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Efficiency of surfactant-enhanced bioremediation of aged polycyclic aromatic hydrocarbon-contaminated soil: Link with bioavailability and the dynamics of the bacterial community

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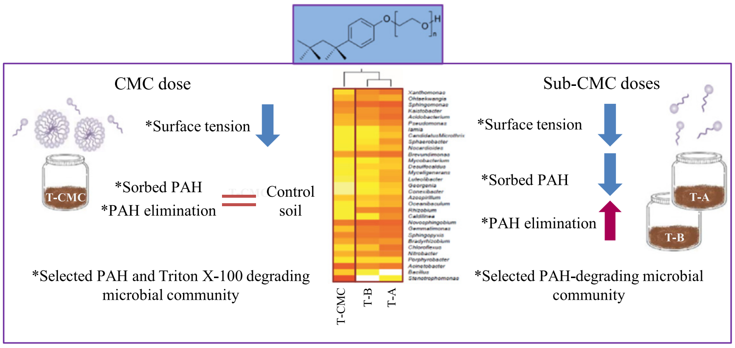
HIGHLIGHTS

- PAH degradation was enhanced by surfactant at sub-CMC doses in aged-contaminated soil.
- Soil bacterial community dynamics were changed by all tested surfactant doses.
- PAH degradation was negatively affected by a selected bacterial community at CMC dose.
- SEBR economic cost would be reduced by the application of sub-CMC doses.

GRAPHICAL ABSTRACT



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ABSTRACT

Shifts in the bacterial-community dynamics, bioavailability, and biodegradation of polycyclic aromatic hydrocarbons (PAHs) of chronically contaminated soil were analyzed in Triton X-100–treated microcosms at the critical micelle concentration (T-CMC) and at two sub-CMC doses. Only the sub-CMC-dose microcosms reached sorbed-PAH concentrations significantly lower than the control: 166 ± 32 and 135 ± 4 mg kg^{-1} dry soil versus 266 ± 51 mg kg^{-1}; consequently an increase in high- and low-molecular-weight PAHs biodegradation was observed. After 63 days of incubation pyrosequencing data evidenced differences in diversity and composition between the surfactant-modified microcosms and the control, with those with sub-CMC doses containing a predominance of the orders Sphingomonadales, Acidobacteriales, and Gemmatimonadales (groups of known PAHs-degrading capability). The T-CMC microcosm exhibited a lower richness and diversity index with a marked predominance of the order Xanthomonadales, mainly represented by the *Stenotrophomonas* genus, a PAHs- and Triton X-100–degrading bacterium. In the T-CMC microcosm, whereas the initial surface tension was 35 mN m^{-1}, after 63 days of incubation an increase up to 40 mN m^{-1} was registered. The previous observation and the gas-chromatography data indicated that the surfactant may have been degraded at the CMC by a highly selective bacterial community with a consequent negative impact on PAHs biodegradation. This work obtained strong evidence for the involvement of physicochemical and biologic influences determining the different behaviors of the studied microcosms. The results reported here contribute significantly to an optimization of, surfactant-enhanced bioremediation strategies for chronically contaminated soil since the application of doses below the CMC would reduce the overall costs.

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1. Introduction

Bioremediation is an economically and environmentally friendly alternative for the clean-up of soil contaminated by recalcitrant pollutants such as polycyclic aromatic hydrocarbons (PAHs) (Lladó et al., 2015). Although most PAHs (especially those of low molecular weight) are biodegradable in the presence of a suitable microbial community, these compounds represent the main chemicals whose particular limitation in bioremediation exists because of low bioavailability (Ortega-Calvo et al., 2013). Bioavailability processes have been defined as the individual physical, chemical, and biologic interactions that determine the exposure of chemicals to the organisms associated with soils and sediments (Ren et al., 2017). Because, a low PAHs bioavailability in a contaminated environment must limit the availability of the substrate to microorganisms during bioremediation, a suitable strategy for enhancing bioavailability becomes necessary.

Surfactant-enhanced bioremediation (SEBR) is a promising chemical technology for improving the accessibility of the pollutants (Ortega-Calvo et al., 2013). When the concentration of a surfactant (amphiphilic molecule) is above the critical micelle concentration (CMC), micellar aggregates provide an additional available hydrophobic area within the central region of the constituent micelles that enhances the solubility of PAHs in an aqueous bulk solution (Mao et al., 2015). In addition to micellar solubilization, certain authors have proposed another possible mechanism involving a modification of the contaminant matrix (Adrion et al., 2016; Bezza and Chirwa, 2017; Singleton et al., 2016). This latter mechanism usually would occur at doses below the CMC through an increase in PAHs diffusivity (Adrion et al., 2016) and a decrease in interfacial tension; thus changing the wettability of the system (Bezza and Chirwa, 2017; de la Cueva et al., 2016), and in so doing, enhancing the separation of the pollutant from the soil particles.

In general, the nonionic surfactants exhibit higher hydrocarbon solubilization than cationic and anionic ones (Lamichhane et al., 2017). Thus, nonionic surfactants are the most frequently used in biodegradation approaches, mainly because of the absence of an electrical charge in the surfactant molecule—minimizing possible toxic effects—along with the generally lower CMC compared to those of cationic or anionic surfactants (Bueno-Montes et al., 2011). The application of nonionic surfactants during soil bioremediation has thus been studied extensively (de la Cueva et al., 2016; Lamichhane et al., 2017). Different SEBR strategies, however, have exhibited inconsistent effects, depending on the properties of the soil and pollutant and on the surfactant type and concentration. Certain studies have reported positive results with SEBR (Adrion et al., 2016; Bueno-Montes et al., 2011; Singleton et al., 2016; Sun et al., 2012; Wang et al., 2016; Yu et al., 2011; Zhu and Aitken, 2010), but others have registered negligible and/or negative effects (Colores et al., 2000; Ghosh and Mukherji, 2016; Liu et al., 2016; Lladó et al., 2013). The possible reasons for the negative findings include the utilization of the surfactant as a carbon and energy source in preference to the contaminants (Colores et al., 2000), a toxicity to the PAHs-degrading bacteria at surfactant supra-CMCs (Lladó et al., 2013; Mao et al., 2015), and a low availability of PAHs to microorganisms once in the micellar phase (de la Cueva et al., 2016; Makkar and Rockne, 2003).

In addition to the possible mechanisms previously described for surfactant action in solubilizing of contaminants, PAHs biodegradation could be affected by the action of surfactants on the dynamics of the microbial community (Adrion et al., 2016; Colores et al., 2000; Zhu and Aitken, 2010). Recently the use of the molecular-biology–fingerprinting technique (PCR-DGGE) along with high-throughput DNA-sequencing have pointed to the conclusion that surfactant addition to PAHs-contaminated soils causes dramatic shifts in the microbial populations present (Colores et al., 2000; Wang et al., 2016), even under sub-CMC conditions (Colores et al., 2000), thus demonstrating the necessity to study the effect of surfactants on the complex microbial communities in PAHs-polluted soils in order to optimize the SEBR.

The aim of this study was therefore to ascertain the link between the population dynamics of the soil bacterial community, as determined by high-throughput sequencing of 16S rRNA amplicons of soil DNA and quantitative real-time PCR, and the efficiency of the SEBR strategy at different concentrations of Triton X-100 in PAHs-aged contaminated soil microcosms. Concentrations of Triton X-100 both at and below the CMC (determined on soil suspensions) were tested.

2. Materials and methods

2.1. Chemicals

The nonionic surfactant used was the octylphenol ethoxylate ether Triton X-100 (ultrapure, USB Corporation, USA), with an average number of ethylene-oxide units around 9.5, corresponding to an average molecular weight of 625 g mol^{-1}. The polymeric adsorbent resin Amberlite XAD-2 (20–60 mesh) was supplied by Sigma Aldrich, USA. The hydrocarbon extraction was performed with acetone and dichloromethane (Sintorgan, Argentina). Dibenzothiophene (Sigma Aldrich, USA) was used as an internal standard for the quantitative analysis of hydrocarbons. The PAHs were identified through comparison with the commercial standard solutions supplied by Restek, USA.

2.2. Aged PAH-contaminated soil

The soil (IPK) used for these assays—removed from a site located within a petrochemical plant in the suburb of the city of Ensenada, Argentina—had been used for a Land-Farming treatment of an area containing petrochemical sludge involving several applications of the treatment over a period of 2 years. The present samples were taken >10 years after that process was over. At the laboratory the sample was sieved (2-mm mesh) and the microcosms assembled within 48 h thereafter.

The physicochemical properties of the soil, upon analysis in the Laboratory of Soil Science at the University of La Plata, were loam at pH 7.71; organic carbon, 2.20%; organic matter, 3.78%; total nitrogen, 0.20%; available phosphorus, 0.00083%; and PAHs, as detected by gas chromatography (GC), 574 ± 138 mg kg^{-1}. The concentration of PAHs of low molecular weight (LMW) and high molecular weight (HMW) was 302 ± 84 mg kg^{-1} and 272 ± 54 mg kg^{-1}, respectively.

2.3. CMC of Triton X-100 in IPK soil

The CMC of Triton X-100 in the soil was assessed by measuring the surface tension of soil suspensions with different surfactant concentrations, as described in (Bueno-Montes et al., 2011). The CMC was determined from supernatants obtained after centrifuging (4000 rpm, 10 min) soil suspensions (2.8 g 70 ml) in distilled water that had been equilibrated with different surfactant concentrations in a 24 h incubation at room temperature with agitation in a rotary shaker. The surface tension was determined at room temperature with a Du Nouy tensiometer (F.B.R., Argentina) for each supernatant in triplicates. The means for each concentration analyzed were plotted in mN m^{-1} as a function of the logarithm of the surfactant concentration in mg l^{-1}. The CMC was calculated as the lowest surfactant concentration not leading to a significant decrease in surface tension.