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# Impacts of *Pantoea agglomerans* strain and cation-modified clay minerals on the adsorption and biodegradation of phenanthrene

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ABSTRACT

Interactions between microorganisms and minerals have the potential contribution to remove polycyclic aromatic hydrocarbons (PAHs) in model systems. In this study, phenanthrene (PHE) was used as a probe molecule to explore the potential adsorption and biotransformation processes in the presence of microorganisms and various reference clays, such as montmorillonite (M), kaolinite (K), and pyrophyllite (P). Equilibrium adsorption experiments and scanning electron microscopy (SEM) technique were used to investigate the sorption of *Pantoea agglomerans* strains on clay minerals saturated with cations ($Na^+$ and $Fe^{3+}$). The adsorption isotherms of PHE and *Pantoea agglomerans* strains on cation-modified clay minerals fitted to Langmuir equation, and their adsorbed amounts both followed the sequence: montmorillonite > kaolinite > pyrophyllite. For six types of cation-modified minerals, the behavior of PHE adsorbed and *Pantoea agglomerans* adhered onto mentioned minerals was in the order of Na(I)-M > Fe(III)-M, Na(I)-K > Fe(III)-K and Fe(III)-P > Na(I)-P, respectively. The biodegradation results showed that cation-modified clay minerals could enhance the biodegradation of PHE, ascribing to their large specific surface area, and cation exchange capability, as well as the difference in zeta potential between minerals and *Pantoea agglomerans* strains. Comparison of biodegradation rates displayed that PHE was degraded the highest in the presence of Na-M (93.285%). In addition, the obtained results suggested that the adhesion of bacteria onto cation-exchanged clay minerals was beneficial to the biodegradation of PHE. Anthracen-9-ylmethanol and 3,4-dimethyl-2-(3-methylbutanoyl)benzoic acid were detected as the main intermediate compounds, which can be further biodegraded into small molecules. The overall results obtained in this study are of valuable significance for the understanding of the behavior of PHE in soil and associated environment.

## 1. Introduction

The widespread production of polycyclic aromatic hydrocarbons (PAHs) via anthropogenic activities has been on the increase since the era of industrial revolution. Soil and sediment are considered as a major sink of these compounds. Due to their resistance to degradation and damage to ecosystem, surface soil contamination by PAHs becomes a great concern among public, scientists and decision-makers (Rani et al., 2009; Ferrarese et al., 2008; Jia et al., 2014). As one of the most commonly detected PAHs, phenanthrene (PHE) is often applied as a model compound to investigate the environmental fate and behaviors in the contaminated sites (Jia et al., 2012; Zhang et al., 2011a).

PAHs in soils may be subjected to various processes including biotic degradation and abiotic transformation. Biodegradation of PAHs has been considered as the main pathway for their disappearance in soil environment (Cheng et al., 2012). As reported previously, PAHs such as PHE, chrysene and pyrene can be transformed into harmless products by employing bacterial strains of *Alcaligenes faecalis*, *Pseudomonas monteilii* and *Pantoea agglomerans* (Greiner, 2004; John et al., 2012; Isaac et al., 2015). It has been demonstrated that *Pantoea agglomerans* exhibit a high biodegradation ability for PHE and strong toxicity tolerant to harsh environment such as various pH, salt, and PHE concentration (Song et al., 2011). During the biotransformation process, adsorption/desorption can play a significant role in the bioavailability of PAHs (Cai et al., 2011; Zhao et al., 2014a). PAHs can be adsorbed onto soil particles owing to the hydrophobic interaction with organic matter and non-hydrophobic interaction including electron donor-acceptor interaction and hydrogen bonding. Therefore, the biotransformation of PAHs is highly related to soil physicochemical properties and components including inorganic minerals and organic

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carbon. As reported previously, the presence of soil inorganic minerals, such as iron oxides and clay minerals, could improve the biotransformation of PAHs significantly (Hwang and Cutright, 2003; Froehner et al., 2009; Chen et al., 2009).

Clay minerals consisting of stacked aluminosilicate layers are seen as one type of active components in soil. The unique properties of clay minerals such as negative charged layers, high specific surface areas (SSA), and high cation exchange capacities (CEC) are able to provide desired active sites for organic pollutants (Jia et al., 2014; Zhang et al., 2011b), and can act as a template or provide a micro-environment for microbes (Biswas et al., 2015). Therefore, clay minerals are generally regarded as a favorable adsorbent and/or transformation reactor for organic compounds in soil (Ray and Okamoto, 2003; Gao et al., 2000; Chen et al., 2008; Luan et al., 2015). In addition, the negative charges on the clay surface can be neutralized by the exchangeable inorganic cations, resulting in the changes in the structure and physicochemical properties of clay surfaces, and thereby influencing their interactions with organic compounds or microbes. However, limited work has been done for the potential transformation process of PHE by employing cation-modified clay minerals and microorganisms (Moody et al., 2001).

In this study, the *Pantoea agglomerans* strain, isolated from local petroleum-contaminated soil, was applied to investigate the adsorption and biodegradation behaviors of PHE on cation-modified clay minerals. The impacts of interfacial interactions among clay minerals, PHE molecules and bacterial strains on PHE biodegradation were assessed. The possible pathways and mechanisms for PHE transformation were proposed and illustrated.

## 2. Experimental

### 2.1. Chemicals and materials

HPLC-grade methanol and PHE (AR, > 99%) were obtained from Sigma-Aldrich. Sodium chloride (NaCl), anhydrous ferric chloride ($FeCl_3$), anhydrous sodium sulfate methylene chloride and acetone (AR, > 99%) were purchased from Sinopharm Chemical Reagent Co., Ltd. Three kind of clay minerals including montmorillonite (M), kaolinite (K) and pyrophyllite (P) were perchased from Zhejiang Fenghong New Material Co., Ltd.

### 2.2. Preparation and characterization of cation-saturated and PHE-contaminated clay minerals

Cation-saturated clay minerals were prepared by following the procedures as reported previously (Rong et al., 2008; Jia et al., 2014). Specifically, 5 g of original clay particles were suspended in 100 mL 0.1 M NaCl solution, and the pH value was adjusted to 6.8 with 0.1 M NaOH and 0.1 M $HNO_3$ solution. The suspension was then treated by ultrasonication for 10 min. Ethanol solution (30%, volume ratio) was employed to wash the mixture until free of chloride ions. The final Na (I)-saturated clay minerals were dried at 60 °C for 24 h, and designated as Na(I)-M, Na(I)-K and Na(I)-P. Similarly, Fe(III)-saturated clay minerals were prepared with $FeCl_3$ solution using the same procedures as that for Na(I)-saturated minerals, and the obtained products were designated as Fe(III)-M, Fe(III)-K and Fe(III)-P. All the prepared minerals were grounded to pass 100 mesh sieves and stored in dark conditions for subsequent use. For X-ray diffraction (XRD) analysis, 1.0 g of each sample powder was weighed and examined by a BRUKER D8 Advance instrument (Germany) with Cu Kα radiation (λ = 1.5406 Å) operating at 40 kV and 40 mA. The XRD signals were recorded with scattering angles ranging from 5° to 80° at a scanning rate of 0.1 ° $s^{-1}$.

The zeta potentials of clay minerals (1.0 wt% of clay in suspension) were measured in electrolytes with different pH values in the range of 2–10 by a ZS90 Zetasizer (Malvern, UK). The electrolytes were prepared using 0.1 mM NaCl and the electric conductivity was adjusted to 40 μS $cm^{-1}$. The required pH value was adjusted by 0.1 M NaOH or HCl. The electrokinetic potential was measured with the zeta meter and the value was obtained by the average of eight repetitions. CEC was measured by $NH_4C_2H_3O_2$ displacement method (Fagbenro and Agboola, 1999). Specially, 30 mL of 0.2 M $NH_4Cl$ was added into a 50-mL polypropylene centrifuge tube containing 4 g of air-dried clay minerals. The tubes were shaken for 1 h on a side-to-side shaker, and then were centrifuged for 10 min at 9000 rpm. The supernatant was collected and the solids were rewashed with $NH_4Cl$ solution for another three times. All supernatants were mixed together to measure the exchangeable cations including $K^+$, $Na^+$, $Ca^{2+}$, $Mg^{2+}$, and $Al^{3+}$ by inductively coupled plasma mass spectrometry (ICP-MS, Agilent 7500a, USA). Then, the CEC value was calculated by the sum of those exchangeable cations. SSA was measured with a Quantachrome Autosorp1C analyzer (USA). $N_2$ was used as an adsorbate. The samples were degassed at 373 K for 5 h before measurements. Measurements were conducted at 77.3 K within $P/P_0$ values of $10^{-5}$ to 0.999 using a volumetric vacuum apparatus. SSA was determined by employing the Brunauer-Emmett-Teller (BET) method. Additionally, clay minerals were digested by a mixture of nitric acid, perchloric acid and hydrofluoric acid, and then the iron contents were determined using an inductively coupled plasma atomic emission spectrometer (VISTA-PRO CCD Simultaneous ICP-OES, Varian, USA).

The zeta potential of bacteria was prepared as following procedures. Cells harvested in the mid-exponential phase were obtained by centrifugation at 10,000 rpm for 10 min at 4 °C. The precipitant was washed using deionized water for three times to remove ions, and then resuspended with 3.4 mM NaCl solution. The pH value was adjusted to 6.8. Specific method can be obtained in previous work (Liu et al., 2015a).

For obtaining PHE-contaminated clays, 1 g of clay minerals was spiked by 1 mL of PHE with a concentration of 1 g $L^{-1}$ in acetone. The detailed method can be referred to our previous report (Jia et al., 2014).

### 2.3. Isolation of Pantoea agglomerans strain

*Pantoea agglomerans* strain used in this study is a gram-negative microorganism capable of utilizing PHE as a sole carbon source, which was isolated from a petroleum-contaminated site at Karamay in Xinjiang in China. Briefly, 5 g of the contaminated soils was added to 50 mL of mineral salt media (MSM, g $L^{-1}$) consisting of $(NH_4)_2SO_4$ 1.0, $K_2HPO_4$ 0.8, $KH_2PO_4$ 0.2, $MgSO_4{\cdot}7H_2O$ 0.2, $CaCl_2{\cdot}2H_2O$ 0.1, NaCl 0.2, pH 7.0–7.2 (Cai et al., 2011), and 20 mg $L^{-1}$ of PHE was supplemented as a sole carbon source for the first cultivation cycle. The culture was incubated at 28 °C in a rotary shaker at 150 rpm. The isolates were domesticated using a gradient method. Namely, 5 mL of bacterial enrichment culture was transferred to 50 mL of fresh MSM with a PHE concentration of 40 mg $L^{-1}$ for the second cultivation cycle, and repeated every 5 d for 6 weeks. The final diluted enrichment was placed onto MSM agar with PHE at a concentration of 120 mg $L^{-1}$. Then the colonies were inoculated into 50 mL of MSM containing LB medium, and cultivated at 28 °C at 150 rpm for 12 h. The enriched cells were harvested by centrifugation, washed twice with deionized water and suspended in sterile MSM for subsequent assays.

The isolated strain was identified with amplified ribosomal DNA restriction analysis (ARDRA) method by amplification of genomic DNA with primers 27 F and 1492 R. The PCR reactions contained a total volume of 50 μL, containing 10 μM of each primer, 2 units of Taq PCR Mix in 25 μL (Dingguo, China), 22 μL DNA-free water, and 1 μL of template genomic DNA. An initial denaturation at 94 °C for 5 min was applied, followed by 35 cycles at 94 °C for 30 s, 54 °C for 30 s, and 72 °C for 90 s, with a final extension stage at 72 °C for 10 min. The PCR products were digested with HaeIII at 37 °C for 3 h. Gel electrophoresis was performed using 2.0% agarose gel at 140 V for 25 min, and then the target fragments were cut. ARDRA patterns were blasted against NCBI