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The influence of sorption on polyaromatic hydrocarbon biodegradation in the presence of modified nonionic surfactant organoclays

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ABSTRACT

This study examined naphthalene (NAP) biodegradation in the presence of organoclays (Ca-Mon) modified by two nonionic surfactants (TX-100 and Brij 35). The nonionic surfactants are adsorbed onto the clay and this provides extra organic matter, which then generates a partition effect that affects NAP biodegradation. NAP biodegradation was found to be affected by the presence of monomer (MN) or micelle (CMC) surfactant-modified organoclays. The *pseudo* first-order rate constants for NAP biodegradation were as follows: NAP-Brij35-MN > NAP-Brij35-CMC > NAP > NAP-TX-100-MN > NAP-TX-100-CMC, exactly opposite to the soil organic carbon–water partitioning coefficient (k_{oc}) values for NAP in the presence of different nonionic surfactants. The bacterial community and physiological characteristics of PAH biodegradation were different between the free-living bacteria and the particle-attached bacteria and was not associated with the surfactant used. The dominant free-living bacteria involved in NAP biodegradation were *Brevundimonas diminuta*, *Caulobacter* spp., *Mycoplana bullata*, *Acidovorax* spp. and *Pseudomonas aeruginosa*. Community-level physiological profiling showed significant differences between free-living bacteria and particle-attached bacteria. Significant extracellular enzyme activities, such as esterases and phosphatases, were associated with the TX-100-MN/CMC modified organoclays. Practically, the results suggest that Brij 35 MN-modified organoclay is the most suitable substrate when using a nonionic surfactant for NAP bioremediation.

Introduction

Hydrophobic organic contaminants (HOCs) are defined as persistent compounds that can be existed in soil for a long time. Polyaromatic hydrocarbon (PAH) is one of the most common HOCs and exposure to this chemical involves biological risk; it is produced during incomplete combustion and is associated with industry and transportation. An integrated approach that combines bioremediation with the surfactant/solvent flushing technique has been identified as a successful approach to the removal of PAH from contaminated sites (Mohan et al., 2006; Chi, 2010). Biodegradation provides an effective and clean treatment method when carrying out PAH removal, as well as having economic advantages. Bacterial species, including both pure stains and mixed cultures, have been shown to be able to degrade PAHs in many successful case studies of *in situ* bioremediation at polluted sites (Haritash and Kaushik, 2009). Nonionic surfactants offer a potential improvement when remedying PAH-contaminated soils via a soil-flushing operation; this is because they are able to enhance the apparent solubility of the PAHs present in the contaminated soil. When microorganisms are able to solely or preferentially utilize dissolved PAHs in the presence of surfactant-modified organoclays, the biodegradation rate of PAH can be either accelerated or inhibited by the presence of non-ionic surfactant. These inconsistent results need to be explored on a case-by-case basis because PAH biodegradation *in situ* will differ between contaminated sites. A number of studies have found that there is a positive effect when nonionic surfactants are used to enhance the solubility and desorption of PAHs (Jin et al., 2007; Fernando Bautista et al., 2009). However, negative effects due to surfactant toxicity and an inhibition of bacterial growth in soil have

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also been found (Allen et al., 1999; Yuan et al., 2000; Chang et al., 2014). Possible mechanisms that influence these effects include physical sorption and chemical interactions, both of which seem to play important roles in PAH biodegradation when different complexes of soil and PAH–nonionic surfactant are present. For example, the cohesive energy density of the PAH–nonionic surfactant complex has been shown to be able to alter the PAH biodegradation rate in aqueous solution (Chang et al., 2007a). More aggressive approaches to the remediation process are needed and more attention needs to be paid to the development of PAH-contaminated soil remediation systems.

Clay minerals, due to their layered structure, high cation exchange capacity and low cost, are widely used as adsorbents when creating solid waste disposal land-filled liners. The modification of this clay by adding organic matter has been paid significant attention because this approach is a possible means of effectively remediating any HOCs present in soil and groundwater. Many methods have been used when preparing organoclays that have been modified by the addition of organic substances (Akbulut et al., 2012; Singla et al., 2012). Specifically, surfactant-modified organoclays, which are able to reduce the hydrophilicity of bacterial surface, have been used as absorbents for the successful removal of organic and inorganic pollutants, including chromate, chlorinated phenols (Michot and Pinnavaia, 1991), herbicide and nitrate (Bagherifam et al., 2014; Dutta and Singh, 2015). However, the adsorption by organoclay of different surfactant structures, namely monomer (MN) or micelle (CMC), is known to affect the distribution of PAH in the soil–water system. These adsorbed surfactants are able to enhance the soil organic matter (SOM) content, while the PAHs present is either partitioned to the SOM/adsorbed surfactants or dissolved in the solution. For example, PAHs in the CMC-modified organoclay have a tendency to partition into solution (Edwards et al., 1994). These mechanisms have an influence on the efficiency of PAH biodegradation. Little research has been reported on PAH biodegradation using organoclays modified by the different concentrations of nonionic-surfactant. The bacterial communities involved, as well as their physiological profiles, have been discussed even less.

The aim of this study is to enhance PAH biodegradation by the use of MN/CMC-modified organoclays. The sorptive influence of nonionic surfactants and their effect on PAH biodegradation are explored in this study. Variation in the bacterial community and in the bacterial population's physiological characteristics, including community-level physiological profile (CLPP), carbon degradation potential (CDP) and extracellular specific enzyme activity, was also measured during PAH biodegradation using both MN-modified and CMC-modified organoclays. Our findings should help to provide alternative processes that can be developed into new green and clean technologies useful for *in situ* PAH bioremediation.

Materials and methods

PAH, nonionic surfactants, clay and chemicals

The target PAH: naphthalene ($C_{10}H_8$, NAP) was purchased from the Acros (99% purity). Two nonionic surfactants: (1) octylphenol [9.5] polyethoxylate (TX-100) with an average ethylene oxide (EO) chain length of $n = 9.5$ and (2) polyoxyethylene [23] lauryl ethers (Brij 35) were obtained from the Merck Company, Germany. Ca-montmorillonite (Ca-Mon) was selected as the material for modification during this study and was purchased from the Source Clays Repository, Purdue University, West Lafayette, IN, U.S.A. The physicochemical characteristics of the Ca-Mon are presented in Table A.1. The Ca-Mon was used directly without pretreatment. All chemical compounds in this paper were of analytical grade or better, with purities >98%.

Microorganisms

The PAH-biodegrading microorganisms were enriched from soil samples that had been collected from several PAH-contaminated sites located in Chung-Li City, Taiwan. Enrichment was carried using a series of batch cultures followed by incubation in a chemostat system (Chang et al., 2006). The chemostat was fed with sterilized PAH-mineral salts medium (MSB) that included a mixture of the selected PAHs as carbon source, namely NAP, phenanthrene (PHE) and pyrene. The concentrations of these PAHs were such that their water solubility allowed complete solubility in the MSB. The concentrations of each PAH at the inlet and the outlet of the chemostat were regularly monitored in order to confirm PAH biodegradation.

Nonionic surfactant-modified organoclays preparation

Table 1 lists nonionic surfactant-modified organoclays that were created by adding different nonionic surfactants to Ca-Mon. The ratios of surfactant-modified Ca-Mon to MSB were set at 2:25 (g:ml). The concentrations of nonionic surfactant in the Ca-Mon material were set by adding different amounts to reach levels below and above the nominal CMC concentration of the MSB, which allowed the formation of either MN-modified organoclay or CMC-modified organoclay. The surfactant-modified organoclays were equilibrated using a reciprocating shaker for 24 h at room temperature. The surfactant-modified systems were adjusted to pH 7.0 in order to allow optimum bacterial growth.

Experimental procedure

Biodegradation experiments begun by transferring a mixed culture of PAH-degrading bacteria from the chemostat into the serum bottles containing NAP in the presence of surfactant-modified organoclays. The initial NAP concentrations in all experiments were adjusted to be 70% of their theoretical water solubility ($21\ \mathrm{mg\ l^{-1}}$) in order to avoid biosorption/desorption of PAH onto the bacterial biomass and NAP solubilization in surfactant-modified organoclays. The initial bacterial density at 590 nm (OD_{590}) for each inoculum was adjusted to the same value 0.02 (about 4.6×10^5 CFU $\mathrm{ml^{-1}}$) for all experiments. Inoculum samples were incubated using a reciprocating shaker at 125 rpm at room temperature, which averaged 28 °C, in a dark environment during the batch experiments. No obvious NAP loss was detected. The experiments were sampled regularly to allow analysis and were always kept at a constant surfactant-modified clay-to-MSB ratio (2g:25 ml)

Table 1

The nonionic surfactant-modified organoclays used in this study.

No.	Surfactant-modified organoclays	Nonionic surfactant			OC (%) by sorption of surfactant	Log k_{oc}[c] (NAP)
		Compound	C_0[a] (mg L^{-1})	C_e[b] (mg L^{-1})		
1	Ca-Mon (control)	–	–	–	<0.03	3.29, a
2	TX-100-MN-Clay	TX-100	1000	65	2.86	3.24, b
3	TX-100-CMC-Clay		4500	195	6.76	3.08, c
4	Brij35-MN-Clay	Brij 35	1200	65	11.60	2.74, d
5	Brij35-CMC-Clay		2400	195	18.56	2.54, e

[a] Initial concentration for the modified organoclay.

[b] Equilibrated concentration of the modified organoclay.

[c] Values are the mean of $n = 3$. Values in the column of Log k_{oc} that differ at $p < 0.05$ are followed by different letters.