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Invited paper

Dual partitioning and attachment effects of rhamnolipid on pyrene biodegradation under bioavailability restrictions

Eleonora Congiu [a], John R. Parsons [b], José-Julio Ortega-Calvo [a, *]

[a] Instituto de Recursos Naturales y Agrobiología (IRNAS), C.S.I.C., Apartado 1052, E-41080, Seville, Spain
[b] Institute for Biodiversity and Ecosystem Dynamics (IBED), University of Amsterdam, Science Park 904, 1098 XE 94248, Amsterdam, 1092 GE, The Netherlands

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ABSTRACT

We investigated the effects of different bioavailability scenarios on the rhamnolipid-enhanced biodegradation of pyrene by the representative polycyclic aromatic hydrocarbon degrader *Mycobacterium gilvum* VM552. This biosurfactant enhanced biodegradation when pyrene was provided in the form of solid crystals; no effect was observed when the same amount of the chemical was preloaded on polydimethylsiloxane (PDMS). An enhanced effect was observed when pyrene was sorbed into soil but not with the dissolved compound. Synchronous fluorescence spectrophotometry and liquid scintillation were used to determine the phase exchange of pyrene. We also investigated the phase distribution of bacteria. Our results suggest that the rhamnolipid can enhance the biodegradation of pyrene by micellar solubilization and increase diffusive uptake. These mechanisms increase substrate acquisition by bacterial cells at exposure concentrations well above the half-saturation constant for active uptake. The moderate solubilization of pyrene from PDMS by the rhamnolipid and the prevention of cell attachment may explain the lack of enhancement for pyrene-preloaded PDMS.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants and may have potentially harmful effects toward human health and the environment. Biodegradation is a key factor in the environmental impact of these hydrophobic organic compounds and is often the basis for the sustainable remediation of contaminated soils and sediments. However, successful bioremediation is often limited by the low bioavailability of these chemicals. PAHs tend to strongly sorb to solid surfaces but desorb slowly. This slow desorption is often the limiting factor in biodegradation (Johnsen et al., 2005; Yang et al., 2011).

A promising strategy to improve the bioavailability of soil-sorbed PAHs is the use of biosurfactants. In recent decades, biosurfactants have become known as environmentally benign alternatives to chemical surfactants (Banat et al., 2010). The anionic, rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* is one of the most studied biosurfactants. This biosurfactant can enhance the biodegradation of PAHs by increasing the dissolution rate of crystalline chemicals (Zhang et al., 1997), in nonaqueous-phase liquids (NAPLs) (Garcia-Junco et al., 2003), and when sorbed into soils (Congiu and Ortega-Calvo, 2014). New approaches are required to enhance the bioavailability of PAHs through biosurfactants in a cost-effective manner without exceeding regulatory concentration levels (Ortega-Calvo et al., 2013). In contaminated soils, the efficiency of biosurfactants for bioremediation is dependent on the physicochemical environment of the soil, the mass transfer rate, and the balance between the solubilization of the chemical and the sorption of the biosurfactant to the soil (Ochoa-Loza et al., 2007). The successful application of biosurfactants should also minimize the risks associated with the increased chemical activity and toxicity of the PAHs and metabolites as a result of solubilization at concentrations in excess of the metabolic potential of microorganisms. These concentrations may be high compared with the affinity constant (K_m) of microbial enzymes and uptake mechanisms, thus saturating the biodegradation process. This effect may be relevant during the treatment of point-source PAH pollution in soils and sediments, which typically have high pollutant concentrations and a wide diversity of desorption patterns (Cornelissen et al., 1998; Gomez-Lahoz and Ortega-Calvo, 2005; Rhodes et al., 2010).

Biodegradable chemical surfactants can enhance bioremediation in soils where biodegradation has already removed the fast-desorbing PAHs, leaving the slowly desorbing residue (Bueno-

* Corresponding author.
E-mail address: jjortega@irnase.csic.es (J.-J. Ortega-Calvo).

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Montes et al., 2011; Zhu and Aitken, 2010). The application of environmentally compatible rhamnolipids instead of chemical surfactants is of considerable interest for removing slowly desorbing PAHs. However, the effect of biosurfactants on the removal of slowly desorbing pollutants may be limited. For example, rhamnolipid efficiency may decrease as a result of intra-aggregate diffusion limitations on the solubilization process due to pollutant aging (Congiu and Ortega-Calvo, 2014). Rhamnolipids may also have antiadhesive activity toward bacteria (Nickzad and Deziel, 2014) and may negatively impact the biodegradation of PAHs because, under bioavailability restrictions, adherent bacteria have better access to hydrophobic pollutants than suspended bacteria (Ortega-Calvo and Alexander, 1994). Few studies have examined the solubilizing effect of rhamnolipids on PAHs for different levels of chemical activity and on bacterial attachment to the pollutant source.

Therefore, the aim of this study was to investigate the effects of different exposure scenarios that result in dissimilar phase exchanges of pyrene on rhamnolipid-enhanced biodegradation. The scenarios used in this study were designed to create wide changes in the exposure concentrations of the contaminant. Therefore, the effect of the rhamnolipid on the biodegradation of pyrene by a representative PAH degrading bacterium, *Mycobacterium gilvum* VM552, was tested with systems in which the chemical was supplied either completely dissolved in an aqueous solution, sorbed to soil, in its crystalline form or partitioned into a preloaded silicone polymer. This partitioning-based method enabled the study of the biotransformation kinetics of hydrophobic organic chemicals under controlled conditions (Smith et al., 2012). The aim was to generate different bioavailability restrictive conditions that involved dissimilar dissolution rates and aqueous phase concentrations, taking as a reference the half-saturation affinity constant for the bacterial uptake of the dissolved compound. We also investigated the phase distribution of bacterial cells in our experimental systems.

2. Experimental section

2.1. Chemicals

^{14}C-pyrene (58.8 mCi/mmol, radiochemical purity >98%) was purchased from Campro Scientific GmbH (Veenendaal, The Netherlands). Unlabeled phenanthrene and pyrene were obtained from Sigma–Aldrich (Madrid, Spain). Analytical grade hexane and acetone were supplied by Panreac (Barcelona, Spain). Polydimethylsiloxane (Silastic MDX4-4210 BioMedical Grade Elastomer Kit) was purchased from Dow Corning Corporation (Midland, MI).

2.2. Bacteria, media, and cultivation

P. aeruginosa 19SJ, a strain originally isolated from a petroleum-contaminated soil, was selected as the rhamnolipid biosurfactant producer. The strain was routinely maintained in a liquid SWF medium containing 2% (w/v) mannitol as the sole source of carbon (Garcia-Junco et al., 2003).

M. gilvum VM552, a strain able to grow with phenanthrene and pyrene, was used as the inoculum for mineralization experiments. The strain was cultured with phenanthrene as the sole source of carbon and prepared for mineralization experiments in an inorganic salt solution (mineralization medium, MM) as previously described (Bueno-Montes et al., 2011). To prevent the precipitation of the rhamnolipid, the pH of the solution was adjusted to 6.7 with 0.05 M sodium bicarbonate.

2.3. Biosurfactant

The rhamnolipid biosurfactant produced by *P. aeruginosa* 19SJ was purified and quantified following previously described procedures (Congiu and Ortega-Calvo, 2014). The biosurfactant is composed mainly of L-rhamnosyl-3-hydroxydecanoyl-3-hydroxydecanoate and L-rhamnosyl-L-rhamnosyl-3-hydroxy-decanoyl-3-hydroxydecanoate, besides a small proportion of other congeners with variable length-hydrocarbon chains (C10–C18). The final rhamnolipid concentration was quantified as rhamnose equivalents (RE) by the orcinol method. The surface tension of the rhamnolipid solutions (in MM) was estimated at 23 °C with a TD1 Lauda ring tensiometer. In our conditions, the critical micelle concentration (CMC) of the biosurfactant was 40 mg/L.

2.4. Sorption onto soil, desorption and solubilization

The sorption of ^{14}C-labeled pyrene onto soil was achieved using a previously described procedure (Congiu and Ortega-Calvo, 2014) and adapted to create different exposure concentrations. The soil sample used in this study was a sandy-loam forest soil originating from Los Alcornocales Natural Park (Cádiz, Spain) with 6.1% organic matter and 5.5% clay. The background concentration of pyrene in the soil was 66 μg/kg, which was present in a highly recalcitrant form (Posada-Baquero and Ortega-Calvo, 2011). Briefly, 16 mg of dry soil was introduced into 50-mL glass bottles (Schott), together with 10 mL MM containing 8.4 ng/mL dissolved ^{14}C-pyrene (5000 dpm/mL). The bottles were incubated for 24 h. This contact period was sufficient to cause restrictions for the biodegradation of the soil-sorbed pyrene (Congiu and Ortega-Calvo, 2014). The concentration of ^{14}C-pyrene in the aqueous solution was determined after centrifugation, by radioactivity measurements using a liquid scintillation counter (Model LS6500, Beckman) while the concentration of the sorbate was calculated by difference. Assuming that equilibrium was achieved after this contact period, the solid-water distribution, *Kd* (in L/kg) was calculated as previously described (Congiu and Ortega-Calvo, 2014). A theoretical *Kd* value was estimated from the organic carbon-normalized distribution coefficient (*Koc*) for the compound (Schwarzenbach et al., 2003).

Desorption experiments with soil-sorbed pyrene were performed in soil suspensions with the Tenax solid-phase extraction method using a previously described procedure (Congiu and Ortega-Calvo, 2014). After selected time intervals, Tenax was separated from the soil suspensions and the same amount of fresh Tenax was added to repeat the cycle. The Tenax was extracted with acetone-hexane (1:1) for subsequent radioactivity measurements. This procedure was not possible with the rhamnolipid because of the interference of the biosurfactant foam on the recovery of the Tenax beads. Instead, the solubilization of sorbed pyrene in the presence of the rhamnolipid was determined by centrifugation of soil suspensions as described elsewhere (Congiu and Ortega-Calvo, 2014).

2.5. Dissolution of crystalline and polydimethylsiloxane-associated pyrene in the presence of rhamnolipid

The effects of the rhamnolipid on the dissolution of crystalline pyrene and pyrene-preloaded polydimethylsiloxane (PDMS) were studied using conditions comparable with those of the mineralization experiments but in the absence of bacteria (Tejeda-Agredano et al., 2014). To measure the dissolution of crystalline pyrene, an acetone solution of ^{14}C-pyrene (50,000 dpm) and of unlabeled pyrene to achieve a final concentration of 5 μg/mL, was evaporated at the bottom of 15-mL Pyrex tubes. Then, 5 mL of MM containing rhamnolipid (400 mg/mL) and bicarbonate was added to the tubes.