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Bacterial mineralization of phenanthrene on thermally activated palygorskite: A ^{14}C radiotracer study

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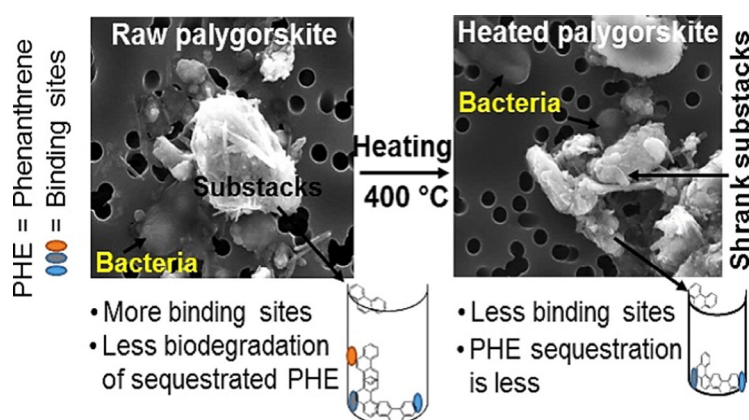
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HIGHLIGHTS

- Heat-treated palygorskite assisted biodegradation of phenanthrene by *Burkholderia sartisoli* was studied.
- Moderate heat (400 °C) altered physicochemical properties without structure destruction.
- Binding sites in palygorskite were reduced and less phenanthrene was sequestered.
- Particle aggregation congenial for bacterial survival was created.
- Phenanthrene biomineralization was enhanced in a simulated soil slurry system.

GRAPHICAL ABSTRACT



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ABSTRACT

Clay-bacterial interaction can significantly influence the biodegradation of organic contaminants in the environment. A moderate heat treatment of palygorskite could alter the physicochemical properties of the clay mineral and thus support the growth and function of polycyclic aromatic hydrocarbon (PAH)-degrading bacteria. By using ^{14}C -labelled phenanthrene and a model bacterium *Burkholderia sartisoli*, we studied the mineralization of phenanthrene on the surface of a moderately heat-treated (up to 400 °C) palygorskite. The heat treatment at 400 °C induced a reduction of binding sites (e.g., by the elimination of organic matter and/or channel shrinkage) in the palygorskite and thus imparted a weaker sequestration of phenanthrene on its surface and within the pores. As a result, a supplement with the thermally modified palygorskite (400 °C) significantly increased (20–30%; $p < 0.05$) the biomineralization of total phenanthrene in a simulated soil slurry system. These results are highly promising to develop a clay mineral based technology for the bioremediation of PAH contaminants in water and soil environments.

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1. Introduction

Organic contaminants such as polycyclic aromatic hydrocarbon (PAH) in soil, sediment and water are a serious concern due to their potential ecological and human health impacts (Cachada et al., 2016). Biodegradation of these contaminants through the action of microorganisms is considered as a potential strategy to remediate contaminated environments (García-Delgado et al., 2015; Megharaj et al., 2011). The associated microorganisms hold a high potential to dictate the microenvironment around them (Vila et al., 2015). However, efficient, faster and complete biodegradation is still a challenge to achieve. In recent practices, natural material supported microbial functions were achieved to boost up the degradation efficiency of some target contaminants (Biswas et al., 2015; Mandal et al., 2016). Clay minerals, one of the most reactive and natural soil components, can serve as an excellent supportive material for the potential degraders (e.g., bacteria). The clay materials can provide a substrate for living and shelter against predators to the microorganisms. They also protect the microorganisms from harsh environmental conditions and supply nutrient supplements for an enhanced proliferation (Biswas et al., 2015; Lünsdorf et al., 2000; Sarkar et al., 2012b). Such a clay-bacterial interaction largely depends on the type of bacterial species, surface and charge properties of clay minerals, and the bioavailability of the target contaminant compounds (Biswas et al., 2015; Ortega-Calvo et al., 2015). The environmental bacteria could grow and function better in the presence of clay minerals containing higher cation exchange capacity (CEC) and surface area (Chaerun et al., 2005), optimum cation-saturation (Na^+ , Ca^{2+} , Fe^{3+}) (Ugochukwu et al., 2014a; Warr et al., 2009) and those form macro-aggregates in soil (Vogel et al., 2014). These bacterial-supportive clay properties can be obtained through different modifications; however, obtaining the most suitable clay product for enhancing the bacterial growth and function warrants in-depth investigations.

Palygorskite has drawn significant attention in environmental applications due to its unique fibrous and elongated nanostructure (Chen et al., 2012; Sarkar et al., 2015), which gives a moderate CEC ($\sim 30\text{--}40\text{ cmol [p}^+ \text{ kg}^{-1}$) and high surface area ($\sim 150\text{--}600\text{ m}^2\text{ g}^{-1}$) (Biswas et al., 2015; Chen et al., 2012). In the fibrous clay mineral group, palygorskite is a better candidate than sepiolite for field application under diverse environmental conditions because the latter holds a lesser stability at pH below 8 (Guggenheim and Krekeler, 2011). However, application of palygorskite as a vehicle that supports microorganisms and thus in environmental bioremediation remains largely unexplored.

Recently, raw palygorskite was reported to support bacterial growth in aqueous media (Biswas et al., In press; Ugochukwu et al., 2014b). Different modifications of palygorskite were also practiced to obtain engineered surface properties. The modified properties enhanced their contaminant adsorption/removal capacities (Álvarez-Ayuso and García-Sánchez, 2003; Ouali et al., 2015; Rusmin et al., 2015; Sarkar et al., 2015; Sarkar et al., 2012a; Zhao et al., 2015). However, the use of such modified palygorskite as a bioremediation supplement could sometime be limited due to the toxic effect of the modifying agent itself (Sarkar et al., 2010). Alternatively, a less vigorous surface modification could be achieved through heat treatment (Boudriche et al., 2012; Cheng et al., 2011), especially in the moderate temperature range ($\leq 400\text{ }^\circ\text{C}$). Heat treatment could change the surface area, pore volume, charge properties (e.g., CEC), dissolution of elements and organic matter content in palygorskite without altering its crystallinity substantially (Biswas et al., In press; Gan et al., 2009). Heat treated palygorskite was successfully used for adsorption of dyes (Chen et al., 2012), metals (Wang et al., 2007), and inorganic ions (Gan et al., 2009). However, how thermally-modified palygorskite products influence bacterial growth and functions in a PAH biodegradation scenario was not tested before.

The biomineralization of PAHs could depend on their adsorption to the surface and interlamellar spaces of clay minerals (McAllister and Semple, 2010). A clay-PAH-bacterial complex or microenvironment

could lead to biofilm formation in a slurry system, which might impart an enhanced biodegradation of PAH (Biswas et al., 2015). However, a completely sequestered PAH compound within the clay interlayer or pores might not be readily available to microorganisms (McAllister and Semple, 2010). The physicochemical properties of heat-treated palygorskite could largely influence the PAH adsorption behavior and thus bacterial mineralization efficacy. In this study, we used a ^{14}C isotopic technique to investigate the mineralization of both total and adsorbed fractions of phenanthrene (a model PAH compound) in a slurry system containing moderately heat-treated palygorskite. A PAH-degrading bacterium *Burkholderia sartisoli* was used as the model biomineralizing agent.

2. Materials and methods

2.1. Material preparation

Sieved ($\leq 75\text{ }\mu\text{m}$) palygorskite (geological source: Western Australia; supplier: Hudson Resource Ltd., Australia) was heated for 2 h aerobically in a muffle furnace (S.E.M. Pty Ltd. Australia) at different pre-set temperatures (100, 200, 300 and $400\text{ }^\circ\text{C}$) ($\pm 5\text{ }^\circ\text{C}$ precision). The raw palygorskite (PaU) served as the control material. Following heating, the samples were cooled immediately at room temperature in a desiccator. The modified materials were named as Pa100, Pa200 and so on. In a previous study, we found that thermal treatment over $400\text{ }^\circ\text{C}$ did not support bacterial proliferation and attachment with the palygorskite surfaces (Biswas et al., In press). Therefore, only modified palygorskite products heated at no higher than $400\text{ }^\circ\text{C}$ was selected for the mineralization experiments.

2.2. Material characterization

The mineralogical, morphological and physicochemical changes in the heat-treated products (up to $400\text{ }^\circ\text{C}$) were examined. X-ray diffraction (XRD) of the finely ground powdered materials was performed in stainless steel sample holders. XRD patterns were obtained using $\text{Cu K}\alpha$ radiation ($\lambda = 1.540598\text{ \AA}$) on a PANalytical Empyrean X-ray diffractometer equipped with PIXcel^{3D} detector (PANalytical Inc., The Netherlands) operating at 40 kV and 40 mA between 5° and $30^\circ 2\theta$ (step size, 0.0130° ; with 0.25° fixed divergence slit and 0.50° anti-scatter slit). The mineral phases were identified by the X'Pert HighScore Plus software using the International Centre for Diffraction Data (ICDD) database. Fourier transform infrared (FTIR) spectra of the materials (discs of clay-KBr mixture; 0.1% w/w) were collected by using an Agilent Cary 600 Series FTIR spectrometer (64 scans, 8 cm^{-1} resolution, $400\text{--}4000\text{ cm}^{-1}$). The morphological changes in palygorskite due to the thermal modification were observed by Scanning Electron Microscopy (FEI Quanta 450 FEG, USA). The powdered samples were prepared as a thin layer on a silicon wafer and coated with 2.5 nm platinum using a Quorum QT150ES coating system. The potential change in the total organic carbon (OC) content due to the heat treatment was also determined in the carbonate-free samples (2 M HCl treated) by a CNS analyzer (LECO[®] Corporation, USA). The total organic carbon is strongly correlated to and a key component of total organic matter (OM) (Jiménez and García, 1992). In this study, OM was calculated from the OC content using the Van Bemmelen factor of 1.72 ($\text{OM} = \text{OC} \times 1.72$). The pore volume of the samples was measured using a surface area analyzer (Gemini 2380, Micromeritics, USA) at a constant temperature using liquid nitrogen. Before analysis, samples were degassed overnight at $70\text{ }^\circ\text{C}$. The CEC of palygorskite products was also measured following the Cu(II) -trien method (Meier and Kahr, 1999).

2.3. Inoculum preparation

B. sartisoli RP007^T (Vanlaere et al., 2008), purchased from German culture collection (DSMZ) was originally isolated from PAH-

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