Published in final edited form as:
Environ Sci Technol. 2010 October 1; 44(19): 7266–7271. doi:10.1021/es100114g.

Effects of Nonionic Surfactant Addition on Populations of Polycyclic Aromatic Hydrocarbon-Degrading Bacteria in a Bioreactor Treating Contaminated Soil

Hongbo Zhu, **David Singleton**, and **Michael D. Aitken***
Department of Environmental Sciences and Engineering, Gillings School of Global Public Health, University of North Carolina, Chapel Hill, NC 27599-7431

Abstract

We studied the effects of two polyethoxylated nonionic surfactants, Brij 30 and $C_{12}E_8$, on populations of polycyclic aromatic hydrocarbon- (PAH-) degrading bacteria from a bioreactor treating PAH-contaminated soil. Each surfactant was evaluated at doses that corresponded to aqueous-phase concentrations both above and below the critical micelle concentration (CMC) after mixing with reactor slurry. Real-time quantitative PCR was used to quantify 16S ribosomal RNA (rRNA) gene sequences representing degraders of salicylate, naphthalene, phenanthrene or pyrene previously identified in the bioreactor community by stable-isotope probing. Sequences representing two groups of organisms associated with degradation of naphthalene and/or salicylate in the bioreactor increased in abundance by more than an order of magnitude after incubation with either surfactant at each dose tested. In contrast, the abundance of a group of uncultivated pyrene-degrading bacteria, whose relative abundance in the soil without surfactant addition was up to 9% of the total 16S rRNA genes, decreased by an order of magnitude or more in the presence of each surfactant at each dose. These results indicate that surfactant addition can have substantial, differential effects on populations of organisms responsible for contaminant degradation within a microbial community.

Introduction

The contamination of soils and sediments by polycyclic aromatic hydrocarbons (PAHs) is a common problem at industrial sites. Although PAHs are known to be biodegradable, the extent to which they are available to microorganisms capable of degrading them in a field-contaminated soil or sediment depends on site-specific conditions, particularly the solid-phase components with which PAHs associate. A number of studies have shown that only a fraction of any given PAH desorbs relatively rapidly from field-contaminated soil or sediment (1,2), which can be comparable to the fraction available for biodegradation (3,4). In situations in which PAH biodegradation is controlled by bioavailability, strategies to release the PAHs from their partitioning compartments in the solid phase might be warranted to meet cleanup goals at a particular site.

Surfactants can increase the release of hydrophobic organic compounds (HOCs) from soil to the liquid phase by solubilization into micelles (5-7). However, only a few studies have been conducted on the effects of surfactants on the biodegradation of PAHs in field-contaminated soil (8-10) and even fewer have evaluated the effects of surfactants on the microbial community

*Corresponding author phone 919-966-1024; mike_aitken@unc.edu.

Supporting Information Available
This information is available free of charge via the Internet at http://pubs.acs.org/

in such systems. Carmichael and Pfaender (11) evaluated the effects of two surfactants on phenanthrene-degrading microorganisms in contaminated soil from a wood-treatment site, using most-probable-number (MPN) enumeration; neither surfactant led to a significant change in the number of phenanthrene degraders after a one-week incubation. Other studies have followed changes in the microbial community as a result of surfactant addition in soils spiked with PAHs, using "fingerprinting" methods such as denaturing-gradient gel electrophoresis (DGGE) analysis (12) and phospholipid fatty acid (PLFA) profiles (13). In neither case was it possible to quantify the effects of the surfactant on the PAH-degrading organisms.

The objectives of the present study were to investigate the effects of surfactant addition on PAH-degrading populations in soil slurry from a bioreactor treating contaminated soil from a former manufactured-gas plant (MGP) site, whether such effects were dose-dependent, and whether they might correspond to observed patterns of PAH biodegradation. Two nonionic, ethoxylated alcohol surfactants with different hydrophile-lipophile balance (HLB) values were used: polyoxyethylene(4) lauryl ether (Brij 30), which is a relatively hydrophobic surfactant (HLB 9.7), and octaethylene glycol mono *n*-dodecyl ether ($C_{12}E_8$), which is a relatively hydrophilic surfactant (HLB 13.1); these surfactants differ only in the length of the polyethoxylate moiety. Three doses were evaluated for each surfactant, corresponding to aqueous-phase concentrations below, slightly above and well above the critical micelle concentration (CMC). In a companion paper (14) we report that Brij 30 enhanced PAH biodegradation in the soil slurry from the bioreactor, whereas $C_{12}E_8$ did not. To evaluate whether differences in PAH biodegradation resulted from differences in the effects of each surfactant on PAH-degrading bacteria, we quantified the 16S rRNA gene sequences representing naphthalene-, salicylate-, phenanthrene-, and pyrene-degrading bacteria in the bioreactor that had been identified previously by DNA-based stable-isotope probing (SIP) (15-17). The targeted bacteria are summarized in Table 1 and include groups that are not related to any previously isolated or characterized species. Because Brij 30 was able to enhance PAH degradation at the lowest (sub-CMC) dose (14), we also evaluated its influence on initial rates of naphthalene, phenanthrene, pyrene and benzo[*a*]pyrene (BaP) mineralization at that dose.

Materials and Methods

Chemicals

Brij 30, $C_{12}E_8$, [ring-UL-^{14}C]salicylate, [UL-^{14}C]naphthalene, [9-^{14}C]phenanthrene, [4,5,9,10-^{14}C]pyrene, and [7-^{14}C]BaP were all purchased from Sigma-Aldrich (St. Louis, Missouri). The specific activities of the ^{14}C-labeled compounds were 10 mCi/mmol, 17.8 mCi/mmol, 8.3 mCi/mmol, and 26.6 mCi/mmol, respectively.

Samples

The soil used in this study was the effluent slurry from a bench-scale, aerobic bioreactor used to treat soil from a former MGP site in Charlotte, NC (20). Concentrations of PAHs in the treated soil before and after surfactant addition are reported elsewhere (14).

Experimental Design

The overall design of the experiments to evaluate the effect of surfactant addition on PAH biodegradation over a period of 18 d is described elsewhere (14). Briefly, for each surfactant we first quantified its sorption to the soil to determine the aqueous-phase concentration as a function of surfactant dose. Three doses were then selected to correspond to an aqueous-phase concentration below, slightly above and well above the CMC. Brij 30 was evaluated at doses of 5, 20 and 50 mg/g dry soil and $C_{12}E_8$ was evaluated at doses of 2, 10 and 25 mg/g dry soil. Biodegradation of PAHs was quantified by comparing their concentrations before and after

incubation relative to inhibited controls. The desorption of PAHs was also quantified at the lowest dose for each surfactant.

Preliminary experiments were performed to evaluate surfactant toxicity by following the mineralization of two water-soluble substrates: acetate as an indicator of general microbial activity (21) and salicylate as an indicator more specific to PAH metabolism. These experiments were conducted immediately after surfactant addition and after 2 d of incubation with surfactant before adding the ^{14}C-labeled substrate.

The same batch of reactor slurry that was used for the biodegradation experiment with a given surfactant (14) was used to quantify specific organisms by real-time quantitative PCR (qPCR), mineralization of salicylate as a function of the selected surfactant doses, and mineralization of PAHs. PAH mineralization was evaluated only at the lowest (sub-CMC) dose of Brij 30 because ^{14}C-PAH partitioning into micelles at the higher doses would have confounded interpretation of the mineralization data. PAH mineralization was not evaluated for $C_{12}E_8$ because it did not enhance PAH degradation at any dose (14).

Mineralization Assays

For mineralization assays on [^{14}C]salicylate, [^{14}C]naphthalene, [^{14}C]phenanthrene, [^{14}C] pyrene, and [^{14}C] BaP, six replicate samples were prepared for each of five conditions for each surfactant: no surfactant, surfactant at each of the three doses, and inhibited controls. Slurry from the bioreactor was first centrifuged, the supernatant discarded, aliquots of the soil pellet distributed to replicate vessels, and the soil re-suspended to 6.35 mL at a solids concentration of 10% (w:w) in phosphate buffer (pH 7). Inhibited controls were prepared by adding sodium azide to a concentration of 0.3 mg/mL. All tubes were shaken at 200 rpm at room temperature. Three tubes for each condition were taken from the shaker for DNA extraction (2 mL) and mineralization assays (4.35 mL) at day 4, and the remaining three tubes were removed for the same analyses at day 18.

Each assay consisted of adding 20,000 disintegrations per minute (dpm) of [^{14}C]salicylate dissolved in water, or ^{14}C-labeled PAH dissolved in dimethyl sulfoxide (DMSO), into soil slurry. Incubations with the radiolabeled substrate were intended to capture the initial period over which mineralization was considered to be linear (20 min each for naphthalene and phenanthrene, 30 min for salicylate, 16 h for pyrene and 24 h for BaP). Other details of mineralization assays are described elsewhere (19). Reported mineralization data are normalized per unit soil dry wt.

Residual Surfactant Concentration

The surface tension of the filtered liquid phase from samples obtained during the PAH biodegradation incubations (14) were analyzed by a Du Nouy tensiometer (CSC Scientific Co., Fairfax, VA). Samples were diluted as necessary to achieve a final surface tension corresponding to a surfactant concentration below the CMC. The concentration of residual surfactant was calculated using a calibration curve of surface tension *vs.* surfactant concentration and adjusting for dilution.

qPCR

DNA was extracted from 2 mL of slurry in triplicate samples without surfactant addition at day 0 and for each incubation condition after 18 d (except the inhibited controls), using the MoBio (Carlsbad, CA) UltraClean Soil DNA kit. For each reaction, 1 L of DNA as template was analyzed in a SmartCycler system (Cepheid, Sunnyvale, CA) with a SYBR®Green PCR kit (Qiagen Inc., Valencia, CA). Total bacterial 16S rRNA genes and the sequences representing 16S rRNA genes associated with the six groups of bacteria shown in Table 1 were

quantified using primers summarized in Table S1 of the Supporting Information and methods described elsewhere (16-18). For each group, the absolute gene copy number and the abundance of that sequence relative to total eubacterial 16S rRNA genes were calculated using group-specific standard curves as described elsewhere (16-18). For any set of triplicate data that contained a value below the quantification limit, the mean and standard deviation were calculated using Cohen's maximum likelihood estimator method for censored data (22).

Results

Preliminary Experiments

Initial rates of mineralization of [^{14}C]acetate were measured to test the potential toxicity of Brij 30 or $C_{12}E_8$ to the general microbial community in the bioreactor. Each surfactant appeared to inhibit acetate mineralization slightly immediately after the surfactant was added to the soil slurry, but mineralization was greatly enhanced if the surfactant was allowed to reach sorption equilibrium with the soil over a 2-d period (Figure S1 in Supporting Information). A similar preliminary experiment with salicylate was performed to determine whether surfactant addition might affect the mineralization of a water-soluble substrate known to be an intermediate in the degradation pathway for several PAHs (23-27). Both surfactants decreased initial rates of salicylate mineralization when they were initially added to the slurry at 40 mg/g. With a 2-d pre-equilibration period, mineralization in samples with $C_{12}E_8$ was comparable to that of the controls without surfactant, whereas Brij 30 substantially increased salicylate mineralization (data not shown).

Salicylate and PAH Mineralization

Any condition that substantially changes the abundance of a specific organism should correspond to changes in observed rates of activity, such as mineralization of a particular substrate. Accordingly, we measured initial rates of mineralization of salicylate and selected PAHs (see Materials and Methods for the periods corresponding to the initial rate for each substrate) after incubating the soil slurry with or without surfactant. The initial rate of salicylate mineralization was quantified at the three surfactant doses selected to evaluate PAH biodegradation (14). As shown in Figure 1, the mineralization of salicylate was inhibited immediately after either surfactant was added to the slurry except for $C_{12}E_8$ addition at the lowest dose (2 mg/g). After 4 d of incubation, salicylate mineralization was still inhibited in the samples incubated with the two higher doses of either surfactant, but it increased at the lowest dose (corresponding to aqueous concentrations below the CMC); for Brij 30 the initial rate was approximately 3-fold higher than in the incubation without surfactant. After 18 d of incubation, salicylate mineralization in all samples containing surfactant was 5-6 times higher than in the control without surfactant for Brij 30 and 3-4 times higher than in the control for $C_{12}E_8$.

Because Brij 30 at the lowest (sub-CMC) dose had the greatest effect on PAH biodegradation (14), we evaluated its effect on the mineralization of naphthalene, phenanthrene, pyrene, and BaP as representative 2-, 3-, 4-, and 5-ring compounds, respectively (Figure 2). Initial rates of mineralization increased significantly (*t*-test, $p < 0.05$) within four days of incubation with Brij 30 for naphthalene and phenanthrene, whereas pyrene and BaP mineralization were both inhibited over this period. After 18 d, however, the initial rate of pyrene mineralization was slightly higher in the presence of Brij 30 than in the surfactant-free control, and BaP mineralization was comparable to that observed in the control.

Residual surfactant

Based on surface tension measurements at the end of the 18-d incubations, the liquid-phase concentrations of Brij 30 at the two lower doses and of $C_{12}E_8$ at all three doses were negligible.

At the highest dose of Brij 30, however, the surface tension was about twice the value that would have corresponded to the predicted equilibrium surfactant concentration in the liquid phase at that dose (14). The residual concentration of either surfactant at the highest dose in the inhibited controls after 18 d was about 30% of the predicted equilibrium concentration (data not shown).

qPCR

The effects of surfactant addition on total bacterial 16S rRNA genes and on 16S genes for six groups of organisms previously identified as degraders of salicylate, naphthalene, phenanthrene or pyrene are summarized in Table 2 for Brij 30 and Table 3 for $C_{12}E_8$. The total bacterial 16S rRNA gene copy number increased slightly after 18 d of incubation with Brij 30 at all three doses relative to controls with no surfactant addition. It also increased by an order of magnitude at the two higher doses of $C_{12}E_8$, but was not affected at the lowest dose.

Sequences representing three groups of organisms in the bioreactor slurry that previously were associated with salicylate degradation (17) were quantified. Two of these groups, referred to as SG2 and SG3, are closely related to naphthalene-degrading bacteria identified in the same bioreactor slurry, whereas the third group, SG1, is not (15,17). The 16S rRNA gene sequences representing SG3 increased one to two orders of magnitude above the quantification limit in the samples that had been incubated for 18 d with either surfactant at all three doses (Tables 2 and 3). Group SG3 was 6-7% of the total bacterial 16S rRNA genes after incubation with Brij 30 at the two lowest doses and at least 4% after $C_{12}E_8$ addition at all three doses. The relative abundance of group SG1 increased by an order of magnitude above the quantification limit, to 1-2% of the total bacterial 16S rRNA genes, in response to the two lower doses of Brij 30 and the two higher doses of $C_{12}E_8$. The 16S rRNA genes representing SG2 organisms were below the quantification limit of 3×10^5 copies/g soil in all samples from all incubations.

In previous work we identified *Acidovorax* spp. as the predominant phenanthrene-degrading bacteria in the bioreactor slurry (15,18). These organisms were below the quantification limit in all samples from the incubations with Brij 30 and near or below the quantification limit in incubations with $C_{12}E_8$ (Table 3).

Two groups of bacteria (PG1 and PG2) unrelated to any cultivated organism were previously identified as the predominant pyrene-degrading bacteria in the bioreactor (16) and also were found to grow on phenanthrene (18). Copies of the 16S rRNA genes representing PG1 were not greatly affected by either surfactant (Tables 2 and 3). Both surfactants, however, decreased the abundance of sequences representing PG2, in most cases by at least an order of magnitude.

Discussion

The effects of surfactant addition on PAH degradation in contaminated soil pretreated in an aerobic, slurry-phase bioreactor are reported elsewhere (14). Briefly, the concentrations of all 3- and 4-ring PAHs were reduced by 20-50% in the slurry amended with Brij 30 at the two lower doses (at which PAH solubilization was negligible), whereas removal of only the 3-ring PAHs was significantly enhanced at the highest Brij 30 dose. In contrast, $C_{12}E_8$ did not enhance PAH removal at any dose. The purpose of the work reported here was to determine whether the two surfactants had differential effects on known PAH-degrading bacteria in the bioreactor as well.

Increases in total bacterial abundance in response to the addition of either surfactant (Tables 2 and 3) could have been due to growth on the surfactant, which has been observed in other studies (7,9,12,28,29), or to growth on contaminants whose availability may have increased as a result of surfactant addition. However, $C_{12}E_8$ did not increase the bioavailability of the PAHs

(14) and the apparent loss of $C_{12}E_8$ (at all three doses) and Brij 30 (at the two lower doses) over the 18-d incubation period is consistent with their possible use as growth substrates.

The enhancement of salicylate mineralization after surfactant addition (Figure 1) was an unexpected finding, given that at neutral pH salicylate is water-soluble and that neither surfactant contains an aromatic moiety whose metabolism might stimulate the growth of salicylate-degrading bacteria. The increase in salicylate mineralization is consistent with the substantial increases in abundance of sequences representing two groups of salicylate-degrading organisms, SG1 and SG3, after incubation with both surfactants (Tables 2 and 3).

From the available data it is not possible to elucidate which substrates were used for growth by SG1 and SG3 organisms during incubations with the surfactants. Group SG1 includes organisms related to *Curvibacter* and *Polaromonas* spp. (Table 1). We are not aware of a prior link of *Curvibacter* organisms to PAH degradation, but *Polaromonas* spp. include organisms associated with growth on pyrene and phenanthrene in a biofilm community (30) and with growth on naphthalene (31); in the latter case, *Polaromonas naphthalenivorans* was not able to degrade either phenanthrene or pyrene (31). Group SG3, which comprises organisms related to *Ralstonia eutropha* and other *Ralstonia* spp. (17), has been associated with naphthalene degradation in the bioreactor by SIP (15). However, naphthalene degradation was not improved at any dose of either surfactant, which was explained by their inability to enhance the desorption of naphthalene in the soil slurry (14). *Ralstonia eutropha* (previously classified as *Alcaligenes eutrophus* (32)) is known to grow on the ethoxylated alcohol surfactant polyoxyethylene(10) lauryl ether (33), which is structurally similar to the surfactants used in this study. *Ralstonia* spp., including *R. eutropha*, are also known to grow on anthracene (34) and phenanthrene (35). In previous work, we observed that the addition of phthalate to the bioreactor slurry led to enrichment of *Ralstonia* spp. in group SG3 (19); the enrichment corresponded to slight enhancements in the mineralization of phenanthrene and pyrene over a 24-h period (19).

The pyrene-degrading group PG2 was a major member of the microbial community in the bioreactor soil slurry before surfactant addition, comprising 5-9% of the total 16S rRNA genes. Incubation for 18 d with either surfactant reduced the abundance of sequences representing PG2 by at least an order of magnitude compared to controls without surfactant addition, except at the lowest dose of $C_{12}E_8$ (Tables 2 and 3). Despite the large decrease in abundance of PG2 organisms, pyrene degradation increased by 35% after Brij 30 addition at the two lowest doses (14) and its mineralization in incubations amended with Brij 30 for 18 d at the lowest dose was comparable to that in incubations without surfactant addition (Figure 2). These results could not be explained by changes in abundance of sequences representing PG1, another pyrene-degrading group that had been identified by SIP in the bioreactor (16). Group PG1 was a relatively minor constituent of the microbial community, with an initial relative abundance < 0.1%, and Brij 30 addition either decreased or did not affect the abundance of these organisms (Table 2). Therefore, it appears that organisms other than these major pyrene degraders previously identified by SIP were responsible for enhanced pyrene removal in the presence of Brij 30.

Overall, both surfactants had comparable effects (positive, negative and neutral) on each group of organisms we quantified (Tables 2 and 3), yet the addition of Brij 30 enhanced the biological removal of PAHs remaining after slurry-phase bioremediation and $C_{12}E_8$ did not (14). This difference was attributed to the observation that only Brij 30 enhanced PAH bioavailability (14), which thus appears to be a necessary condition for enhanced PAH removal. A second necessary condition is that surfactant addition not adversely affect the microbial community, which could negate any positive effect of the surfactant on contaminant bioavailability. Such an effect was not observed, as each surfactant generally increased total bacterial abundance as well as the two groups of organisms designated SG1 and SG3. In addition, based on

mineralization data (Figure 2), Brij 30 increased the capacity for naphthalene and phenanthrene degradation by the bioreactor community. The substantial increase in abundance of SG3 organisms could account for the stimulation in naphthalene mineralization, as these organisms are known naphthalene degraders (15). Enhanced phenanthrene mineralization in response to Brij 30 suggests that there was a significant increase in the concentration of organisms that were collectively capable of mineralizing phenanthrene. However, the abundance of *Acidovorax* organisms, the predominant phenanthrene degraders in bioreactor community as determined by SIP (15), was not above the quantification limit at any dose of Brij 30. Of the bacterial groups we followed by qPCR, the only two that increased by at least an order of magnitude after Brij 30 addition, SG1 and SG3, did not appear to be associated with phenanthrene mineralization (17).

It is clear that there were PAH-degrading organisms in the bioreactor community that we did not quantify. We focused on the organisms that were previously identified by SIP, but it should be noted that SIP would have selected for organisms that grew most rapidly on the added ^{13}C-labeled PAH. Other organisms capable of metabolizing the same PAH, albeit more slowly, could have been responsible for increases in PAH removal and/or phenanthrene mineralization activity in response to Brij 30 addition. Note that increases in PAH removal would not necessarily have been coupled to growth-related metabolism. For example, partial metabolism that would not have led to growth or to mineralization could explain the discrepancy between increases in pyrene removal (14) and lack of stimulation of pyrene mineralization (Figure 2) in response to Brij 30.

Despite the prevalence of molecular tools in microbial community analysis, there are still relatively few studies in which these tools have been used to evaluate biological treatment of field-contaminated soil or sediment. In studies on PAH-contaminated systems, investigators have used DGGE or thermal-gradient gel electrophoresis coupled to identification of 16S rRNA gene sequences of selected bands (36-38); PLFA profiles (39); clone libraries (40); and qPCR of functional genes relevant to aromatic hydrocarbon metabolism (38,39). To our knowledge, the present study and our previous work on enrichment of the bioreactor slurry with salicylate (17) or phthalate (19) are the only studies to evaluate the effects of bioremediation strategies on field-contaminated soil by quantification of relevant organisms that had been identified by SIP.

Surfactants can serve as growth substrates for microorganisms, especially for the easily degradable surfactants (41); therefore, the addition of a surfactant to soil can lead to enrichment of surfactant-degrading organisms (7,9,12,28,29,33). Our two-stage approach of adding the surfactant in a batch treatment process after conventional slurry-phase biological treatment would preclude the enrichment of surfactant degraders during the primary bioremediation stage (14). We have demonstrated that surfactant addition in a second stage can lead to substantial changes in populations of indigenous organisms capable of degrading the target contaminants, both positively and negatively. Although the application of molecular tools such as SIP and qPCR has improved our ability to study population dynamics in field-contaminated soil, more work still needs to be done to fully understand the responses of indigenous microorganisms to remediation strategies. Such understanding can lead to better predictive capabilities for bioremediation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the NIEHS Superfund Research Program (5 P42 ES005948).

Literature Cited

1. Loehr RC, Lamar MR, Poppendieck DG. A protocol to estimate the release of anthropogenic hydrocarbons from contaminated soils. Environ. Toxicol. Chem 2003;22(9):2202–2208. [PubMed: 12959552]
2. Lei L, Khodadoust AP, Suidan MT, Tabak HH. Biodegradation of sediment-bound PAHs in field-contaminated sediment. Water Res 2005;39(2-3):349–361. [PubMed: 15644243]
3. Breedveld GD, Karlsen DA. Estimating the availability of polycyclic aromatic hydrocarbons for bioremediation of creosote contaminated soils. Appl. Microbiol. Biotechnol 2000;54(2):255–261. [PubMed: 10968642]
4. Lei L, Bagchi R, Khodadoust AP, Suidan MT, Tabak HH. Bioavailability prediction of polycyclic aromatic hydrocarbons in field-contaminated sediment by mild extractions. J. Environ. Eng. (ASCE) 2006;132(3):384–391.
5. Yeom IT, Ghosh MM, Cox CD. Kinetic aspects of surfactant solubilization of soil-bound polycyclic aromatic hydrocarbons. Environ. Sci. Technol 1996;30:1589–1595.
6. Walters GW, Aitken MD. Surfactant-enhanced solubilization and anaerobic biodegradation of 1,1,1-trichloro-2,2-bis(p-chlorophenyl)-ethane (DDT) in contaminated soil. Water Environ. Res 2001;73 (1):15–23. [PubMed: 11558297]
7. Kim HS, Weber WJ. Polycyclic aromatic hydrocarbon behavior in bioactive soil slurry reactors amended with a nonionic surfactant. Environ. Toxicol. Chem 2005;24(2):268–276. [PubMed: 15719985]
8. Deschênes L, Lafrance P, Villeneuve JP, Samson R. Adding sodium dodecyl sulfate and *Pseudomonas aeruginosa* UG2 biosurfactants inhibits polycyclic aromatic hydrocarbon biodegradation in a weathered creosote-contaminated soil. Appl. Microbiol. Biotechnol 1996;46(5-6):638–646. [PubMed: 9008894]
9. Tiehm A, Stieber M, Werner P, Frimmel FH. Surfactant-enhanced mobilization and biodegradation of polycyclic aromatic hydrocarbons in manufactured gas plant soil. Environ. Sci. Technol 1997;31 (9):2570–2576.
10. Torres LG, Rojas N, Bautista G, Iturbe R. Effect of temperature, and surfactant's HLB and dose over the TPH-diesel biodegradation process in aged soils. Process Biochem 2005;40(10):3296–3302.
11. Carmichael LM, Pfaender FK. Polynuclear aromatic hydrocarbon metabolism in soils: relationship to soil characteristics and preexposure. Environ. Toxicol. Chem 1997;16(4):666–675.
12. Colores GM, Macur RE, Ward DM, Inskeep WP. Molecular analysis of surfactant-driven microbial population shifts in hydrocarbon-contaminated soil. Appl. Environ. Microbiol 2000;66(7):2959–2964. [PubMed: 10877792]
13. Joner EJ, Johansen A, Loibner AP, dela Cruz MA, Szolar OHJ, Portal JM, Leyval C. Rhizosphere effects on microbial community structure and dissipation and toxicity of polycyclic aromatic hydrocarbons (PAHs) in spiked soil. Environ. Sci. Technol 2001;35(13):2773–2777. [PubMed: 11452608]
14. Zhu H, Aitken MD. Surfactant-enhanced desorption and biodegradation of polycyclic aromatic hydrocarbons from contaminated soil. submitted.
15. Singleton DR, Powell SN, Sangaiah R, Gold A, Ball LM, Aitken MD. Stable-isotope probing of bacteria capable of degrading salicylate, naphthalene or phenanthrene in a bioreactor treating contaminated soil. Appl. Environ. Microbiol 2005;71(3):1202–1209. [PubMed: 15746319]
16. Singleton DR, Sangaiah R, Gold A, Ball LM, Aitken MD. Identification and quantification of uncultivated proteobacteria associated with pyrene degradation in a bioreactor treating PAH-contaminated soil. Environ. Microbiol 2006;8:1736–1745. [PubMed: 16958754]
17. Powell SN, Singleton DR, Aitken MD. Effects of enrichment with salicylate on bacterial selection and PAH mineralization in a microbial community from a bioreactor treating contaminated soil. Environ. Sci. Technol 2008;42(11):4099–4105. [PubMed: 18589972]

18. Singleton DR, Hunt M, Powell SN, Frontera-Suau R, Aitken MD. Stable-isotope probing with multiple growth substrates to determine substrate specificity of uncultivated bacteria. J. Microbiol. Methods 2007;69:180–187. [PubMed: 17267058]
19. Singleton DR, Richardson SD, Aitken MD. Effects of enrichment with phthalate on polycyclic aromatic hydrocarbon biodegradation in contaminated soil. Biodegradation 2008;19:577–587. [PubMed: 17990065]
20. Zhu H, Roper JC, Pfaender FK, Aitken MD. Effects of anaerobic incubation on the desorption of polycyclic aromatic hydrocarbons from contaminated soils. Environ. Toxicol. Chem 2008;27(4): 837–844. [PubMed: 18333673]
21. Tsitonaki A, Smets BF, Bjerg PL. Effects of heat-activated persulfate oxidation on soil microorganisms. Water Res 2008;42(4-5):1013–1022. [PubMed: 17942135]
22. Berthouex, PM.; Brown, LC. Statistics for Environmental Engineers. Lewis Publishers; Boca Raton, Fla.: 1994.
23. Sutherland, JB.; Rafii, F.; Khan, AA.; Cerniglia, CE. Mechanisms of polycyclic aromatic hydrocarbon degradation. In: Young, LY.; Cerniglia, CE., editors. Microbial Transformation and Degradation of Toxic Organic Chemicals. Wiley-Liss; New York, NY: 1995. p. 269-306.
24. Chen SH, Aitken MD. Salicylate stimulates the degradation of high-molecular weight polycyclic aromatic hydrocarbons by *Pseudomonas saccharophila* P15. Environ. Sci. Technol 1999;33(3):435–439.
25. Prabhu Y, Phale PS. Biodegradation of phenanthrene by *Pseudomonas* sp. strain PP2: novel metabolic pathway, role of biosurfactant and cell surface hydrophobicity in hydrocarbon assimilation. Appl. Microbiol. Biotechnol 2003;61(4):342–351. [PubMed: 12743764]
26. Demanèche S, Meyer C, Micoud J, Louwagie M, Willison JC, Jouanneau Y. Identification and functional analysis of two aromatic-ring-hydroxylating dioxygenases from a *Sphingomonas* strain that degrades various polycyclic aromatic hydrocarbons. Appl. Environ. Microbiol 2004;70(11): 6714–6725. [PubMed: 15528538]
27. Poonthrigpun S, Pattaragulwanit K, Paengthai S, Kriangkripipat T, Juntongjin K, Thaniyavarn S, Petsom A, Pinphanichakarn P. Novel intermediates of acenaphthylene degradation by *Rhizobium* sp. strain CU-A1: evidence for naphthalene-1,8-dicarboxylic acid metabolism. Appl. Environ. Microbiol 2006;72(9):6034–6039. [PubMed: 16957226]
28. Kim HS, Weber WJ Jr. Preferential surfactant utilization by a PAH-degrading strain: effects on micellar solubilization phenomena. Environ. Sci. Technol 2003;37(16):3574–3580. [PubMed: 12953868]
29. Makkar RS, Rockne KJ. Comparison of synthetic surfactants and biosurfactants in enhancing biodegradation of polycyclic aromatic hydrocarbons. Environ. Toxicol. Chem 2003;22(10):2280–2292. [PubMed: 14551990]
30. Eriksson M, Dalhammar G, Mohn WW. Bacterial growth and biofilm production on pyrene. FEMS Microbiol. Ecol 2002;40(1):21–27. [PubMed: 19709207]
31. Jeon CO, Park W, Ghiorse WC, Madsen EL. Polaromonas naphthalenivorans sp. nov., a naphthalene-degrading bacterium from naphthalene-contaminated sediment. Int. J. Syst. Evol. Microbiol 2004;54 (1):93–97. [PubMed: 14742464]
32. Yabuuchi E, Kosako Y, Yano I, Hotta H, Nishiuchi Y. Transfer of two *Burkholderia* and an *Alcaligenes* species to *Ralstonia* gen. Nov.: Proposal of *Ralstonia pickettii* (Ralston, Palleroni and Doudoroff 1973) comb. Nov., *Ralstonia solanacearum* (Smith 1896) comb. Nov. and *Ralstonia eutropha* (Davis 1969) comb. Nov. Microbiol. Immunol 1995;39(11):897–904. [PubMed: 8657018]
33. Layton AC, Lajoie CA, Easter JP, Muccini M, Sayler GS. An integrated surfactant solubilization and PCB bioremediation process for soils. Bioremediation Journal 1998;2(1):43–56.
34. Ilori MON, Amund D-I. Degradation of anthracene by bacteria isolated from oil polluted tropical soils. Z. Naturforsch. C 2000;55(11-12):890–897. [PubMed: 11204192]
35. Widada J, Nojiri H, Kasuga K, Yoshida T, Habe H, Omori T. Molecular detection and diversity of polycyclic aromatic hydrocarbon-degrading bacteria isolated from geographically diverse sites. Appl. Microbiol. Biotechnol 2002;58:202–209. [PubMed: 11876413]

36. Del Panno MT, Morelli IS, Engelen B, Berthe-Corti L. Effect of petrochemical sludge concentrations on microbial communities during soil bioremediation. FEMS Microbiol. Ecol 2005;53(2):305–316. [PubMed: 16329950]

37. Viñas M, Sabaté J, Espuny MJ, Solanas AM. Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil. Appl. Environ. Microbiol 2005;71(11):7008–7018. [PubMed: 16269736]

38. Cebron A, Beguiristain T, Faure P, Norini M-P, Masfaraud J-F, Leyval C. Influence of vegetation on the in situ bacterial community and polycyclic aromatic hydrocarbon (PAH) degraders in aged PAH-contaminated or thermal-desorption-treated soil. Appl. Environ. Microbiol 2009;75(19):6322–6330. [PubMed: 19633127]

39. Ringelberg DB, Talley JW, Perkins EJ, Tucker SG, Luthy RG, Bouwer EJ, Fredrickson HL. Succession of phenotypic, genotypic, and metabolic community characteristics during in vitro bioslurry treatment of polycyclic aromatic hydrocarbon-contaminated sediments. Appl. Environ. Microbiol 2001;67:1542–1550. [PubMed: 11282603]

40. Ni Chadhain SM, Norman RS, Pesce KV, Kukor JJ, Zylstra GJ. Microbial dioxygenase gene population shifts during polycyclic aromatic hydrocarbon biodegradation. Appl. Environ. Microbiol 2006;72(6):4078–4087. [PubMed: 16751518]

41. Singer AC, Gilbert ES, Luepromchai E, Crowley DE. Bioremediation of polychlorinated biphenyl-contaminated soil using carvone and surfactant-grown bacteria. Appl. Microbiol. Biotechnol 2000;54 (6):838–843. [PubMed: 11152078]

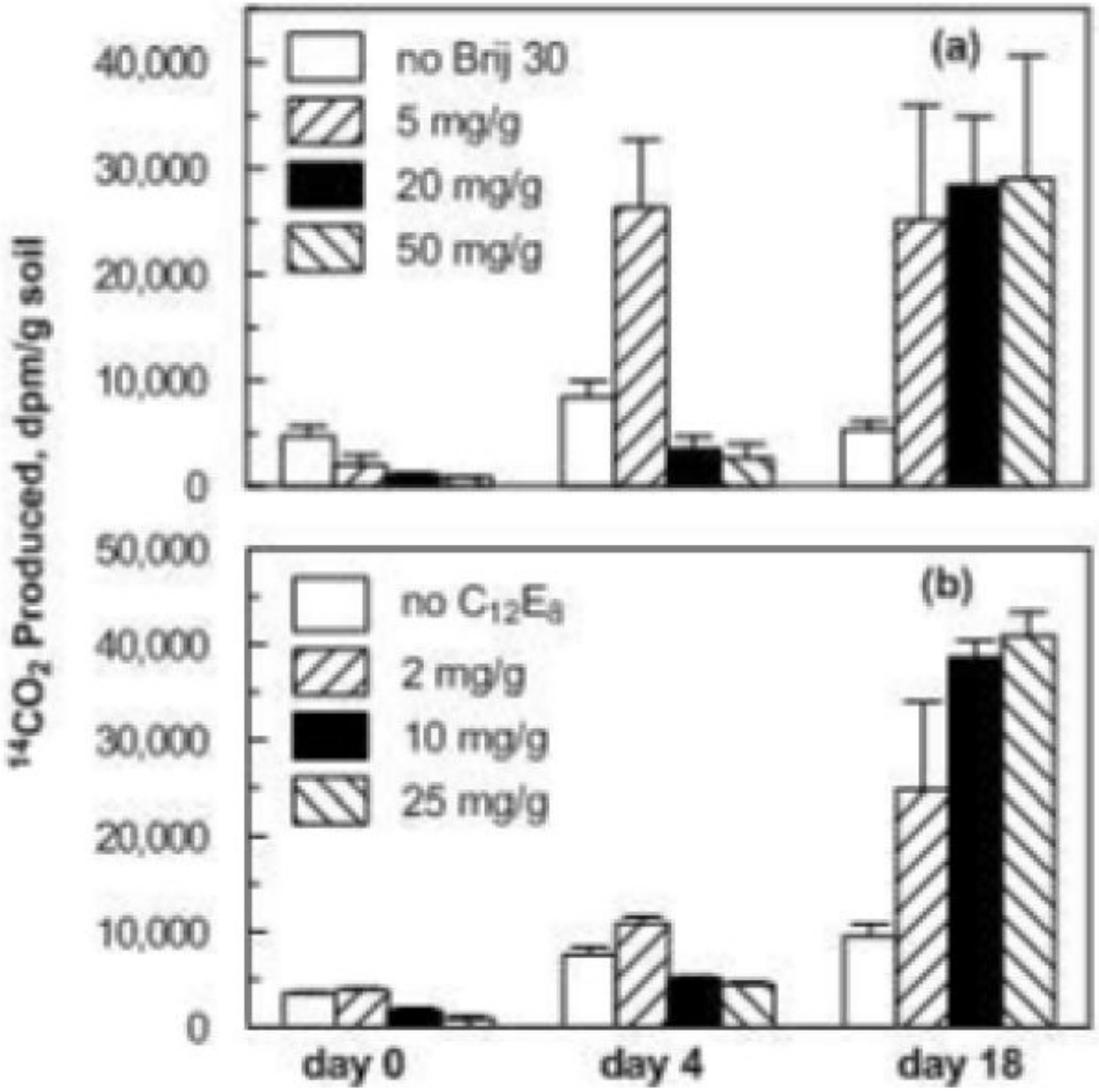

Figure 1.

Mineralization of [^{14}C]salicylate by the microbial community in soil slurry incubated with various doses of **(a)** Brij 30 or **(b)** $C_{12}E_8$ for different periods of time (day 0 is immediately after adding the surfactant). Values are means and standard deviations of triplicate incubations and represent initial rate measurements for aliquots of soil slurry taken at the indicated incubation time.

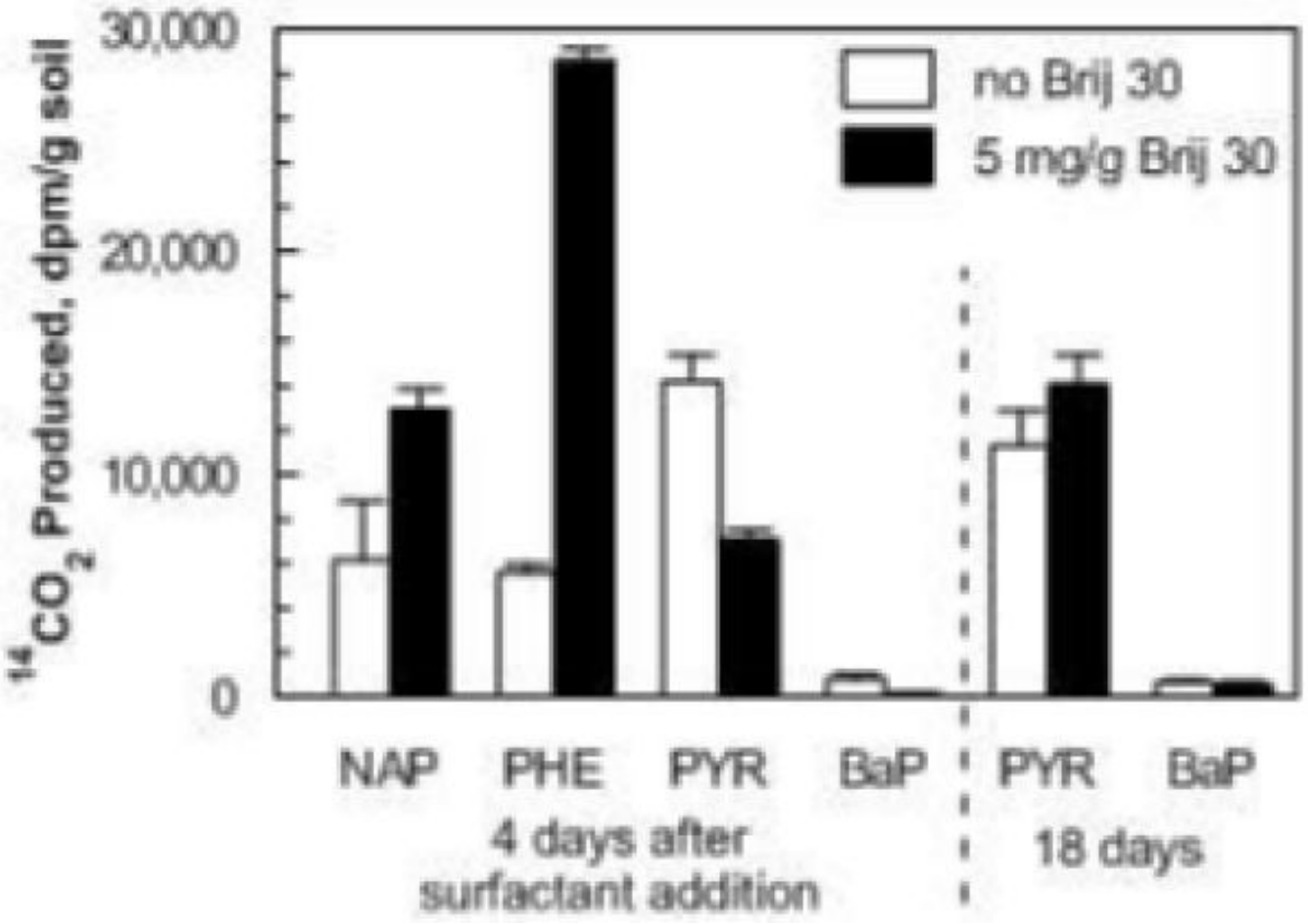

Figure 2.
Mineralization of [^{14}C]naphthalene (NAP), [^{14}C]phenanthrene (PHN), [^{14}C]pyrene (PYR) and [^{14}C]BaP in soil slurry with or without Brij 30 addition at 5 mg/g. Values are means and standard deviations of triplicate incubations and represent initial rate measurements for aliquots of soil slurry taken at the indicated incubation time. Data to the left of the dashed line are for mineralization assays that were conducted after 4 days of incubation with or without Brij 30, and data to the right of the dashed line are for assays conducted after 18 days of incubation.

Table 1

Bacterial groups previously identified by SIP that were quantified before and after amendment of bioreactor slurry with surfactants

Target group	Growth substrate(s)[a]	Closely related species
Salicylate Group 1 (SG1)	salicylate (17)	*Curvibacter* spp., *Polaromonas* spp.
Salicylate Group 2 (SG2)	naphthalene, salicylate (17)	*Pseudomonas* spp.
Salicylate Group 3 (SG3)	naphthalene (15), salicylate (15,17), phthalate (19)[b]	*Ralstonia* spp.
Acidovorax spp.	phenanthrene (15)	*Acidovorax* spp.
Pyrene Group 1 (PG1)	pyrene (16), phenanthrene (18)	uncultivated β-Proteobacteria
Pyrene Group 2 (PG2)	pyrene, (16), phenanthrene (18)	uncultivated γ-Proteobacteria

[a] Determined by DNA-based SIP except as noted otherwise.

[b] Inferred by large increase in abundance in response to enrichment of the bioreactor slurry with phthalate (19).

Table 2

Total bacterial 16S rRNA genes and 16S rRNA genes representing specific bacterial groups in soil slurry before ("initial") and after incubation with various doses of Brij 30 for 18 days

	Log gene copy #/g soil at indicated Brij 30 dose (mg/g soil) [a]				
Group	**Initial**	**0**	**5**	**20**	**50**
Total bacteria	7.8 ± 0.4	7.9 ± 0.3	8.3 ± 0.4	8.5 ± 0.3	8.4 ± 0.4
SG1	< 5.5 [a]	< 5.5	**6.9 ± 0.4**	**6.7 ± 0.3**	5.6 ± 0.1
SG3	< 5.9	< 5.9	**7.4 ± 0.5**	**7.6 ± 0.4**	6.7 ± 0.2
PG1	< 3.7	3.9 ± 0.1	3.9 ± 0.2	< 3.7	< 3.7
PG2	6.4 ± 0.2	6.6 ± 0.1	***5.2 ± 0.3***	***< 4.9***	***< 4.9***

[a] "less than" values are below the qPCR quantification limit for that group. Increases of the mean by an order of magnitude or greater compared to incubations without surfactant are in bold, and decreases of an order of magnitude or greater are in bold italics. Sequences representing *Acidovorax* spp. were below the quantification limit of 1.2×10^4 gene copies/g soil in all samples.

Environ Sci Technol. Author manuscript; available in PMC 2011 October 1.

Table 3

Total bacterial 16S rRNA genes and 16S rRNA genes representing specific bacterial groups in soil slurry before ("initial") and after incubation with various doses of $C_{12}E_8$ for 18 days

	Log gene copy #/g soil at indicated $C_{12}E_8$ dose (mg/g soil) [a]				
Group	**Initial**	**0**	**2**	**10**	**25**
Total bacteria	8.1 ± 0.4	7.6 ± 0.4	7.4 ± 0.6	9.0 ± 0.5	8.7 ± 0.5
SG1	< 5.5	< 5.5	5.8 ± 0.6	**7.6 ± 0.4**	**7.2 ± 0.1**
SG3	< 5.9	< 5.9	6.7 ± 0.7	**7.9 ± 0.4**	**7.9 ± 0.4**
Acidovorax	4.8 ± 0.1	4.3 ± 0.1	< 4.1	4.7 ± 0.4	< 4.1
PG1	4.7 ± 0.2	4.1 ± 0.4	< 3.7	4.4 ± 0.5	4.5 ± 0.3
PG2	6.4 ± 0.1	5.9 ± 0.3	5.4 ± 0.3	***< 4.9***	***< 4.9***

[a] Notes as in Table 1.