Environmental Pollution 242 (2018) 462–469

Contents lists available at ScienceDirect

Environmental Pollution

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

Oxidation of benzo[*a*]pyrene by laccase in soil enhances bound residue formation and reduces disturbance to soil bacterial community composition☆

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ARTICLE INFO

Article history:
Received 3 November 2017
Received in revised form
9 May 2018
Accepted 21 June 2018
Available online 6 July 2018

Keywords:
Benzo[*a*]pyrene
Bound residue
Environmental fate
Laccase
Pyrosequencing

ABSTRACT

Laccases are capable of rapidly oxidizing benzo[*a*]pyrene. It is thought that the metabolites with an increase in water solubility caused by the oxidation of benzo[*a*]pyrene may stimulate the subsequent mineralization. However, to date, there has been no experimental evidence to support this. In this study, the fate of benzo[*a*]pyrene in soil affected by laccase amendment and the resulting soil bacterial responses were investigated. Laccase amendment promoted benzo[*a*]pyrene dissipation (15.6%) from soil, accompanied by trace mineralization (<0.58 ± 0.02%) and substantial bound residue formation (~80%). An increase of ~15% in the bound residue fraction was observed by laccase amendment, which mainly resulted from covalent binding of the residues to humin fraction. During the incubation, the abundance of bacterial 16S rRNA and polycyclic aromatic hydrocarbon ring-hydroxylating dioxygenase genes did not change markedly. In contrast, benzo[*a*]pyrene treated with laccase resulted in a smaller shift in the bacterial community composition, indicating a reduced disturbance to the soil microbial communities. These results here suggest that benzo[*a*]pyrene contaminated soil can be detoxified by laccase amendment mainly due to the enhanced bound residue formation to soil organic matter via covalent binding.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are of great environmental concern in view of their potential toxicity, mutagenicity, and carcinogenicity. Over 5.2×10^5 tons year^{-1} of the priority PAHs are released globally, and most of these compounds enter the soil or sediment via atmospheric deposition (Zhang and Tao, 2009).

The high-molecular-weight (HMW) PAHs with thermodynamic stability and low water solubility are recalcitrant to microbial degradation in soil. Previous work has shown that soil bacteria effectively mineralize PAHs with up to four rings to CO_2, whereas five-ring PAHs, such as benzo[*a*]pyrene, are rarely used as the sole carbon source (Peng et al., 2008). By contrast, some fungi can degrade HMW PAHs more effectively, but mainly through polarization of metabolites rather than mineralization to CO_2 (Schmidt et al., 2010). The work in Kotterman et al. (1998) and Boonchan et al. (2000) showed that co-culture of fungi and bacteria promotes benzo[*a*]pyrene mineralization, and the fungal transformation can be considered as the initial step to breakdown PAHs while the bacteria provide intact enzymes to mineralize the PAH metabolites successively. Laccases produced by white-rot fungi rapidly oxidize benzo[*a*]pyrene forming metabolites with an increase in water solubility (Potin et al., 2004; Canas et al., 2007). It was thought that oxidization of benzo[*a*]pyrene by laccase may stimulate sequential mineralization by soil bacteria, and our previous work revealed that benzo[*a*]pyrene metabolites oxidized by

☆ This paper has been recommended for acceptance by Dr. Joerg Rinklebe.

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https://doi.org/10.1016/j.envpol.2018.06.075
0269-7491/

laccase can be successively transformed by PAH-degrading mycobacteria (Zeng et al., 2013), suggesting a natural mechanism for PAH transformation by laccase and bacteria. However, it is still unknown if co-transformation by laccase and PAH degraders promote the mineralization of benzo[*a*]pyrene. We hypothesized that the metabolites with an increase in water solubility caused by the laccase catalyzed transformation may promote the mineralization benzo[*a*]pyrene in soil via cooperation with soil bacteria.

The xenobiotics entering soil undergo complex turnover processes, such as up-take by living organisms, degradation by soil microorganisms, or immobilization as non-extractable residues (Kästner et al., 2014). Among these processes, bound residue formation is also considered a detoxification process in soil due to its stability and recalcitrance to breakdown by microbes and transport (Richnow et al., 1994; Li et al., 2015; Possberg et al., 2016). The fate of xenobiotics in soil has been investigated extensively to assess their nature and potential hazard to ecosystems. Meanwhile, due to that soil microbes are related to soil biogeochemical processes (Bardgett et al., 2008), the transformation of xenobiotics pollution in soil directly accompanied by changes in microbial communities, such as enrichment of indigenous degrading bacteria (Hazen et al., 2010) or of functional gene like that encoding aromatic ring-hydroxylating dioxygenase (Park and Crowley, 2006). In this study, a ^{14}C-labelled radioactive substrate was used to characterize the fate of benzo[*a*]pyrene affected by laccase amendment in terms of mineralization and bound residue formation. We also investigated the changes in the abundance and composition of a bacterial community in response to benzo[*a*]pyrene contaminated soil with laccase amendment.

2. Materials and methods

2.1. Chemicals

Laccase of *Trametes versicolor*, 2,2′-azino-bis-(3-ethyl-benzothiazoline-6- sulfonic acid) diammonium salt (ABTS), phenanthrene, pyrene and benzo[*a*]pyrene were obtained from Sigma-Aldrich (Shanghai, China). [7-^{14}C]benzo[*a*]pyrene (9.84×10^8 Bq mmol^{-1}) was purchased from the American Radiolabeled Chemicals, Inc., and the reported radiochemical purity was ≥99%.

2.2. Soil sampling and analysis

Soil sample was taken from the 0–10 cm layer of a farmland in Nanjing, Jiangsu Province, China (31°53′ N, 118°36' E). The soil was sieved to 5 mm and stored at 4 °C in darkness prior to use. Soil organic carbon was determined by the Tyurin method (Nelson and Sommers, 1975). Soil total carbon, total nitrogen, total phosphorus and total potassium were determined by combustion (CNS-2000; LECO, St. Joseph, MI, USA). PAHs of the soil were analyzed by ultra-fast liquid chromatography (UFLC-20 system; Shimadzu, Kyoto, Japan) (Zeng et al., 2010). The soil characteristics are listed in Table 1.

2.3. Soil incubation experiments

The experiments were performed with an indigenous microbial community (Group I) and specific exogenous microorganisms (Group II). It was previously reported that an effective microbial consortium resulted from a pre-culture was important for a rapid benzo[*a*]pyrene mineralization (Kanaly and Watanabe, 2004). Therefore, the soil (100.0 g for each) in Group I was pre-incubated with individual PAHs in a 250-ml flask, to induce diverse indigenous PAH-degrading microbial community. Treatments of Enz-Phe, Enz-Pyr and Enz-BaP were pre-incubated with 100 mg kg^{-1} phenanthrene, 100 mg kg^{-1} pyrene and 5 mg kg^{-1} benzo[*a*]pyrene, respectively; Enz was pre-incubated under the same condition without PAHs (Table 2). The soils were brought up to 60% water holding capacity using sterile dH_2O, and incubated at 28 °C in the dark for 2 months. In Group II, the soil was autoclaved (120 °C for 30 min twice on two consecutive days) and inoculated with a specific pyrene-degrading *Mycobacterium* sp. NSG-1B (Enz-bac) (Zeng et al., 2013). The preparation of mycobacterial inoculum is described previously (Zeng et al., 2010). Controls without laccase or mycobacterial inoculation were also set up, including (1) boiled enzyme (BoE) in Group I, (2) boiled enzyme and inoculation (BoE-bac) in Group II, and (3) enzyme and boiled inoculum (Enz-Kbac) in Group II. The treatments including controls were performed in triplicate.

The introduction of PAHs into the soil, including pre-incubation and ^{14}C-substrate experiments, was performed using the method in Brinch et al. (2002) to minimize the effects of solvent on soil microorganisms; that is, the solvent containing PAHs is added 20% of the soil sample, followed by evaporation of the solvent and mixing with the remaining 80% of the soil sample. In ^{14}C-substrate experiments, aliquots of acetone solution containing ^{14}C-benzo[*a*]

Table 2
Experimental treatments.

Treatments[a]	Sterilization	Pre-incubation[b]	*Mycobacterium*	Laccase
Group I:				
BoE	−	+ (CK)	−	−
Enz	−	+ (CK)	−	+
Enz-Phe	−	+ (Phe)	−	+
Enz-Pyr	−	+ (Pyr)	−	+
Enz-BaP	−	+ (BaP)	−	+
Group II:				
BoE-bac	+	−	+	−
Enz-bac	+	−	+	+
Enz-Kbac	+	−	−	+

[a] The experiment was carried out with laccase amendment cooperated with indigenous microbial community (Group I) or specific exogenous microorganism (Group II). Enz, Enz-Phe, Enz-Pyr and Enz-BaP were pre-incubated with different PAHs to induce diverse indigenous PAH-degrading microbial community, while Enz-bac was treated with a pyrene-degrading *Mycobacterium* sp. NSG-1B. BoE, BoE-bac and Enz-Kbac served as controls.

[b] Soils were pre-incubated with PAHs or without PAHs. Phe: phenanthrene (100 mg kg^{-1}); Pyr: pyrene (100 mg kg^{-1}); BaP: benzo[*a*]pyrene (5 mg kg^{-1}); CK: without PAHs.

Table 1
Soil characteristics.

pH		7.0
Organic carbon	%	0.88
Total carbon	%	1.20
Total nitrogen	%	0.12
Total phosphorus	mg g^{-1}	0.64
Total potassium	mg g^{-1}	18.3
∑16 EPA PAHs[a]	mg kg^{-1}	1.30

[a] Total 16 US Environmental Protection Agency priority (EPA) PAHs.