

# Bioremediation and Biodegradation

## 9,10-Phenanthrenequinone Photoautocatalyzes its Formation from Phenanthrene, and Inhibits Biodegradation of Naphthalene

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### ABSTRACT

Polycyclic aromatic hydrocarbons (PAHs) have earned considerable attention due to their widespread environmental distribution and toxicity. In the environment, PAHs decompose by a variety of biotic and abiotic pathways. In both polar and nonpolar environments, phenanthrene (Phe, a common, three-ring PAH) is converted by sunlight to more polar products such as 9,10-phenanthrenequinone (PheQ) and subsequent oxidation products such as the corresponding opening dicarboxylic acid product. Biodegradation of phenanthrene also usually leads to oxidative metabolites, and eventually ends in mineralization. Our experimental objective was to investigate the photodegradation of phenanthrene and determine the effect of reaction products such as PheQ on microbial biodegradation of two- and three-ring PAHs. Abiotic experiments were performed to examine the photolytic breakdown of Phe; Phe was converted to PheQ, which catalyzed its own formation. In biodegradation experiments PheQ (0.04–4 mg/L) caused marked inhibition of naphthalene (Nap) biodegradation by a *Burkholderia* species; Phe did not. Only 20% of the naphthalene was degraded in the presence of PheQ compared with 75% in the control culture with no PheQ added. No PAH-degrading cultures were able to use PheQ as sole carbon source; however, the Phe-degrading enrichment culture dominated by a *Sphingomonas* species was able to degrade PheQ cometabolically in the presence of Phe. These results may explain why photooxidized phenanthrene-containing mixtures can resist biodegradation.

POLYCYCLIC AROMATIC HYDROCARBONS have widespread environmental distribution and display a variety of toxic effects, which have repeatedly been demonstrated in humans, other animals, and plants (LaVoie et al., 1979). These effects vary significantly between PAHs and include acute toxicity, carcinogenicity, and teratogenicity. In the environment, PAHs decompose by a variety of biotic and abiotic pathways. For example, many PAHs show strong absorbance in the solar ultraviolet region and undergo rather favorable photolysis reactions in air, water, or solid phases (Larson and Weber, 1994). Polycyclic aromatic hydrocarbons can also be degraded by bacteria and fungi (Cerniglia, 1984).

Phenanthrene (Fig. 1) is a three-ringed PAH commonly found in petroleum distillates and coal-derived products such as creosote. In both polar and nonpolar environments, it is converted by sunlight to more polar

products such as PheQ (Fig. 1) and subsequent oxidation products such as the corresponding ring-opened dicarboxylic acid product (Sawaki, 1983; Barbas et al., 1996). 9,10-Phenanthrenequinone has additional interesting photochemical properties, such as cycloaddition to olefins to form 1,4-dioxane derivatives (Chow et al., 1970) and photoreduction by H- or electron-donors (alcohols, hydrocarbons, ethers, etc.) that are in turn oxidized (Hong and Kim, 1993). This indicates that PheQ may also interact with a variety of biomolecules.

Whereas photomodification has been shown to increase the toxicity of some PAHs (McConkey et al., 1997), in some cases it has made PAHs more susceptible to biodegradation (Larson and Berenbaum, 1988). Photooxidation generally increases the toxicity of PAHs to eukaryotes (Arfsten et al., 1996) due to the production of toxic products such as quinones. Toxicity (including genotoxicity) of many quinones has been reviewed (Chesis et al., 1984; Heldal et al., 1984) and several mechanisms of action proposed. Intracellular participation of naturally occurring quinones in phosphorylation and metabolic redox cycles may present target sites for xenobiotic quinones. 9,10-Phenanthrenequinone, in particular, has been described as being toxic to a greater extent than its parent compound Phe in a bacterium (*Photobacterium phosphoreum*) and a plant [duckweed (*Lemna gibba* L.)] (Bucker et al., 1979; McConkey et al., 1997). 9,10-Phenanthrenequinone also inhibited endogenous respiration in an activated sludge while Phe did not (Meulenber et al., 1997). Given the result that phenanthrene's toxicity to plants was shown to increase during exposure to ultraviolet radiation (Huang et al., 1995), it is likely that transformation of Phe to PheQ has potential for increasing toxicity.

Many microorganisms can quickly mineralize or partially degrade small PAHs such as Nap (Fig. 1) and Phe (Cerniglia, 1984). Phenanthrene, for example, is converted to PheQ by at least one fungus species (Hammel et al., 1992). Some metabolites of PAHs are readily biodegraded by natural communities of microorganisms (Kotterman et al., 1998). Several studies of marine systems, for example, have shown increased biodegradation of illuminated PAHs compared with parent compounds (Dutta and Harayama, 2000; Rontani et al., 1985). This has also been shown in soil and sewage sludge (Miller et al., 1988) and laboratory cultures (Ni'matuzahroh et al., 1999; Lehto et al., 2000). In other cases, however, photooxidation products have been shown to

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**Abbreviations:** BSM, basal salts medium; HPLC, high performance liquid chromatography; Nap, naphthalene; PAH, polycyclic aromatic hydrocarbon; Phe, phenanthrene; PheQ, 9,10-phenanthrenequinone.

be less amenable to biodegradation, and may be actively toxic (Meulenberg et al., 1997).

Polycyclic aromatic hydrocarbons are subject to transformation by biodegradation and abiotic reactions such as photooxidation. Interactions between these biological and abiotic processes can occur in synergistic or antagonistic ways (Miller et al., 1988; Lehto et al., 2003; Meulenberg et al., 1997). Situations clearly arise whereby PAHs are exposed to solar radiation (railroad crossties, telephone poles, contaminated surface soils). In such a situation, photooxidation could occur, and photoproducts could be washed into PAH-contaminated soils or waters by rainwater. If biodegradation is the dominant mechanism of PAH loss from a soil, the effects of photoproducts on biodegradation could control the rates of PAH loss. This work examines one interaction between photooxidation and biodegradation by studying the formation of phenanthrene's major photoproduct, PheQ, examining its effects on biodegradation of naphthalene, and determining its own biodegradability. Elucidation of such interactions gives us insights into the fate of complex chemical mixtures in complex environments like coal tar creosote in soil.

## MATERIALS AND METHODS

### Photooxidation

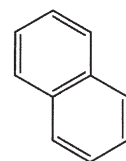
The light source used was a 2500-W xenon arc lamp, filtered and positioned to simulate the intensity of midsummer midday sunlight at 40° latitude. Pyrex tubes containing 1:1 acetonitrile to water and 0.01 mM Phe were mounted in a sample rotator for a period of 4 h. One of these tubes was removed for each hour of the test. To determine initial concentrations, another tube was kept in dark conditions (wrapped in aluminum foil on the sample rotator) throughout the test. The loss of Phe and the formation of PheQ were monitored by high performance liquid chromatography (HPLC) (see Analyses section, below). Chemicals used in photooxidation experiments were 98% pure (Phe), 99% pure (Nap), or greater than 99% pure (PheQ). The HPLC analyses detected no trace amounts of PheQ in the Phe.

### Culture Media

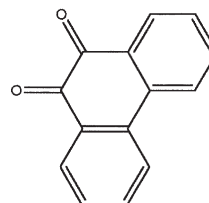
All cultures were grown in basal salts medium (BSM) as described in Sanford et al. (2002). The Phe, Nap, and PheQ were 98, 99, and >99% pure, respectively.

### Enrichment Cultures

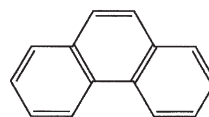
Primary PAH-degrading enrichments were originally inoculated from creosote-contaminated soil (5 g of contaminated soil in 100 mL of BSM). After incubation in the dark at 30°C until visibly turbid, they were transferred (1% inoculum by volume) to fresh BSM containing 200 mg/L PAH. Solid PAHs were added directly to culture flasks, and all cultures were kept on a shaker. Subsequent enrichment cultures were incubated under identical conditions until disappearance of visible PAH crystals, at which point they were again transferred to fresh BSM (1% inoculum by volume). Enrichment culture volumes were selected to ensure adequate aeration and were 100 or 200 mL in 250- or 500-mL in Erlenmeyer flasks, respectively. Enrichment cultures were capped with aluminum foil closures.



Naphthalene (Nap)



9,10-Phenanthrenequinone (PheQ)



Phenanthrene (Phe)

Fig. 1. Structures of various polycyclic aromatic hydrocarbons (PAHs).

### Biodegradation Culture Details

Cells for biodegradation experiments were grown in 4-L flasks. These "stock" cultures were inoculated from PAH-degrading enrichment cultures and were aerated using aquarium air pumps and diffusers. Three weeks before each experiment, a small inoculum (less than 2% of the final volume) of the Nap-degrading community was added to BSM, along with solid Nap at 200 mg/L loading. Cells were harvested for experimentation after Nap crystals were not visible for 3 to 5 d. The Nap-degrading stock cultures at this stage had an optical density at 600 nm ( $OD_{600}$ ) of approximately 0.2, corresponding to  $7.7 \times 10^7$  colony forming units (CFU) per mL.

Biodegradation cultures were prepared as follows. Saturated Phe and PheQ cultures were incubated with solid PAH for 12 h at room temperature before inoculation to allow dissolution of PAHs. Naphthalene and PheQ were added to other cultures from saturated aqueous stock solutions (nominally 1000 mg/L), which were stored in the dark at 30°C for at least 1 wk, stirred vigorously for at least 1 h, and then filtered (glass fiber, 3- $\mu$ m average pore size) to remove PAH crystals. Polycyclic aromatic hydrocarbon stock solution concentrations were 4.0 mg/L for PheQ and variable for Nap depending on the filtration method. Vacuum filtration resulted in Nap concentrations of 6.5 mg/L and was replaced by gravity filtration, which gave 20.0 mg/L, in later experiments. These concentrations were quantified by HPLC (below, Analyses section).

Serum bottles (160 mL; Wheaton, Millville, NJ) were used for all biodegradation cultures unless stated otherwise. Bottles were sealed with butyl stoppers held in place with aluminum crimp caps. In biodegradation experiments, microorganisms, BSM, Phe, and PheQ were added 12 h before the experiments began. Experimental clocks began on addition of Nap. All biodegradation cultures were incubated on a shaker table at 30°C in the dark.

### Biodegradation Experiments

To investigate the effects of PheQ on Nap biodegradation, Nap-degrading cultures were exposed to varying concentrations of PheQ. The Nap concentrations were quantified before and after a 2-h incubation period using fluorescent spectroscopy (see Analyses section, below). Initial Nap concentration was 3.25 mg/L; cells were present at  $6.4 \times 10^7$  CFU/mL. There were eight cultures of each treatment; these cultures contained 4.0 (saturated), 0.4 (10% saturated), 0.04 (1% saturated), or 0 mg/L (no PheQ) PheQ in a total volume of 40 mL. An additional two cultures at each PheQ concentration were kept as sterile controls; their mean Nap concentration was interpreted as an overall sterile control.

To elucidate the effects of PheQ vs. Phe on Nap biodegradation, Nap-degrading cultures were exposed either to varying concentrations of PheQ, or to saturated Phe. The Nap concentrations were quantified via HPLC over 6 h (see Analyses section, below). Initial Nap concentration was 10.0 mg/L; cells were present at  $4.8 \times 10^7$  CFU/mL in a total volume of 80 mL. 9,10-Phenanthrenequinone was added to eight cultures each at a loading of 4.0 (saturated) or 0.25 (low PheQ) mg/L. Phenanthrene was added to eight cultures at 0.25 mg/L (saturated). An additional eight cultures received no three-ring PAHs (Nap only). Finally, an additional two cultures from each treatment were kept as sterile controls; their mean Nap concentration was used as an overall sterile control.

Finally, the Phe-degrading community was screened for the ability to degrade PheQ in the presence of Phe. In a 250-mL Erlenmeyer flask, a 100-mL enrichment of Phe degraders was created as described above. In addition to 50 mL of filtered saturated PheQ stock solution, solid Phe was added to this culture at a loading of 200 mg/L. The PheQ concentration was measured by HPLC until it reached a stable concentration. The culture was then allowed to incubate at 30°C until the Phe crystals were no longer easily visible (4 wk). Next it was removed from the shaker and incubated at 30°C. After several more months, Phe was again added, this time to loading of 300 mg/L, and PheQ concentration was again measured, this time until the PheQ concentration dropped below the HPLC method detection limit.

### Analyses

Optical density at 600 nm was measured relative to a blank of BSM on a Spectronic 20 (Bausch & Lomb, Rochester, NY). Fluorescent spectroscopy was performed using a Series II luminescence spectrometer (AMINCO-Bowman, Beverly, MA) calibrated with standard aqueous Nap solutions. Excitation and emission wavelengths were 282 and 346 nm, respectively. Fluorescence contribution due to PheQ was measured with mixtures of Nap and PheQ. The PheQ contributions to fluorescence were found to be insignificant.

The HPLC analysis of PAH biodegradation experiments was performed using a Model 1090 HPLC (Hewlett-Packard, Palo Alto, CA) equipped with a photodiode array detector and Hi-Pore RP-318 reversed phase column (Bio-Rad, Hercules, CA). Mobile phase was methanol, water, and  $H_3PO_4$ , 80:20:0.1. Injection volumes were 100  $\mu$ L. Polycyclic aromatic hydrocarbons were identified by absorbance spectra and retention time relative to external standards. Retention time for Nap was 4.3 min.

High performance liquid chromatography of Phe photodegradation experiments was performed with a Model 250 pump (PerkinElmer, Wellesley, MA) and a Spectroflow Model 757 absorbance detector (Kratos, Chestnut Ridge, NY) at 254 nm. The column used was an Alltima 18 Rocket 3 (Alltech

Associates, Deerfield, IL), with 1:1 acetonitrile to water the eluting solvents. Injection volumes were 200  $\mu$ L. The average retention time for Phe was 18.5 min, while that for PheQ was 3.25 min.

During sampling of large culture sets, all cultures were stored in an ice and water bath to suspend degradation activity. Samples (1.5 mL) were removed from cultures using sterile syringes and needles, then filtered with 0.22- $\mu$ m-pore-size nitrocellulose membranes and stored in sealed glass sample vials. A typical sampling period required roughly 2 h; immediately afterward, the culture bottles were placed in a 30°C water bath for 30 min. Sample vials were stored at 4°C until analysis, no more than 3 d later.

### Genetic Analyses of Polycyclic Aromatic Hydrocarbon-Degrading Cultures

DNA was extracted from cell pellets of PAH-degrading isolates using the UltraClean Microbial DNA extraction kit (MoBio Laboratories, Carlsbad, CA) according to the manufacturer's recommendations with one modification. Instead of a special vortex adaptor, a bead beating machine (Mini Beadbeater 8; Biospec Products, Bartlesville, OK) was used to beat the cells in the bead solution provided for the recommended time. After centrifugation, tubes were decanted and remaining pellets were frozen at -20°C until DNA extraction.

The 16S rDNA gene was amplified by polymerase chain reaction (PCR) using the 27F (5' AGA GTT TGA TCM TGG CTC AG 3') forward and 1492R (5' GGT TAC CTT GTT ACG ACT T 3') reverse primers, which are specific to the bacterial domain and universal, respectively (Operon Technologies, Alameda, CA). The forward primer was labeled at the 5' end with phosphoramidite fluorochrome 5-carboxyfluorescein (FAM). Reactions were performed using a PTC-200 thermal cycler with a heated lid (MJ Research, South San Francisco, CA) and the following reagents from Takara Shuzo Co. (Shiga, Japan): 0.05 units/ $\mu$ L Ex Taq Polymerase, 0.2 mM each dNTP, 2.0 mM  $MgCl_2$ , and Ex Taq buffer (manufacturer's recommended concentration). Primers were each present at 0.2  $\mu$ M, and template concentrations varied. Total reaction volumes were 50  $\mu$ L, with a 2-min hot start at 94°C followed by 30 cycles of denaturation (1 min, 94°C), annealing (1 min, 55°C), and elongation (90 s, 72°C).

The PCR products were purified using QIAquick spin columns (Qiagen, Valencia, CA). Purified PCR products were separated into three aliquots, each digested with one of the following enzymes using buffers supplied by its manufacturer: Hha1, Rsa1 (both from Gibco BRL Invitrogen, Carlsbad, CA), or Msp1 (Roche, Basel, Switzerland). All digestions were performed for 3 h at 37°C. Digested fragments were analyzed on a polyacrylamide sequencing gel at the Core Sequencing Facility of the W.M. Keck Center for Comparative and Functional Genomics on the University of Illinois, Urbana-Champaign campus. Terminal fragments were compared with those from theoretical digestions performed using the Terminal Restriction Fragment Length Polymorphism Analysis Program (TAP-TRFLP) simulation tool on the website of the Ribosomal Database Project, maintained by the Center for Microbial Ecology at Michigan State University (<http://rdp.cme.msu.edu/index.jsp> [verified 11 Nov. 2004], described in Marsh et al., 2000).

## RESULTS AND DISCUSSION

### Photooxidation Behavior

Phenanthrene underwent photodegradation by an apparent two-phase process in 1:1 acetonitrile to water

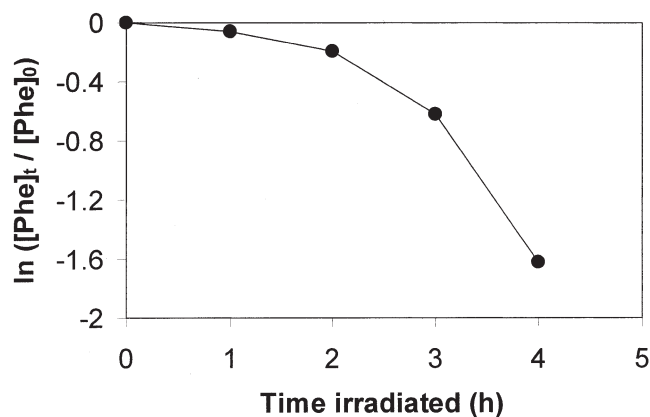


Fig. 2. Photodegradation of phenanthrene in 1:1 acetonitrile to water.

mixture in the absence of added PheQ. A slow lag period was followed after several hours of illumination by a faster reaction, with PheQ as the major identified product (Fig. 2). Additional minor products were 9-phenanthrol and 9,10-phenanthrenediol. The lag phase was extended in the presence of phenol and halophenols (presumed to be free-radical quenchers), but was eliminated if PheQ was present at the beginning of the reaction (Fig. 3). The loss rate after the characteristic lag period (PheQ initially absent) appeared to be very similar to the loss rate with PheQ present at the beginning of the reaction. The promoting effect of PheQ could be due to the formation of an exciplex between excited Phe and ground-state PheQ, which would stabilize electron transfer redox processes by exciton resonance and charge transfer interactions (Dabestani and Sigman, 2000). 9,10-Phenanthrenequinone illuminated by itself photoreacts slowly under these conditions, but in the presence of Phe it is also being formed, and what is observed is a gradual increase of PheQ concentration.

In addition, there were apparent oxygen-dependent and oxygen-independent photoprocesses. When the solution was purged with argon before illumination, the lag period was decreased substantially (Fig. 4). In the absence of oxygen, PheQ was still the principal product, although 9-phenanthrol appeared to be a transient intermediate in its formation. The oxygen-dependent photolysis mechanism for Phe may involve photoionization or internal electron transfer within a Phe-O<sub>2</sub> complex, with the released electron scavenged by molecular O<sub>2</sub>, and subsequent recombination of the radical cation of Phe with superoxide (Fasnacht and Blough, 2003). Slow rearrangement of the resulting peroxy radical could lead to formation of PheQ, which would then induce a faster photolysis reaction. Both the oxygen-independent and -dependent photolysis of Phe may involve the photooxidation of water by PheQ; a hydroxyl radical would be produced, by analogy with other quinones (Alegría et al., 1997) and by naturally occurring dissolved organic matter (Vaughan and Blough, 1998).

### Enrichment Cultures

Enrichment cultures were established with creosote-contaminated soil near telephone poles in Urbana, IL,

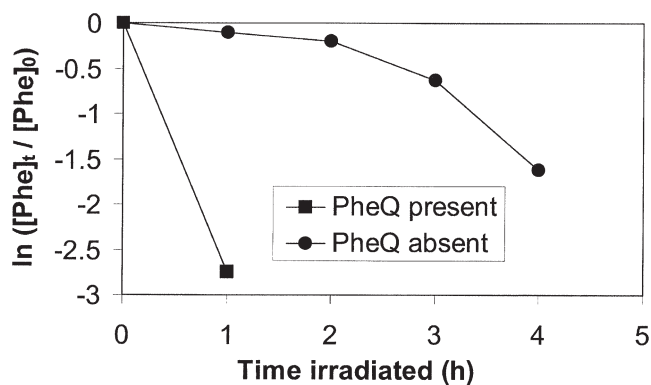


Fig. 3. Comparison of photodegradation of phenanthrene (0.01 mM) with and without an equimolar amount of phenanthrenequinone added at the beginning of the reaction.

using Nap or Phe as sole carbon source. After 200 mg/L PAH was degraded, Nap and Phe enrichments were visibly turbid. Typical optical densities at 600 nm (OD<sub>600</sub>) for Nap and Phe enrichments were 0.21 and 0.25, respectively.

### Inhibition of Naphthalene Biodegradation in the Presence of 9,10-Phenanthrenequinone

Naphthalene removal in the presence of PheQ was measured over 2 h (Fig. 5). All cultures initially contained 3.3 mg/L Nap. After 2 h, sterile controls demonstrated little or no decrease in Nap concentration, while Nap-only cultures degraded more than 75% of their initial Nap. Cultures saturated with PheQ (4.0 mg/L) lost only 20%. Two other culture sets contained 0.4 and 0.04 mg/L PheQ (10% saturated and 1% saturated, respectively); these cultures showed intermediate Nap biodegradation. These data imply that increased PheQ concentration caused decreased rates of Nap biodegradation.

### Naphthalene Biodegradation Inhibition by Phenanthrene versus 9,10-Phenanthrenequinone

The effects of PheQ and Phe on Nap degradation were tested as well (Fig. 6). In this experiment, Nap concentra-

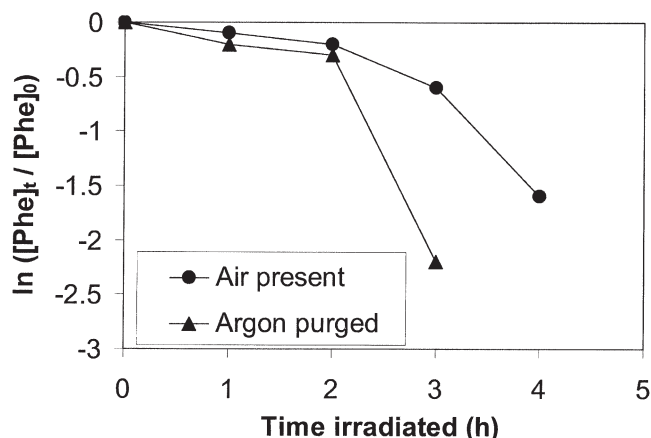


Fig. 4. Comparison of photodegradation of phenanthrene in air-saturated and argon-purged solution.

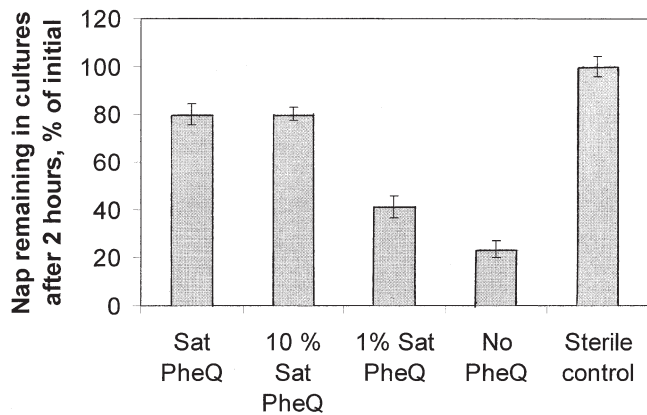


Fig. 5. Percent removal of naphthalene (Nap) by a Nap-degrading community in the presence of various 9,10-phenanthrenequinone (PheQ) concentrations. The terms "Sat PheQ," "10% Sat PheQ," and "1% sat PheQ" indicate PheQ concentrations at saturation (4 mg/L), 10% of saturation (0.4 mg/L), and 1% of saturation (0.04 mg/L), respectively. "No PheQ" contained no PheQ, while "sterile control" contained no inoculum. Error bars represent two standard errors.

tion (initially 10.0 mg/L) was quantified repeatedly by HPLC for each culture. Sterile control Nap concentrations were constant over the course of the experiment; all other sets of cultures experienced noticeable loss of Nap.

This experiment demonstrated an obvious difference in degradation rate between Phe-containing cultures and those containing PheQ at the same concentration (0.25 mg/L); PheQ apparently caused a decrease in Nap degradation rate. Saturated PheQ cultures showed even slower biodegradation, while Phe apparently had no effect. These data clearly reinforce results presented above; again, PheQ caused inhibition of Nap biodegradation while Phe, PheQ's parent compound, did not have any effect.

Studies that examine interactions between photooxidation and biodegradation of PAHs have demonstrated

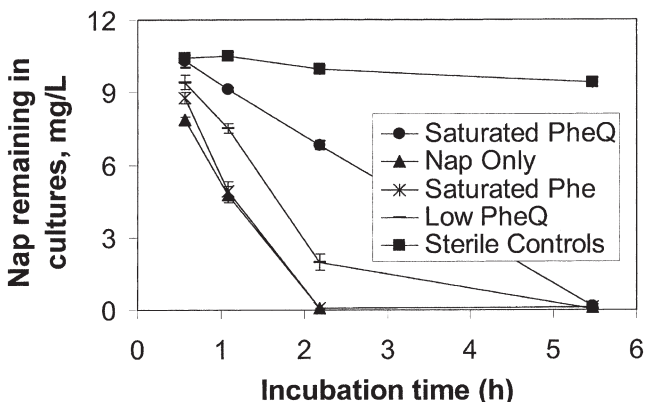


Fig. 6. Naphthalene (Nap) concentrations in Nap-degrading communities in the presence of 9,10-phenanthrenequinone (PheQ) or phenanthrene (Phe). The Nap-containing cultures were amended as follows with Phe or PheQ: "Saturated PheQ," PheQ at saturation (4 mg/L); "Nap only," no PheQ or Phe; "Saturated Phe," Phe at saturation (0.25 mg/L); "Low PheQ," PheQ at 0.25 mg/L; "Sterile controls," two representatives of each previously mentioned treatment containing no inoculum. Error bars represent two standard errors.

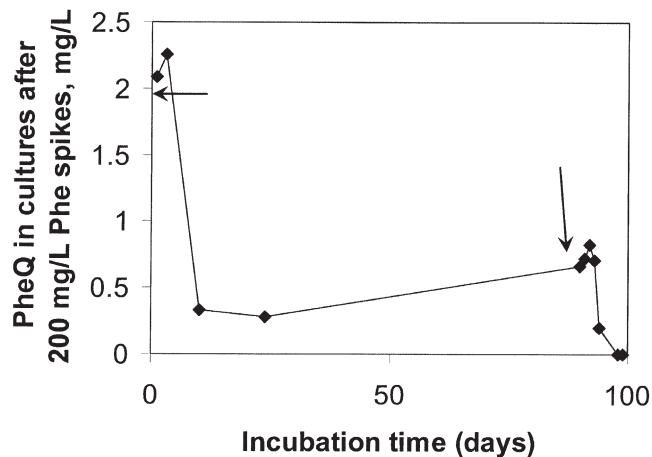


Fig. 7. 9,10-Phenanthrenequinone (PheQ) remaining in phenanthrene (Phe)-degrading enrichment as a function of time. Culture was spiked (arrows) twice; first with 200 and then 300 mg/L Phe.

synergistic relationships between these processes. Studies of seawater (Dutta and Harayama, 2000; Rontani et al., 1985), soil and sewage sludge (Miller et al., 1988), and laboratory cultures (Ni'matuzahroh et al., 1999; Lehto et al., 2000) have consistently shown that photo-degradation can facilitate biodegradation in PAHs or creosote. This is not unexpected, because PAH oxidation products are more soluble than their parent compounds and are therefore expected to be more bioavailable. This is not necessarily the case for Phe, however. As shown by Meulenberg et al. (1997) using oxygen uptake rates and the above work using Nap degradation rates, PheQ appears to be less biodegradable than Phe, its parent compound.

Recent work by Lehto et al. (2003) demonstrated a tendency for photooxidation to increase the potential for biodegradation of PAHs in synthetic mixtures by enrichment cultures and individual *Sphingomonas* strains. An interesting facet of this work was a demonstration that photooxidation of creosote inhibited biodegradation of creosote PAHs. This was attributed to formation of toxic products such as PheQ. 9,10-Phenanthrenequinone toxicity has been shown to affect a bacterium, *Photobacterium phosphoreum*, a plant, duckweed (McConkey et al., 1997), and an activated sludge microbial community (Meulenberg et al., 1997). The results presented above clearly support the idea that PheQ, a photoproduct of Phe, could inhibit bacterial-mediated biodegradation.

### Degradation of 9,10-Phenanthrenequinone by a Phenanthrene-Degrading Community

The Phe-degrading enrichment was screened for ability to degrade PheQ in the presence of Phe. First, the Phe enrichment was used to inoculate cultures containing PheQ as sole carbon source. No turbidity increase or crystal-associated biomass was observed in PheQ enrichments, and the solid quinone was still present after 6 mo. No growth was observed on PheQ crystals, even though such growth was clearly evident in Phe-degrading cultures. These results are consistent with previous

studies in which Phe-mineralizing inocula, a soil, and an activated sludge were unable to oxidize PheQ (Meulenberg et al., 1997).

The Phe-degrading community was then exposed to Phe and PheQ simultaneously; PheQ concentrations were measured over time (Fig. 7). Once the PheQ concentration stabilized, the culture was allowed to incubate for 2 mo, after which the culture was spiked with 300 mg/L Phe. At this time, PheQ measurements were resumed until PheQ was completely degraded. The disappearance of PheQ was clearly linked to the presence of Phe. Though this apparent cometabolism has not been described before, several different types of interactions between PAHs have been reported (Bauer and Capone, 1988; Bouchez et al., 1995).

### Molecular Characterization of Enrichments

Both Phe- and Nap-degrading cultures were analyzed by T-RFLP. Samples were taken on the first evidence of turbidity, then again after all substrate had been removed (11 and 27 d for Nap and Phe cultures, respectively). For both cultures, a single peak dominated each profile at both time points, indicating that a single phylogenetic group was predominant. The Nap-degrading dominant peak accounted for 87.7% of fluorescence signal, while the Phe-degrading dominant peak accounted for 70.3%.

Virtual digestions were performed on genomic DNA sequences from the Ribosomal Database; these digestions yielded peaks similar in fragment size to those seen in the physical digestions of our PAH-degrading communities. Thirty-one profiles matched that of the predominant Phe degrader; 14 represented *Sphingomonas* strains. Seven profiles matched that of the Nap-degrading predominant peak; all seven were members of the genus *Burkholderia*. We suspect, based on this analysis, that the predominant Phe-degrader is a *Sphingomonas* or related organism, and that the predominant Nap-degrading organism is related to *Burkholderia*. Both genera have been associated with PAH degradation. Members of *Sphingomonas*, for example, have been shown to degrade high and low molecular weight PAHs (Lehto et al., 2003; Janikowski et al., 2002; Nadalig et al., 2002; Ye et al., 1996) including Nap and Phe. *Burkholderia* species have also been shown to degrade Nap and Phe (Laurie and Lloyd-Jones, 1999).

### CONCLUSIONS

The data presented above demonstrate several previously undescribed phenomena associated with Phe and its main photoproduct, PheQ. 9,10-Phenanthrenequinone catalyzed its own formation by two different probable mechanisms: electron transfer within an exciplex with Phe, and photolysis of water to produce hydroxyl radical. It also inhibited biodegradation of Nap by a *Burkholderia*-dominated community, while Phe itself did not. This particular result was demonstrated using a simple, rapid assay that required, at most, 6 h of incubation time. 9,10-Phenanthrenequinone was not utilized by a Phe-degrading *Sphingomonas*-dominated com-

munity, though this community apparently degraded PheQ cometabolically in the presence of Phe. These results are unique, and could also explain the observation by Lehto et al. (2003) that creosote mixtures lost potential for biodegradation when subjected to photooxidation. Given PheQ's self-catalyzing formation and apparent toxicity, it is easy to imagine a scenario whereby the presence of Phe in a creosote mixture causes inhibition of the creosote's biodegradation.

### ACKNOWLEDGMENTS

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