Environmental Pollution 242 (2018) 778–787

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Uranium adsorption and subsequent re-oxidation under aerobic conditions by *Leifsonia* sp. - Coated biochar as green trapping agent[☆]

Lei Ding [a], Wen-fa Tan [a, b, *], Shui-bo Xie [b, c], Kathryn Mumford [d], Jun-wen Lv [a], Hong-qiang Wang [a], Qi Fang [a], Xiao-wen Zhang [a], Xiao-yan Wu [a], Mi Li [a]

[a] *Environmental Protection and Safety Engineering, University of South China, Hengyang 421001, China*
[b] *Key Laboratory of Pollution Control and Resource Technology of Hunan Province, Hunan University, Hengyang 421001, China*
[c] *Key Laboratory of Uranium Metallurgy and Biotechnology, University of South China, Hengyang 421001, China*
[d] *Department of Chemical and Biomolecular Engineering, The University of Melbourne, Australia*

ARTICLE INFO

Article history:
Received 5 May 2018
Received in revised form
11 July 2018
Accepted 11 July 2018
Available online 17 July 2018

Keywords:
Uranium adsorption
Re-oxidation
Leifsonia sp.
Trapping agent
Aerobic conditions

ABSTRACT

It has generally been assumed that the immobilization of U(VI) via polyphosphate accumulating microorganisms may present a sink for uranium, but the potential mechanisms of the process and the stability of precipitated uranium under aerobic conditions remain elusive. This study seeks to explore the mechanism, capacity, and stability of uranium precipitation under aerobic conditions by a purified indigenous bacteria isolated from acidic tailings (pH 6.5) in China. The results show that over the treatment ranges investigated, maximum removal of U(VI) from aqueous solution was 99.82% when the initial concentration of U(VI) was 42 μM, pH was 3.5, and the temperature was with 30 °C much higher than that of other reported microorganisms. The adsorption mechanism was elucidated via the use of SEM-EDS, XPS and FTIR. SEM-EDS showed two peaks of uranium on the surface. A plausible explanation for this, supported by FTIR, is that uranium precipitated on the biosorbent surfaces. XPS measurements indicated that the uranium product is most likely a mixture of 13% U(VI) and 87% U(IV). Notably, the reoxidation experiment found that the uranium precipitates were stable in the presence of Ca^{2+} and Mg^{2+}, however, U(IV) is oxidized to U(VI) in the presence of NO_3^- and Na^+ ions, resulting in rapid dissolution. It implies that the synthesized *Leifsonia* sp. coated biochar could be utilized as a green and effective biosorbent. However, it may not a good choice for in-situ remediation due to the subsequent re-oxidation under aerobic conditions. These observations can be of some guiding significance to the application of the bioremediation technology in surface environments.

1. Introduction

Decades of anthropogenic activities including uranium mining and milling have produced vast quantities of waste ores and mill tailings, leaving a legacy of environmental contamination (Gavrilescu et al., 2009; Santos-Frances et al., 2018). Soils and waters that are heavily contaminated with uranium within these environments contain many kinds of microorganisms (Qiu et al., 2011; Favas et al., 2016; Pacheco et al., 2018).

These microorganisms possess interesting mechanisms for interactions between uranium and the environment. It is assumed that the ability of many microorganisms to accumulate and immobilize uranium may offer an efficient alternative to conventionally used approaches, such as physical screening, extraction and ion exchange for remediation of uranium contaminated environments (Pollmann et al., 2006).

Enzymatically catalyzed reduction is amongst the most popular discovered mechanisms (Lovley and Phillips, 1992; Lovley et al., 1991). Here, bacteria, such as *Escherichia coli* and sulfate-reducing bacteria, reduce soluble hexavalent uranium to the tetravalent state, resulting in the precipitation of the less toxic mineral uraninite (UO_2). However, it is known that UO_2 is stable only in reducing environments (Hua et al., 2018). Previous researches indicated that UO_2 is easily reoxidized by oxygen, nitrate, and other oxidative species to form aqueous uranyl complexes in oxidizing environments (Fredrickson et al., 2002; Luo and Gu, 2011; Sani et al., 2005;

[☆] This paper has been recommended for acceptance by Dr. Jorg Rinklebe.

[*] Corresponding author. Environmental Protection and Safety Engineering, University of South China, Hengyang 421001, China.
E-mail address: 430000471800@usc.edu.cn (W.-f. Tan).

https://doi.org/10.1016/j.envpol.2018.07.050
0269-7491/

Senko et al., 2002; Spycher et al., 2011; Wan et al., 2005; Wang et al., 2013; Wu et al., 2006). Therefore, its long term effectiveness is constrained by the prevailing oxidation conditions in soil or water surface areas.

Alternatively, some microorganisms, like the genera *Pseudomonas* and *Bacillus*, have been investigated for their potency to immobilize U(VI) as uranyl phosphate minerals. In contrast to reduced UO_2, they are not susceptible to reoxidations in oxidizing contaminated environments over a broad pH range. Also, uranium phosphate minerals have been identified in sediments and the Hanford 300 Area facility, WA in the US (Stubbs et al., 2006; Wellman et al., 2007; Catalano et al., 2005; Arai et al., 2007), among others. Rufyikiri et al. (2006) investigated the effect of phosphorus fertilization in the range 0–500 mg P kg^{-1} soil in terms of U extractability and U uptake. It is demonstrated that adding phosphorus resulted in reduced U solubility and extractability in soil, thus decreasing risks of U dispersion. However, researches showed that it is not an efficient strategy by direct addition of inorganic phosphate to contaminated environments, as it readily adsorbs to soils and precipitates as minerals, and then ultimately decreases the hydraulic conductivity of soils. Thus, research has turned to stimulate microbially-mediated phosphate production such as glycerol-2-phosphate (Beazley et al., 2007; Martinez et al., 2007; Beazley et al., 2007; Shelobolina et al., 2009). Salome et al. (2013) show that in the presence of glycerol-2-phosphate, the role of anaerobic respiration in the immobilization of uranium is based on bio-reduction and bio-mineralization under oxygen-free conditions. The authors suggest that biomineralization of U(VI)-phosphate minerals may be preferable to bioreduction in certain environments due to its utility in a wide range of chemical and redox conditions. Also, Rui et al. (2013) indicate that the ratio of bioreduced U(IV) and U(VI)-phosphate precipitates is strongly controlled by the redox status of the system, and phosphate is shown to inhibit the formation of reduced U(IV) species. Merroun et al. (2011) investigated that the autunite mineral was present on the cells of *Bacillus sphaericus* JG-7B and *Sphingomonas* sp. at pH 3.0 and 4.5 under acidic conditions.

These studies indicate that polyphosphate-accumulating microorganisms could induce the precipitation of uranium and have a role in its immobilization in the environment. However, the respective mechanisms were mainly investigated under anaerobic conditions. Potential changes in the oxidation-reduction potential (ORP) or ions present in the natural water systems may impact on the adsorption of U(VI) via competition and/or by changing ion activities (Malamis and Katsou, 2013). Hence, the study of the potential mechanisms and the subsequent re-oxidation of uranium by typical microorganisms under aerobic conditions are of great importance.

In the present study, an indigenous phosphoric bacteria was purified in order to determine the feasibility of polyphosphate-accumulating microorganisms (PAOs) as a trapping agent of uranium in aerobic environments. The microorganisms were isolated from a uranium mine waste pile and examined for its uranium bioremoval ability. From this, the best performing strain was selected and further purified. Prior to the U(VI) removal experiments, the selected strain was blended with biochar to synthesize a composite trapping agent (PAOs-BC) in order to accommodate adsorption capacity and stability. In addition, the re-oxidation of uranium precipitate with different concentrations of HNO_3 and ions was elucidated. The overall novelty objective of this study lies in the fact that: i) a new bio-trapping agent was synthesized; ii) the adsorption stability and its mechanism of depositing uranium on the compound sorbent were analysed under different aerobic conditions.

2. Materials and methods

2.1. Isolation and culture of indigenous bacteria

The microorganisms used in this study were initially isolated from acidic uranium tailings (pH 6.5) of a tailings pond near the city of Hengyang, China. A mixed culture of bacteria was obtained from 10 g of collected tailings by inoculating 100 mL of sterile basal mineral medium which was prepared according to Xu et al.'s study (Xu et al., 2017). The glassware used was pre-washed.

2.2. Purification, selection and identification of indigenous bacteria

Microorganisms were purified by using the ten times dilution method to obtain individual bacterial colonies in preparation for the 16S rRNA sequence identification. The different diluted cell suspensions were incubated onto nutrient agar plates for 24 h at 25 °C. A purified bacterium was used to test for its ability of uranium adsorption, with the strain with the highest removal selected for the preparation of the sorbent material.

Fingerprinting was performed and 16S rRNA genes were then amplified by 27F and 1492R primers. The polymerase chain reaction (PCR) products were analyzed by electrophoresis, recovered, sequenced, and then analyzed by searching of the National Center for Biotechnology Information gene library (Chabalala and Chirwa, 2010).

2.3. The synthesis of selected strains and biochar

Biochar was produced by pyrolysis of sawdust at 600 °C in a pipe type oven. The resulting chars were allowed to cool to room temperature under nitrogen atmosphere.

The previously isolated cultures (PAO) were grown in 100 ml of beef extract peptone medium in a 300-ml flask mixed with 0.3g biochar (BC) to synthesize PAOs-BC, with continuous shaking (150 rpm) and at a temperature of 30 °C. In order to obtain a sufficient amount after separation from the growth medium, the cultures were grown for 18 h. The PAOs-BC underwent lyophilization after filtration and separation, and was then used in the removal experiments.

2.4. Batch U(VI) removal and re-oxidation experiments

The stock U(VI) solution (4201 μM) was prepared according to (Wang et al., 2012). Uranium working solutions having concentrations of 8.4–126.05 μM for uranium removal experiments were obtained by diluting 4201 μM stock solution as required.

Experiments were performed with 100 mL Erlenmeyer flasks. 0.05 g dry weight of PAOs-BC and 50 mL of 42 μM U(VI) solution were added to each flask and the flasks were put on the shaker and kept shaken at 150 rpm and 30 °C for 12 h. All experiments were conducted in triplicate and repeated three times.

The effects of the initial pH of solution, contact time, concentration of initial uranium and biomass dosage were analyzed. The effect of initial pH ranging from 2.5 to 7.5 of solution on the adsorption processing of U(VI) onto the immobilized PAOs-BC was investigated, the pH of the working solutions was adjusted initially to the required value with HCl (0.1 M) or NaOH (0.1 M) and not controlled during the experiment.

The effect of adsorption contact time on the removal rate of U(VI) onto the immobilized PAOs-BC was investigated at an initial pH of 3.5. At prescribed time intervals, samples were withdrawn and centrifuged at 15,000 rpm for 1 min and the liquid supernatant was taken to determine concentration of U(VI).

The concentration of the initial U(VI) ranging from 8.4 to