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The large-scale process of microbial carbonate precipitation for nickel remediation from an industrial soil☆

Xuejiao Zhu, Weila Li, Lu Zhan, Minsheng Huang, Qiuzhuo Zhang, Varenyam Achal*

Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration (SHUES), School of Ecological and Environmental Sciences, East China Normal University, Shanghai 200241, China

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ABSTRACT

Microbial carbonate precipitation is known as an efficient process for the remediation of heavy metals from contaminated soils. In the present study, a urease positive bacterial isolate, identified as *Bacillus cereus* NS4 through 16S rDNA sequencing, was utilized on a large scale to remove nickel from industrial soil contaminated by the battery industry. The soil was highly contaminated with an initial total nickel concentration of approximately 900 mg kg^{-1}. The soluble-exchangeable fraction was reduced to 38 mg kg^{-1} after treatment. The primary objective of metal stabilization was achieved by reducing the bioavailability through immobilizing the nickel in the urease-driven carbonate precipitation. The nickel removal in the soils contributed to the transformation of nickel from mobile species into stable biominerals identified as calcite, vaterite, aragonite and nickelous carbonate when analyzed under XRD. It was proven that during precipitation of calcite, Ni^{2+} with an ion radius close to Ca^{2+} was incorporated into the $CaCO_3$ crystal. The biominerals were also characterized by using SEM-EDS to observe the crystal shape and Raman-FTIR spectroscopy to predict responsible bonding during bioremediation with respect to Ni immobilization. The electronic structure and chemical-state information of the detected elements during MICP bioremediation process was studied by XPS. This is the first study in which microbial carbonate precipitation was used for the large-scale remediation of metal-contaminated industrial soil.

1. Introduction

Heavy metals are toxic pollutants that contaminate various ecosystems. Several studies reported an elevated level of metals near industrial areas (Jung, 2008). These metals mostly contaminate nearby agricultural soils and enter into the ecological cycle. The heavily polluted soils become a long-term source of pollution to groundwater and the ecosystem (Giannis et al., 2010). The contamination of soils with heavy metals affects human health directly or indirectly and causes great economic losses (Zinjarde et al., 2014). The risks are related to the mobility and the bioavailability of the metals and consequently to their speciation in soil (Jean et al., 2007). To control the dispersion and biomagnification of metals, various physico-chemical techniques, including oxidation, reduction, a membrane process and precipitation, have been used; however, there are many disadvantages associated with them (Krishna and Philip, 2005).

There have also been various reports of the bacterial decontamination of soils polluted with heavy metals. The fundamental processes that enable bioremediation include changes in pH and/or redox reactions, increases or decreases in solubility by complexation, and adsorption or uptake of pollutants in the environment (BMI, 1994). The different oxidation states of many heavy metals have varying degrees of toxicity, making bioremediation involving oxidation or reduction ineffective. Furthermore, different soil redox potentials affect bioremediation differently because the activity of microbes is affected by a prevailing redox potential and may result in failure to stabilize a heavy or trace metal in the contaminated soil (Achal et al., 2012a). These disadvantages lead to opt for better bioremediation technology and microbially induced calcite precipitation (MICP) as an emerging technology is receiving wide attention.

In MICP, bacterial urease hydrolyzes urea into ammonia and carbamate, which, upon subsequent hydrolysis, release ammonia and carbonic acid. Because these bacteria precipitate Ca in the form of $CaCO_3$, they could be efficiently utilized to trap other heavy

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* Corresponding author.
E-mail address: varenyam@re.ecnu.edu.cn (V. Achal).

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metals and form immobilized heavy metals containing carbonates (Achal et al., 2012a, 2011, 2012b; Li et al., 2014). The process of MICP has recently been investigated in the efficient removal of arsenic (As), cadmium (Cd), copper (Cu), and lead (Pb) from contaminated soils (Achal et al., 2012a, 2011, 2012b; Kumari et al., 2014). In this process, some microbes accelerate the transformation of heavy metals from mobile species into stable minerals (Achal et al., 2012a; Govarthanan et al., 2013; Kumari et al., 2014).

With the rapid industrial and urban development in China, vehicle production has increased rapidly. Electric bicycles are becoming popular across the country, resulting in the construction of many battery factories. Nickel (Ni) is a common pollutant produced by such industries. Nickel compounds induce respiratory tract irritation, chemical pneumonia, emphysema, varying degrees of pulmonary cell hyperplasia, fibrosis (pneumoconiosis), and can be a potential carcinogen. Despite the toxic properties associated with Ni, most bioremediation technologies focus on other heavy metals. In addition, most of the research on MICP focuses on metal remediation from soil at the flask level. Thus, in the present study, a large-scale microbially induced calcite precipitation process was used for the first time to remove nickel from contaminated soil. The process of MICP would not only significantly decrease the bioavailability of Ni but also recycle Ni in the natural system in the form of minerals.

In this research, *Bacillus cereus* NS4 was used to immobilize Ni from industrial soil collected from a battery factory. The MICP bioremediation process was evaluated using SEM-EDS (scanning electron microscopy and energy-dispersive X-ray spectroscopy), Raman and FTIR (Fourier transform infrared) spectroscopy, and XRD (X-ray diffraction) analysis. Further, XPS (X-ray photoelectron spectroscopy) was used to provide the chemical-state information of the detected elements during MICP bioremediation process.

2. Methodology

2.1. Microorganism

Nickel-resistant bacteria were isolated from the industrial soil of a battery factory in Shanghai, China. Ten (10) grams of the sample were inoculated in 100 ml of distilled water with 4% urea and incubated in a shaker (200 rpm) at 28 °C for 7 days. For the isolation and enumeration of nickel-resistant bacterial isolates, the supernatant from the enrichment flask was diluted and spread on a nutrient-rich agar medium supplemented with 100 mM $NiCl_2 \cdot 6H_2O$. All of the plates were incubated at 28 °C for 24 h. Subsequently, the colonies were transferred onto a urea agar base to check the production of urease. One of the bacterial isolates, NS4, was selected for further studies.

The bacterium was identified via 16S rDNA sequencing. An NBU (Nutrient Broth-Urea) medium containing nutrient broth, 2% urea and 25 mM $CaCl_2$ was used throughout this study.

2.2. Industrial soil remediation

Industrial soils were collected near the effluent of a battery factory in Shanghai, China, and analyzed to determine their physico-chemical properties (Table 1). For the remediation of Ni from this soil, a 2 m × 2 m plot with a thickness of 7.5 cm was prepared in the laboratory. The bacterial isolate, NS4, was grown in NBU media for 48 h prior to irrigating the contaminated industrial soil. A sprayer was manually used to spray the bacterial culture homogenously throughout the plot at an interval of 12 h for one week and was then left for one month. Control plots were also prepared in similar manner where bacteria were not added. The soils were manually aerated weekly using agricultural tool used for digging soil. After one month, soil samples from the plot were randomly collected and were analyzed for Ni concentrations.

Table 1
Physicochemical properties of industrial soil.

Parameter	Content
Sand (%)	36.2
Silt (%)	26.8
Clay (%)	37.0
Moisture content (%)	29.2
pH	5.96
Ca (mg Kg^{-1})	15.4
Ni (mg Kg^{-1})	898

2.3. Enzyme activities

The two key enzymes in the process of bioremediation, urease and dehydrogenase, were measured in the soil samples at regular time intervals. The non-buffer method based on NH_3-N formation in the urea-amended soil sample was used to measure urease activity (Zantua and Bremner, 1975). The soil dehydrogenase activity was determined according to Achal et al. (2012b).

2.4. Nickel analysis

The nickel concentration in the soil was analyzed by AAS after drying the samples at 105 °C for 24 h in an oven. The chemical fractionation of Ni in the soils was determined using the sequential extraction procedure for four different fractions: soluble-exchangeable, organic matter-bound, carbonate-bound, and residual fractions (McGrath and Cegarra, 1992). Briefly, one gram of dried industrial soil was treated with 0.1 M $CaCl_2$ for 16 h, and the extracts were used as the soluble-exchangeable fractions of Ni. The residue was treated with 0.5 M NaOH for 16 h to obtain the organic matter bound fractions of Ni. The carbonated bound fraction was obtained after treatment with 0.05 M Na_2EDTA for 1 h, and the extracts were digested after the addition of aqua regia to determine the residual fraction of Ni.

The concentrations of Ni in all these fractions were measured using an atomic absorption spectrometer (AAS). Three replicates were measured in all cases, and the background correction was obtained using blank samples.

2.5. SEM-EDS

After completing the bioremediation experiments, industrial soil was collected and dried at room temperature for a week. The dried soil samples were mounted on an aluminum holder with a carbon conductive tape, followed by a gold coating with a thickness of approximately 200 A. The gold sputter coating was put on the carbon to increase the conductivity and reduce the charge of the specimen. The coated specimens were examined at 15 kV using an S4800 (Hitachi, Japan) electron microscope and an EDS system for elemental analysis.

2.6. Raman and FTIR spectroscopy

To understand the molecular structure change of the soil components after bacterial treatment, Raman spectrum scanning was performed at a range of 100–3500 cm^{-1} with a Raman shift interval of 0.96 cm^{-1}. Soil samples (50 mg) were washed thrice with distilled water, followed by lyophilization at −80 °C for 20 h. The sample was carefully ground in an agate mortar before the test. A trace of the prepared sample was fixed on a coverslip and subjected to Raman spectroscopy.