Available online at www.sciencedirect.com

ScienceDirect

www.elsevier.com/locate/jes

JES JOURNAL OF ENVIRONMENTAL SCIENCES

www.jesc.ac.cn

Bio-remediation of acephate–Pb(II) compound contaminants by *Bacillus subtilis* FZUL-33

*Wenting Lin, Zhen Huang, Xuezhen Li, Minghua Liu, Yangjian Cheng**

The College of Environment and Resources, Fuzhou University, Fuzhou 350108, China. E-mail: jasmine1113@hotmail.com

ARTICLE INFO

Article history:
Received 10 September 2015
Revised 18 December 2015
Accepted 22 December 2015
Available online 1 February 2016

Keywords:
Acephate–Pb^{2+}
Bacillus subtilis
Biodegradation
Biomineralization

ABSTRACT

Removal of Pb^{2+} and biodegradation of organophosphorus have been both widely investigated respectively. However, bio-remediation of both Pb^{2+} and organophosphorus still remains largely unexplored. *Bacillus subtilis* FZUL-33, which was isolated from the sediment of a lake, possesses the capability for both biomineralization of Pb^{2+} and biodegradation of acephate. In the present study, both Pb^{2+} and acephate were simultaneously removed via biodegradation and biomineralization in aqueous solutions. Batch experiments were conducted to study the influence of pH, interaction time and Pb^{2+} concentration on the process of removal of Pb^{2+}. At the temperature of 25°C, the maximum removal of Pb^{2+} by *B. subtilis* FZUL-33 was 381.31 ± 11.46 mg/g under the conditions of pH 5.5, initial Pb^{2+} concentration of 1300 mg/L, and contact time of 10 min. Batch experiments were conducted to study the influence of acephate on removal of Pb^{2+} and the influence of Pb^{2+} on biodegradation of acephate by *B. subtilis* FZUL-33. In the mixed system of acephate–Pb^{2+}, the results show that biodegradation of acephate by *B. subtilis* FZUL-33 released PO_4^{3+}, which promotes mineralization of Pb^{2+}. The process of biodegradation of acephate was affected slightly when the concentration of Pb^{2+} was below 100 mg/L. Based on the results, it can be inferred that the *B. subtilis* FZUL-33 plays a significant role in bio-remediation of organophosphorus-heavy metal compound contamination.

Introduction

Lead is a toxin, accumulating in the body through the food chain, which can harm the liver, kidney, nervous system, and blood vessels (Bai et al., 2014; Beier et al., 2013; Yang et al., 2013). Especially for children, excess levels of lead in the body can cause movement disorders, reduction of attention, hyperactivity, barriers to cognitive ability and other issues, seriously affecting the growth of children and threatening human health (Adriano, 2001; Rainbow, 2002). Nowadays, people can become easily exposed to lead pollution due to its wide sources, including air, water, soil, dust and food (Gloag, 1981).

Many chemical methods have been used to reduce the bioavailability and mobility of Pb^{2+} to avoid its entry into the food chain. However, most of the chemical methods have the disadvantages of high operating cost, incomplete precipitation (Kobya et al., 2005) and inhibition of soil fertility (Lasat, 2002). Recently a number of studies on removal of lead by plants, macro-algae, fungi, and bacteria (Fein et al., 1997; Lin and Rayson, 1998; Klimmek et al., 2001; Vasconcelos and Leal, 2001; Zang et al., 2014) have been conducted. Several studies

* *Corresponding author*. E-mail: yjcheng@fzu.edu.cn (Yangjian Cheng).

http://dx.doi.org/10.1016/j.jes.2015.12.010

have reported that a variety of bacteria are considered to have the ability to affect heavy-metal speciation, bioavailability and mobility by biosorption, biomineralization and redox reactions (Rizlan et al., 2003; Gadd, 2004; Huang et al., 2002). Muthusamy et al. (2013) reported the biomineralization of lead in mine tailings by *Bacillus* sp. KK1 and indicated the versatility of autochthonous *Bacillus* sp.KK1 for bioremediation of mine tailings. Bai et al. (2014) have studied the mechanism of soil Pb^{2+} immobilization by *Bacillus subtilis* DBM and reported the ability of the strain to convert Pb^{2+} into more stable forms, which showed the value of biomineralization for phytostabilization of multi-heavy metals in soil. Huang and Liu (2013b) reported that the biosorption capacity of the bacterium *Pseudomonas* sp. LKS06 for Pb^{2+} was found to be 77.9 mg/g under optimum conditions, and that the biomass of *Pseudomonas* sp. LKS06 can be evaluated as an alternative biosorbent. Guo et al. (2010) reported the excellent adaptation ability and remediation of entophytic *Bacillus* sp. L14 at the initial Pb^{2+} concentration of 10 mg/L, which proved the superiority of this endophyte in heavy metal bio-remediation at low concentrations.

On the other hand, organophosphorus pesticides (OPs) have become common pollutants due to their extensive use in agriculture, which could be seriously harmful to humans and lead to extremely serious environment issues. Acephate, O,S-dimethyl acetylphosphoramidothioate, as one of the most widely used organophosphorus pesticides, has gained public attention. In recent years, it has been reported frequently that OPs could be degraded by various bacteria under different conditions. *Bacillus cereus*, *B. subtilis*, *Brucella melitensis*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens*, and *Serratia marcescens* are all found to have the characteristic of being able to utilize Ops as carbon sources in aqueous media (Lakshmi et al., 2008). *Pseudomonas* sp. S-2 separated from a methamidophos workroom was found to possess an enzyme that is able to degrade methamidophos (Wu et al., 2005). Ramu and Seetharaman (2014) reported that *P. aeruginosa* Is-6 has the ability to degrade acephate, methamidophos, methyl parathion, dimethoate, and malathion. Xie et al. (2008) reported that *Chryseobacterium* sp. can use acephate as the sole carbon and nitrogen sources and that the biodegradation rate of acephate by this strain was up to 87.76% at the concentration of 500 mg/L acephate.

Although studies on removal of Pb^{2+} and biodegradation of organophosphorus have been conducted respectively, bioremediation of Pb^{2+}-organophosphorus remains largely unexplored. Human activities such as misuse of pesticides and fertilizers, and mining, industrial, and vehicle emissions often lead to complex environmental problems of Pb^{2+}–acephate (Cao et al., 2010; Liu et al., 2013). Some studies on bio-remediation of multiple contaminants have been reported in recent years. Zang et al. (2014) studied the removal of acephate and Hg^{2+} with an immobilized microorganism. Wang et al. (2014) revealed that *B. subtilis* 38, a mutant species acquired by UV irradiation, could be an ideal bio-adsorbent for adsorption of multiple heavy metals. However, there are few reports on bio-remediation of Pb^{2+}–acephate so far. The objectives of this article were: (1) to investigate the process of removal of Pb^{2+} and the characteristics of biodegradation of acephate by *B. subtilis* FZUL-33 under different conditions; (2) to reveal the influence of acephate on biomineralization of Pb^{2+} and the influence of Pb^{2+} on biodegradation of acephate by *B. subtilis* FZUL-33; and (3) to prove that biodegradation of acephate by *B. subtilis* FZUL-33 releases PO_4^{3+}, which promotes mineralization of Pb^{2+}.

1. Materials and methods

1.1. Preparation of bacterial suspensions

The lead-resistant bacterium was isolated from sediment in a lake located in Fuzhou University, China. Identification of the strain was conducted by 16S rDNA sequence homology analysis. The process includes extraction of bacterial DNA according to the standard procedure of Sambrook et al. (1982), amplification of bacterial 16S rDNA via PCR with universal primers (27f: AGA GTT TGA TCM TGG CTC AG and 1492r: TAC GGY TAC CTT GTT ACG ACT T) and conducting sequence homology alignment in GenBank using the BLAST program.

The isolated bacterium was cultured in LB medium (Tryptone1%; Yeast 0.5; NaCl 1%) for 48 hr at 37°C. The bacterial cells were obtained via centrifugation at 8000 r/min for 5 min at 4°C. The supernatant was discarded and the pellet was washed with 0.9% NaCl after centrifugation.

1.2. Lead solution and analysis

The stock solution of Pb^{2+} (10,000 mg/L) was prepared by dissolving analytical grade $Pb(NO_3)_2$ in deionized water. Other concentrations of Pb^{2+} ranging from 100 to 1000 mg/L as working solutions were prepared by diluting the stock solution. The initial pH of working solutions was adjusted by adding HCl or NaOH solution. The concentration of Pb^{2+} was measured by inductively coupled plasma optical emission spectrometry (ICP-OES) (Optima 7000 DV, PerkinElmer, USA). The concentration of Pb^{2+} was analyzed at 220.356 nm and samples were analyzed within 7 days after collection (Dunham-Cheatham et al., 2011).

1.3. Acephate solution and analysis

Analytical-grade acephate (99% purity) was purchased from Micxy Reagent (Shanghai, China). A 10,000 mg/L stock solution of acephate was prepared using 30% acephate, emulsifiable, purchased from Pesticide Factory in Yongtai, Fujian, China and was used for preparing the desired concentrations of acephate in aqueous solution. Acephate residues were analyzed by a Hitachi high performance liquid chromatography (HPLC) (1260 Infinity, Agilent, USA) equipped with a C18 column (XB-C18, 5 μm, 4.6 × 250 mm, Welth, Shanghai, China). For the HPLC analysis of acephate, aliquots (1 mL) of the acephate aqueous solution were extracted by ethyl acetate (2 mL) in a rotary shaker for 2 hr at room temperature. The ethyl acetate layer was centrifuged at 8000 r/min for 10 min, dried over anhydrous Na_2SO_4 and evaporated using a stream of nitrogen (Tang and You, 2012). The samples used for injection were

prepared by dissolving residues with 2 mL of acetonitrile and filtering through a 0.45 μm syringe filter. Water–acetonitrile (95:5 by volume) mobile phase was used at a flow rate of 1 mL/min and the samples were detected at 210 nm with injection volume of 20 μL.

1.4. Analysis of phosphate

A 1000 mg/L phosphate stock solution was prepared by dissolving an accurately weighed quantity of analytical grade Na_2HPO_4 in deionized water. Standard solutions of phosphate were prepared from the phosphate stock solution. Samples were collected by filtering with a 0.45 μm microporous membrane and stored at 4°C. Phosphate residues were detected by ion chromatography with a Metrosep A SUPP 5-250 column (Dahllöf et al., 1997). Chromatographic conditions were as follows: eluent: 3.6 mmol/L Na_2CO_3 with 2% acetone; column temperature: 40°C; flow velocity: 0.7 mL/min; and pretreated samples mixed with eluent at volume ratio of 1:99 (pretreated samples: eluent) before use (Shotyk et al., 1995; Fein et al., 1997).

1.5. X-ray diffractometry

The powder samples from experiments of biomineralization for X-ray diffractometry (XRD) analysis (MiniFlex600, Rigaku, Japan) were prepared via centrifugation of bacterial suspensions, dehydrated by lyophilization and ground into a fine powder by using a mortar. The powder samples were measured under the conditions of Cu Ka incident beam monochromated by a nickel filter at a tube voltage of 40 kV and tube current of 20 mA. The diffractograms were collected by scanning the region of 2θ from 5° to 80° at 8°/min.

1.6. Batch experiments

1.6.1. The effects of pH, time and initial Pb^{2+} concentration on removal of Pb^{2+}

The effects of pH, time and initial Pb^{2+} concentration on removal of Pb^{2+} were investigated by adding the pellet (1 g/L) to 30 mL Pb^{2+} solution in 100 mL Erlenmeyer flasks and shaking at 180 r/min at 25°C for 120 min. After shaking, the pellet was separated by centrifugation. The supernatant was used for the analysis of Pb^{2+} concentration.

The pellet was added to 100 mg/L Pb^{2+} solution and shaken for 120 min at pH values ranging from 1.0 to 5.5 to determine the optimum pH for the removal of Pb^{2+}. The effect of time on removal of Pb^{2+} was determined by batch experiments conducted at regular intervals of time up to 120 min with initial Pb^{2+} concentration of 100 mg/L and pH of 5.5. The effect of initial Pb^{2+} concentration on removal of Pb^{2+} was investigated by varying the Pb^{2+} concentration at pH 5.5 and shaking for 120 min.

1.6.2. The interaction between acephate and Pb^{2+} in the process of bioremediation of acephate–Pb^{2+} by bacteria

The influence of acephate on removal of Pb^{2+} by bacteria was researched by adding the pellet to a mixed solution of acephate (380 mg/L) and Pb^{2+} (100 mg/L) at pH 5.5, shaking at 180 r/min at 25°C for 2 days. The influence of Pb^{2+} on biodegradation of acephate by bacteria was researched by adding the pellet to mixed solutions with different concentrations of Pb^{2+} and acephate (380 mg/L) at pH 5.5, shaking at 180 r/min at 25°C for 2 days.

2. Results and discussion

2.1. Removal of Pb^{2+} by B. subtilis FZUL-33

The isolated strain FZUL-33 was identified as *B. subtilis* based on its 16S rDNA sequence homology analysis. The removal of Pb^{2+}, as affected by pH, interaction time and Pb^{2+} concentration, was investigated in this section. The results are shown in Fig. 1. The pH played a significant role in the process of removal of Pb^{2+}. The biosorption of Pb^{2+} rose as the pH value increased, due to the metal ions competing with protons for available binding sites on the cell surfaces at lower pH values (Fig. 1a). The experiments were only conducted at pH values ranging from 1.0 to 5.5, because Pb^{2+} begins to precipitate at pH 6.0 due to the high concentration of OH^- ions. As Fig.1b shows, the process of removal of Pb^{2+} by *B. subtilis* FZUL-33 was very fast, and it reached equilibrium within 10 min. The removal amount of Pb^{2+} by *B. subtilis* FZUL-33 rose as the initial Pb^{2+} concentration increased from 100 to 1300 mg/L in the solution. Maximum removal was reached when the initial Pb^{2+} concentration was 1300 mg/L (Fig. 1c). The maximum removal volume of Pb^{2+} by this strain was 381.31 ± 11.46 mg/g under the conditions of pH 5.5, initial Pb^{2+} concentration of 1300 mg/L, interaction time of 10 min and temperature of 25°C.

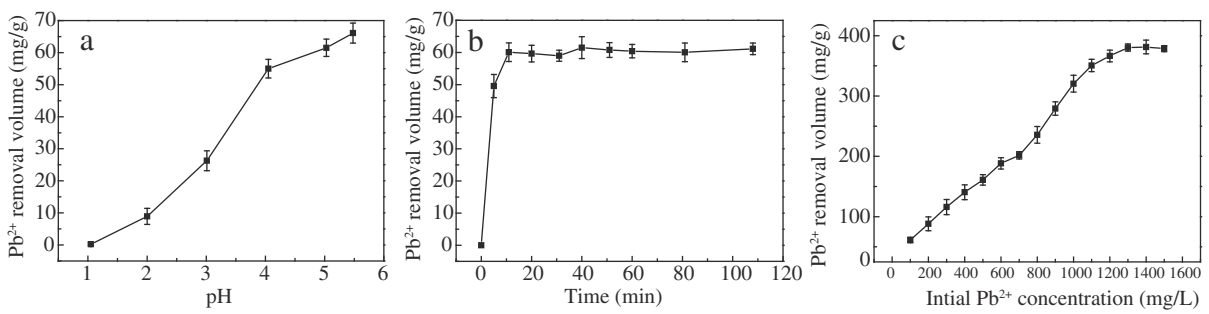

Fig. 1 – The bacteria removal of Pb^{2+} effects by pH (a), interaction time (b) and Pb^{2+} concentration (c).

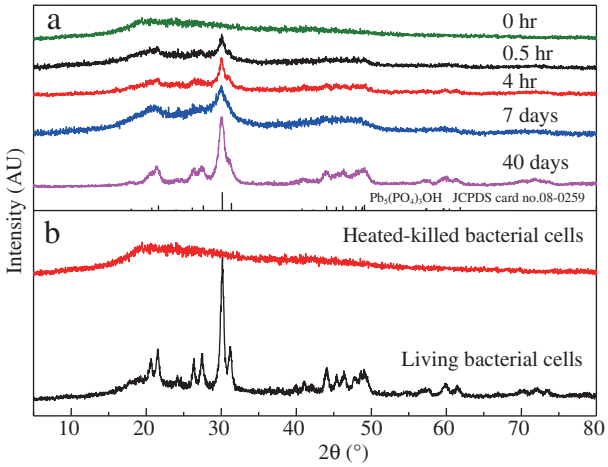

Fig. 2 – X-ray diffraction (XRD) diffractograms of *Bacillus subtilis* FZUL-33 after and before mixing with Pb^{2+}: (a) the connection between contact time and biomineralization of Pb^{2+}; (b) the effect of bacterial cell activity on biomineralization of Pb^{2+}.

2.2. X-ray diffraction analysis

After interaction between Pb^{2+} and bacterial cells, the pellets were collected at different times for XRD analysis. As Fig. 2a shows, no distinct pattern was observed at the beginning. After mixing bacteria cells with Pb^{2+} for 0.5 hr, an XRD pattern of peaks corresponding to pyromorphite ($Pb_5(PO_4)_3OH$) (JCPDS card no. 08-0259) became gradually apparent. The pattern became more apparent as the time increased. Conversion of the amorphous phase to the crystal phase required only 0.5 hr, which indicates that the process of the Pb^{2+} biomineralization by *B. subtilis* FZUL-33 occurs rapidly.

Fig. 2b shows that the heat-killed *B. subtilis* FZUL-33 could not form $Pb_5(PO_4)_3OH$, which suggests that the activity of bacteria is required for biomineralization of Pb^{2+}. In addition, the acid stability of biomineralization products was investigated. It was found that the deposit still remained stable after immersion in 10^{-3} mol/L HCl and 5×10^{-4} mol/L H_2SO_4 solution, which is of significance for environmental problems.

2.3. The influence of acephate on removal of Pb^{2+} by *B. subtilis* FZUL-33

In the mixed system of acephate–Pb^{2+}, the process of biodegradation of acephate by *B. subtilis* FZUL-33 reached equilibrium within 24 hr and the degradation rate was 81.7% (Fig. 3). In addition, the increase of phosphate concentration tracked the release of phosphate group after degradation of organophosphorus. The process of removing Pb^{2+} by the strain reached equilibrium within 12 hr and the removal rate of Pb^{2+} was 87.4%, which was increased by 20% compared with the control without acephate. The rise in $Pb_5(PO_4)_3OH$ concentration also matched the reduction of Pb^{2+} during the 0–6 hr period, which is the same as the reaction of bacteria and Pb^{2+} without acephate. Further, it was found that the concentration of acephate decreased greatly and the phosphate group concentration increased greatly in the 6–12 hr range, while the Pb^{2+} concentration reduced from 32.73 to 12.65 mg/L and the $Pb_5(PO_4)_3OH$ concentration increased from 102.53 to 141.97 mg/g simultaneously (Fig. 3). The results suggest that some part of the phosphate and Pb^{2+} transformed to $Pb_5(PO_4)_3OH$ during this phase.

As can be seen in Fig. 4, in period from 0 to 6 hr, the concentration of Pb^{2+} in both groups (control group and experimental group) decreased greatly. From 6 to 12 hr, the concentration of Pb^{2+} in the control group (without acephate)

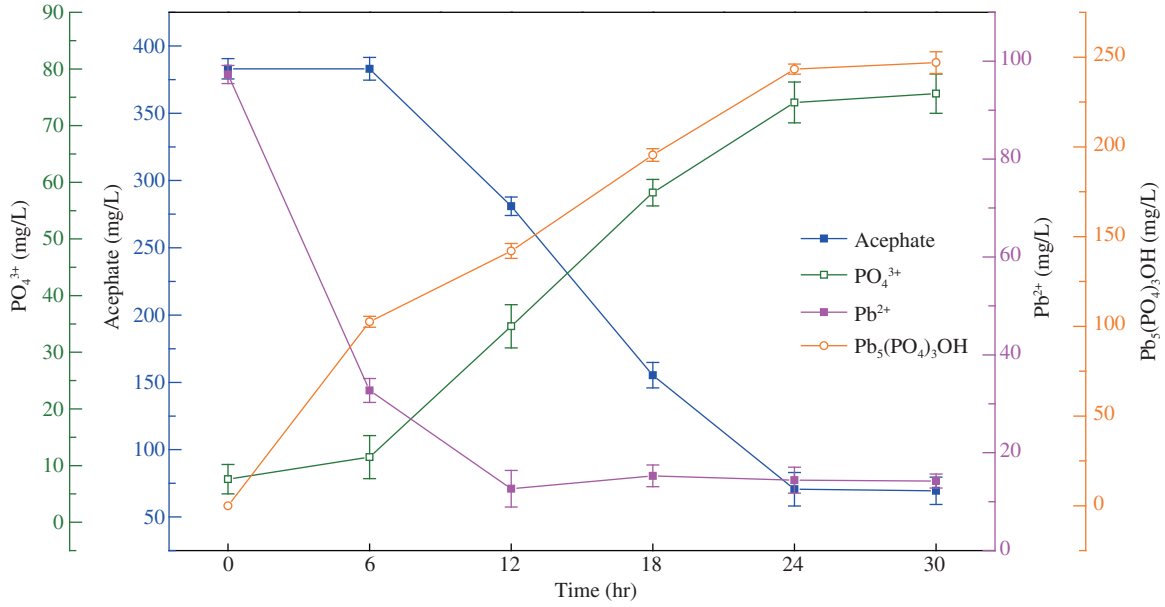

Fig. 3 – Variety of concentration of acephate, phosphate, Pb^{2+} and $Pb_5(PO_4)_3OH$ in mixed system of Pb^{2+}–acephate mixing with bacteria. (pH: 5.5; contact time: 30 hr; temperature: 25°C; initial Pb^{2+} concentration: 100 mg/L; initial acephate concentration: 380 mg/L).

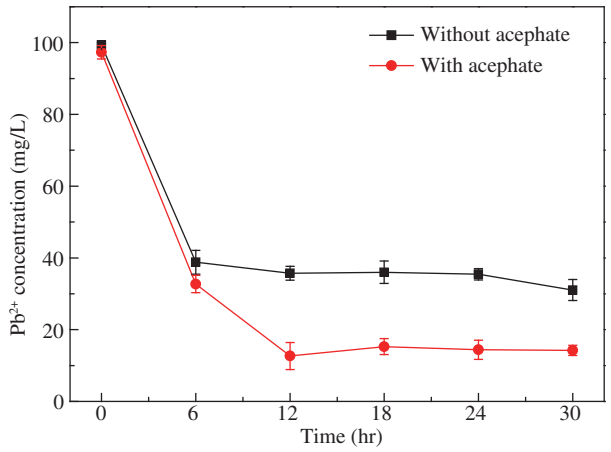

Fig. 4 – The processes of removing Pb^{2+} by *Bacillus subtilis* FZUL-33 with acephate and without acephate. (pH: 5.5; contact time: 30 hr; temperature: 25°C; initial Pb^{2+} concentration: 100 mg/L).

did not decrease any further, while the concentration of Pb^{2+} in the experimental group (with acephate) still showed an obvious decline. Consequently, it can be inferred that the bioremediation process by the strain in the mixed acephate–Pb^{2+} system could be divided into 2 phases. In Phase 1, a certain amount of Pb^{2+} was removed in a short time in this mixed system; in Phase 2, the PO_4^{3-} released from the biodegradation of acephate occurring later contributed to the biomineralization of Pb^{2+}, which improved the removal of Pb^{2+} in this mixed system.

2.4. *The influence of Pb^{2+} on biodegradation of acephate by B. subtilis*

In the mixed acephate–Pb^{2+} system, when bacteria was added, the results show that the variation of acephate concentration was affected slightly when the Pb^{2+} concentration was less than 100 mg/L, and the acephate concentration significantly decreased within 24 hr. When the Pb^{2+} concentration added reached 200 mg/L, the reduction of acephate also decreased obviously, and when it reached 300 mg/L, there was almost no reduction of acephate (Fig. 5a). Similarly, the variation of phosphate concentration showed no significant change in the mixed acephate–Pb^{2+} system with bacteria cells. When Pb^{2+} concentrations were 0 or 100 mg/L, the phosphate concentration significantly increased within 24 hr; and the concentration of phosphate had no change when concentration of Pb^{2+} added reached 300 mg/L, which provided evidence that acephate cannot be degraded under the condition of 300 mg/L Pb^{2+} in solution. Thus it could be deduced that high concentrations of Pb^{2+} could inhibit the biodegradation of acephate by *B. subtilis* FZUL-33.

3. Conclusions

This study investigated the removal of Pb^{2+} and acephate by *B. subtilis* FZUL-33 respectively and focused on the removal of acephate–Pb^{2+} mixture by the strain. The conclusions we made were: (1) The maximum removal of Pb^{2+} of *B. subtilis* FZUL-33 was 381.31 ± 11.46 mg/g under optimal conditions. (2) This strain has the ability for biomineralization of Pb^{2+}. The product biomineralization is $Pb_5(PO_4)_3OH$, and the crystals become more apparent as the time increases. (3) In the mixed acephate–Pb^{2+} system combined with *B. subtilis* FZUL-33, the PO_4^{3-} released from the biodegradation of acephate contributed to the biomineralization of Pb^{2+}, and the process of biodegradation of acephate was inhibited when the concentration of Pb^{2+} reached 300 mg/L. (4) The activity of bacteria is required for the biomineralization and the product of biomineralization has the characteristic of acid stability. The results reveal that *B. subtilis* FZUL-33 has great ability for removal of acephate and Pb^{2+} simultaneously, which showed its potential for application in bio-remediation of compound pollution.

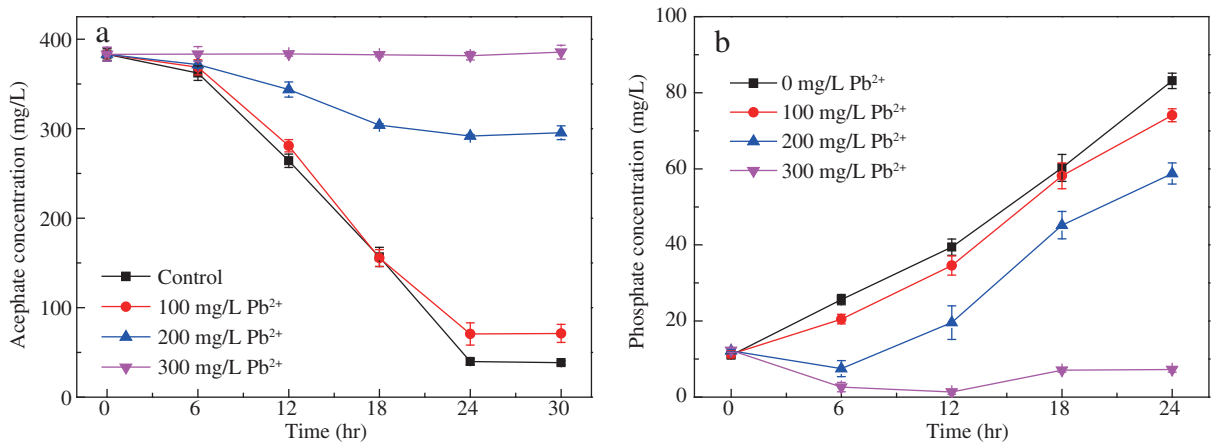

Fig. 5 – The influence of Pb^{2+} on biodegradation of acephate by *Bacillus subtilis*. (a) The acephate concentrations change over time in the processes of biodegradation of acephate by *Bacillus subtilis* FZUL-33; (b) the phosphate concentrations change over time in the processes of biodegradation of acephate by *Bacillus subtilis* FZUL-33.

Acknowledgments

This work was supported by the National Basic Research Program (973) of China (No. 2014CB846003) and the National Natural Science Foundation of China (Nos. 41372346; 21577018).

REFERENCES

Adriano, D.C., 2001. Trace Elements in Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals. Springer.

Bai, J., Yang, X.H., Du, R.Y., Chen, Y.M., Wang, S.Z., Qiu, R.L., 2014. Biosorption mechanisms involved in immobilization of soil Pb by *Bacillus subtilis* DBM in a multi-metal-contaminated soil. J. Environ. Sci. 26, 2056–2064.

Beier, E.E., Maher, J.R., Sheu, T.J., Cory-Slechta, D.A., Berger, A.J., Zuscik, M.J., et al., 2013. Heavy metal lead exposure, osteoporotic-like phenotype in an animal model, and depression of Wnt signaling. Environ. Health Perspect. 121, 97–104.

Cao, H.B., Chen, J.J., Zhang, J., Zhang, H., Qiao, L., Men, Y., 2010. Heavy metals in rice and garden vegetables and their potential health risks to inhabitants in the vicinity of an industrial zone in Jiangsu, China. J. Environ. Sci. 22, 1792–1799.

Dahllöf, I., Svensson, O., Torstensson, C., 1997. Optimising the determination of nitrate and phosphate in sea water with ion chromatography using experimental design. J. Chromatogr., A 771, 163–168.

Dunham-Cheatham, S., Rui, X., Bunker, B., Menguy, N., Hellmann, R., Fein, J., 2011. The effects of non-metabolizing bacterial cells on the precipitation of U, Pb and Ca phosphates. Geochim. Cosmochim. Acta 75, 2828–2847.

Fein, J.B., Daughney, C.J., Yee, N., Davis, T.A., 1997. A chemical equilibrium model for metal adsorption onto bacterial surfaces. Geochim. Cosmochim. Acta 61, 3319–3328.

Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma 122, 109–119.

Gloag, D., 1981. Sources of lead pollution. Br. Med. J. 282, 41–44.

Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., et al., 2010. Bioremediation of heavy metals by growing hyperaccumulator endophytic bacterium *Bacillus* sp. L14. Bioresour. Technol. 101, 8599–8605.

Huang, W., Liu, Z.M., 2013b. Biosorption of Cd(II)/Pb(II) from aqueous solution by biosurfactant-producing bacteria: isotherm kinetic characteristic and mechanism studies. Colloids Surf. B: Biointerfaces 105, 113–119.

Huang, Q.Y., Chen, W.L., Guo, X.J., 2002. Sequential fractionation of Cu, Zn and Cd in soils in the absence and presence of rhizobia. Proceedings of 17th WCSS, August 14–21, Thailand, p. 1453.

Klimmek, S., Stan, H.J., Wilke, A., Bunke, G., Buchholz, R., 2001. Comparative analysis of the biosorption of cadmium, lead, nickel, and zinc by algae. Environ. Sci. Technol. 35, 4283–4288.

Kobya, M., Demirbas, E., Senturk, E., Ince, M., 2005. Adsorption of heavy metal ions from aqueous solutions by activated carbon prepared from apricot stone. Bioresour. Technol. 96, 1518–1521.

Lakshmi, C.V., Kumar, M., Khanna, S., 2008. Biotransformation of chlorpyrifos and bioremediation of contaminated soil. Int. Biodeterior. Biodegrad. 62, 204–209.

Lasat, M.M., 2002. Phytoextraction of toxic metals: a review of biological mechanisms. J. Environ. Qual. 31, 109–120.

Lin, S., Rayson, G.D., 1998. Impact of surface modification on binding affinity distributions of *Datura innoxia* biomass to metal ions. Environ. Sci. Technol. 32, 1488–1493.

Liu, X.M., Song, Q.J., Tang, Y., Li, W.L., Xu, J.M., Wu, J.J., et al., 2013. Human health risk assessment of heavy metals in soil–vegetable system: a multi-medium analysis. Sci. Total Environ. 463-464, 530–540.

Muthusamy, G., Kui-Jae, L., Min, C., Jae Su, K., Seralathan, K.K., Byung-Taek, O., 2013. Significance of autochthonous *Bacillus* sp. KK1 on biomineralization of lead in mine tailings. Chemosphere 90, 2267–2272.

Rainbow, P.S., 2002. Trace metal concentrations in aquatic invertebrates: why and so what? Environ. Pollut. 120, 497–507.

Ramu, S., Seetharaman, B., 2014. Biodegradation of acephate and methamidophos by a soil bacterium *Pseudomonas aeruginosa* strain Is-6. J. Environ. Sci. & Heal. 49, 23–34.

Rizlan, B.L., Leckie, J.O., Bargar, J.R., 2003. Fate of uranyl in a quaternary system composed of uranyl, citrate, goethite, and *Pseudomonas fluorescens*. Environ. Sci. Technol. 37, 3555–3559.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1982. Molecular cloning: a laboratory manual. Cold Spring Harb. Lab.

Shotyk, W., Immenhauser-Potthast, I., Vogel, H.A., 1995. Determination of nitrate, phosphate and organically bound phosphorus in coral skeletons by ion chromatography. J. Chromatogr., A 706, 209–213.

Tang, M.Q., You, M.S., 2012. Isolation, identification and characterization of a novel triazophos-degrading *Bacillus* sp. (TAP-1). Microbiol. Res. 167, 299–305.

Vasconcelos, M.T.S.D., Leal, M.F.C., 2001. Seasonal variability in the kinetics of Cu, Pb, Cd and Hg accumulation by macroalgae. Mar. Chem. 74, 65–85.

Wang, T., Sun, H., Mao, H., Zhang, Y.F., Wang, C.P., Zhang, Z.Y., et al., 2014. The immobilization of heavy metals in soil by bioaugmentation of a UV-mutant *Bacillus subtilis* 38 assisted by NovoGro biostimulation and changes of soil microbial community. J. Hazard. Mater. 278, 483–490.

Wu, X.X., Wu, Z.G., Wang, Y.M., Dong, C., Zhang, L.P., Shi, Y.M., et al., 2005. Study on degradation of methamidophos by *Pseudomonas* sp.S-2. Biotech 15, 77–79.

Xie, K.Z., Xu, P., Chen, Z., J.S., Tang, S.H., Zhang, F.B., Yang, H.H., et al., 2008. Isolation and identification acephate-degrading bacteria XP-3 and studies on its physiological characterization. Guangdong Agric. Sci. 8, 79–82.

Yang, X.F., Shen, Z.G., Zhang, B., Yang, J.P., Hong, W.X., Zhuang, Z.X., et al., 2013. Silica nanoparticles capture atmospheric lead: implications in the treatment of environmental heavy metal pollution. Chemosphere 90, 653–656.

Zang, S.Y., Yin, L.L., Ma, Y., Wang, T.T., Wu, H.X., 2014. Removal of acephate and Hg^{2+} in compounding wastewater with immobilized microorganism. Adv. Mater. Res. 838, 2400–2403.