# Impact of Rhamnolipid Addition on Two Phenanthrene (PHE) Spiked Soils: Sorption, Degradation Kinetics and Phenanthrene Degrading Bacteria

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## **Extended Abstract**

Polycyclic aromatic hydrocarbons (PAHs) are persistent toxic pollutants which present geoaccumulation characteristics. They are more or less strongly sequestrated in soils, and the effectiveness of bioremediation processes is closely linked to their bioavailability for the soils degrading bacterial communities. Rhamnolipids are glycolipidic biosurfactants produced by *Pseudomonas aeruginosa*, which are used in bioremediation processes because of their ability to remobilize PAHs bounded to soil particles. In this study, the objective was to study the impact of addition of biosurfactants, and especially rhamnolipid, (i) on phenanthrene (PHE) sorption to soil, (ii) on PHE degradation kinetics and (iii) on PHE degrading bacteria on two dissimilar soils ( $P_V$  soil having markedly higher contents of clays and organic matter (OM) than PPY soil).

- (i) Sorption isotherms were performed with PHE on both soils, with or without rhamnolipid addition. To compare, isotherms were also realized with a cyclolipopeptidic biosurfactant (produced by *Pseudomonas fluorescens* PFa7b).
- (ii) Degradation kinetics were performed on both soils spiked with PHE (300 mg/kg), and PHE dissipation was monitored for 60 days. Remaining PHE in soils was analyzed after microwave-assisted extraction and analyzed by GC-MS.
- (iii) To assess the impact of rhamnolipid addition on PHE degrading bacteria, DNA stable isotope probing (SIP) method was used. Two sets of microcosms were established, and spiked at 300 mg/kg with <sup>12</sup>C PHE or <sup>13</sup>C PHE, with and without rhamnolipid addition. The <sup>12</sup>C PHE microcosms allowed us to control PHE dissipation and to determine sampling dates (after 20% and 80% PHE dissipation) for DNA analyzes in <sup>13</sup>C PHE microcosms. After microbial DNA extraction and quantification, a separation of <sup>12</sup>C and <sup>13</sup>C labelled DNA allowed us to characterize bacteria having consumed <sup>13</sup>C PHE.

 $K_d$  partition coefficients, obtained from sorption isotherms, were quite similar in the two soils, showing that in the first sorption phase, bioavailability of PHE did not depend on clays and OM contents in soils. By contrast, rhamnolipid allowed a greater desorption of PHE from PPY soil than from Pv soil, showing that PHE was more easy to mobilize by biosurfactants from soils poorer in clays and OM.

Comparatively to rhamnolipid, the cyclolipopeptidic biosurfactant had no impact on PHE desorption from soil.

Kinetic studies on PHE dissipation showed very different behaviors for the two soils. PHE dissipation was significantly faster on Pv soil, showing that in the first rapid degradation phase, the presence of large amounts of geosorbents does not limit its bioavailability and its degradation by natural soils microorganisms. By contrast, dissipation in PPY soil was characterized by a long lag phase before degradation. The rapid degradation of PHE in Pv soil was not influenced by the addition of rhamnolipid while PHE degradation in PPY soil was accelerated, only if the rhamnolipid amount was sufficient.

Finally, DNA SIP experiments allowed us to differentiate bacterial communities in the two dissimilar soils, particularly PHE degrading bacteria which have very different biodegradation behavior. These experiments allowed determining the impact of rhamnolipid addition on degrading bacteria, particularly on PPY soil.