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Research paper

Effects of *Sphingomonas* sp. GY2B on the structure and physicochemical properties of stearic acid-modified montmorillonite in the biodegradation of phenanthrene

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ABSTRACT

The objective of this study was to investigate the interaction between stearic acid-modified montmorillonite (SA-Mt) and *Sphingomonas* sp. GY2B and its effect on the physical and chemical properties of montmorillonite in the 2 days' biodegradation of phenanthrene. Due to bacterial activities, surface and colloidal properties of the bacteria-treated sample evidently changed in comparison with the untreated SA-Mt. Changes in surface morphology and the agglomerate state of SA-Mt were clearly observed by scanning electron microscopy (SEM) after interaction with GY2B for 2 days. Although the interlayer space of SA-Mt structure remained basically unchanged, the decrease of the structural order and the appearance and shift of several absorbance bands in the Fourier transform infrared (FTIR) spectra verified the structural changes of SA-Mt after bacterial treatment. Besides, the second derivative infrared spectra indicated that bacterial growth and metabolism largely affected the microstructure in SA-Mt. Dissolution rates of major elements were obtained by inductively coupled plasma optical emission spectroscopy (ICP-OES) in the solution, which revealed that bacterial activities enhanced the release of major elements and brought about a significant higher dissolution of Si preferentially from the tetrahedral sheet edges of SA-Mt than Al. The improvement of elemental dissolution in the biotic experiment was attributed to the combined action of pH effect and ligand-promoted process. These results substantiated that microorganisms have a considerable influence on the physicochemical properties and structure in the clay-modulated biodegradation of organic compounds. This study has profound impacts on the clay-modulated biodegradation of hydrophobic organic contaminants in the environment.

1. Introduction

Microorganisms, which are abundant in natural soils and sediments, have coevolved with clay minerals over much of Earth's history (Dong, 2012; Dong and Lu, 2012). Interactions between minerals and microbes are of fundamental importance in natural environments by influencing the geochemical process and biogeochemical cycling of a large number of elements (Dong and Lu, 2012). Such processes therefore have provided a potential clay-modulated microbial remediation approach for removing environmental contaminants (Sarkar et al., 2012a; Zhao et al., 2014; Biswas et al., 2015a; Biswas et al., 2016; Wen et al., 2016; Du et al., 2016a).

A large numbers of studies have shown that clay minerals could enhance bacterial proliferation and hydrocarbon degradation in organic-contaminated environment (Quintelas et al., 2013; Mangwani et al., 2014; Ugochukwu et al., 2014; Luo et al., 2015; Mandal et al., 2016; Biswas et al., 2017a). The clay minerals can provide a protective habitat to the microorganisms by forming biofilm and protect them from toxic substances under harsh environmental conditions (Warr et al., 2009; Wen et al., 2016). As a result, the adsorption of organic compounds and the attachment of microorganisms onto the surface of clay minerals can accelerate more efficient degradation of hydrocarbons (Biswas et al., 2017b). However, efficiency of clay-modulated biodegradation still depends on many factors including type of clay

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minerals and contaminants, microbial community structure, specific environmental conditions, and other predominating factors (Biswas et al., 2015a).

It is unquestionable that clays as the most dominant minerals in the natural environment play a significant role in the clay-modulated bacterial degradation process by providing a substrate and habitat for microorganisms. In return, microorganisms also take part in this interaction between microbes and clays. It has been reported that microbes may greatly affect mineral dissolution, precipitation, weathering and transformation (Maurice et al., 2001; Balogh-Brunstad et al., 2008; McMaster, 2012). Many bacteria, such as *Aeromonas hydrophila* (Tao et al., 2017), *Thermoanaerobacter ethanolicus* (Zhang et al., 2007), *Bacillus mucilaginosus* (Yang et al., 2016) and *Shewanella oneidensis* (Kim et al., 2004), have shown abilities in significantly enhancing the elements release and dissolution of minerals. Besides, microbial activities may also result in significant changes in physical and chemical properties of minerals, including surface area, swelling behavior, surface charge and Zeta potential (Kim et al., 2005; Liu et al., 2012, 2014; Mueller, 2015; Hong et al., 2016). However, studies relating to possible changes caused by such interaction in the clay mineral structure and physicochemical properties in the biodegradation of organic pollutants are few in the literature. It is particularly important to understand whether and how bacteria induce any changes in the structure and properties of the clay mineral in the clay-modulated bacterial degradation process.

Montmorillonite (Mt), as one of the most abundant naturally occurring clay minerals, has been widely used in environmental remediation on account of its excellent physical and chemical properties, such as adsorption, ion exchange and co-precipitating capacities (Wang et al., 2017). By increasing the surface hydrophobicity of montmorillonite, organic modification has become an important method in using montmorillonite for cleaning up organic contaminants, such as hydrophobic polycyclic aromatic hydrocarbons (PAHs) (Biswas et al., 2016; Mandal et al., 2016). Cationic surfactants, one of the best known is quaternary ammonium cations (QACs), are commonly used to prepare organoclays, which are proved to be a kind of excellent hydrophobicmaterials and could be used as efficient adsorbents for organic contaminants (de Paiva et al., 2008; Froehner et al., 2009; Sarkar et al., 2011, 2012a, 2012b; Zhu et al., 2016). However, commonly used surfactants like hexadecyl trimethylammonium (HDTMA) and octadecyl trimethylammonium (ODTMA) are toxic to microorganisms and have been proved to inhibit microbial activity of degrading bacteria (Sarkar et al., 2010, 2013; Abbate et al., 2013; Reeve and Fallowfield, 2017). Conversely, naturally occurring long chain fatty acids, for example, stearic acid (SA), are non-toxic and compatible with microorganisms. Therefore, preparation of SA-Mt could reduce organic toxicity and improve PAH-degrading bacterial proliferation in the clay-modulated biodegradation of PAHs (Biswas et al., 2015b).

In this study, *Sphingomonas* sp. GY2B as a kind of efficient degradation bacteria of PAHs was used to degrade a certain concentration of phenanthrene in the culture solution. The main objective of our present study is to investigate the surface morphological, structural and physicochemical changes of SA-Mt after interaction with GY2B for 2 days. And interaction between SA-Mt and *Sphingomonas* sp. GY2B in the process of phenanthrene biodegradation was evaluated by some analytical techniques and characterization methods, including XRD, FTIR, SEM, XPS and ICP-OES.

2. Materials and methods

2.1. Preparation of modified montmorillonite

The initial Mt sample used in this study was purchased from Feilaifeng Nonmetal Mineral Material Co. Ltd. (Sihui, Guangdong Province, China). The Mt powder was washed with deionized water for many times, and centrifuged at 8000 rpm for 10 min in a TGL-16 M high speed refrigerated centrifuge (Yancheng, China). Only the precipitated particles was collected. The above steps were replicated for a couple of times until the supernatant was clean. All collected samples were dried in a DHG-9240A vacuum drying oven (Keelrein Instrument Co. Ltd., Shanghai) at 60 °C for 24 h. Then dry solid products in agate mortar were ground into powder (less than 75 μm in diameter) through 200 mesh sieve and stored in tubes at room temperature to be used for further experiments and analysis.

The SA-Mt complex was prepared using a procedure similar to other researchers used for the preparation of organoclay complexes (Malakul et al., 1998; Biswas et al., 2015b). Analytical grade stearic acid (SA) was supplied by Damao Chemical Reagent Factory (Tianjin, China). The highly purified Mt powder was added to ethanol-dissolved SA (0.91 g SA in 100 mL ethanol-water mixture (1:1)). The quantity of SA was far more than 2-fold CEC of the clay. The treatment medium was gently stirred for 4 h on a DF-101S magnetic stirrer (Zhengzhou, China) at 50 °C. The solid product was collected by centrifugation at 8000 rpm for 10 min and stored after extensive washing with anhydrous ethanol to eliminate excess of stearic acid and drying at 60 °C. The product was termed SA-Mt (SA-montmorillonite).

2.2. Bacterial culture

Sphingomonas sp. GY2B used in this study was isolated from crude oil contaminated soils collected at a site near Guangzhou Petrifaction Company, China. The bacterium was identified on cell and colony morphology, gram reaction, standard physiological and biochemical tests, and 16S rRNA sequence analysis. The strain was routinely growth at 30 °C in mineral salts medium (MSM) consisting of following (per liter): 5 mL phosphate buffer solution (KH_2PO_4, 8.5 $g{\cdot}L^{-1}$; $K_2HPO_4{\cdot}H_2O$, 21.75 $g{\cdot}L^{-1}$; $Na_2HPO_4{\cdot}12H_2O$, 33.4 $g{\cdot}L^{-1}$; NH_4Cl, 5.0 $g{\cdot}L^{-1}$); 3.0 mL $MgSO_4$ solution (22.5 $g{\cdot}L^{-1}$); 1.0 mL $FeCl_3$ solution (0.25 $g{\cdot}L^{-1}$); 1.0 mL $CaCl_2$ solution (36.4 $g{\cdot}L^{-1}$); 1.0 mL trace element solution ($MnSO_4{\cdot}H_2O$, 39.9 $mg{\cdot}L^{-1}$; $ZnSO_4{\cdot}H_2O$, 42.8 $mg{\cdot}L^{-1}$; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 34.7 $mg{\cdot}L^{-1}$). Its pH was adjusted to 7.0–7.2 with 5 $mol{\cdot}L^{-1}$ HCl and NaOH solutions (Tao et al., 2009).

An enrichment culture was developed using a beef extract peptone liquid medium at pH 7 containing the following components: 10 $g{\cdot}L^{-1}$ peptone, 5 $g{\cdot}L^{-1}$ beef extract, 5 $g{\cdot}L^{-1}$ NaCl. 1 g/L phenanthrene (analytical grade, Aladdin Industrial Corporation, China) stock solutions were prepared for the desired concentration in n-hexane before each experimental run. The solutions were always kept in brown volumetric flasks inside a dark cabinet to avoid light oxidation of the phenanthrene at 4 °C refrigeration. About 250 mL culture of the strain was made using the liquid medium and 100 $mg{\cdot}L^{-1}$ phenanthrene in a flat-bottom flask at 150 rpm under dark condition for 24 h. Cultivation was carried out at 30 °C. Following cultivation, the bacterial cells in the supernatant were collected and centrifuged at 8000 rpm for 15 min. The precipitated amount from centrifugation which contains the harvested cells were washed three times with sterilized 0.9% saline and finally suspended in a small volume of sterilized MSM. Optical density of bacterial suspension was adjusted to 1.00 at 600 nm by UV–Vis spectroscopy (Shimadzu UV-2450, Japan) to ensure the equal amount of bacteria for parallel experiments and then bacterial solutions were kept in the refrigerator at 4 °C for subsequent research.

2.3. Batch experiments of mineral-bacteria interaction

The interaction experiments were conducted in 100 mL glass culture flasks with aseptic sealing films (about 0.3 μm polytetrafluoroethylene membrane). All medium and minerals used in this study were autoclaved at 121 °C in advance. For the experimental group, 0.10 g sample powder (SA-Mt) and 10 mL GY2B suspension were added into flat-bottom flasks containing 40 mL of sterile MSM. 2 mL phenanthrene stock solutions (1 g/L) were added into the dispersion to provide the source of carbon and energy for bacteria growth. In order to check the