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Induced biotransformation of lead (II) by *Enterobacter* sp. in SO_4-PO_4-Cl solution

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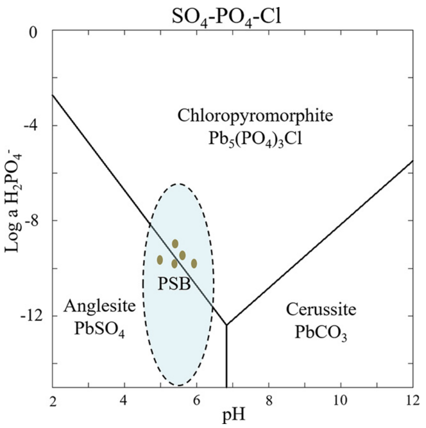
GRAPHICAL ABSTRACT



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ABSTRACT

Pb is a toxic heavy metal in contaminated soil and water, resulted from industrial activities, mine exploration, etc. Phosphate solubilizing bacteria are able to secrete organic acids and further to enhance the solubility of phosphates. *Enterobacter.* sp and geological fluorapatite (FAp) were applied to investigate the biotransformation of Pb^{2+} in solution with SO_4^{2-}, PO_4^{3-}, and Cl^- species by ICP-OES, ATR-IR, XRD, and SEM. *Enterobacter.* sp can lower pH of the medium to ~4. Meanwhile, > 90% mobile Pb (declining from 1000 to 30 ppm) was immobilized via the combination of *Enterobacter.* sp and FAp. With the addition of FAp and Pb, pyromorphite was precipitated, but with relatively low content. In contrast, abundant anglesite mineral was formed in such weakly acidic system. These anglesite crystals can even absorb phosphates particles onto their surface. Additionally, geochemical modeling confirms the formation of anglesite and cerussite under weekly acidic and alkalic condition respectively, especially when $H_2PO_4^-$ concentration $< 10^{-8}$ mM. Furthermore, the presence of Cl^- in solution leads to the formation of chloropyromorphite when $H_2PO_4^-$ concentration $> 10^{-12}$ mM, especially

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under neutral environment. This study explored the biotransformation of Pb in SO_4-PO_4-Cl aqueous system and hence provided guidance on bioremediation of Pb by bacteria and FAp.

1. Introduction

Pollution of heavy metals in aquatic as well as terrestrial ecosystem is of greatly environmental concern due to their high toxicity, i.e., its affinity for protein thiols, long-term persistence, and bioaccumulation in environment [1,2]. Lead (Pb) requires special attention as it is a serious environmental pollutant in its most chemical forms [1,3,4]. Furthermore, extensive industrial activities and widespread exploitation of mines have currently resulted in numerous cases of Pb-contaminated water, wastes, and soils [5].

Various methods of remediation have been applied to remove Pb from contaminated soils and water, including physical/chemical and biological treatments [2,3,6–8]. A well-accepted strategy is to form pyromorphite [$Pb_5(PO_4)_3X$ (X = F, Cl or OH)] via adding inorganic calcium phosphate, such as apatite [1,2,9]. Pyromorphite, with low solubility product constants [$Ksp = 10^{-(70–80)}$] in nature, is thermodynamically stable over a wide variety of environmental conditions [10]. However, anglesite ($PbSO_4$), cerussite ($PbCO_3$), lead oxalate (PbC_2O_4), and $Pb(OH)_2$ (all with relatively high solubility) are also usually formed during Pb remediation [6,11].

Apatite [$Ca_5(PO_4)_3$(F, OH, Cl)] is the most common calcium phosphate mineral on Earth [3,12–14]. Therefore, it is the primary resource for Pb remediation via the formation of pyromorphite. Geological fluorapatite (FAp) is by far the most common apatite phase [15,16], but with relatively low *Ksp* ($< 10^{-60}$) [17,18]. Acidic environment could significantly enhance the solubility of apatite [14,19,20]. There are many phosphate-solubilizing microorganisms (PSMs) which are able to produce amounts of organic acids and thereby cause acidic microenvironment [21–23]. Additionally, it is desirable for these PSMs to have resistance to Pb toxicity and remain active in Pb-contaminated soils by inducing the formation of stable Pb minerals [3,6,24,25]. The application of PSMs combined with phosphates for Pb modification, therefore, has been widely investigated [26,27].

Bacteria, the most active biological microorganism in soil, have a fast rate of reproduction. Phosphate-solubilizing bacteria (PSB) constitute from 0.5 up to 50% of the total bacteria populations in typical soils [28,29]. The activity of PSB and their subsequent effect on P solubilization are regulated by level of soluble P [30]. Although the ability to secrete organic acids from bacteria is usually one tenth of phosphate-solubilizing fungi [31], they still have great potentials to dissolve insoluble P compounds and further to induce Pb immobilization due to their high rate of reproduction.

Sulfates and chlorides are the two most common types of anions in soil. Air precipitation and fertilization increase the concentration of sulfates in current soils. In addition, PSB are able to dissolve insoluble phosphates and release more available P in the environment with abundant sulfates (sulfate is necessary for PSB incubation). Moreover, the high concentration of SO_4^{2-} in solution can drive P dissolution and P release from Fe-P minerals via anion exchange [32]. However, that how PSB assist formation of Pb minerals during the coexistence of sulfate, chloride, and phosphate remains unknown.

The aim of this study is to explore the transformation of Pb in solution containing SO_4^{2-}, PO_4^{3-}, and Cl^- by PSB. The concentrations of soluble P and Pb^{2+} were analyzed by inductively coupled plasma optic emission spectrometry (ICP-OES). In addition, attenuated total reflection infrared spectroscopy (ATR-IR), X-ray diffraction (XRD), and scanning electron microscopy (SEM) were applied to observe the mineralogical properties of the formed minerals after reaction.

2. Materials and methods

2.1. Sample preparation

The natural geological FAp rock was collected from Xiangyang, Hubei Province, China, which contains ~5 wt. % quartz and the estimated FAp content is ~90 wt. % based on our previous study [3]. FAp mineral was ground to the powder and filtered by 150 μm for the following experiments.

Enterobacter sp. is one of the most efficient PSB. The PSB strains were isolated from the soybean rhizosphere soil in Nanjing, China. They were incubated in Pikovskaya (PVK) agar medium using the serial dilution counting method. The prominent colonies were picked and purified in PVK agar medium. *Enterobacter* sp. were identified based on their microscopic, phenotypic, and morphological characteristics, and were then classified depending on their capability to solubilize P in PVK medium. After isolation from soil, *Enterobacter* sp. were selected and stored on Luria-Bertani (LB) agar medium at 4 °C.

Enterobacter sp. were activated in beef extract-peptone agar medium at 30 °C for three days. Then, 6 × 6 mm diameter inoculum plugs were cut from actively growing bacterial colonies using a sterile cork borer (autoclaved at 121 °C for 20 min). They were used as inoculum and inoculated into 100 mL liquid beef extract-peptone medium (30 °C, 180 rpm for 24 h) until the *Enterobacter* sp. achieved logarithmic growth phase. This mixed medium was denoted as SOL for the following experiments.

2.2. Solubilization of FAp and transformation of Pb induced by PSB

Abundant PO_4^{3-}, SO_4^{2-} (5.2 mM), and Cl^- (9.2 mM) were all included in PVK liquid medium which contains 10.0 g glucose, 0.03 g $MgSO_4.7H_2O$, 0.3 g $MgSO_4$. $7H_2O$, 0.3 g NaCl, 0.3 g KCl, 0.5 g $(NH_4)_2SO_4$, 0.03 g $MnSO_4.7H_2O$, and 0.5 g $Ca_3(PO_4)_2$ (TCP) in 1000 ml distilled water. The pH values were adjusted to the range of 7.2–7.4 by 0.1 M NaOH. Then, 1 mL SOL was transferred to a 250 mL Erlenmeyer flask containing 100 mL PVK liquid medium (treatment: San + TCP). The treatment incubated in modified PVK liquid medium with 0.5 g TCP substituted by 0.5 g FAp (as P source) was denoted as San + FAp. In addition, the treatment incubated in the PVK medium without FAp or TCP was denoted as San.

In parallel experiments, the treatments San + FAp + Pb and San + Pb were incubated with 1000 ppm Pb by adding $Pb(NO_3)_2$ powder, compared with the treatments of San + FAp and San respectively. The organic acids secreted by *Enterobacter* sp. in PVK liquid medium could arrived the maximum on the second day, and the soluble P concentration achieved a stable value on the third day. These five treatments were hence all shaken in an orbital incubator at 125 rpm at 30 °C in the dark for three days.

In all above treatments, soluble P and Pb^{2+} concentrations were analyzed by ICP-OES after centrifugation at 4000 rpm for 30 min and filtration through 0.22 μm membrane. The centrifugal precipitates after centrifugation and air dry were analyzed by SEM, XRD, and ATR-IR. For the SEM analyses of *Enterobacter* sp., precipitates of San + FAp were fixed in 2.5% (v/v) distilled glutaraldehyde in 0.2 M phosphate buffer (PB) (pH = 7.2, 24 h at room temperature). The pH of 0.2 M PB was adjusted using 1 M NaOH. After fixation, sample was rinsed twice in 0.2 M PB (pH = 7.2, 15 min per rinse) and then freeze-dried for 48 h.

To investigate the ability of organic acid secretion by *Enterobacter* sp., the mediums of San + TCP and San after three-days incubation were filtered and mixed with FAp powder at the ratio of 100 ml: 0.5 g.