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Multiple-pathway remediation of mercury contamination by a versatile selenite-reducing bacterium

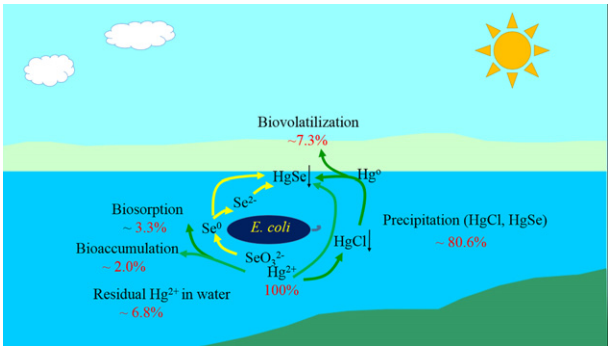
Xiaonan Wang [a,c], Zhanfei He [b], Hongwei Luo [b], Ming Zhang [b], Daoyong Zhang [a,b], Xiangliang Pan [a,b,*], Geoffrey Michael Gadd [d]

[a] Xinjiang Key Laboratory of Environmental Pollution and Bioremediation, Xinjiang Institute of Ecology and Geography, Chinese Academy of Sciences, Urumqi 830011, China
[b] Key Laboratory of Microbial Technology for Industrial Pollution Control of Zhejiang Province, College of Environment, Zhejiang University of Technology, Hangzhou 310014, China
[c] University of Chinese Academy of Sciences, Beijing 100049, China
[d] Geomicrobiology Group, School of Life Sciences, University of Dundee, Dundee, DD15EH, Scotland, UK

HIGHLIGHTS

- First report on removal of Hg^{2+} by *Escherichia coli* via HgSe, HgCl and Hg^0.
- Superoxide is involved in reducing selenite to selenide to immobilize Hg^{2+}.
- The versatile *E. coli* is a promising candidate for treatment of Hg^{2+} wastewater.

GRAPHICAL ABSTRACT



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ABSTRACT

Mercury contamination is a global concern because of its high toxicity, persistence, bioaccumulative nature, long distance transport and wide distribution in the environment. In this study, the efficiency and multiple-pathway remediation mechanisms of Hg^{2+} by a selenite reducing *Escherichia coli* was assessed. *E. coli* can reduce Hg^{2+} to Hg^{+} and Hg^0 and selenite to selenide at the same time. This makes a multiple-pathway mechanisms for removal of Hg^{2+} from water in addition to biosorption. It was found that when the original Hg^{2+} concentration was 40 μg L^{-1}, 93.2 ± 2.8% of Hg^{2+} was removed from solution by *E. coli*. Of the total Hg removed, it was found that 3.3 ± 0.1% was adsorbed to the bacterium, 2.0 ± 0.5% was bioaccumulated, and 7.3 ± 0.6% was volatilized into the ambient environment, and most (80.6 ± 5.7%) Hg was removed as HgSe and HgCl precipitates and Hg^0. On one hand, selenite is reduced to selenide and the latter further reacts with Hg^{2+} to form HgSe precipitates. On the other hand Hg^{2+} is successively reduced to Hg^{+}, which forms solid HgCl, and Hg^0. This is the report on bacterially transformation of Hg^{2+} to HgSe, HgCl and Hg^0 via multiple pathways. It is suggested that *E. coli* or other selenite reducing microorganisms are promising candidates for mercury bioremediation of contaminated wastewaters, as well as simultaneous removal of Hg^{2+} and selenite.

* Corresponding author at: College of Environment, Zhejiang University of Technology, Hangzhou 310014, China.
E-mail address: panxl@zjut.edu.cn (X. Pan).

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1. Introduction

Mercury mainly exists as either elemental (Hg^0) or oxidized mercuric (Hg^{2+}) forms in nature. Hg^{2+} is the primary form occurring in water (Ní Chadhain et al., 2006). The toxicity of Hg^{2+} mainly results from the binding of Hg^{2+} to sulfhydryl groups or disulfide groups in proteins and amino acids, leading to inactivation of enzymes (Zahir et al., 2006). In addition, mercury can be methylated to methyl mercury compounds aerobically and also under anoxic conditions by certain sulfate- and iron-reducing bacteria (Gadd and Griffiths, 1978; Bravo et al., 2014). Methylated derivatives are the most toxic species of mercury owing to their lipid solubility, bioaccumulation and biomagnification through food webs (Ní Chadhain et al., 2006)., not only because of its toxicity and bioaccumulation, but also because of its persistence and wide distribution in the environment (Tavares et al., 2016).

Mercury is one of the most toxic metal elements which is not essential to organisms, and the U.S. Environmental Protection Agency (USEPA) has placed it on the primary list of 129 hazardous chemical substances (Zhang, 2014).

The commonly used model bacterium *Escherichia coli*, including genetically engineered strains, has been widely investigated in environmental microbiology, including in the area of mercury and selenium transformations (Pazirandeh et al., 1998). For instance, a modified strain of *E. coli* possessed enhanced uptake limitations for mercury (Bae et al., 2001). *E. coli* can also mediate selenium transformations such as the reduction of selenite to selenide (Turner et al., 1998). If Cd^{2+} is transported into *E. coli*, subsequent reaction with Se^{2-} can result in synthesis of CdSe (Yan et al., 2014). Mercury and cadmium are in the same column in the Periodic table, and have some similar chemical properties. It can be hypothesized that the bioreduced Se^{2-} produced by *E. coli* could be employed to capture Hg^{2+} in solution.

However, the reaction of Hg^{2+} with selenide is more rapid and therefore may be a more effective mechanism for Hg^{2+} removal from water. Moreover, it is of great interest to capture Hg^{2+} with Se^{2-} or remove selenium and mercury simultaneously, because it should not be overlook that selenium is an important co-existing element with mercury in mercury mining area (Zhang, 2014; Zhang et al., 2014).

Mercury contamination is a global concern because of its high toxicity and global transport. Microbial bioremediation methods have often been proposed as a potential approach to remove mercury ions from water, because of assumed low cost and high efficiency especially at low concentrations. Some microbiological bioremediation methods have already been successfully used to remove heavy metals including Hg^{2+} (Herrero et al., 2005; Gadd, 2010; Yin et al., 2016). Although Biosorption and bioaccumulation of Hg^{2+} have been extensively studied (Gadd, 1993), these technologies have not been successfully applied to engineering application so far because serious problems with separation of small size microbial cells from water and release of mercury from the bioadsorbent after cell death. Much research has also focused on MerA-mediated bacterial reduction of Hg^{2+} to Hg^0 and subsequent volatilization of the Hg^0. However, a major problem is that unless trapped, mercury usually recycles back to the environment in the form of mercury vapour (Wang et al., 2012). Thus, better methods for mercury remediation should involve trapping of elemental mercury or precipitation which would prevent volatilization (Xiong et al., 2009; Sinha and Khare, 2012). Recently, a few studies show that bacterially generated Se^0 can been used to immobilize Hg^0 based on their reaction (Belzile et al., 2006; Johnson et al., 2008; Lee et al., 2009; Fellowes et al., 2011; Yang et al., 2011; Jiang et al., 2012; Wang et al., 2017).

In the present study, a versatile bacterium *Escherichia coli* that can remove mercury from water via multiple pathways was reported. This special *E. coli* can effectively remediate Hg^{2+} contamination by biosorption, bioaccumulation, bioreduction-volatilization immobilization of by Se^0, precipitation as HgCl, and formation of HgSe by reaction of Hg^{2+} and Se^{2-} at the same time.

In this study, removal of Hg^{2+} through selenite reduction by *E. coli* was investigated. Changes in Hg^{2+} concentration in solution and Hg^0 in the ambient environment were measured. Mercury biosorbed or bioaccumulated by *E. coli* was also extracted and measured. The mercury-containing precipitate was collected and characterized by scanning electron microscopy and energy-dispersive X-ray spectrometry (SEM-EDS), X-ray diffraction (XRD) and X-ray photoelectron spectroscopy (XPS). This research shows that most of the supplied Hg^{2+} was removed from solution through the formation of HgCl, Hg^0 and HgSe, and only a very small fraction was biosorbed or bioaccumulated. Moreover, only a small fraction of mercury was volatilized into the ambient environment. It can be concluded that *E. coli*, or microorganisms with similar properties, may be excellent candidate species for Hg^{2+} bioremediation and the simultaneous removal of mercury and selenite from contaminated waters.

2. Materials and methods

2.1. Incubation of bacterium

The facultative anaerobe *Escherichia coli* (GIM1.223), purchased from Guangdong Microbiology Culture Centre, was anaerobically cultivated at 30 °C in nutrient broth in serum bottles. Anaerobic conditions were achieved using a Whitley DG250 anaerobic workstation (Don Whitley Scientific, Shipley, England). After a 5% (v/v) inoculation, growth of *E. coli* under anaerobic conditions was recorded.

2.2. Resistance of the bacterium to Hg^{2+} toxicity

Resistance of *E. coli* to Hg^{2+} was examined. Bacterial cells were grown at 30 °C in the nutrient broth with various concentrations of Hg^{2+} (applied in the form of $HgCl_2$ of analytical grade) and the absorbance at 600 nm of the culture after 18 h exposure to mercury was measured (Cui et al., 2009). The OD_{600} of *E. coli* cultures was comparatively analyzed to assess its resistance to Hg^{2+} toxicity.

2.3. Hg^{2+} removal by E. coli from water

E. coli (5%, v/v) was incubated in 100 mL nutrient broth for 18 h at 30 °C to reach the stationary phase (OD_{600} = 0.88). 5 mL aliquots of the *E. coli* culture were transferred to 93 mL fresh nutrient broth in serum bottles, cultured in an anaerobic workstation for 18 h, prior to 1 mL Na_2SeO_3 and 1 mL $HgCl_2$ being added to the medium. The final concentration of Na_2SeO_3 was 15.8 mg L^{-1} (200 μM) and the final concentration of $HgCl_2$ was 40 and 200 μg L^{-1}. Na_2SeO_3 and $HgCl_2$ stock solutions were sterilized by filtering through a 0.22 μm hydrophilic polyestersulfone membrane filter (Xingya, Shanghai, China). At different time intervals, samples were collected and filtered with 0.22 μm hydrophilic polyestersulfone membranes. SeO_3^{2-} and Hg^{2+} in solution were determined by Liquid Chromatography Hydride Generation Atomic Fluorescence Spectrometry (LC-HGAFS) (Jitian, Beijing, China). Mercury- and selenite-containing nutrient broth without inoculation of *E. coli* was used as a control.

2.4. Hg^0 volatilization

To check whether Hg^{2+} was bioreduced to mercury vapour and volatilized into the ambient environment, an experiment was carried out in a 500 mL jar to measure such Hg^0 using a mercury analyzer (Lumex RA915 +, Saint Petersburg, Russia) (Fig. 1). Firstly, *E. coli* (5 mL) was transferred into 93 mL fresh nutrient broth containing 200 μM selenite and 40 μg L^{-1} $HgCl_2$. The jar was then sealed with a rubber stopper and removed from the anaerobic chamber. The Hg^0 vapour in the jar was measured every 2 h by recording the concentration of Hg^0 for 5 min at each sampling time to calculate an average value. A $KMnO_4$ solution (5%, w/v) was used to capture mercury-containing waste gas. For