International Biodeterioration & Biodegradation 94 (2014) 12–18

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

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

Influence of an arsenate-reducing and polycyclic aromatic hydrocarbons-degrading *Pseudomonas* isolate on growth and arsenic accumulation in *Pteris vittata* L. and removal of phenanthrene

Tiancai Feng [a], Hanping Lin [a], Qiaofeng Guo [b], Yaoyu Feng [a, *]

[a] State Environmental Protection Key Laboratory of Environmental Risk Assessment and Control on Chemical Process, School of Resources and Environmental Engineering, East China University of Science and Technology, Shanghai 200237, China
[b] Yangtze River Pharmaceutical Group Shanghai Haini Pharmaceutical Co., Ltd, 3999 Hunan Road, Shanghai 201318, China

ARTICLE INFO

Article history:
Received 21 March 2014
Received in revised form
25 May 2014
Accepted 9 June 2014
Available online 9 July 2014

Keywords:
Co-contamination
Phenanthrene
Arsenic
Pteris vittata
Bioremediation

ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) and arsenic often co-occur at polluted sites, but remediation strategies for this are scarce. In this study, the effect of bacterial inoculation on plant growth and arsenic uptake by *Pteris vittata* and phenanthrene dissipation was investigated hydroponically using an arsenate-reducing and PAH-degrading *Pseudomonas* isolate. In a 12-d experiment, despite reduced dry weight in some cases, the isolate generally promoted the growth of *P. vittata*. The aboveground and belowground biomass increased by 21.0–38.7% and 3.5–66.3%, respectively. In addition, bacterial inoculation greatly enhanced arsenic uptake by *P. vittata* compared to un-inoculated treatments (from 246.7–438.9 and 102.6–231.4 to 754.1–1425.7 and 121.5–351.4 mg kg^{-1} As in aboveground and belowground biomass, respectively). Accordingly, arsenic transfer factor increased by 116–315%. The enhancement was attributed to the bacteria-mediated As(V) reduction in growth media. A dissipation of phenanthrene from growth media was observed and attributed to the degradation of the chemical by the isolate, as the contribution of *P. vittata* in phenanthrene removal was negligible. The present results demonstrated the versatile arsenate-reducing and PAH-degrading bacteria can effectively enhance arsenic uptake and translocation by *P. vittata* and remove phenanthrene.

© 2014 Elsevier Ltd. All rights reserved.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) and arsenic (As) are listed as priority pollutants by USEPA, and are of great health concerns due to their documented acute toxicity, mutagenicity, and carcinogenicity (Bhattacharya et al., 2007; Haritash and Kaushik, 2009). Anthropogenic activities, such as the simultaneous utilization of creosote oil and chromated copper arsenate (CCA) salts and incomplete combustion of fossil fuels, have significantly contributed to the co-contamination of the two categories of hazardous substances (Maier et al., 2002; Elgh-Dalgren et al., 2011). Over the past decades, the scientific literature has seen a substantial number of remediation studies on environmental pollution by arsenic or PAHs alone, employing approaches such as pollutant-stabilization and containment, and decontamination technologies (Haritash and Kaushik, 2009; Camacho et al., 2011). However, few efforts are made to remediate PAHs and arsenic co-contamination. In fact, only two decontaminating methods are available: soil washing, where single or mixtures of different additives are used to simultaneously remove PAHs and arsenic from the soil (Elgh-Dalgren et al., 2009); and phytoremediation, where arsenic-hyperaccumulator *Pteris vittata* (Chinese Brake fern) is employed to remove contaminants from co-contaminated systems (Sun et al., 2011). Nevertheless, limitations exist in both approaches. For example, soil washing often generates large amounts of soil leachates with increased toxicity. Although arsenic uptake by *P. vittata* roots and its subsequent translocation to the aboveground biomass are efficient, only a negligible PAHs phytoextraction potential was observed in *P. vittata*, which may be due to the low water solubility of PAHs (Smith et al., 2011). Thus, there is a need to develop new technologies for the remediation of arsenic and PHAs co-contamination.

The choice of remediation methods largely depend on the nature of the target contaminant. Microbial degradation is the primary removal mechanism for PAHs in polluted environment (Haritash and Kaushik, 2009), but when PAHs are present together

* Corresponding author. Tel./fax: +86 21 6425 0664.
E-mail address: yyfeng@ecust.edu.cn (Y. Feng).

http://dx.doi.org/10.1016/j.ibiod.2014.06.005
0964-8305/© 2014 Elsevier Ltd. All rights reserved.

with arsenic, the selection of an appropriate PAHs-degrader must take into consideration of its arsenic tolerance ability, since the extent and rate of PAHs dissipation might be negatively influenced if the PAH-degrader cannot withstand the arsenic stress at co-contaminated sites.

A bacterial isolate reported previously by us, *Pseudomonas* isolate PAHAs-1, showed an excellent dual capacity to degrade PAHs and reduce arsenic simultaneously (Feng et al., 2014). In addition, it has been reported that *P. vittata* can behave as As-accumulator and accumulate up to 2.3% total arsenic in its dry biomass (Ma et al., 2001). In addition to arsenic, a recent field survey showed that *P. vittata* exhibits a strong tolerance to high levels of PAHs and several other heavy metals including Pb, Cd, and Ni (Sun et al., 2011). Thus, the integrated usage of PAHAs-1 and *P. vittata* could potentially represent an alternative strategy to remediate arsenic and PAHs co-contaminated sites.

In this study, we examined the effect of PAHAs-1 inoculation on PAHs consumption and arsenic transformation in growth media, as well as growth performance and contaminant accumulation in *P. vittata* under hydroponic culture condition. The objectives were to (1) examine the potential role of microbes and plants in PAHs removal and arsenate reduction in growth media; (2) investigate the arsenic and PAHs distribution and speciation in plant tissues.

2. Materials and methods

2.1. Plants and inoculum preparation

P. vittata ferns used were kindly provided by Dr. Xiaoyong Liao, Institute of Geographic Sciences and Natural Resources Research, Chinese Academy of Science, Beijing. All plants (3–4 months old) were first transferred to 0.2 × Hoagland solution (pH 6.5) for acclimation for 2 weeks. They were then grown in a greenhouse under controlled conditions (27/19°Cday/night temperature, 12-h photoperiod with 350 μmol m^{-2} s^{-1} light intensity, and 60–70% relative humidity). The nutrient solution was aerated constantly and renewed weekly.

The arsenate-reducing and PAH-degrading *Pseudomonas* isolate PAHAs-1 was cultured in chemically defined medium (CDM) at 30 °C and 150 rpm on a rotary shaker. After 2 days of incubation, cells were harvested by centrifugation (8000 ×*g*, 10 min), washed three times with fresh Hoagland solution, and re-suspended in solution. The cell suspension was used as the inoculum, and the inoculation level was approximately 10^7 colony-forming units ml^{-1} growth solution.

2.2. Experimental design

P. vittata plants were cultured in opaque containers through drill holes in the cap in 1 × culture solution spiked with different concentrations of As(V) (as $Na_3AsO_4.12H_2O$; Beijing Chemical Reagent Co., China) and phenanthrene (Fluka, Germany). The experiment consisted of three As(V) levels [As(0): As(V) at 0 mM; As(0.5): As(V) at 0.5 mM; As(1): As(V) at 1 mM)], and two phenanthrene levels [(PHE(25): phenanthrene at 25 mg l^{-1}; PHE(75): phenanthrene at 75 mg l^{-1})], and 5 treatment groups: the culture solution spiked with different concentrations of As(V) and phenanthrene without bacterial inoculation and plant cultivation (CK), bacterial inoculation (B), plant cultivation (P), B + P, negative controls (plants exposed to solution without arsenic and phenanthrene). Methanol was used as co-solvent, and its concentration in all solutions was 0.1% (v/v). The solution was sonicated for 2 h to obtain a homogeneous suspension. Efforts were made to ensure visual uniformity of ferns used in all experiments. Two plants were cultivated per pot with three replicates for each treatment. Any solution loss in containers during the experiment was replenished with the same volume of de-ionized water every day. The greenhouse conditions were the same as described above. Aqueous samples were taken at day 3, 8 and 12. Plants were harvested and weighed after 12 d exposure. Dry weights are used throughout the report unless otherwise specified.

2.3. Chemical analyses

Upon harvest, the roots were shaken gently to release particulate phenanthrene on their surface into the growth media. The plants were then washed thoroughly with de-ionized water, and separated into the aboveground (fronds) and belowground (roots plus rhizomes). To remove adsorbed arsenic, plant roots were rinsed with ice-cold phosphate solution containing 1 mM Na_2HPO_4, 0.5 mM $Ca(NO_3)_2$ and 10 mM 2-(N-morpholino) ethanesulfonic acid (Mathews et al., 2010), and washed three times with de-ionized water. Parts of plant tissues were flash-frozen in liquid nitrogen and stored at −80 °C for arsenic arsenic species determination, parts were freeze-dried at 4 °C for phenanthrene determination, and parts were oven-dried at 65 °C to a constant weight for total arsenic analysis. The aqueous samples were analyzed immediately for arsenic and phenanthrene using methods described below.

2.3.1. Phenanthrene analysis

For the analysis of phenanthrene in plants, samples were extracted by ultrasonication with 1:1 (v/v) acetone and hexane for 1 h. The solvent layer was collected. After the replenishment of fresh extraction solvent, the samples were sonicated for another 1 h. This process was repeated three times. The extracts were combined and dried with anhydrous Na_2SO_4, then evaporated at 40 °C under gentle nitrogen flow. The residue was dissolved in hexane, followed by a clean-up procedure through a silica gel column with an elution of hexane and dichloromethane (v/v = 1:1). The eluate was exchanged by high performance liquid chromatography (HPLC) grade methanol, and analyzed by HPLC. The HPLC analysis was performed on a Shimadzu LC-2010HT equipped with a UV detector and a 4.6 × 250 mm XDB-C18 column using methanol-water (80/20, v/v) as the mobile phase at a flow rate of 1 ml min^{-1} (Feng et al., 2012). The recovery of phenanthrene obtained by spiking plant samples averaged over 95% ($n = 5$, RSD < 2.91%).

For the analysis of phenanthrene in growth media, a 5-ml aliquot of the hydroponic growth media taken after a thorough mixing (Flocco et al., 2002) was diluted with 5-ml of HPLC-grade methanol, followed by filtration through a 0.22-μm syringe filter (Nuclepore Corp., CA, USA). The concentration of phenanthrene in the filtrate was measured by HPLC as described above.

2.3.2. Arsenic analysis

The level of total arsenic in plants was determined using a hot block digestion method based on USEPA Method 3050B. Samples of the oven-dried and ground plant tissue were digested with HNO_3, followed by 30% H_2O_2. For the determination of As(III) and As(V) in plants, the samples were crushed in a mortar and extracted ultrasonically twice with methanol/water (1:1, v/v) for 4 h. The residue was rinsed three times with de-ionized water. All extracts were combined and diluted as necessary.

Total arsenic concentrations in growth media and digested solution were measured by hydride generation-double channel atomic fluorescence spectrophotometry (HG-AFS; AFS-9130, Beijing Titan Instruments Co., Ltd., Beijing, China). For the selective analysis of As(III) in growth media and plant extracts, the carrier liquid in HG-AFS was replaced with 0.5 M sodium citrate buffer (pH 5.0) for hydride generation (Feng et al., 2014). Only As(III) is

Download English Version:

https://daneshyari.com/en/article/4364731

Download Persian Version:

https://daneshyari.com/article/4364731

Daneshyari.com