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Aerobic and anaerobic biosynthesis of nano-selenium for remediation of mercury contaminated soil

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HIGHLIGHTS

- A new promising bioremediation technology for soil Hg was reported.
- Hg was stably immobilized as HgSe by bacteriogenic nano-Se^0.
- Aerobically and anaerobically produced nano-Se^0 can be applied to surface and subsurface soils.
- Biogenic nano-Se^0 is better than chemically synthesized nano-Se^0 for Hg remediation.
- Sodium dodecyl sulfonate can increase Hg remediation using biogenic nano-Se^0.

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ABSTRACT

Selenium (Se) nanoparticles are often synthesized by anaerobes. However, anaerobic bacteria cannot be directly applied for bioremediation of contaminated top soil which is generally aerobic. In this study, a selenite-reducing bacterium, *Citrobacter freundii* Y9, demonstrated high selenite reducing power and produced elemental nano-selenium nanoparticles (nano-Se^0) under both aerobic and anaerobic conditions. The biogenic nano-Se^0 converted 45.8–57.1% and 39.1–48.6% of elemental mercury (Hg^0) in the contaminated soil to insoluble mercuric selenide (HgSe) under anaerobic and aerobic conditions, respectively. Addition of sodium dodecyl sulfonate enhanced Hg^0 remediation, probably owing to the release of intracellular nano-Se^0 from the bacterial cells for Hg fixation. The reaction product after remediation was identified as non-reactive HgSe that was formed by amalgamation of nano-Se^0 and Hg^0. Biosynthesis of nano-Se^0 both aerobically and anaerobically therefore provides a versatile and cost-effective remediation approach for Hg^0-contaminated surface and subsurface soils, where the redox potential often changes dramatically.

1. Introduction

Mercury (Hg) is a naturally occurring non-essential highly toxic metal in the Earth's crust, and it is widely used in many industries such as the extraction of gold from ores, production of NaOH and chlorine in the chlor-alkali industry, and manufacture of compact fluorescent lamps, cosmetics, insecticides and herbicides (Boening, 2000). In some cases, improper use has led to extensive mercury pollution of soil. For example, mercury concentrations in the soil around a chlor-alkali plant in the Netherlands reached up to 1150 mg kg^{-1} (Bernaus et al., 2006). Mercury emissions were also detected in surrounding soils and sealed waste ponds near a chlor-alkali factory (Southworth et al., 2004).

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Mercury speciation in contaminated soil can be classified into water soluble, elemental, exchangeable, strongly-bound, organic, sulfide and residual fractions. Normally, elemental mercury comprises a small proportion of the total mercury in soil whereas in mercury or gold mining regions and in chlor-alkali plant soil, elemental mercury may account for a much larger part of the total mercury. In the Idrija mercury mine region, Slovenia, HgS is the predominant mercury fraction, followed by Hg^0 (Kocman et al., 2004). Elemental mercury accounted for ~95% of the total mercury in soils heavily contaminated with mercury in Venezuela (García-Sánchez et al., 2006). Soil beneath and adjacent to the Pavlodar Chemical Plant in Kazakhstan was also contaminated by mercury, and ~88–98% of the total mercury can be present as elemental mercury (Neculita et al., 2005). Therefore, there is an urgent need to treat elemental mercury-contaminated soil, particularly that caused by the industries mentioned above.

Selenium (Se) is in the same group as sulfur in the Periodic Table, and has an extremely high affinity for mercury with $\triangle G^0 = -38.1$ kJ mol^{-1}, which is higher than that for sulfur (Ho et al., 2015). A large amount of work has been carried out on detection of mercury and selenium in fish, marine mammals and humans. The molar ratio of mercury to selenium in such samples was approximately 1, which suggested detoxification of mercury into less toxic mercuric selenide (HgSe) (Southworth et al., 2000; Squadrone et al., 2015). Selenium nanoparticles have already been shown to be effective for mercury removal from off gases, and unstabilized amorphous nano-Se^0 showed a strong mercury capture capacity of 188 mg g^{-1} dry weight (Johnson et al., 2008; Lee et al., 2009). Biogenic red amorphous nano-Se^0 has also been applied to sequester mercury vapour released from mercury-contaminated museum specimens, the historic mercuric chloride treatment to preserve specimens leading to mercury volatilization (Fellowes et al., 2011). Nano-Se^0 therefore appears to be a promising mercury-trapping agent for cleanup, disposal, recycling and packaging applications (Ralston, 2008).

Most of these examples of mercury removal by selenium are concerned with mercury vapour in the atmosphere. However, this technique can also be applied to the aquatic environment. For example, *Pseudomonas fluorescens* could reduce SeO_3^{2-} and Hg^{2+} into elemental forms, the interaction between these two elements resulting in the formation of Hg-Se complexes within the cells with a Hg:Se molar ratio close to 1 (Yang et al., 2011). Bioreduced Hg^0 by a strain of *Shewanella putrefaciens* was captured as HgSe by extracellular biogenic amorphous selenium nanospheres (Jiang et al., 2012). However, no studies have been carried out which have tested the capacity of biogenic nano-Se^0 to immobilize mercury in soil.

Bioremediation of contaminated soil can be limited by the redox potential and the performance of the remediating bacteria in aerobic and anaerobic conditions. Surface soil layers are usually aerobic while subsurface soil layers may be anoxic, which means that both aerobic and anaerobic processes may be required. In addition, the soil redox potential during bioremediation may change drastically as bacterial cultures and substrates are applied. This may increase the cost, complexity and performance of bioremediation. Therefore, an ability for microbes to produce nano-Se^0 both aerobically and anaerobically may be relevant for the bioremediation of mercury-contaminated soils. Using versatile facultative bacteria to remediate soils with quite different redox potentials could be simpler and more effective.

In the present study, the performance of the facultative anaerobe *Citrobacter freundii* Y9, which can produce amorphous nano-Se^0 under anaerobic and aerobic conditions, in sequestering elemental mercury in soil was evaluated. Sequential soil extraction of mercury was carried out to determine changes in mercury speciation, and the reaction products were characterized by scanning electron microscopy with energy-dispersive X-ray spectrometry (SEM-EDS), X-ray diffraction (XRD), transmission electron microscopy (TEM) and X-ray photoelectron spectroscopy (XPS).

2. Materials and methods

2.1. Bacteriogenic nano-Se^0

Citrobacter freundii Y9, isolated from sludge from an anaerobic sulfate-reducing bioreactor in Urumqi, China was used in this study, and the sequence has been submitted to Gene Bank (number KF781347). The growth medium contained the following components: 1.0 g K_2HPO_4, 0.1 g $MgCl_2$, 0.2% yeast extract, 10 mM sodium citrate in 1 L Milli-Q water. The medium was adjusted to pH 7.0–7.2 using 0.1 M HCl, and sterilized in a vertical heating pressure stream (LDZX-75KBS, Shanghai, China). The bacteria were cultured at 26 °C in 500 ml serum bottles in a Whitley DG250 anaerobic workstation (Don Whitley Scientific, West Yorkshire, England), and aerobically in 250 ml flasks with constant shaking at 150 rpm.

To measure the selenite reduction activity of *C. freundii* Y9, late logarithmic phase cells (5%) were inoculated into fresh medium containing 1 mM sodium selenite, added from a sterile 500 mM sodium selenite stock solution. At appropriate time intervals, samples were collected and filtered using 0.22 μm hydrophilic polyestersulfone membranes. Selenite in the filtrates was analyzed by LC-HGAFS (Liquid Chromatography-Hydride Generation Atomic Fluorescence Spectrometry) (Jitian, Beijing, China). Determination of the number of viable cells (colony-forming units, CFU) was conducted as follows to measure the growth of bacteria (Tugarova et al., 2014). A series of consecutive ten-fold dilutions of bacterial suspensions were made using sterile physiological saline (0.87% NaCl); 200 μl of the corresponding diluted samples were then spread on solid nutrient broth medium and cultured for 4–5 d at 26 °C. Abiotic nano-Se^0 was prepared using L-ascorbic acid as a reductant to reduce H_2SeO_3, polyvinyl alcohol (PVA, 0.05%) was used as a soft template. The abiotic nano-Se^0 was centrifuged at 10,000 × *g* for 10 min and then re-suspended in PVA solution (0.05%). Biogenic and abiotic selenium were characterized by SEM-EDS and XRD.

2.2. Elemental mercury immobilization in soil

Biogenic and abiotic nano-Se^0 were used to capture mercury in contaminated soil under aerobic and anaerobic conditions. Soil was collected from farmland near Urumqi, China, sterilized in a vertical heating pressure stream (LDZX-75KBS, Shanghai, China), and air-dried, sieved (1 mm), and sterilized again under UV light irradiation for 2 h. Liquid mercury was added to the soil directly which was then aged for two months. The mercury immobilization tests were performed in centrifuge tubes which contained 25 *g* of elemental mercury contaminated soil and 25 ml medium containing 4 mM elemental selenium. When biogenic nano-Se^0 was used to treat mercury contaminated soil, one group contained 1% sodium dodecyl sulfate (SDS) to lyse the bacteria and release intracellular Se^0. The original concentration of soil mercury was analyzed using a mercury analyzer (Lumex RP91C, Saint Petersburg, Russia). After one week, the different mercury fractions in the soil samples were analyzed. A control without addition of nano-Se^0 was also treated in the same way. The elemental selenium in the medium or in the PVA suspensions was centrifuged at 12,000 × *g* for 10 min and then the Se-free supernatant was added to the control. Anaerobic and aerobic immobilization were performed inside a Whitley DG250 anaerobic workstation or in a fume hood, respectively.