsubscript{3}−, HCO\textsubscript{3}−, and PO\textsubscript{4}\textsuperscript{3−}. Microbial growth and activity also may well be affected by such salt concentrations.

The copper refinery anode slimes are composed largely of Cu, Se, and precious metals. After recovery of precious metals, Se is leached from the slime using Cl\textsubscript{2} gas, followed by reduction of aqueous Se(VI) to Se(0) by SO\textsubscript{2} gas. Consequently, the wastewater containing the remaining Se(VI) together with Cl\textsuperscript{−} and SO\textsubscript{4}\textsuperscript{2−} ions is produced.

With the aim to search for an effective approach to overcome the negative effect caused by elevated concentrations of Cl\textsuperscript{−} and SO\textsubscript{4}\textsuperscript{2−} on Se(VI) reduction, this study investigated the possible synergistic effect of combination of Se(VI)-reducing bacterium, \textit{T. selenatis} and ZVI.

2. Experimental

2.1 Microorganism

\textit{T. selenatis} (ATCC 55363) was maintained micro-aerobi
cally in 50 mL Falcon tubes containing 35 mL of the basal medium composed of (g\textsuperscript{−1}): NaCl: 1.2, KCl: 0.3, NH\textsubscript{4}Cl: 0.3, KH\textsubscript{2}PO\textsubscript{4}: 0.2, Na\textsubscript{2}SO\textsubscript{4}: 0.3, MgCl\textsubscript{2}·6H\textsubbox{O}: 0.6, CaCl\textsubscript{2}·2H\textsubbox{2}O: 0.15 (pH 7.0), supplemented with 0.4 g\textsuperscript{−1} yeast extract, 10 mM CH\textsubscript{3}COONa and 8 mg\textsuperscript{−1} Se(IV) (added as Na\textsubscript{2}SeO\textsubscript{3}). To adapt cells to high concentrations of SO\textsubscript{4}\textsuperscript{2−} and Cl\textsuperscript{−} ions, \textit{T. selenatis} was sub-cultured by gradually elevating the concentrations of basal salts components, Na\textsubscript{2}SO\textsubscript{4} and KCl. Cultures were incubated still at 25°C.

2.2 Chemical Se(VI) removal experiment using ZVI

The effect of SO\textsubscript{4}\textsuperscript{2−} and Cl\textsuperscript{−} ions on Se(VI) reduction capacity of ZVI (ETI-CC-1004, Connelly-GPM; 1.0 m\textsuperscript{2}·g\textsuperscript{−1}) was evaluated. Higher concentration of Se(VI) solutions (added as Na\textsubscript{2}SeO\textsubscript{4}; 30 mg\textsuperscript{−1}) were prepared to clearly see the effect of the anions. One hundred milliliter flasks containing 50 mL of the above Se(VI) solutions (pH 6.0) with different concentrations of SO\textsubscript{4}\textsuperscript{2−} (0, 0.1, 1, 10 g\textsuperscript{−1}, added as Na\textsubscript{2}SO\textsubscript{4}) or Cl\textsuperscript{−} (0, 0.1, 1, 10 g\textsuperscript{−1}, added as NaCl) were prepared, followed by N\textsubscript{2} gas purge for 10 min. After addition of 1.7% (w/v) ZVI (washed with 100% ethanol), flasks were sealed with rubber stoppers and incubated at 25°C, shaken at 100 rpm. Samples were taken at regular intervals and filtered (0.20 µm) to determine total soluble Se concentrations by ICP-AES (SEIKO Vista-MPX).

2.3 Se(VI) removal experiment using combination of \textit{T. selenatis} and ZVI

\textit{T. selenatis} cells which had gained tolerance with 200 mM SO\textsubscript{4}\textsuperscript{2−} and 300 mM Cl\textsuperscript{−} during sub-culturing process were used for the experiment. For micro-aerobic experiment, \textit{T. selenatis} cells were inoculated into 50 mL Falcon tubes containing 35 mL of the above mentioned liquid medium. Concentrations of SO\textsubscript{4}\textsuperscript{2−} and Cl\textsuperscript{−} were set at 200 mM and 300 mM by adjusting Na\textsubscript{2}SO\textsubscript{4} and KCl concentrations, respectively. Ethanol-washed ZVI (electrolytic iron powder; through 150 µm 95%, Wako Chemicals) was used at 1.5% (w/v). For anaerobic experiment, 35 mL of the same liquid medium (concentrations of SO\textsubscript{4}\textsuperscript{2−} and Cl\textsuperscript{−} were set at 200 mM and 300 mM, or at 4 mM and 30 mM, by adjusting Na\textsubscript{2}SO\textsubscript{4} and KCl concentrations, respectively) was purged with N\textsubscript{2} gas and poured into 50 mL vial bottles under strictly anaerobic atmosphere. Cells were incubated to a final cell concentration of 1×10\textsuperscript{7} cells mL\textsuperscript{−1}. Different initial pHs (pH 7, 6 and 5) and incubation temperatures (25, 30 and 35°C) were tested. All cultures were incubated still. Samples were regularly taken and filtered (0.20 µm) to determine total soluble Se concentrations by ICP-AES (SEIKO Vista-MPX). Changes in pH, Eh (vs NHE), dissolved oxygen (DO) and cell numbers were also monitored. As control, cultures containing either ZVI alone, or \textit{T. selenatis} cells alone, were prepared.

2.4 Characterization of Se precipitates

The precipitates generated from Se(VI) reduction were collected and freeze-dried overnight, and analyzed by Raman spectroscopy (Thermo Scientific DXR Smart equipped with YAG laser (532 nm, 10 mW) or JASCO NRS 2000 equipped with Ar ion laser (514.5 nm; 38 mW)). An aliquot of bacterial culture suspension was casted on a copper grid and air dried for transmission electron spectroscopy (TEM; Tecnai-20, FEI). For observation with scanning electron microscopy (SEM; VE-9800, KEYENCE), the Se precipitates were collected and fixed with 2.5% glutaraldehyde, followed by ethanol dehydration and t-butanol freeze-drying (VFD21-S, Vacuum device).

3. Results and Discussion

The effect of varying concentrations of SO\textsubscript{4}\textsuperscript{2−} and Cl\textsuperscript{−} ions on chemical Se(VI) removal by ZVI was first investigated (Fig. 1). Se(VI) removal efficiency was increasingly hindered at elevated SO\textsubscript{4}\textsuperscript{2−} and Cl\textsuperscript{−} concentrations: While 91% of Se(VI) was removed in the absence of both anions, only 11% or 34% of Se(VI) was removed in the presence of 100 mM...
SO$_4^{2-}$ or 300 mM Cl$^-$, respectively (Fig. 1). Relatively greater inhibition effect of SO$_4^{2-}$ on Se(VI) removal than of Cl$^-$ may be due to the chemical properties of SO$_4^{2-}$ being similar to Se(VI). SO$_4^{2-}$ may compete with SeO$_4^{2-}$ during the reduction and adsorption process in the presence of ZVI. Cl$^-$ ions can corrode the surface of ZVI, which may hinder its reduction ability of Se(VI). The results indicate that the utilization of ZVI in the presence of high concentrations of SO$_4^{2-}$ and Cl$^-$ has a limited effect on Se(VI) reduction.

Secondly, effect of a combination of Se(VI) reducing bacterium T. selenatis and ZVI on Se(VI) removal, was investigated. Since T. selenatis is known to anaerobically utilize Se(VI) as a terminal electron acceptor, cultures were firstly set up strictly anaerobically. The DO values remained <0.25 mg·l$^{-1}$ (even lower in the presence of ZVI; <0.1 mg·l$^{-1}$) throughout the experiment. When cultures were inoculated with T. selenatis alone at lower concentrations of SO$_4^{2-}$ (4 mM) and Cl$^-$ (30 mM), cell growth was observed, accompanied by Se(VI) reduction to produce red Se precipitates (Fig. 2). However at higher concentrations of SO$_4^{2-}$ (200 mM) and Cl$^-$ (300 mM), a different trend was apparent: Cell growth was inhibited, especially noticeably in the presence of ZVI (Fig. 2(b)). Under this condition, no chemical Se(VI) removal by ZVI was observed. Likewise, microbial Se(VI) reduction by T. selenatis was severely retarded, with this phenomenon being even more apparent in the presence of ZVI (Fig. 2(a)). From the results obtained, it became clear that under strictly anaerobic conditions chemical as well as microbiological Se(VI) removal in the presence of such elevated concentrations of SO$_4^{2-}$ and Cl$^-$, is ineffective.

Finally the experiment was carried out under micro-aerobic conditions. Different temperatures (25, 30, 35°C) were first tested and the following observations were noted: (i) Similarly to the strictly anaerobic condition, chemical Se(VI) removal by ZVI alone was strictly inhibited in the presence of SO$_4^{2-}$ (200 mM) and Cl$^-$ (300 mM) (Fig. 3(a)). (ii) In contrast to the strictly anaerobic condition, Se(VI) reduction by T. selenatis alone still progressed at all temperatures tested. Optimal growth temperature for T. selenatis is reported to be 25–30°C (its growth is inhibited at 39°C). At 35°C, which is over the range of optimal growth temperature for T. selenatis, less Se(VI) reduction was observed (Fig. 3(a)). (iii) Se(VI) reduction was effectively facilitated by combining T. selenatis with ZVI at all temper-

![Fig. 2 Changes in total soluble Se concentration (a) and cell number (b) during Se(VI) reduction experiment by T. selenatis, ZVI or T. selenatis plus ZVI under strictly anaerobic condition at 25°C. Initial SO$_4^{2-}$ and Cl$^-$ concentrations were 200 mM and 300 mM, respectively (solid lines) or 4 mM and 30 mM, respectively (broken lines).](image1)

![Fig. 3 Changes in total soluble Se concentration (a), cell number (b) and DO (c) during Se(VI) reduction experiment at different temperatures: 25°C (○, ●), 30°C (△, ▲) or 35°C (■, □). Cultures included T. selenatis alone (broken lines; ○, △, ■), ZVI alone (solid lines; ●, ▲, □) or T. selenatis plus ZVI (solid lines; ●, ▲, ■) under micro-aerobic condition. Initial SO$_4^{2-}$ and Cl$^-$ concentrations were 200 mM and 300 mM, respectively.](image2)
atures tested. This synergistic effect became more apparent as the temperature increased (Fig. 3(a)), even though a decrease in cell number after the stationary phase was more significant at 35°C (Fig. 3(b)). Under micro-aerobic conditions, the dissolved oxygen present at the beginning of the experiment was quickly utilized during the first 20 hours (Fig. 3(c)). This indicates that growth of *T. selenatis* initiates by respiring O₂ which is then switched to Se(VI) respiration under O₂ depletion. Although anaerobic growth was strictly inhibited (Fig. 2), *T. selenatis* was shown able to grow readily aerobically in the presence of high concentrations of SO₄²⁻ (200 mM) and Cl⁻ (300 mM). Whilst Se(VI) removal by ZVI alone was limited due to the high salt concentrations, it can indirectly serve as a reducing agent to maintain low DO levels, consequently facilitating Se(VI) respiration by *T. selenatis*. Higher temperature (even though sub-optimal for microbial growth) also likely results in a quicker depletion of O₂, thus facilitating Se(VI) respiration. In the presence of *T. selenatis* alone at 35°C, only 55% of Se(VI) was removed. However, when the cultures contained both *T. selenatis* and ZVI, Se(VI) removal was significantly improved to 98%, within 65 hours.

Effect of pH on Se(VI) removal was then evaluated (Fig. 4). At pH 7 and 6 a similar pattern in change of Se(VI) reduction and cell number was observed (Fig. 4(a), (b)): Although no chemical Se(VI) removal was shown by ZVI alone, a synergistic effect on Se(VI) removal was observed in the presence of *T. selenatis* and ZVI. Under an acidic condition (pH 5.0), chemical Se(VI) removal by ZVI alone was observed to some extent, whereas this pH was too acidic for neutrophilic *T. selenatis*, resulting in lower cell numbers and the inhibition of Se(VI) reduction. Nonetheless, the synergistic effect by *T. selenatis* and ZVI on Se(VI) removal was still observed, probably in this case owing to the increased pH in the presence of ZVI (Fig. 3(d)), according to the following reaction: 2Fe⁰ + 2H₂O + O₂ → 2Fe²⁺ + 4OH⁻.⁶ The results indicate that although acidic pH is more suitable for chemical Se removal by ZVI alone, as was reported by Yoon et al.,⁵ to optimize the condition for the synergistic effect by *T. selenatis* and ZVI, the pH should be set at around 7 which is optimal for growth of *T. selenatis.*²²

Figure 4(c) again indicates that aerobic respiration by *T. selenatis* quickly consumes dissolved oxygen during the first 20 hours, which is then switched to anaerobic Se(VI) respiration.

Se precipitates formed by a combination of *T. selenatis* and ZVI, under micro-aerobic conditions, were examined by Raman spectroscopy. A broad Raman peak consisting of two possible components corresponding to Se(-II) (ZnSe; with the peak at 245 cm⁻¹) and Se(0) (elemental Se; with the peak at 237 cm⁻¹), was observed (Fig. 5). Se precipitates formed anaerobically by *T. selenatis* alone is known to be Se(0).²⁷ The results suggest that advantages of adding ZVI in *T. selenatis* culture is to provide the bacterium with an anaerobic atmosphere after allowing the initial microbial growth by O₂ respiration. This sequential reaction consequently enables rapid Se(VI) reduction in the presence of high concentration of SO₄²⁻ and Cl⁻. Results with SEM (Fig. 6(a), (b)) and TEM (Fig. 6(c), (d)) observation indicated that Se deposition by *T. selenatis* alone consists solely of 100–400 nm Se nano-spheres (Fig. 6(a), (c)) which then tend to aggregate extracellularly (Fig. 6(b), (d)). On the other hand, in the presence of *T. selenatis* and ZVI, Se nano- and micro-structures with different morphologies (hexagonal, needle-like, and honeycomb-like crystal structures) were
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Fig. 5 Raman spectra of Se precipitates formed by T. selenatis plus ZVI (a) and Se standards: ZnSe and Se⁰ (b).

Fig. 6 TEM (a, b) and SEM (c–f) images of Se nano-spheres deposited by T. selenatis alone (a–d), Se nano-structures with different morphologies deposited in the presence of T. selenatis and ZVI (e), and the surface of ZVI (electrolytic iron powder) without Se deposition (f).

The proposed mechanism for this synergistic effect can be summarized as follows: (i) Micro-aerobic condition allows more energetically favorable aerobic growth of T. selenatis during the first hours, enabling the bacterium to overcome the inhibitory effect of Cl⁻ and SO₄²⁻; (ii) ZVI indirectly serves as a reducing agent to keep the low DO level, readily switching the respiration mode of T. selenatis from aerobic (O₂) to anaerobic (Se(VI)); (iii) ZVI may also directly act for Se deposition by reducing microbially-produced intermediate Se(IV), which is more reactive than Se(VI). The results from the present study suggest that the bacterial treatment system combined with ZVI as reducing agent can effectively remove Se(VI) from copper refinery wastewaters.

4. Conclusion

This study investigated the utility of a combination of Se(VI)-reducing bacterium, T. selenatis and ZVI for treatment of Se-bearing copper refinery wastewater with high Cl⁻ and SO₄²⁻ ion contents. In the presence of SO₄²⁻ (200 mM) and Cl⁻ (300 mM) the following observations were made: (i) ZVI alone was incapable of Se(VI) removal both under strictly aerobic and micro-aerobic conditions. (ii) T. selenatis alone was unable to reduce Se(VI) under strictly anaerobic conditions. (iii) On the other hand, T. selenatis was capable of growth and Se(VI) reduction under micro-aerobic conditions. (iv) Combination of T. selenatis and ZVI under micro-aerobic conditions showed synergistic effect on Se(VI) reduction; the Se(VI) removal of 55% at 35°C by T. selenatis alone, was readily improved to 98% by adding ZVI to T. selenatis cultures.

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