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Surface tailored organobentonite enhances bacterial proliferation and phenanthrene biodegradation under cadmium co-contamination

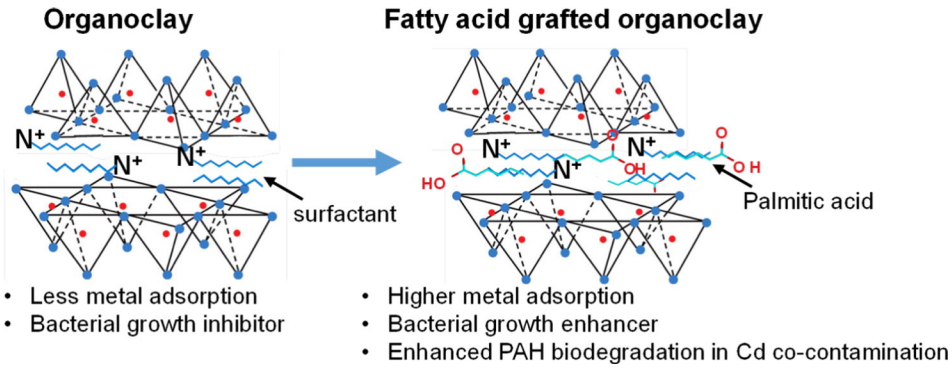
Asit Mandal [a,b], Bhabananda Biswas [a,c], Binoy Sarkar [a,c,*], Ashok K. Patra [b], Ravi Naidu [c,d,**]

[a] Future Industries Institute (formerly Centre for Environmental Risk Assessment and Remediation), University of South Australia, Mawson Lakes, SA 5095, Australia
[b] Indian Council of Agricultural Research (ICAR), Indian Institute of Soil Science, Bhopal, India
[c] Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), ACT Building, University of Newcastle, Callaghan, NSW 2308, Australia
[d] Global Centre for Environmental Remediation (GCER), Faculty of Science and Information Technology, University of Newcastle, Callaghan, NSW 2308, Australia

HIGHLIGHTS

- Surface tailored organobentonite synthesised and characterised
- Modified clay adsorbs Cd and reduces toxicity to *Mycobacterium gilvum*.
- It creates congenial microenvironment for bacterial survival.
- It enhances phenanthrene biodegradation in metal co-contaminated condition.

GRAPHICAL ABSTRACT



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ABSTRACT

Co-contamination of soil and water with polycyclic aromatic hydrocarbon (PAH) and heavy metals makes biodegradation of the former extremely challenging. Modified clay-modulated microbial degradation provides a novel insight in addressing this issue. This study was conducted to evaluate the growth and phenanthrene degradation performance of *Mycobacterium gilvum* VF1 in the presence of a palmitic acid (PA)-grafted Arquad® 2HT-75-based organobentonite in cadmium (Cd)-phenanthrene co-contaminated water. The PA-grafted organobentonite (ABP) adsorbed a slightly greater quantity of Cd than bentonite at up to 30 mg L^{-1} metal concentration, but its highly negative surface charge imparted by carboxylic groups indicated the potential of being a significantly superior adsorbent of Cd at higher metal concentrations. In systems co-contained with Cd (5 and 10 mg L^{-1}), the Arquad® 2HT-75-modified bentonite (AB) and PA-grafted organobentonite (ABP) resulted in a significantly higher (72–78%) degradation of phenanthrene than bentonite (62%) by the bacterium. The growth and proliferation of bacteria were supported by ABP which not only eliminated Cd toxicity through adsorption but also created a congenial microenvironment for bacterial survival. The macromolecules produced during ABP–bacteria interaction could form a stable

* Correspondence to: B. Sarkar, Future Industries Institute (formerly Centre for Environmental Risk Assessment and Remediation), University of South Australia, Mawson Lakes, SA 5095, Australia.
** Correspondence to: R. Naidu, Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE), ACT Building, University of Newcastle, Callaghan, NSW 2308, Australia.
E-mail addresses: binoy.sarkar@unisa.edu.au (B. Sarkar), ravi.naidu@newcastle.edu.au (R. Naidu).

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clay-bacterial cluster by overcoming the electrostatic repulsion among individual components. Findings of this study provide new insights for designing clay modulated PAH bioremediation technologies in mixed-contaminated water and soil.

1. Introduction

Polycyclic aromatic hydrocarbon (PAH) is one of the commonly encountered contaminants in the environment posing carcinogenic, mutagenic, and teratogenic effects on human health (Lemieux et al., 2015). This group of contaminants frequently coexists with toxic heavy metals at sites like former gasworks (Duan and Naidu, 2013; Olaniran et al., 2013; Thavamani et al., 2012). Finding novel clean-up technology for these contaminants is urgently needed because of the increased awareness among community about their harmful effects and stringent regulations evolving around the globe.

PAHs, specially the 4–5 ring compounds, are challenging to remediate because of the compounds' toxicity to microorganisms and their limited bioavailability. Where PAHs are present together with metals as mixtures, metals may exert additional toxic stress to microorganisms and make microbial degradation extremely difficult (Lu et al., 2013; Vig et al., 2003). One possible strategy in such scenario would be reduction of metal toxicity and improvement of PAH bioavailability.

A range of natural or modified adsorbents including clay minerals could be used for reducing metal bioavailability and hence toxicity to bacteria (Gupta and Bhattacharyya, 2006; Gupta et al., 2014; Mohan et al., 2014). However, preconditions of successful remediation in a mixed-contaminated scenario include the following: (a) the adsorbent would reduce metal bioavailability but improve PAH bioavailability and degradation, and (b) the adsorbent is fully compatible to bacterial growth and proliferation (Biswas et al., 2015a; Sarkar et al., 2012).

Clay minerals can be modified in several ways for adsorbing environmental contaminants including PAHs and heavy metals (Sarkar et al., 2012). Adsorbent prepared by grafting long chain fatty acid chelates, e.g., palmitic acid (PA) and stearic acid (SA), on clay minerals and the product's application in reducing heavy metal (cadmium) toxicity to *Pseudomonas putida* was reported previously (Malakul et al., 1998b). The degree of biocompatibility of such organoclay adsorbents is likely to vary according to the organoclay constituents (Sarkar et al., 2013; Sarkar et al., 2010a) and individual bacterial species. However, report on such interactions and their plausible effect on bacterial proliferation and PAH biodegradation under mixed-contaminated situation is scant in the literature.

The current study therefore was conducted to evaluate the growth performance of *Mycobacterium gilvum* VF1, a well-known PAH degrading bacterium (Kästner et al., 1994; Mutnuri et al., 2005; Pagnout et al., 2007), in the presence of a PA grafted di(hydrogenated tallow) dimethylammonium based organobentonite (Sarkar et al., 2011) under an aqueous condition co-contaminated with cadmium (Cd) and phenanthrene. The adsorption of Cd and biodegradation of phenanthrene were thoroughly investigated under the above conditions.

2. Materials and methods

2.1. Preparation of adsorbents and their characterisation

An Australian bentonite (B) (CEC = 85.8 cmol $[p^+]$ kg^{-1}; conductivity = 3.74 dS m^{-1}; clay content = 83.4%; silt content = 2.5%) was used in this study. This clay is a montmorillonite-type bentonite mixed with traces of dolomite and halite (see the X-ray diffraction (XRD) pattern in Fig. 1); the material is mainly composed of 47.06% SiO_2, 25.28% Al_2O_3, 6.51% TiO_2, 2.34% Na_2O, 2.81% Fe_2O_3, 0.67% CaO, 0.23% K_2O (analysed by Energy Dispersive X-ray Absorption Spectroscopy (EDAX), image not shown).

Bentonite (B) was modified with the surfactant (Arquad® 2HT-75 which is a di(hydrogenated tallow) dimethylammonium type cationic surfactant; purchased from Sigma-Aldrich, Australia) at a loading rate 100% of CEC of the clay (Sarkar et al., 2011). The surfactant modified bentonite was denoted as AB.

In the next step, the surfactant modified bentonite (AB) was added to palmitic acid (PA) (purchased from Sigma-Aldrich, Australia; purity >98%; dissolved 10.99 g (≈100% CEC of the clay) in 1000 mL ethanol–water mixture (1:1 v/v) at pH 8.0–8.5) (Biswas et al., 2015a). The above mixture was agitated gently over 4 h on a magnetic stirrer. The final product was collected by centrifugation at 3400 ×*g* for 20 min, washed thoroughly with Milli-Q water (resistivity = 18.2 Ω cm^{-1}), dried at 60 °C and stored in an air-tight container. The PA-grafted organobentonite was termed as ABP.

XRD patterns of the original bentonite (B) and its modified products (AB and ABP) were obtained using CuK_α radiation (λ = 1.540598 Å) on a PANalytical Empyrean X-ray diffractometer equipped with PIXcel^{3D} detector (PANalytical Inc., The Netherlands). The instrument was operated at 40 kV and 40 mA between 2° and 50° 2θ at a step size of 0.0263°. The basal spacing (d) was calculated from the 2θ value using Bragg's equation ($n\lambda = 2d\sin\theta$, where, n = an integer, λ = wavelength and θ = the scattering angle).

Fourier Transform Infrared (FTIR) spectra of the adsorbents were collected by using an Agilent Cary 600 Series FTIR Spectrometer. Finely powdered samples were mixed with dehydrated KBr and discs were prepared with the aid of a hydraulic press. Spectra were recorded in the range of 4000 cm^{-1} to 400 cm^{-1} with a 4 cm^{-1} resolution.

2.2. Adsorption of Cd

In a batch experiment, 0.1 g of each of the adsorbents (B, AB, ABP) in duplicate was added into 20 mL of Cd solutions (prepared with $Cd(NO_3)_2$ (Scharlab) of various concentrations (0–75 mg L^{-1}) in Milli-Q water in a 50 mL polypropylene tube. The screw-tight tubes were then incubated in an end-over-end shaker for 24 h at 25 °C temperature. The Cd concentration was measured by ICP-MS (Model 7500c, Agilent Technologies, Japan) in the clear supernatant obtained by centrifugation at 3400 ×*g* for 20 min. The quantity of Cd adsorbed was calculated using the following Eq. (1):

$$q_e = V\frac{C_i - C_e}{M \times 1000} \quad (1)$$

Where, q_e is the amount of solute adsorbed on the adsorbent (mg g^{-1}), C_i the initial liquid phase concentration of the solute (mg L^{-1}), C_e the equilibrium liquid phase concentration of the solute (mg L^{-1}), V the volume of liquid phase (mL) and M the mass of the adsorbent (g).

2.3. Bacterial culture and inoculum preparation

M. gilvum VF1, which is able to degrade PAHs, was purchased from the German collection of microorganisms and cell cultures (DSMZ, Germany) (Kästner et al., 1994). Freeze-dried cells were revived, grown and stored at −80 °C until further use. Prior to conducting