JOURNAL OF ENVIRONMENTAL SCIENCES 45 (2016) 94–99

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