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Research paper

Influence of thermally modified palygorskite on the viability of polycyclic aromatic hydrocarbon-degrading bacteria

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ABSTRACT

Thermal activation of palygorskite is considered as a simple and cost-effective method for modifying its structural and surface properties, which can be congenial for the adsorptive removal of environmental contaminants. However, for a more efficient removal of organic contaminants like polycyclic aromatic hydrocarbons (PAH), clay-microbial synergy combining both adsorption and biodegradation is an emerging strategy. In this study, we investigated the compatibility of heat treated palygorskite products (100–900 °C) with a PAH-degrading soil bacterium *Burkholderia sartisoli*. The mineralogical and physico-chemical properties were characterised in detail, and the bacterial adhesion to the substrate and their growth were observed in relation to these properties. The major variation in the cation exchange capacity (CEC), surface area, water content and the elemental dissolution in the aqueous medium occurred in the palygorskite products heated at extreme temperature (700–900 °C). These changes significantly influenced the bacterial growth and attachment. The maximum viability was imparted by the palygorskite product obtained at 400 °C. Dissolution of Al from products heated above 500 °C also posed inhibitory effect on bacterial growth in the aqueous media. This study provided valuable information about the mechanisms of bacterial viability as affected by modified clay minerals, which is important for developing a novel clay-modulated-bioremediation technology.

1. Introduction

Clay minerals, one of the most reactive soil components in nature, can serve as an excellent adsorptive material due to their congenial surface and charge properties (Sarkar et al., 2012a). Manipulation of these surface properties can extend the application of this material in adsorption-based clean-up of environmental contaminants (Hofstetter et al., 2006; Sarkar et al., 2010b; Xi et al., 2010; Sarkar et al., 2013; Wang et al., 2015).

Palygorskite, among the clay minerals, has drawn considerable attention in cleaning up both organic and inorganic contaminants due to its high surface area, moderate cation exchange capacity (CEC) and unique fibrous orientation of elongated one dimensional nano-structure (Murray, 2000; Chen et al., 2012; Ugochukwu et al., 2014b). The surface properties of palygorskite can be changed by employing different modification techniques in order to improve the adsorbent's affinity to a particular contaminant compound. Some of these modifying agents include surfactant molecules (Sarkar et al., 2010b; Sarkar et al., 2012b), polymers (Alcântara et al., 2014; Rusmin et al., 2015), nanoparticles (Ouali et al., 2015; Sarkar et al., 2015), inorganic acids (Barrios et al., 1995; Ugochukwu et al., 2014a), etc. However, many of these treatments engage in using chemical agents whose environmental fate and reactions are either harmful (Sarkar et al., 2010a) or still unknown. Heat treatment is considered as a simple, less vigorous, but effective modification technique (VanScoyoc et al., 1979). Also, it is cheaper than many chemical modification processes. Without employing a chemical agent, heat treatment can alter the orientation of different water molecules, structural elements and pores of palygorskite and other fibrous clay minerals (e.g., sepiolite) (Kuang et al., 2004). Surface area of raw palygorskite can be increased significantly by heating it up to 300 °C (Chen et al., 2011), and no substantial changes in the clay mineral structure occur up to 400 °C due to a reversible dehydration process (Gan et al., 2009). However, skewness is expected in the mineralogical properties of palygorskite originating from different geological sources. This fact has been taken into account for characterizing the surface and charge properties of thermally treated palygorskite over a series of temperatures (VanScoyoc et al., 1979; Kuang et al., 2004; Boudriche et al., 2012), and assessing their removal capacity of inorganic (Wang et al., 2007; Gan et al., 2009) and organic (Chen et al., 2011; Chen et al., 2012) contaminants. Most of these previous studies concentrated on

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the adsorption behaviour of raw and modified palygorskite products for contaminants removal (Wang et al., 2007; Gan et al., 2009; Chen et al., 2011; Chen et al., 2012).

Complete clean-up of organic contaminants, such as polycyclic aromatic hydrocarbons (PAH), requires adsorption followed by degradation which eliminates the risk of redispersal of the compound back into the environment (Sarkar et al., 2012a; Biswas et al., 2015b). Degradation of organic compounds can be achieved cost-effectively via active involvement of microorganisms (Megharaj et al., 2011). Certain raw and modified clay minerals are known to improve the viability, growth and proliferation of microorganisms (Chaerun and Tazaki, 2005; Warr et al., 2009; Biswas et al., 2015a; Mandal et al., 2016). However, to achieve the most efficient biodegradation of organic contaminants, the clay-bacterial interaction warrants assessment under specific conditions involving various contaminants, clay minerals and their modification types and the species of microorganisms (Biswas et al., 2015b). The surface structure and charge properties of clay minerals can influence the viability of hydrocarbon-degrading bacteria in clay-mediated culture media (Chaerun and Tazaki, 2005; Warr et al., 2009). For example, Ugochukwu et al. (2014b) reported that the higher surface area of a raw palygorskite than an organically modified palygorskite might enhance the biodegradation of crude oil by supporting the growth of a hydrocarbon-degrading microbial consortium. The lower pH in acid-treated palygorskite and the surfactant toxicity in organo-palygorskite might inhibit such microbial proliferation (Ugochukwu et al., 2014b). However, no scientific information is available whether a heat treated palygorskite can support microbial proliferation and functions. Therefore, this study assessed the viability of a model PAH-degrading bacterium (*Burkholderia sartisoli*) with respect to the changes of mineralogical and surface properties, and elemental composition of a palygorskite which underwent thermal treatment over a wide range of temperatures.

2. Materials and methods

2.1. Material preparation

A palygorskite (Pal) sample collected from Western Australia (supplied by Hudson Resource Limited, Australia) was selected for thermal modification. Sieved palygorskite (<75 μm) was subjected to thermal modification at a wide range of heating temperatures (100, 200, 300, 400, 500, 550, 600, 650, 700, 750, 800, 850 and 900 °C). The raw clay mineral (PalU) served as the control material. The incubation time for heating was set 2 h (Gan et al., 2009) by taking the sample (10 g) in an alumina crucible. Samples were heated aerobically in a muffle furnace (S.E.M. Pty Ltd. Australia) with ±5 °C precision of the set temperature and cooled immediately at room temperature in a desiccator. The modified materials were named as Pal100, Pal200 and so on.

2.2. Material characterisation

2.2.1. X-ray diffraction (XRD)

The powdered materials were pressed in stainless steel sample holders. XRD patterns were obtained using Cu K_α radiation ($\lambda = 1.540598$ Å) 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 90° 2θ at a step size of 0.0130° using a 0.25° fixed divergence slit and 0.50° anti-scatter slit. The basal spacing (*d*-value) was calculated from the 2θ value using Bragg's equation ($n\lambda = 2d \sin\theta$, where, n is an integer determined by the order given, λ the wavelength, and θ the scattering angle). The mineral phases were identified by analysing the XRD patterns using X'Pert HighScore Plus software combined with the International Centre for Diffraction Data (ICDD) database.

2.2.2. Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared (FTIR) spectra of the materials were collected by using an Agilent Cary 600 Series FTIR spectrometer. Discs of finely powdered clay-KBr mixture (1:240 clay:KBr, w/w) were prepared with the aid of a hydraulic press. Spectra were recorded in the range of 4000 to 400 cm^{-1} by co-addition of 16 scans with an 8 cm^{-1} resolution.

2.2.3. Thermogravimetric analysis (TGA)

Thermogravimetric analysis of powdered raw palygorskite (PalU) was conducted on a Mettler-Toledo DSC-1 thermogravimetric analyser (Mettler-Toledo International Inc., USA). Approximately 18 mg of finely ground sample was heated in an open platinum crucible. The temperature for TGA was raised at a rate of 10 °C min^{-1} over the range of 25 to 1000 °C with a resolution of 6 °C under continuous N_2 flow (50 mL min^{-1}).

2.2.4. Surface area and charge properties

For specific surface area (SSA) analysis, Brunauer-Emmett-Teller (BET)-N_2 adsorption technique (Brunauer et al., 1938) was applied using a Gemini 2380 surface area analyser (Micrometrics, USA) under an isothermal condition achieved via liquid nitrogen. The raw palygorskite had a SSA of 117.29 $m^2\ g^{-1}$. For measuring the surface charge, a zeta potential analyser (Nicomp 380 ZLS, USA) was used to obtain the zeta potential (ζ) value (mV) of 0.05% clay dispersion (w/v) in Milli-Q water (18.2 Ω) in duplicate. The corresponding pH of the clay dispersion was measured directly by a pH electrode (Orion 2 star plus, Thermo Scientific™). Also, the CEC of the duplicate of all clay minerals was measured by Cu(II)-trien method (Meier and Kahr, 1999).

2.2.5. Elemental profiling

Based on XRD patterns, FTIR spectra, surface area and pore size results, selected samples were further analysed for elemental composition using a FEI Quanta 450 FEG Environmental Scanning Electron Microscope equipped with an Apollo EDX detector (TEAM™ EDS (energy dispersive X-ray spectroscopy) analysis, EDAX®, USA), and a semi-quantitative analysis was performed by eZAF using TEAM™ EDS software suite.

2.3. Viability of B. sartisoli

2.3.1. Culture preparation

The bacterium *Burkholderia sartisoli* RP007^T was described as a highly efficient degrader of PAHs, especially the low molecular weight (LMW) PAHs (Vanlaere et al., 2008). It could use the PAH compounds as the sole carbon source (Vanlaere et al., 2008). For this reason, *Burkholderia sartisoli* RP007^T was used in this study as a model PAH degrading bacterial species which was isolated from PAH-contaminated field soil in New Zealand (Vanlaere et al., 2008), and was purchased from German culture collection (DSMZ). This bacterium was grown in TSB media and preserved at −80 °C until further use. Prior to conducting bacterial viability assessment, the frozen cells were reactivated and grown in Brunner salt media ($Na_2HPO_4 = 2.44$ g, $KH_2PO_4 = 1.52$ g, $(NH_4)_2SO_4 = 0.50$ g, $MgSO_4{\cdot}7H_2O = 0.20$ g, $CaCl_2{\cdot}2H_2O = 0.05$ g, trace element solution supplemented with vitamin solution, pH = 7.0) (see Supplementary information for the full recipe of the trace salt solution). As the sole carbon source, dimethylformamide-dissolved phenanthrene stock solution (≥98%; Sigma-Aldrich, Australia) (5000 mg L^{-1}) was added into the media to obtain a final concentration of phenanthrene 20 mg L^{-1}. Under similar media compositions and conditions, five continuous subcultures were performed before using the bacterium in the viability test with the clay minerals. The cells were harvested at late log phase by centrifugation at 3200 ×g for 10 min and washed twice with 50 mM phosphate buffer solution (PBS). The washed cells were passed through sterile packed glass wool filter and washed again with 0.02% PBS to remove

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