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Bioremediation of lead contaminated soil with *Rhodobacter sphaeroides*

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HIGHLIGHTS

- *Rhodobacter sphaeroides* showed a certain remediation effect on Pb-contaminated soil.
- More available fractions were transformed to less accessible and inert fractions.
- Pb phytoavailability was reduced in amended soils.

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ABSTRACT

Bioremediation with microorganisms is a promising technique for heavy metal contaminated soil. *Rhodobacter sphaeroides* was previously isolated from oil field injection water and used for bioremediation of lead (Pb) contaminated soil in the present study. Based on the investigation of the optimum culturing conditions and the tolerance to Pb, we employed the microorganism for the remediation of Pb contaminated soil simulated at different contamination levels. It was found that the optimum temperature, pH, and inoculum size for *R. sphaeroides* is 30–35 °C, 7, and 2×10^8 mL^{-1}, respectively. *Rhodobacter sphaeroides* did not remove the Pb from soil but did change its speciation. During the bioremediation process, more available fractions were transformed to less accessible and inert fractions; in particular, the exchangeable phase was dramatically decreased while the residual phase was substantially increased. A wheat seedling growing experiment showed that Pb phytoavailability was reduced in amended soils. Results inferred that the main mechanism by which *R. sphaeroides* treats Pb contaminated soil is the precipitation formation of inert compounds, including lead sulfate and lead sulfide. Although the Pb bioremediation efficiency on wheat was not very high (14.78% root and 24.01% in leaf), *R. sphaeroides* remains a promising alternative for Pb remediation in contaminated soil.

1. Introduction

Lead (Pb) contamination in soil is one of the major public concerns in recent years, as Pb can accumulate in plant or human body leading to irreversible damage to human health, especially for children. Such damage includes impaired development, reduced intelligence, short-term memory loss, disabilities in learning and coordination problems, and risk of cardiovascular disease (Dixit et al., 2015). Heavy metal contamination in soil can be remediated through various mobilization and immobilization techniques (Fan et al., 2012). Compared with physicochemical methods, biotechnological approaches are gaining increasing prominence in the remediation of a variety of environmental matrices because they are cost effective, environmentally friendly, and are associated with fewer side effects. They have therefore emerged as potentially useful alternative technologies for restoring contaminated sites and removing contaminants from the environment (Dhankhar and Hooda, 2011; Mani and Kumar, 2013; Merugu et al., 2014; Aryal and Liakopoulou-Kyriakides, 2015; Dixit et al., 2015; Fonti et al., 2015).

A wide variety of microorganisms (fungi, algae, bacteria, etc.) are already used as tools for heavy metal bioremediation, whose mechanisms mainly include valence transformation, volatilization and extracellular chemical precipitation (Wu et al., 2010; Marques et al., 2011). *Rhodobacter sphaeroides* is a Gram-negative, phototropic purple non-sulfur bacterium exhibiting several metabolic pathways depending on the growth conditions (Calvano et al., 2014). This versatile bacterium has drawn considerable attention

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in energy and environment researches; for example, it has been reported to be important in hydrogen production and photo-bioelectrochemical fuel cell development (Zhu et al., 2002; Rosenbaum et al., 2005; Hakobyan et al., 2012). In addition, it has been widely applied to treat wastewater because of its strong survivability under abiotic stress conditions and high tolerance to carbon starvation (Kanno et al., 2014), herbicides (Zhang et al., 2012), salt (Panwichian et al., 2010b), heavy metals (Buccolieri et al., 2006; Giotta et al., 2006; Panwichian et al., 2011; Volpicella et al., 2014), and organic and eutrophication (Nagadomi et al., 2000; Kim et al., 2004; Kantachote et al., 2005; Takeno et al., 2005; Madukasi et al., 2010; Merugu et al., 2014). The *R. sphaeroides* strains had been used to degrade various contaminants from soil and sediment mud, such as phosphorus, atrazine, salts and radionuclide (cesium), whilst the removal efficiency, impacting factors and mechanisms of which had also been discussed (Takeno et al., 1999; Du et al., 2011; Panwichian et al., 2012; Sasaki et al., 2012a, b). However, the studies concentrated on bioremediation of heavy metal in soil using *R. sphaeroides* has rarely been reported. Fan et al. (2012) and Panwichian et al. (2012) had used the strain to remove heavy metals from soil and sediment mud, respectively, and plant growth experiments were performed to evaluate the phytotoxicity after bioremediation. But, the bioremediation mechanism for heavy metal contaminated soil has not been well understood until now.

R. sphaeroides had been employed to remedy cadmium (Cd) contaminated soil in our former research (Fan et al., 2012), which concluded that the bacterium could redistribute the geo-speciation of Cd and reduce the Cd phytoavailability in amended soils. In addition, it was noticed that the geo-speciation of Pb also changed remarkably during the bioremediation process. Thus, in this paper, we further investigate the optimum culturing condition of *R. sphaeroides* and its tolerance to Pb. The remediation efficiency and mechanisms are also discussed in details.

2. Materials and methods

2.1. Isolation and identification of R. sphaeroides

The strain isolated from the oil field injection water in DaQing was identified as *R. sphaeroides* (Fan et al., 2012). Postgate C liquid medium was selected as the culture medium for *R. sphaeroides* (Postgate, 1979). The culture medium was prepared with oil field injection water. Optical density at 420 nm (OD_{420}) measured by an ultraviolet–visible spectrophotometer (UV-754) was examined for cell counting because a significant positive correlation ($r = 0.9850$, $p < 0.01$) was obtained between OD_{420} and bacterial cells (Fig. S1a).

2.2. Cultivation of R. sphaeroides

According to the typical logarithm growth curve (Logistic, $r = 0.9965$) of *R. sphaeroides* (Fig. S1b), the bacteria reaches logarithmic phase and stationary phase after 5 h and 32 h, respectively. The bacteria was sampled during the logarithmic and stationary phases (24–48 h) to conduct subsequent experiments. The growth curves of *R. sphaeroides* at different temperatures (20–40 °C), pH (5–9), and inoculum size (1×10^8–5×10^8 mL^{-1}) were investigated to determine the optimum culture condition. The pH values of the culture medium were adjusted with NaOH or HCl before autoclaving. In addition, different concentrations of $PbNO_3$ solution (0, 20, 50, 100, 150 mg L^{-1}) were prepared to study the tolerance of *R. sphaeroides* to Pb under anaerobic dark conditions for 5 d.

2.3. Bioremediation experiments

2.3.1. Background soil pre-treatment

The background soil used for bioremediation was collected from a test field in the Chinese Academy of Agriculture and Science in Beijing, China. Its characteristics and element concentrations were previously described in Fan et al. (2012). The sampled soils were air-dried, pre-treated to remove stones, gravels, and leaves and then ground to pass a 0.83-mm sieve and sub-divided. Each soil sub-sample weighed 1.5 kg and was put into a 2-L beaker.

A $PbNO_3$ spike solution was added into the beaker to simulate soil contamination. The beaker was then filled with deionized water to reach a volume of 1800 mL and maintained at room temperature for 30 d of spiking, during which the submerged soil was stirred once daily. The concentration of Pb in spiked soil was designed from 0 to 1500 mg kg^{-1}. The maximum concentration of 1500 mg kg^{-1} was set to three times of the environmental quality standard for soils in China (500 mg kg^{-1} in Grade III). There were totally seven test groups with different Pb concentration in soils, named group 1, 2, 3 4, 5, 6 and 7, respectively. As the culture medium contains sulfate, which can precipitate with Pb, so three control samples added only the same content of culture medium without *R. sphaeroides* were used to determine the influence of the culture medium in the process. Pb concentrations of control samples were 0, 500, and 1000 mg L^{-1} (the same designed concentrations as groups 1, 3, and 5) and were named $1^{\#}$, $3^{\#}$, and $5^{\#}$, respectively. The experiments were carried out in triplicates. After spiking, the equilibrated concentration of Pb in the soil was determined by acid digestion (HNO_3:$HClO_4$ = 3:2) and inductively coupled plasma-atomic emission spectrometry (ICP-AES, IRIS Intrepid-II) analysis. The designed pollution levels of Pb in soils and its content after 30 d spiking are summarized in Table 1.

2.3.2. Bioremediation process

Rhodobacter sphaeroides (CFU = 2×10^8 mL^{-1}) cultured at 30 °C, pH 7, was inoculated into 300 mL liquid culture medium and added into each beaker. Each sample was stirred, sealed, and placed in an incubator at 30 °C for 31 d. The soil was sampled at 0, 16 and 30 d. These experiments were also carried out in triplicates and the total content of Pb in soil and its speciation in collected samples were also both analyzed.

2.3.3. Wheat seedling experiments

Wheat seedling growing experiments were conducted to investigate the bioremediation performance of *R. sphaeroides*. Wheat seeds (Lunxuan 798) were purchased from the Chinese Academy of Agricultural Sciences. Soils used for wheat seeding experiments were sampled from before bioremediation (no strain and no culture medium added) and after 30 d bioremediation, including both test groups (1, 2, 3, 4, 5, 6 and 7) and control groups ($1^{\#}$, $2^{\#}$ and $3^{\#}$). A total of 100 seeds were sowed into each plastic pot where 100 g soil was put in and covered with quartz sand. The pretreatment of seeds and the wheat seedling experiments were conducted according to the procedures described in Fan et al. (2012). After 14 d, the leaves and roots of the wheat seedlings in each group were washed, separated, dried, weighed, and then acid digested with HNO_3/$HClO_4$ (3:2 v/v) at 140 °C. The Pb concentration was measured to study the changes in Pb phytoavailability in soil. The experiments were conducted in triplicates.

2.4. Analysis of Pb content and speciation

Soil samples collected during remediation were air dried after removal from beakers and then ground to pass a 0.1-mm sieve. The total content of Pb in soil was determined by acid digestion with