Rhamnolipid-enhanced solubilization and biodegradation of PAHs in soils after conventional bioremediation

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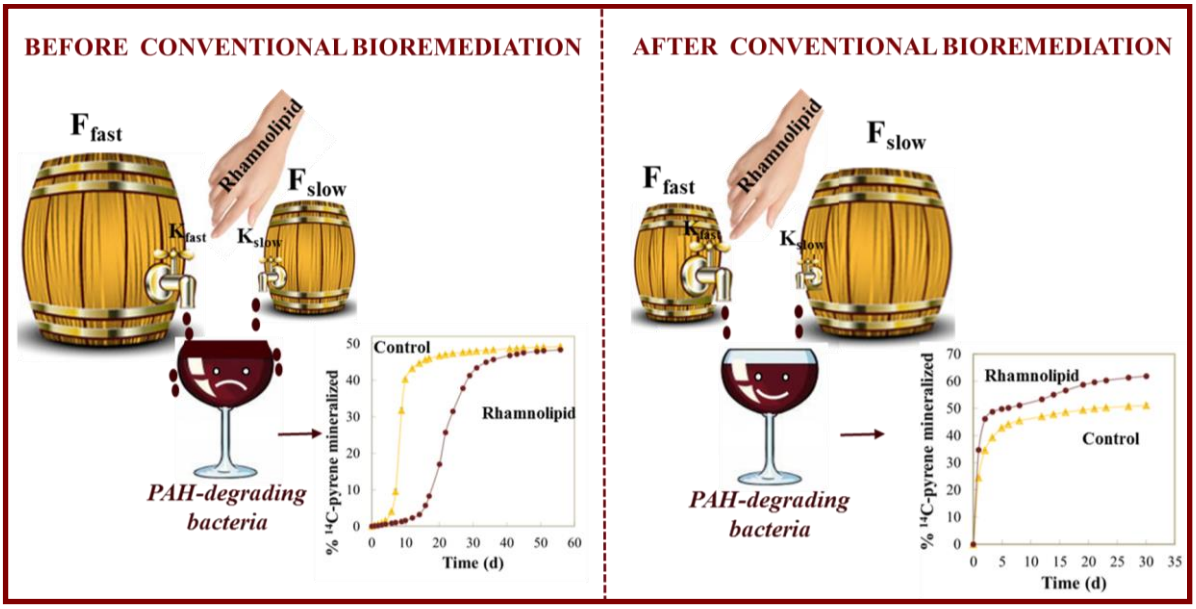
Abstract

The application of a rhamnolipid biosurfactant for enhanced solubilization and biodegradation of slowly desorbing polycyclic aromatic hydrocarbons (PAHs) in contaminated soils was determined in this study. The soil samples exhibited different levels of pollution and different bioremediation stages: the first soil originated from a creosote-polluted site, contained 4370 mg kg^{-1} of PAHs and had not been bioremediated; the second soil was the same as the first but had received bioremediation treatment with nutrient amendment in biopiles for a period of 5 months and contained 580 mg kg^{-1} of PAHs after this treatment; the third soil was treated by bioremediation for several years to reduce the concentration of PAHs to 275 mg kg^{-1}. The kinetics of PAH desorption were determined to assess the magnitude of the slowly desorbing fractions present in the polluted soil and to optimize the biosurfactant effectiveness in terms of biodegradation. The soils that had been treated by bioremediation were enriched in slowly desorbing PAHs. The rhamnolipid at a concentration above its critical micelle concentration enhanced biodegradation in the soils that had been bioremediated previously. The measurement of residual concentrations of native PAHs showed the promoting effect of the biosurfactant on the biodegradation of the slowly desorbing fractions. Interestingly, benzo(a)pyrene was biodegraded in the soil that had been bioremediated for a long time. Rhamnolipid can constitute a valid alternative to chemical surfactants in promoting the biodegradation of slow-desorption PAHs, which is one of the most important problems in bioremediation, but the efficiency depends strongly on the bioremediation stage in which the biosurfactant is applied.

47 **GRAPHICAL ABSTRACT**



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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are the best representatives of chemicals for which specific limitations in bioremediation exist due to low bioavailability. These compounds have a low solubility and high hydrophobicity, and for this reason, an unacceptably high concentration of these organic compounds can remain sorbed to the soil after conventional bioremediation [1-3]. The impact of the biphasic desorption kinetics of these compounds on their biodegradation is well known [4,5], where the second, slow phase of desorption, with half-lives of up to months or even years, is often the limiting factor. Currently, the slow desorption of PAHs and other nonpolar contaminants in soils still represents a challenge for bioremediation, because the remaining pollutants may follow exposure routes specific for humans and ecological targets, therefore affecting environmental risk assessment and management decisions [6, 7].

To improve the bioremediation of PAH-polluted soils, the bioavailability of slowly desorbing PAHs can be enhanced with the use of chemical surfactants. The few studies reporting precise measurements of biodegradation rates of slowly desorbing PAHs in polluted soils show an enhancement of the transformation in the presence of nonionic surfactants, although the enhancement usually occurs after the removal of the fast desorbing fraction through traditional bioremediation [8, 9]. However, chemical surfactants have potential disadvantages when used in bioremediation due to their possible toxicity, effects on soil quality, and economic impact. A promising alternative is the use of biologically produced surfactants. Among these, rhamnolipid, an anionic glycolipid biosurfactant produced by *Pseudomonas aeruginosa*, is the most extensively studied. Its nontoxic, biodegradable and environmentally benign nature and its possibilities for large-scale production make this biosurfactant a reference for environmental applications, including bioremediation [10, 11]. Despite these advancements, studies focused on the biodegradation of slowly desorbing PAHs in the presence of rhamnolipid and other biosurfactants are very scarce. The only available assessment of rhamnolipid was a recent report on pyrene aged in soil under laboratory

conditions [12]. Using well-controlled sorption and aging conditions for ^{14}C-pyrene, the study demonstrated biosurfactant-enhanced desorption and biodegradation of the aged compound. However, the rhamnolipid efficiency decreased, compared with non-aged conditions as a result of intra-aggregate diffusion limitations to the solubilization process. To date, no studies exist about rhamnolipid, as well as other biosurfactants, that combine measurements of slow desorption and biodegradation of native PAHs in contaminated soils, which is necessary for the definitive integration of biosurfactants into bioremediation.

The present study investigated the effect of rhamnolipid in the solubilization and biodegradation of slowly desorbing PAHs in three field-contaminated soils selected for their different pollution profiles. One of these soils was polluted by creosote originating directly from a field site. The second soil was the same as the first but had received bioremediation treatment for five months, and the third soil was a manufactured gas plant (MGP) soil that had been previously treated extensively by conventional bioremediation for several years. We studied five target PAHs, having three to five benzene rings, and differing in their physicochemical properties and susceptibility for microbial attack. The objectives of this study were to 1) characterize the desorption kinetics of native PAHs in these soils, determining the exact magnitude of the slow-desorbing fractions, 2) determine the effect of rhamnolipid on the solubilization and biodegradation of these fractions, and 3) propose, on the basis of these results, ways to improve the rhamnolipid efficiency to reduce the residual pollutant concentrations resulting from bioremediation of PAH-polluted soils.

2. Materials and methods

2.1 Chemicals

[4,5,9,10-^{14}C]-pyrene (58.8 mCi/mmol, radiochemical purity >98 %) was purchased from Campro Scientific GmbH (Veenendaal, The Netherlands). Analytical grade dichloromethane, acetonitrile, hexane and acetone were supplied by Fischer Chemical (Canada). Tenax (60-80 mesh) 177-250 µm

was supplied by Buchem BV (Netherlands). Rhamnolipid biosurfactant (R90, 90 % pure) was supplied by AGAE Technologies (Oregon, USA).

2.2 Soils

Three soils were used in this study: one untreated polluted soil and two bioremediated soils. The first soil (soil 1) was obtained by combining heavily polluted soil (silty clay loam) from a historical wood-treating facility in southern Spain with soil (sandy loam) from the agricultural experimental station of the University of Barcelona (this agricultural soil was previously treated with three cycles of autoclaving). Before combining them, both soils were air dried and sieved (2 mm sieve). A total of 20 kg of agricultural soil was combined with 10 kg of soil from the creosote site, and the mixture was homogenized in a tumbler mixer for 24 h. This creosote-polluted soil 1 had the following properties: 24.8 % clay, an organic carbon content of 7.1 %, a pH of 8.1 and 4370 mg kg^{-1} total PAH (sum of 16 EPA PAH). One of the bioremediated soils (soil 2) originated from the creosote-polluted soil 1 which was amended with urea and K_2HPO_4 to reach a C:N:P ratio of 300:10:1 and was subjected to bioremediation in dynamic biopiles for 5 months, where the water content was maintained at 40 % of the water holding capacity. After this time, this soil had an organic carbon content of 5.6 %, a similar clay content and pH as soil 1, and contained 580 mg kg^{-1} total PAH.

The other bioremediated soil (soil 3) originated from a Danish MGP site. This soil was obtained from a remediation company (Soilrem, Kalundborg, Denmark) that treated the soil in biopiles for several years to reduce the total PAH concentration to 275 mg kg^{-1} (sum of 16 EPA PAH). These residual PAHs exhibited high resistance to dissipation, according to further bioremediation efforts performed by the company that included organic amendments and composting [13]. The soil had 2.1 % organic carbon, 28.8 % clay and a pH of 7.96 [9]. These soils were air-dried, sieved (2 mm mesh) and stored in glass containers in the dark at 4 ºC until use.

2.3 Desorption

The method used for desorption experiments with soils 1, 2, and 3 used Tenax as an infinite sink and was similar to a previously described method [9]. Tenax desorption allows a permanent PAH aqueous concentration of almost zero, and therefore, sorption of the PAH back to the soil can be neglected. Briefly, 0.5 g dry soil, 35 ml milli-Q water, 0.2 ml formaldehyde and 0.7 g Tenax were placed in 50 ml stainless steel centrifuge tubes equipped with a stainless steel seal and were kept at room temperature and 120 rpm on a rotary shaker.

To obtain desorption data the following first-order, two-compartment kinetic model [5] was used:

$$S_t/S_0 = F_{fast}\exp(-k_{fast}t) + F_{slow}\exp(-k_{slow}t) \quad (1)$$

In this equation, S_t and S_o (mg) are the soil-sorbed amounts of PAHs at time t (h) and at the start of the experiment, respectively. F_{fast} and F_{slow} are the fast- and slow-desorbing fractions, and k_{fast} and k_{slow} (h^{-1}) are the rate constants of fast and slow desorption, respectively. To calculate the values of the different constants and fractions (F_{fast}, F_{slow}, k_{fast}, and k_{slow}) exponential curve fitting was used. The ln form of equation 1 was subjected to curve fitting. The fits were obtained by minimizing the squares of the differences between experimental and calculated values of ln (S_t/S_0) (Solver option in Microsoft Excell). When evaluating desorption data, half-lives for fast and slow desorption were calculated as ln $2/k_{fast}$ and ln $2/k_{slow}$, respectively.

Because our goal was to use the size of the slow desorption fractions as a benchmark to evaluate the performance of the rhamnolipid in its solubilization and biostimulation roles, we used this approach, which allowed us to satisfactorily capture the biphasic nature of the desorption process. However, we did not use the model to draw conclusions about the mechanism of desorption in each of these kinetic fractions, which was not the objective of this specific study.

149 **2.4 Solubilization of soil PAHs by rhamnolipid solutions**

150 The solubilization of PAHs in the presence of aqueous-phase rhamnolipid was determined in 50 mL
151 steel centrifuge tubes having a suspension that contained 0.5 g dry soil, 35 mL inorganic aqueous
152 solution, 0.2 mL formaldehyde (40 %), and a rhamnolipid solution to give a final surfactant
153 concentration of 1 g L^{-1}. The inorganic aqueous solution used in these experiments, called
154 mineralization medium (MM), contained KH_2PO_4 (0.9 g L^{-1}), K_2HPO_4 (0.1 g L^{-1}), NH_4NO_3 (0.1 g
155 L^{-1}), $MgSO_4 \cdot 7H_2O$ (0.1 g L^{-1}), $CaCl_2$ (0.080 g L^{-1}), $FeCl_3 \cdot 6H_2O$ (0.01 g L^{-1}), and 1 mL $L^{-1}$of a
156 microelement stock solution to obtain final concentrations of 0.0014 g L^{-1} for $Na_2MoO_4 \cdot 2H_2O$ and
157 0.002 g L^{-1} for each of the following: $Na_2B_4O_7 \cdot 10H_2O$, $ZnSO_4 \cdot H_2O$, $MnSO_4 \cdot H_2O$, and
158 $CuSO_4 \cdot 5H_2O$. The pH of this solution was adjusted to 6.7 to prevent the precipitation of the
159 rhamnolipid by adding 0.05 M sterilized sodium bicarbonate.

160 The suspensions were maintained under the same conditions as Tenax desorption
161 experiments. After certain time intervals, the tubes were centrifuged for 10 min at 17,212 g, and an
162 aliquot of the supernatant was analyzed for PAHs. The remaining supernatant was carefully
163 decanted without disturbing the soil pellet; then fresh rhamnolipid solution was added to the tube,
164 and the washing cycles were repeated for approximately 10 days. Solubilization results were
165 expressed as the percentage of compound extracted by the rhamnolipid solutions at certain time
166 intervals (F_{rhamn}, %). No attempt was done to perform a further kinetic analysis of solubilization
167 data, directly comparable to that performed with Tenax desorption, given the inherent difficulties
168 caused by the progressive sorption of the biosurfactant onto soil during continued sequential
169 extractions.The surface tension of the rhamnolipid solutions (in MM with pH 6.7) was estimated at
170 23 °C with a TD1 Lauda ring tensiometer. Under our conditions, the critical micelle concentration
171 (CMC) of the rhamnolipid in MM solution was 31.6 mg L^{-1}, which is approximately 30 times lower
172 than the surfactant concentration used in these solubilization experiments, i.e., 1 g L^{-1}.
173

174 **2.5 Biodegradation experiments**

175 Mineralization experiments were performed in slurries: 1 g of sample was placed in 250-mL
176 Erlenmeyer flasks, and 67.6 mL of MM solution and 1 mL of the same solution containing 80,000
177 dpm of radiolabeled pyrene was added. The flasks were closed with Teflon-lined stoppers, from
178 which a 5-mL vial containing 1 mL of 0.5 M NaOH was suspended to trap $^{14}CO_2$. The flasks were
179 incubated at 23 ± 2°C on an orbital shaker operating at 120 rpm for 1 day for equilibration (during
180 this period of time the mineralization of pyrene was insignificant).

181 After equilibration, the rhamnolipid was added (0.4 mL of a 0.05 M sodium bicarbonate stock
182 solution, to give a final concentration of 1 g L^{-1}). Although this minimal volume of solution added
183 did not affect the pH of the buffered MM medium, the same volume of a sodium bicarbonate
184 solution (0.05 M) was added to the rhamnolipid-free controls. These suspensions were inoculated
185 with *Mycobacterium gilvum* VM552. The strain was cultured with phenanthrene as the sole source
186 of carbon and prepared for mineralization experiments in the mineralization medium as previously
187 described [9]. Each flask received 1 mL of this inoculum (containing approximately 10^8 cells g^{-1} soil).
188 All biodegradation experiments were performed with inoculated suspensions. To prevent the
189 precipitation of the rhamnolipid, the pH of the mineralization medium was adjusted to 6.7 with 0.05
190 M sodium bicarbonate. The final volume was 70 mL in every flask to maintain the same soil-
191 aqueous phase ratio as in the desorption and solubilization experiments. Measurements of the
192 mineralization of radiolabeled pyrene were carried out as described elsewhere [14]. To estimate the
193 biodegradation, the residual contents of native PAHs were determined in separate, duplicate flasks
194 that were incubated under the same conditions but that contained no ^{14}C-labeled compound. At the
195 end of the assays, the flasks were sacrificed and kept frozen at -80 ºC until analysis of the PAH
196 content by HPLC. Under the same conditions, control experiments in the absence of the
197 rhamnolipid biosurfactant were also run.

In the experiment with delayed biosurfactant addition to soil 2, ^{14}C-pyrene was added to two different sets of soil suspensions, which were incubated in parallel under the same conditions: one at the initial stage of the experiment, to confirm the achievement of the mineralization plateau after 7 days, and a second one for the soil suspensions that received the rhamnolipid at 1 g L^{-1}. These suspensions were incubated for another 13 days, in parallel to suspensions that received no ^{14}C and were sacrificed for the measurement of native PAH concentrations, as described above.

2.6 Analysis of native PAHs

To measure the PAH concentration, the soil samples were separated from the supernatant by decantation. The surface tension was measured on the supernatant to semiquantively determinethe rhamnolipid in solution. The soil samples (1 g) were mixed and ground with 1 g of anhydrous sodium sulfate and then extracted in a Soxhlet apparatus for 8 h with 100 mL of 1:1 (v/v) dichloromethane/acetone. The extracted volumen was reduced with a rotary evaporator and then cleaned with a Sep-pak Fluorisil cartridge. The cleaned extract was taken near dryness under a gentle stream of nitrogen. The residue was then dissolved in acetonitrile and filtered through a syringe filter of nylon. The analysis of native PAHs was carried out using a Waters HPLC system with two detectors (2690 photo diode array and fluorescence). The mobile phase used in this system was an acetonitrile/milli-Q water gradient. More specifications of this method are described elsewhere [15]. In the samples from the rhamnolipid biosurfactant treatments, acetone was used for extraction instead of dichloromethane/acetone.

3. Results and discussion

3.1 Desorption kinetics of PAHs

The desorption kinetics of phenanthrene, anthracene, fluoranthene, pyrene and benzo[a]pyrene were determined by Tenax extraction. Figure 1 shows the results of representative desorption

223 experiments. In this figure, we can observe that the model (eq. 1) allows a good prediction of
224 spontaneous desorption in all cases. The values of the residual sum of squares from exponential
225 curve fitting are shown in Table S1. The results for the five compounds and three soils were
226 successfully fitted, and the kinetic parameters are shown in Tables 1, 2 and 3. With the exception of
227 benzo(a)pyrene, the fast-desorbing fraction in soil 1 was the highest (up to 73.4 %), compared with
228 the other two soils. This desorption profile is typical for a soil that has not been bioremediated. This
229 fraction was significantly reduced in soil 2 and, especially, soil 3. This is in agreement with
230 previous observations that have shown traditional remediation to preferentially remove the fast
231 desorbing fraction present in PAH-polluted soils [9] and with previous studies on the recalcitrance of
232 background PAH pollution in soil [15]. Furthermore, the direct comparison of Tables 1 and 2 shows
233 that, for the five studied PAHs, k_{fast} and k_{slow} values also decreased after bioremediation, resulting in
234 a longer half-life for desorption. For example, the half-lives of the phenanthrene slow-desorbing
235 fraction were 9.2 and 335 days for soil 1 and 2, respectively.

236 **3.2 Solubilization of slowly desorbing PAHs by the biosurfactant**

237 The effect of the rhamnolipid on the solubilization of PAHs was determined. The rhamnolipid
238 biosurfactant concentration was 30 times higher than the CMC to ensure the presence of micelles in
239 the aqueous phase, what is the main factor in the solubilization process [16]. Figure 2 shows the
240 results of the solubilization of pyrene, taken as an example for a representative PAH, in the
241 presence of the rhamnolipid biosurfactant compared to Tenax-driven desorption. The direct
242 comparison of Figures 2A and B shows that the slow desorption profile of the bioremediated soil 2
243 reduced the mobilization of slowly desorbing pyrene in this soil compared with soil 1. However,
244 solubilization was significant in soil 3. The fraction of pyrene extracted by the biosurfactant in this
245 soil after 312 h was 50.7 %, double the fast-desorbing fraction (20.0 %). The effect of the
246 rhamnolipid biosurfactant on solubilization, expressed as F_{rhamn}, of the five studied PAHs is shown
247 in Tables 1-3. In general, the same trends that were observed with pyrene occurred with the other

four PAHs, independently from their physicochemical properties. The capacity of rhamnolipid to act on a significant fraction of slowly desorbing PAHs in these field-contaminated materials suggests the interaction with the compartments eventually responsible for slow desorption, such as non-aqueous-phase-like materials [17] and aged residues [12]. The relative contribution of either of these compartments to slow desorption in these field-polluted soils will be the subject of future research.

3.3 Biodegradation

The effect of the rhamnolipid biosurfactant ata concentration of 1 g L^{-1}on the biodegradation of PAHs in the three soils was tested in suspensions inoculated with *M. gilvum* VM552 cells. The mineralization of ^{14}C-pyrene, shown in Figure 3 and Table S2, was used here as a physiological indicator of the biodegradation process occurring with the native PAHs. In soil 1, ^{14}C-pyrene mineralization had a longer acclimation phase in the presence of the rhamnolipid biosurfactant and occurred at a lower maximum rate than the biosurfactant-free control (Table S2). Pyrene mineralization in soil 2 did not show any acclimation phase, but the biosurfactant still caused a lower rate and extent of mineralization. In soil 3, the effect of the biosurfactant was opposite to that in soil 2, i.e., an increase in the rate and extent of $^{14}CO_2$ production. A direct comparison of the results of solubilization and mineralization of pyrene (Figures 2 and 3, respectively), shows that the capacity of the rhamnolipid to solubilize a significant fraction of the slowly desorbing chemicals present in the soils is not always correlated with enhancements in the biodegradation rates. These results can be explained by postulating a dissimilar outcome caused by the combined effects of the enhanced solubilization of PAHs, on the one hand, and the initial concentration and desorption profile, on the other hand. In soil 1, the solubilization of PAHs initially present at high concentrations possibly caused an increased bioavailability that eventually exceeded, by competition, the metabolic potential of degrading bacteria, or, alternately, even caused a transient, toxicity-related effect on pyrene mineralization. In soil 2, which was treated, the solubilizing

potential of the biosurfactant was diminished due to its slow-desorption profile, but the relatively high initial concentrations of PAHs still may explain, in accordance with this mechanism, the observed inhibition of the mineralization of pyrene. Only in residual soil 3, having relatively low PAH concentrations, typical for a soil subjected to bioremediation for several years, was the moderate solubilization caused by the surfactant compatible with an enhanced rate of pyrene mineralization.

The residual contents on native PAHs were determined once ^{14}C-pyrene mineralization plots reached a plateau (approximately 60 days for soil 1 and 30 days for soils 2 and 3, Figure 3). These results are shown in Tables 4 to 6 and are compared with the sum of predicted concentrations of the five PAHs assuming that biodegradation acted only upon fast-desorption PAHs. In the three soils, the residual concentrations in the absence and presence of the rhamnolipid biosurfactant were in most cases lower than the predicted concentration from F_{fast}, thus evidencing the biodegradation of slow-desorbing PAHs. The impacts of the rhamnolipid observed on ^{14}C-pyrene mineralization rates (inhibition in soils 1 and 2 and stimulation in soil 3) were not reflected in the residual concentrations of native pyrene, which remained very similar with or without biosurfactant for the three soils. However, the rhamnolipid caused an effect in the case of soil 1 by reducing the residual concentration of anthracene. No effects were observed on the residual concentrations of native PAHs in soils 2 and 3. The measurement of the surface tension of the aqueous phase at the end of these treatments indicated in all cases the disappearance of the biosurfactant from the aqueous phase (60 ± 0.5 dyn cm^{-1}).

In the case of soil 2, biodegradation was also studied when the addition of rhamnolipid (1 g L^{-1}) was carried out after an incubation period of 7 days to reach the pyrene mineralization plateau (Figure 3B) to enhance the biodegradation of the slowly desorbing fractions. In this experiment, the second addition of ^{14}C-pyrene, performed together with the biosurfactant, resulted in the immediate achievement of a new plateau, at a mineralization extent of approximately 40 % after 2 days (Fig.

298 S1), although the suspensions were incubated for 13 additional days. The residual concentrations of
299 native PAHs after this 20-day treatment (Table 5) evidenced the enhanced biodegradation of
300 phenanthrene, anthracene, fluoranthene and pyrene, but this did not occur in the case of
301 benzo(a)pyrene. The measurement of the surface tension of the aqueous phase at the end of the
302 treatment evidenced the disappearance of the biosurfactant. These results indicate that the
303 biosurfactant action can be optimized through strategies oriented towards the prior removal of fast-
304 desorbing PAHs initially present in the soil, with potential negative interactions with the degrading
305 bacteria. In this sense, the rhamnolipid can be similar to a chemical surfactant, such as Brij 35,
306 which has been shown to behave similarly during bioremediation of slow-desorbing PAHs in field-
307 contaminated soils [9].

308 A higher rhamnolipid biosurfactant concentration (20 g L^{-1}) was also tested in soil 3, under
309 comparable conditions to our previous study to determine the effect of Brij 35 on the
310 biodegradation of PAHs in this soil [9]. The optimization did not include in this case a delayed
311 application of the rhamnolipid, given the low starting concentrations of the PAHs, as compared with
312 the other two soils (Tables 4-6). The maximum rate and extent of ^{14}C-pyrene mineralization were
313 significantly lower than in the control in the presence of this high rhamnolipid biosurfactant
314 concentration (Figure S2 and Table S2). However, the residual concentrations revealed a clear
315 enhancing effect of the biosurfactant on biodegradation of the five PAHs studied (Table 6). The
316 reduction in benzo(a)pyrene concentration is especially relevant, given the regulatory implications
317 of this persistent, toxic and carcinogenic compound [6]. Indeed, the residual concentration achieved
318 with rhamnolipid (4.3 mg kg^{-1}) was lower than the residual concentration resulting from the
319 application of a chemical surfactant to this soil, i.e., 9.7 mg kg^{-1} [9], and was very close to the lower
320 regulatory threshold for soil pollution, set as health investigation levels for this chemical (which
321 ranges from 3 to 40 mg kg^{-1}, depending on land use [18]). The surface tension of the aqueous phase at
322 the end of these experiments was 25 ± 8.5 dyn cm^{-1}, indicating the presence of the biosurfactant in

solution. For this reason, the PAH concentrations were measured in the aqueous phase. These concentrations were very low (i.e., lower than 1 %), although they were included in the calculation of final residual concentrations. The increase in biosurfactant dosage eventually caused a higher solubilization of slow-desorbing PAHs and compensated biosurfactant losses due to biodegradation.

4. Conclusions.

This work demonstrates the importance of characterizing of the kinetics of PAH desorption to assess the biosurfactant role in bioremediation. The magnitude of the different desorbing fractions present in the polluted soils is a useful benchmark for understanding the rhamnolipid–enhanced biodegradation of PAHs. Our results indicate that the potential of rhamnolipid biosurfactants to enhance the bioaccesibility of recalcitrant PAHs from contaminated soils can be increased through approaches oriented towards the achievement of soils with a slow desorption profile, prior to biosurfactant application. In this way, the negative effects on biodegradation by the solubilization of fast desorbing PAHs, initially present at high concentrations, can be avoided. When applied properly, the rhamnolipid was useful in enhancing pollutant removal, even reaching decontamination levels (as exemplified by the benzo(a)pyrene losses in bioremediated soil 3), which are demanded by current regulatory frameworks. Therefore, the data suggest that rhamnolipid can constitute a valid alternative to chemical surfactants in promoting the biodegradation of slow-desorption PAHs during bioremediation.

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Table 1.
Kinetic parameters for desorption with Tenax, and solubilization of PAHs by rhamnolipid in soil 1.

PAH	Initial (mg kg^{-1})	k_{fast} [a] (h^{-1})	k_{slow} [a] ($10^{-3}h^{-1}$)	F_{fast} [a] (%)	F_{rhamn} [b] (%)
Phenanthrene	843.10 ± 17.17	0.14 ± 0.08	3.1 ± 1.7	73.4 ± 5.0	127.5 ± 15.5
Anthracene	264.96 ± 12.6	0.16 ± 0.02	2.4 ± 1.6	60.1 ± 3.0	77.2 ± 11.0
Fluoranthene	1246.6 ± 250	0.11± 0.05	0.13 ± 0.008	29.2 ± 0.3	64.9 ± 4.6
Pyrene	362.01 ± 28.6	0.10 ± 0.03	0.41 ± 0.1	48.1 ± 1.2	84.5 ± 8.9
Benzo(a)pyrene	56.49 ±0.9	0.035± 0.003	0.13 ± 0.002	16.7 ± 3.7	54.6 ± 4.6

[a]Kinetic parameters for desorption obtained with Tenax extraction. [b]PAH fraction extracted with the rhamnolipid (1 g L^{-1}) after 168 hours.

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Table 2.
Kinetic parameters for desorption with Tenax , and solubilization of PAHs by rhamnolipid in soil 2.

PAH	Initial [a] (mg kg^{-1})	k_{fast} [a] (h^{-1})	k_{slow} [a] ($10^{-3}h^{-1}$)	F_{fast} [a] (%)	F_{rhamn} [b] (%)
Phenanthrene	45.98 ± 9.3	0.027 ± 0.005	0.086 ± 0.04	16.8 ± 0.9	53.4 ± 2.0
Anthracene	140.29 ± 25.31	0.014 ± 0.003	0.025 ± 0.002	27.4 ± 6.4	51.2 ± 6.5
Fluoranthene	72.88 ± 3.1	0.02 ± 0.002	0.11 ± 0.002	16.0 ± 0.3	22.4 ± 2.3
Pyrene	42.45 ± 4.75	0.16 ± 0.01	0.08 ± 0.02	16.0 ± 1.0	23.9 ± 3.3
Benzo(a)pyrene	35.71 ± 2.5	0.015 ± 0.002	0.1 ± 0.02	23.2 ± 2.3	57.5 ± 1.1

[a]Kinetic parameters for desorption obtained with Tenax extraction. [b]PAH fraction extracted with the rhamnolipid (1 g L^{-1}) after 215 hours.

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Table 3.
Kinetic parameters for desorption with Tenax , and solubilization of PAHs by rhamnolipid in soil 3.

PAH	Initial [a] (mg kg^{-1})	k_{fast} [a] (h^{-1})	k_{slow} [a] ($10^{-3}h^{-1}$)	F_{fast} [a] (%)	F_{rhamn} [b] (%)
Phenanthrene	42.3 ± 5.6	0.11± 0.01	0.6 ± 0.1	16.8 ± 0.9	55.3 ± 3.1
Anthracene	15.4 ± 1.5	0.07 ± 0.03	0.2 ± 0.02	4.2 ± 0.5	9.5 ± 2.6
Fluoranthene	54.8 ± 4.3	0.15 ± 0.01	0.1 ± 0.01	8.3 ± 0.7	75.2 ± 9.5
Pyrene	48.3 ± 6.7	0.08 ± 0.01	0.2 ± 0.05	20.0 ± 3.5	50.7 ± 3.0
Benzo(a)pyrene	22.4 ± 0.1	0.07± 0.02	0.03 ± 0.003	7.0 ± 2.5	18.1 ± 2.2

[a]Kinetic parameters for desorption obtained with Tenax extraction. [b]PAH fraction extracted with the rhamnolipid (1 g L^{-1}) after 312 hours.

Table 4.
Effect of the rhamnolipid on the biodegradation of PAHs in suspensions of soil 1.

PAH concentration (mg kg^{-1})	Initial [a]	Predicted [b]	Control [c]	Rhamnolipid [d]
Phenanthrene	843.1 ± 17.2	224.3	11.9 ± 0.9	7.2 ± 0.5
Anthracene	264.9 ± 12.6	105.7	20.9 ± 1.9	5.5 ± 0.6
Fluoranthene	1246.6 ± 25.0	882.6	9.2 ± 1.1	6.2 ± 1.0
Pyrene	362.0 ± 28.6	187.9	9.0 ± 1.8	11.5 ± 5.9
Benzo(a)pyrene	56.5 ± 0.9	47.1	20.2 ± 1.8	23.5 ± 1.4
∑PAH[e]	2773.2 ± 84.3	1447.6	71.3 ± 7.6	53.9 ± 9.5

[a] Initial PAH concentration. [b] Predicted concentration assuming that biodegradation acted only on fast-desorption PAHs. [c,d] final concentration obtained without rhamnolipid and with rhamnolipid (1 g L^{-1}) respectively. [e] Sum of five PAH : phenanthrene, anthracene, fluoranthene, pyrene and benzo(a)pyrene.

Table 5.
Effect of the rhamnolipid on the biodegradation of PAHs in suspensions of soil 2.

PAH concentration (mg kg $^{-1}$)	Initial [a]	Predicted [b]	Control [c]	Rhamnolipid Biosurfactant	
				1 g L^{-1} [d]	1 g L^{-1} delayed [e]
Phenanthene	45.9 ± 9.3	38.2	9.8 ± 0.4	14.2 ± 4.7	6.7 ± 0.8
Anthracene	140.3 ± 25.3	101.8	24.7 ± 4.7	20.6 ± 0.2	5.2 ± 0.2
Fluoranthene	72.9 ± 3.1	61.2	13.8 ± 0.8	15.9 ± 4.7	10.3 ± 0.5
Pyrene	42.4 ± 4.7	35.7	6.72 ± 0.6	8.2 ± 2.5	5.7 ± 1.2
Benzo(a)pyrene	35.7 ± 2.5	27.4	22.9 ± 1.3	27.8 ± 0.9	23.1 ± 2.8
∑PAH[f]	337.3 ± 44.9	236.9	77.9 ± 7.8	86.8 ± 12.7	51.0 ± 5.6

[a] Initial PAH concentration. [b] Predicted concentration assuming that biodegradation acted only on fast-desorption PAHs. [c,d,e] final concentration in the respective control, with the rhamnolipid, and resulting from the delayed addition of the rhamnolipid after an incubation period (7 days) . [f] Sum of five PAHs: phenanthrene, anthracene, fluoranthene, pyrene and benzo(a)pyrene.

Table 6.
Effect the rhamnolipid on the biodegradation of PAHs in suspensions of soil 3.

PAH concentration (mg kg $^{-1}$)	Initial [a]	Predicted [b]	Control [c]	Rhamnolipid Biosurfactant	
				1 g L^{-1} [d]	20 g L^{-1} [e]
Phenanthrene	42.3 ± 5.6	35.2	10.0 ± 0.4	10.6 ± 4.3	2.4 ± 0.8
Anthracene	15.4 ± 1.5	14.7	2.4 ± 0.2	2.6 ± 0.5	0.9 ± 0.2
Fluoranthene	54.8 ± 4.3	50.2	16.3 ± 0.2	13.1 ± 0.5	7.3 ± 2.5
Pyrene	48.3 ± 6.7	38.6	12.2 ± 0.6	8.8 ± 0.9	5.4 ± 1.5
Benzo(a)pyrene	22.4 ± 0.1	20.8	9.9 ± 0.2	11.7 ± 0.8	4.3 ± 0.3
∑PAH[f]	183.2 ± 18.2	159.5	50.9 ± 1.1	48.5 ± 8.0	20.4 ± 5.3

[a] Initial PAH concentration. [b] Predicted concentration assuming that biodegradation acted only on fast-desorption PAHs. [c,d,e] final concentration obtained without rhamnolipid, with rhamnolipid (1 g L^{-1}) and with rhamnolipid (20 g L^{-1}), respectively. [f] Sum of five PAHs: phenanthrene, anthracene, fluoranthene, pyrene and benzo(a)pyrene.

Figure legends

FIGURE 1. Kinetics of desorption, determined by Tenax extraction, of polycyclic aromatic hydrocarbons: phenanthrene (white triangles), anthracene (black square), fluoranthene (white square), pyrene (black triangles) and benzo(a)pyrene (black circle) from soil 1 (A), soil 2 (B) and soil 3 (C). The dashed lines represent model fitting desorption results to equation 1.

FIGURE 2. Effect of the rhamnolipid biosurfactant (1 g L^{-1}) on the solubilization (circles) of pyrene from soil 1 (A), soil 2 (B) and soil 3 (C) compared with the desorption of the chemical with Tenax extraction (triangles).

FIGURE 3. Mineralization of pyrene in the absence (triangles) and presence (circles) of 1 g L^{-1} rhamnolipid. A soil 1, B soil 2 and C soil 3. Error bars represent one standard deviation. When error bars are not evident, they were smaller than the size of the symbols.

Figure 1. Posada-Baquero et al.

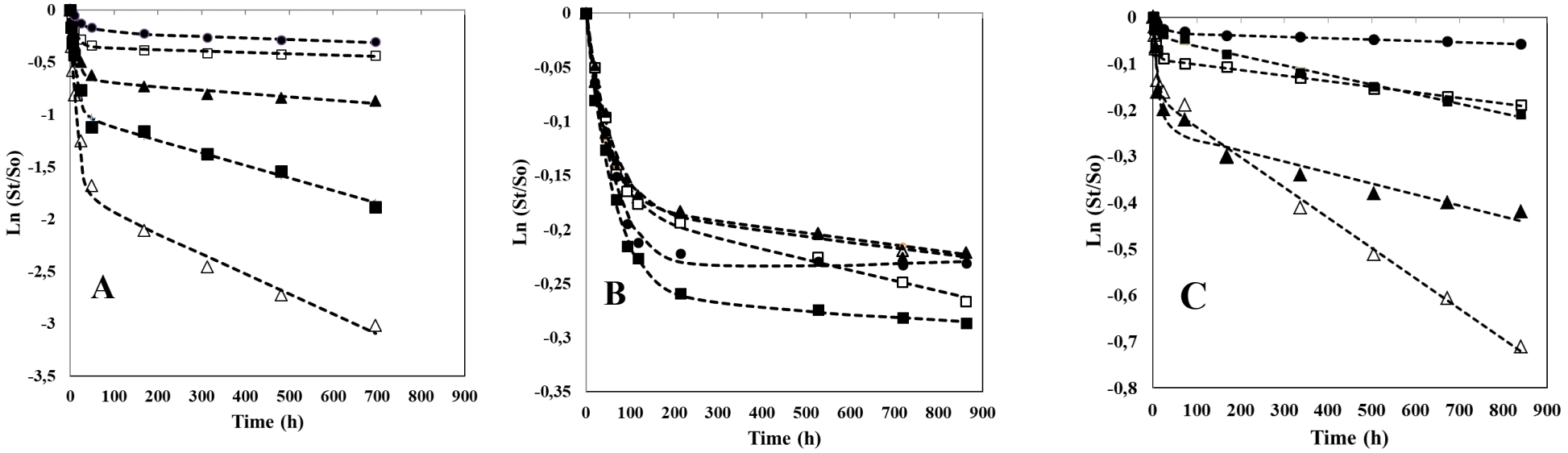

Figure 2. Posada-Baquero et al.

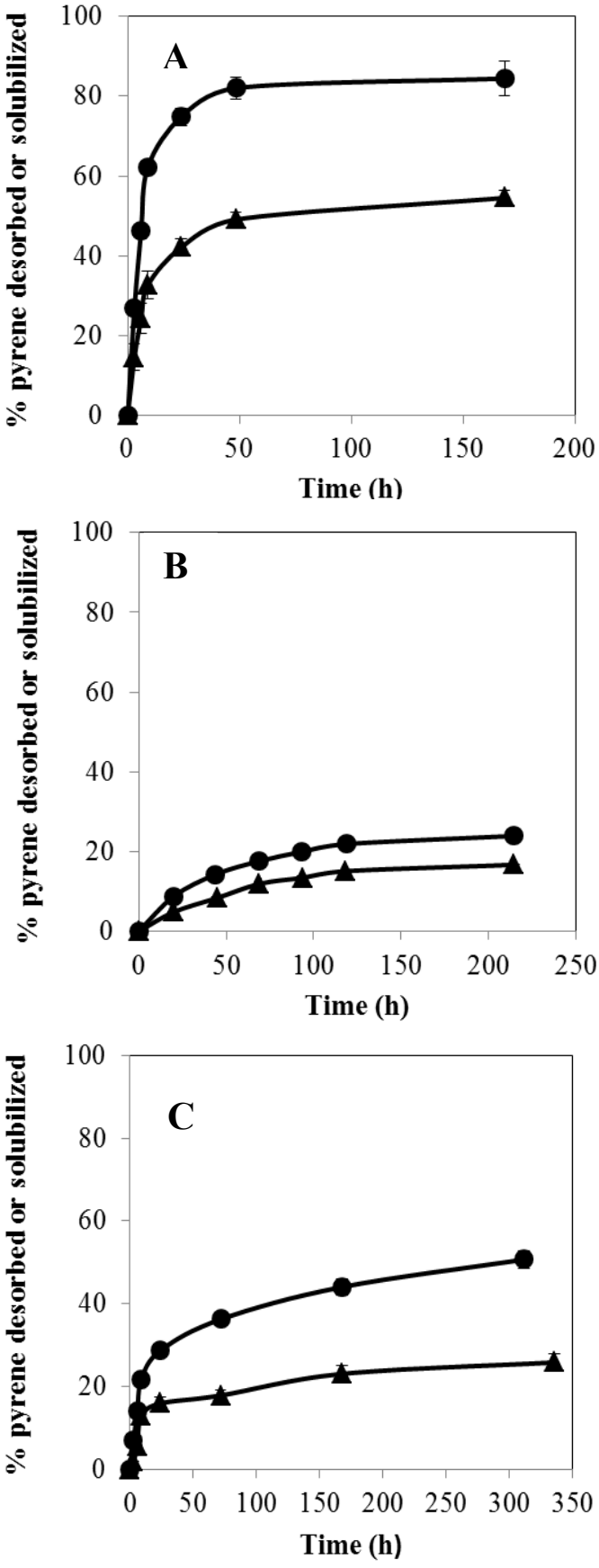

Figure 3. Posada-Baquero et al.