



# Effect of heavy metal co-contaminants on selenite bioreduction by anaerobic granular sludge



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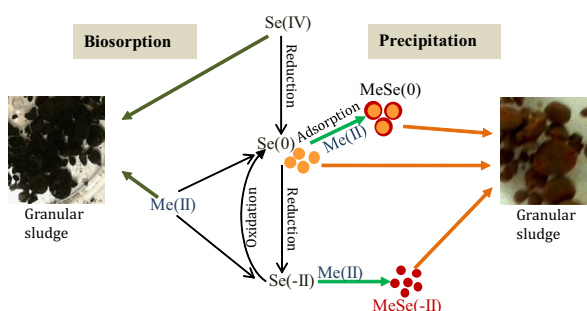
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## HIGHLIGHTS

- Cd inhibited selenite bioreduction at concentration >150 mg/L.
- Fate of bioreduced selenium was influenced in the presence of Cd.
- Selenite reduction was not inhibited up to 150 mg/L of Pb and 400 mg/L of Zn.
- Biosorption kinetics of heavy metals followed a pseudo-second order kinetic model.
- First report on anaerobic selenite bioreduction in the presence of heavy metals.

## GRAPHICAL ABSTRACT



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## ABSTRACT

This study investigated bioreduction of selenite by anaerobic granular sludge in the presence of heavy metals and analyzed the fate of the bioreduced selenium and the heavy metals. Selenite bioreduction was not significantly inhibited in the presence of Pb(II) and Zn(II). More than 92% of 79 mg/L selenite was removed by bioreduction even in the presence of 150 mg/L of Pb(II) or 400 mg/L of Zn(II). In contrast, only 65–48% selenite was bioreduced in the presence of 150–400 mg/L Cd(II). Formation of elemental selenium or selenide varied with heavy metal type and concentration. Notably, the majority of the bioreduced selenium (70–90% in the presence of Pb and Zn, 50–70% in the presence of Cd) and heavy metals (80–90% of Pb and Zn, 60–80% of Cd) were associated with the granular sludge. The results have implications in the treatment of selenium wastewaters and biogenesis of metal selenides.

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## 1. Introduction

Selenium and sulfur belong to the chalcogen group (periodic table group 16), and have similar chemical behavior. Often, selenium oxyanion contamination occurs concomitantly with

sulfate and heavy metals in different waste streams such as acid mine drainage, acid seeps, and agricultural drainage (Table S1, Supplementary data). There are limited studies on the microbial transformation of selenium oxyanions as compared to sulfur (Nancharaiah and Lens, 2015a). Bioreduction of selenium oxyanions, particularly selenite, to elemental selenium can be achieved using both aerobic and anaerobic microorganisms (Nancharaiah and Lens, 2015a). Thus, selenate (Se(VI)) and selenite (Se(IV)) reducing microorganisms could be potentially used for the bioremediation of selenium contaminated soils, sediments, industrial

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effluents, and agricultural drainage waters (Lenz et al., 2008; Nancharaiah and Lens, 2015a,b). However, the use of this strategy for practical applications may have important limitations because the microbial reduction processes as well as the fate of bio-reduced selenium can be affected by the presence of co-contaminants such as heavy metals.

Heavy metals are toxic for microorganisms and cannot be biodegraded like organic pollutants. However, they can be transformed from mobile and toxic forms into immobile and less or non-toxic forms (Beyenal and Lewandowski, 2004; Nancharaiah et al., 2015, 2016). Both adsorption and redox conditions essentially control the mobility of these chemical species in natural environments. It has been well documented that heavy metals such as Cu(II), Zn(II) and Cd(II) can either be adsorbed (Demirbas, 2008) or precipitated as metal sulfide in anaerobic environments (Prasad and Jha, 2010). In contrast, their fate in selenium containing environment is poorly documented.

There is an increasing interest in the potential biotechnological applications of bacterial selenium oxyanion reduction as a green method for the production of metal selenide quantum dots (Ayano et al., 2013; Fellowes et al., 2013; Nancharaiah and Lens, 2015b). Among several metal selenides, mainly cadmium selenide (CdSe), zinc selenide (ZnSe) and lead selenide (PbSe) have attracted considerable attention due to their quantum confinement effects and size-dependent photoemission characteristics (Fellowes et al., 2013). Hence, microbial reduction of selenium oxyanions in the presence of heavy metals (e.g. Cd, Zn, and Pb) is very important for the development of efficient bioremediation processes and for the microbial synthesis of metal selenide quantum dots (Nancharaiah and Lens, 2015b).

The use of sulfate reducing bacteria in metal bioremediation processes has been widely reported, for example, bioprecipitation as metal sulfide for cadmium (White and Gadd, 1998), zinc and lead (Guo et al., 2010; Hien Hoa et al., 2007). However, to the best of our knowledge, there is no study on the effect of heavy metal co-contaminants on selenium oxyanion bioreduction or vice versa. Therefore, the objective of this work was to investigate microbial reduction of selenite in the presence of heavy metals. In this study, experiments were performed on selenite reduction by anaerobic granular sludge in the presence of different concentrations of three heavy metals, i.e. Cd(II), Zn(II) and Pb(II). Time course profiles of selenite removal along with the fate of bio-reduced selenium and heavy metals were analyzed.

## 2. Methods

### 2.1. Source of biomass

Anaerobic granular sludge was collected from a full-scale upflow anaerobic sludge blanket (UASB) reactor treating paper mill wastewater (Industriewater Eerbeek B.V., Eerbeek, The Netherlands) and was utilized as the inoculum for all experiments. The anaerobic granular sludge was characterized in detail by Roest et al. (2005). The sludge was stored at 4 °C in an air tight jar under anaerobic conditions and used for selenite reduction experiments. All the experiments were performed in serum bottles under anaerobic conditions.

### 2.2. Selenite reduction experiments

The mineral medium used in selenite reduction experiments contained (mg/L): NH<sub>4</sub>Cl (300), CaCl<sub>2</sub>·2H<sub>2</sub>O (15), KH<sub>2</sub>PO<sub>4</sub> (250), Na<sub>2</sub>HPO<sub>4</sub> (250), MgCl<sub>2</sub> (120), and KCl (250). Nitrilotriacetic acid (1 mg/mg heavy metal) was used as the chelating agent to prevent heavy metal precipitation. Sodium lactate (10 mM) was used as the

carbon and electron source. Sodium selenite (Na<sub>2</sub>SeO<sub>3</sub>, 1 mM = 79 mg/L) was used as the source of selenium. The pH of the medium was adjusted to 7.3 with 1 M NaOH. The medium was distributed into 100 mL volume glass serum bottles as 70 mL aliquots. The serum bottles were inoculated with 0.7 g wet weight (0.2 g dry weight) of anaerobic granular sludge. The bottles were purged with N<sub>2</sub> gas for ~5 min and incubated at 30 °C on an orbital shaker set at 150 rpm for 9 d. All the experiments were performed either in duplicate or triplicate.

### 2.3. Effect of heavy metals on selenite reduction

Selenite reduction experiments described above were performed in the presence of heavy metals. Stock solutions of heavy metals were prepared by dissolving 1 g/L of CdCl<sub>2</sub>, ZnCl<sub>2</sub>, or PbCl<sub>2</sub> in ultrapure water. Heavy metals were added to serum bottles individually at different concentrations (10, 30, 50, 70, 90, 150, 300 and 400 mg/L). To avoid precipitation, Pb concentrations were used up to a maximum of 150 mg/L. Liquid samples were collected at regular time intervals for analyzing lactate, selenite, elemental selenium, total selenium and heavy metals. After 9 d of incubation, biomass was subjected to microwave-assisted acid digestion for measuring the total metal (Cd, Zn or Pb) and selenium concentration. Appropriate controls were setup by incubating serum bottles containing only mineral medium and sodium lactate with selenite and heavy metals, but without the biomass.

### 2.4. Kinetics of heavy metal removal

The heavy metal (Cd, Zn and Pb) removal kinetics were analyzed by fitting the data using the pseudo-first order rate expression based on the solid capacity as given below:

$$\frac{dq_t}{dt} = k_1(q_e - q_t) \quad (1)$$

where  $q_e$  and  $q_t$  are the amount of metal biosorbed per unit weight of biosorbent (mg/g dry weight) at equilibrium and at any time  $t$ ; respectively, and  $k_1$  is the rate constant of pseudo-first order sorption ( $\text{min}^{-1}$ ). After applying the initial and boundary conditions, for  $t = 0$  and  $q_t = 0$ , the integrated form of the above equation becomes:

$$\log(q_e - q_t) = \frac{\log q_e - k_1}{2.303 \times t} \quad (2)$$

In contrast to the pseudo-first order model, the pseudo-second order kinetic model predicts the behavior over the whole range of adsorption and is widely used by many researchers because it provides a more appropriate description than the first order equation (Volesky and Holan, 1995). It can be expressed in linear form as:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (3)$$

where  $q_t$  is the amount of the sorbate on the sorbent at time  $t$  (mg/g),  $k_2$  is the equilibrium rate constant of pseudo-second order sorption kinetics (g/mg.min) and  $q_e$  is the equilibrium uptake (mg/g) (Volesky and Holan, 1995). The pseudo-first and -second order constants were determined by plotting  $\log(q_e - q_t)$  against  $t$  and  $t/q$  against  $t$ , respectively (Cordero et al., 2004).

### 2.5. Analytical methods

The concentration of Cd, Zn and Pb was analyzed using an atomic absorption spectrophotometer (AAS) (PerkinElmer Model Analyst 200). Liquid samples were first filtered through a 0.45 μm cellulose acetate syringe filter (Sigma Aldrich, USA) and then the filtrate was analyzed for residual heavy metals after

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