Chemosphere 184 (2017) 482–488

Contents lists available at ScienceDirect

Chemosphere

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

Evaluation of bacterial biodegradation and accumulation of phenanthrene in the presence of humic acid

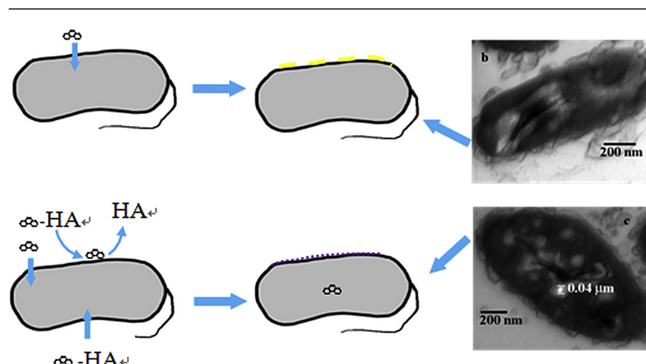
Yun Xie [a, b], Zhenggui Gu [a], H.M.S.K. Herath [c], Minfen Gu [a], Chang He [a], Fang Wang [b], Xin Jiang [b], Jie Zhang [a, **], Yinping Zhang [a, b, *]

[a] *Nanjing Normal University, School of Chemistry and Materials Science, Center for Analysis and Testing, Nanjing 210046, China*
[b] *Key Laboratory of Soil Environment and Pollution Remediation, Institute of Soil Science, Chinese Academy of Sciences, Nanjing 210008, China*
[c] *Department of Export Agriculture, Faculty of Animal Science and Export Agriculture, Uva Wellassa University, Passara Road, Badulla, 90 000, Sri Lanka*

HIGHLIGHTS

- Both biodegradation and bio-accumulation of PAHs were affected by HA.
- Addition of HA enhanced bacterial surface sorption capacity.
- The interactions between bacteria and HA favored internalizing PHE.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 January 2017
Received in revised form
17 May 2017
Accepted 7 June 2017
Available online 8 June 2017

Handling Editor: T Cutright

Keywords:
Polycyclic aromatic hydrocarbons (PAHs)
Humic acid (HA)
Bacteria
Biodegradation
Bioaccumulation

ABSTRACT

This study evaluated the effect of humic acid (HA) on physicochemical properties of bacterial surfaces and on mass transfer of polycyclic aromatic hydrocarbons (PAHs) from aqueous phase into intracellular bacteria. Due to this process' potential for bacterial degradation, using *Sphingobium* sp. PHE3, degradation of phenanthrene (PHE) was compared in HA and non-HA sets. The results showed that approximately 51.1% of PHE at a concentration of 102.0 mg L^{-1} was biodegraded in the non-HA sets, whereas almost all PHE was biodegraded with HA after 72 h. Interestingly, PHE that accumulated in the intracellular bacteria reached 3.80 mg L^{-1} for the HA sets, which was significantly higher than that of non-HA. Lipid inclusion bodies appeared when *Sphingobium* sp. PHE3 was treated with HA. The results were further confirmed by the enhanced bacterial surface sorption capacity for the HA sets. Therefore, we concluded that added HA not only act as carriers and biosurfactants facilitating PHE uptake but also adjust bacteria cell wall properties for internalizing PHE, which ultimately overcame the PHE bioavailability resulting in enhanced biodegradation.

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1. Introduction

At present, polycyclic aromatic hydrocarbons (PAHs) are increasing in the natural environment due to dry and wet

* Corresponding author. Nanjing Normal University, School of Chemistry and Materials Science, Center for Analysis and Testing, Nanjing 210046, China.
** Corresponding author.
E-mail addresses: 40389@njnu.edu.cn, 40042@njnu.edu.cn (J. Zhang), zhangyinpingnjnu@163.com (Y. Zhang).

http://dx.doi.org/10.1016/j.chemosphere.2017.06.026
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deposition caused by incomplete combustion of organic materials (Braguglia et al., 2016). Given the potential risks on human health and the ecosystem, concerns on PAHs have increasingly become important. A number of methods have been tested examined for their ability to remove PAHs from the environment (Hamdi et al., 2007; Gadd, 2009), a key finding from this research is the important role naturally available bacteria play. This is due to the bacteria using PAHs as a carbon and energy source, nevertheless PAHs still persist in the soil environments at a significant level (Yin et al., 2008; Aranda, 2016).

A comprehensive understanding on bioavailability of PAHs may depend on (i) estimation of their speciation in solution, (ii) how they interact with inorganic or organic ligands, and (iii) how these species interact with cellular targets (Gu et al., 2016). Among different PAHs, dissolved organic matter (DOM) is one of the most widely distributed complexing agents of PAHs in soil solution (Poerschmann et al., 2007). It is also known as a multi-component mixture of aromatic and aliphatic hydrocarbon structures with polar functional groups, such as carboxyl, hydroxyl, amine, amide, ketone, imidazole and sulfhydryl (Polubesova and Chefetz, 2014). These functional groups provide shuttle molecules, which facilitate toxic Hg uptake by *Escherichia coli* HMS174 (Chiasson-Gould et al., 2014) and 2-Nitrophenol reduction (Zhu et al., 2014). Over the last few decades, studies aimed on predicting bioavailability of PAHs focus on DOM, which is associate with clusters of relatively small and chemically diverse organic molecules linked by H-bonds, and polar and hydrophobic interactions (Ounnas et al., 2009). Factually, it is difficult to describe the interactions between DOM-interacted PAH and bacteria. In the aqueous phase, the DOM clusters exhibit micellar-like properties and act as one kind of surfactants. PAHs in the micellar surfactants directly interact with bacterial surface, thereby becoming more bioavailable. Simultaneously, DOM can lower biodegradation and bioavailability by reducing the concentration of freely dissolved PAHs via sorption. It is still not clear whether bacterial surface transport and uptake of DOM-interacted PAH is related to the enhancement of its bioavailability and thereby improved biodegradation.

In general, cellular uptake of PAHs is an important step for subsequent intracellular PAHs metabolism. For example, PHE enters into the cells of *Mycobacterium* sp. strain RJGII-135 by passive diffusion for intracellular metabolism (Miyata et al., 2004). As Kallimanis et al. (2007) observed, *Arthrobacter* sp. strain Sphe3 internalize PAH with two mechanisms: a passive diffusion when cells are grown on glucose, and an inducible active transport system when cells are grown on PHE as a sole carbon source. Furthermore, elimination of cell walls facilitates accessibility of substrates to the plasma membrane and results in enhanced metabolic reactions (Kallimanis et al., 2007). However, knowledge of the effects of DOM on bacterial transportation and uptake of PAHs under natural environments is still quite limited.

The present study was conducted to evaluate the hypothesis that DOM can alter bacterial cell surface properties, which facilitate the mass transfer of PAHs from aqueous phase into extracellular and intracellular bacteria, thus availing the compounds for biodegradation.

2. Materials and methods

2.1. Chemicals and materials

PHE was selected as a model PAH given its significant sorption behavior and detectable concentrations in aqueous phase with respect to the solubility (1.28 mg L^{-1}) and hydrophobicity (log K_{ow}, 4.45). It was obtained from Supelco Corporation (purity 95%, USA). Acetonitrile was purchased from Tedia Company (USA), while methanol, acetone, and all other chemicals were obtained from Nanjing Chemicals Reagent (Nanjing, China). Humic acid sodium salt (HA), a common DOM, was purchased from Beijing Kaien Company (Beijing, China). Its elemental composition (C, H, N, S), without pH adjustment, was determined by an elemental analyzer (Vario Micro, Elementar, Germany) using the high-temperature combustion method. The percentage values of C, H, N, and S were 54.32, 2.95, 1.08, and 0.47%, respectively.

2.2. Bacteria, medium, and cultivation

A PHE-degrading strain, *Sphingobium* sp. PHE3 (strain PHE3, Zhang et al., 2011a), deposited in China Center for Type Culture Collection with No. CCTCC AB 2010361, was employed to investigate the associated mechanisms of DOM on PAHs mass transfer and uptake. Mineral medium (MM) for bacterial growth was prepared as described by Zhang et al. (2011b) and the pH was adjusted to 7.0 ± 0.2. Phosphate buffered saline (PBS) was prepared following two steps: first, 7.164 g of $Na_2HPO_4 \cdot 12H_2O$ and 3.121 g of $NaH_2PO_4 \cdot 2H_2O$ were dissolved in 100.0 mL of distilled water, separately. Subsequently, the former solution was neutralized by the latter at a final pH of approximately 7.0 ± 0.2. A nutrient broth medium (NB), which contains 10.0 g peptone, 3.0 g beef extract, and 5.0 g NaCl in one liter of water, was also prepared. Biomass was produced by growing bacteria in nutrient broth medium overnight at 28 °C, and harvested at the exponential growth phase (OD_{600}, 0.8). By washing in PBS, the bacteria were re-suspended in MM with a cell density of approximately 1.6×10^8 CFU mL^{-1}.

2.3. PHE biodegradation and bioaccumulation kinetics

To unravel the effect of HA on PHE biodegradation and bioaccumulation, it was necessary to determine PHE biodegradation in the absence of HA. Therefore, PHE biodegradation experiments with non-HA was conducted. Firstly, an aliquot of 1.0 mL of PHE stock solution (2×10^3 mg L^{-1}) dissolved in acetone were pipetted into 50 mL sterile tubes. After evaporating acetone, 18.0 mL of sterile MM was added in, and 2.0 mL of re-suspended bacterial biomass was then inoculated to obtain the initial PHE concentration and bacterial density of 100.0 mg L^{-1} and 1.6×10^7 CFU mL^{-1}, respectively. Control tubes were not inoculated to evaluate PHE abiotic loss. PHE biodegradation kinetic experiments in the HA sets followed as similar protocol as described above. Following the evaporation of acetone, 2.0 mL of sterile HA stock solution (0.5 mg mL^{-1}), 16.0 mL of sterile MM and 2.0 mL of re-suspended bacterial biomass was successively added to give equivalent cell density and PHE concentrations, as above. The inoculated sets without PHE were the control for evaluating the influence of HA on bacterial growth. The sets without inoculation were employed to assess the abiotic loss of PHE in the HA sets.

All tubes were shaken at 160 rpm and at 28 °C in the dark. As the low aqueous solubility of PHE, destructive sampling was conducted to ensure better determination of PHE concentration in the whole system (Zhang et al., 2015). Approximately, at 12 h time intervals, six tubes were used for different measurements: three tubes to measure PHE residuals in the whole culture, and the other three to determine intracellular PHE levels and bacterial growth. After 36 h of incubation, three different sets that contained bacteria were taken out to prepare samples for the observation by Scan electron microscopy (SEM) and transmission electron microscopy (TEM).

2.4. PHE sorption behavior

To determine the amount of PHE adsorbed to the bacterial surface at each experimental point, the experiment was designed

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