Products from the Incomplete Metabolism of Pyrene by Polycyclic Aromatic Hydrocarbon-Degrading Bacteria

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Pyrene is a regulated pollutant at sites contaminated with polycyclic aromatic hydrocarbons (PAH). It is mineralized by some bacteria but is also transformed to nonmineral products by a variety of other PAH-degrading bacteria. We examined the formation of such products by four bacterial strains and identified and further characterized the most apparently significant of these metabolites. Pseudomonas stutzeri strain P16 and Bacillus cereus strain P21 transformed pyrene primarily to cis-4,5-dihydro-4,5-dihydroxypyrene (PYRdHD), the first intermediate in the known pathway for aerobic bacterial mineralization of pyrene. Sphingomonas yanoikuyae strain R1 transformed pyrene to PYRdHD and pyrene-4,5-dione (PYRQ). Both strain R1 and Pseudomonas saccharophila strain P15 transform PYRdHD to PYRQ nearly stoichiometrically, suggesting that PYRQ is formed by oxidation of PYRdHD to 4,5-dihydroxypyrene and subsequent autoxidation of this metabolite. A pyrene-mineralizing organism, Mycobacterium strain PYR-1, also transforms PYRdHD to PYRQ at high initial concentrations of PYRdHD. However, strain PYR-1 is able to use both PYRdHD and PYRQ as growth substrates. PYRdHD strongly inhibited phenanthrene degradation by strains P15 and R1 but had only a minor effect on strains P16 and P21. At their aqueous saturation concentrations, both PYRdHD and PYRQ severely inhibited benzo[a]pyrene mineralization by strains P15 and R1. Collectively, these findings suggest that products derived from pyrene transformation have the potential to accumulate in PAH-contaminated systems and that such products can significantly influence the removal of other PAH. However, these products may be susceptible to subsequent degradation by organisms able to metabolize pyrene more extensively if such organisms are present in the system.

Polycyclic aromatic hydrocarbons (PAH) are known to be degradable by a variety of soil bacteria (40). Consequently, the bioremediation of PAH contamination with naturally occurring microorganisms has been attempted at a number of sites (43, 45). Most of the interest in the biodegradation of PAH in the field has been in the removal of the parent compounds, while most research on pure cultures of PAH-degrading bacteria has focused on their ability to grow on or mineralize specific PAH substrates. Relatively little attention has been paid to the potential formation of products from the partial transformation of PAH.

Most of the information that does exist on PAH metabolites has been obtained in the context of identifying transient metabolites formed by isolates during growth on the parent compound (13, 33, 35, 40) or metabolites formed by mutants of wild-type degraders (4, 40). Some bacteria, however, are capable of transforming one or more PAH despite an inability to grow on or mineralize the PAH in question (1, 20, 31, 47). It is important to evaluate the products of such incomplete PAH metabolism because of their potential effects on PAH-degrading microorganisms or on potentially exposed human populations (44). Identification of common products of incomplete metabolism can also be important in assessing the extent to which natural attenuation may be occurring at contaminated sites (44).

In a recent study (1), we described the broad PAH substrate ranges of 11 bacteria isolated from PAH-contaminated soils by enrichment on phenanthrene as a sole carbon source. None of these organisms is capable of mineralizing pyrene, yet all could remove pyrene from solution, and all that were examined further transformed pyrene to unidentified metabolites (1). The objectives of this study were to isolate and identify the major products from the incomplete metabolism of pyrene by four of these organisms, evaluate the potential degradability of these metabolites by an organism known to grow on pyrene as a sole carbon source, and determine the effects of these metabolites on the degradation of phenanthrene, a representative growth substrate for many PAH degraders, and benzo[a]pyrene, a representative carcinogenic PAH that is not known to serve as a growth substrate for any organism.

MATERIALS AND METHODS

Chemicals and media. Pyrene (99%), osmium tetroxide (>98%), ruthenium dioxide (99.9%), sodium periodate (99.8%), and acetone-d₆ (99.9%) were obtained from Aldrich (Milwaukee, Wis.). Phenanthrene (>96%), [7-¹⁴C]benzo[a]pyrene (7-¹⁴C-labeled BaP) (>98%; specific activity, 26.8 mCi mmol⁻¹), and [4,5,9,10-²⁰¹C]pyrene (>98%; specific activity, 61 mCi mmol⁻¹) were obtained from Sigma (St. Louis, Mo.). All solvents used were high-pressure liquid chromatography (HPLC) grade or the equivalent. Mineral salts buffer (MSB) was as described in Stucki et al. (39).

cis,4,5-Dihydro-4,5-dihydroxypyrene (PYRdHD) was synthesized by oxidation of pyrene with osmium tetroxide (10, 23). The crude product was extracted with 200 ml of hexane to remove unreacted pyrene, recrystallized from acetone, and dried at 50°C for 24 h. The purity of the synthesized compound was established by the presence of a single peak in chromatograms from HPLC, and the authenticity was established by ¹H nuclear magnetic resonance (NMR) spectroscopy. PYRdHD exhibits UV absorbance maxima at 219 and 261 nm. The ¹H NMR shifts recorded for PYRdHD were (in parts per million) 7.90 (d, 2), 7.84 (s, 2), 7.50 (d, 2), 7.46 (t, 2), and 7.33 (t, 2). Pyrene-4,5-dione (PYRQ) was synthesized by the oxidation of pyrene with ruthenium dioxide and sodium periodate (17). The crude product was dissolved in 50 ml of methylene chloride and passed through a column of silica gel, which was eluted successively with hexane (to remove unreacted pyrene) and methylene chloride. Methylene chloride eluted PYRQ first and then eluted pyrene-1,6-dione and pyrene-1,8-dione. The purity and authenticity of synthesized PYRQ...
were established by HPLC and 1H NMR spectroscopy, respectively. PYRQ exhibits UV-visible light absorbance maxima at 238, 295, and 432 nm. The 1H NMR shifts for PYRQ were (in parts per million) 8.43 (d, 2), 8.38 (d, 2), 8.05 (s, 2), and 7.88 (d, 2).

Aqueous solubleis of PYRHD and PYRQ at room temperature were determined by adding an excess of either compound to 10 ml of MSB in triplicate vials. After 24 h, the samples were filtered through a 0.2-μm-pore-size filter, acidified to pH 2.5 with 20% H3PO4, and analyzed through a column of octadecyl silica (Bellefonte, Pa.). The residue was dissolved in 1 ml of acetonitrile and separated by thin-layer chromatography (TLC) (1000-μm diameter silicon, 20 by 20 cm; F254; Whatman) with a 50/50 mixture of hexane and benzene, followed by a 90/10 mixture of benzene and acetone and finally a 85/10/5 mixture of benzene, acetone, and acetic acid. Bands that fluoresced strongly under UV light (254 nm) were scraped off the plates, resolvolized in acetonitrile, and filtered through a 0.02-μm-pore-size alumina filter (Whatman). The filtrates were evaporated under a gentle stream of N2. The residue was dissolved in 1 ml of acetonitrile and separated by HPLC. Injections (150 μl each) were made on a Supelcosil C18 semipreparative column (25 cm by 10 mm; particle size, 5 μm; Whatman) with a 50/50 mixture of hexane and benzene, followed by a 90/10 mixture of benzene and acetone and finally a 85/10/5 mixture of benzene, acetone, and acetic acid. Bands that fluoresced strongly under UV light (254 nm) were scraped off the plates, resolvolized in acetonitrile, and filtered through a 0.02-μm-pore-size alumina filter (Whatman). The filtrates were evaporated under N2, and then the residue was dissolved in 300 μl of acetonitrile and analyzed by HPLC. Injections (150 μl each) were made on a Supelcosil C18 semipreparative column (25 cm by 10 mm; particle size, 5 μm; Whatman). The filtrates were evaporated under N2, and then the residue was dissolved in 0.5 ml of acetonitrile, DMSO, and water mixture 50:50:0.5. A number of pyrene metabolites were identified by the same method used in earlier work (1). Phenanthrene was added as a control and was identified as phenanthrene-1,3,4-triol (1). The effects of PYRQ on BaP mineralization by strains P15 and R1 were determined with triplicate washed-cell suspensions (5 ml each) of strain R1 or P15 in vials containing 10 μM PYRQ. Reactions were terminated after 3 days by adding 1 ml of acetonitrile, and the supernatants were analyzed for residual PYRQ by HPLC.

The effects of PYRQ on BaP mineralization by strains P15 and R1 were determined with triplicate washed-cell suspensions (5 ml) in 20-ml scintillation vials containing PYRQ or BaP at concentrations identified in a preliminary experiment. Each vial contained a 1-ml solution of filter paper soaked with 0.5 μl of 2 N KOH as a CO2 trap (1). Approximately 20,000 dpm of 14C-labeled BaP (equivalent to 0.064 μM) was added to each vial, which was then capped with a Teflon-lined septum. Positive controls did not contain pyrene or pyrene metabolites, and controls containing BaP and 14C-labeled BaP were incubated under the same conditions as described above. To avoid autocatalysis of the reactions, the vials were shaken for an additional 24 h, and then the filter paper strips were placed in 5 ml of scintillation cocktail and another 5 ml of scintillation cocktail was added directly to the sample vials. Samples were analyzed by scintillation counting.

**RESULTS**

**Isolation of pyrene metabolites.** Growth of strains P15, P16, P21, and R1 in a medium containing phenanthrene as a sole carbon source and in the presence of pyrene in excess of its aequous solubility resulted in the extracellular accumulation of a number of pyrene metabolites. The relative quantities of these metabolites were assessed by the size and strength of the fluorescent bands on TLC plates. The most significant bands that were isolated by TLC were purified further by semi-preparative HPLC, and the purified compounds were analyzed by 1H NMR spectroscopy.

The most significant metabolite from isolates P16 and P21 was identified as PYRHD (Fig. 1a), which is the first intermediate in the aerobic bacterial pathway for metabolism of pyrene (13, 24, 33, 35, 49). PYRHD was one of two significant products from strain R1, which also transformed pyrene to PYRQ (Fig. 1b). No other isolated TLC bands were identified, including all those from strain P15, either because there were insufficient amounts for NMR analysis or the NMR spectra could not be characterized. Optical spectra obtained for the unidentified bands did not match published spectra of known intermediates in the bacterial degradation of pyrene (33).

Identification of PYRHD and PYRQ was confirmed by comparing the NMR spectra of the bacterial metabolites to...
Metabolism of PYRdHD and PYRQ. Resting cells of strains R1 and P15 both converted PYRdHD nearly stoichiometrically to PYRQ, whereas neither strain P16 nor P21 was able to transform PYRdHD to PYRQ (Table 1). Mycobacterium PYR-1, an organism known to mineralize pyrene, also transformed PYRdHD to PYRQ to various extents depending on the initial concentration of PYRdHD (Table 2). Strain PYR-1 converted all of the PYRdHD to PYRQ at an initial PYRdHD concentration of 200 \( \mu M \), whereas at an initial concentration of 50 \( \mu M \) only 4% of the diol was converted to the quinone. This organism was able to grow on either PYRdHD or PYRQ as a sole carbon source in MSB medium, as determined by significant increases in optical density and protein concentration relative to inoculated vessels with no carbon source (results not shown). The protein yield from PYRQ was the same as that from 500 \( \mu M \) pyrene under the same conditions, while that from PYRdHD was about one-third the yield from pyrene or PYRQ.

Effects of pyrene metabolites on phenanthrene degradation. Apparent first-order rates of phenanthrene degradation were measured in the presence and absence of PYRdHD and PYRQ at an initial phenanthrene concentration of 5.6 \( \mu M \), which is less than its aqueous solubility. PYRdHD strongly inhibited phenanthrene degradation by strains P15 and R1, modestly inhibited phenanthrene degradation by strain P21, and had a negligible effect on strain P16 (Table 3). PYRQ strongly inhibited phenanthrene degradation by strain R1 and had a negligible-to-minor effect on the remaining strains.

In contrast to the observed inhibitory effects of pyrene metabolites on rates of dissolved phenanthrene degradation by some of the strains, the yield of protein after growth on 560 \( \mu M \) phenanthrene for 3 days in the presence of PYRQ or PYRdHD was equivalent to the yield obtained after growth on

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**TABLE 1. Conversion of PYRdHD to PYRQ by resting cells after 3 days**

<table>
<thead>
<tr>
<th>Strain</th>
<th>PYRdHD removed (( \mu M ))^a</th>
<th>PYRQ detected (( \mu M ))^b</th>
</tr>
</thead>
<tbody>
<tr>
<td>P15</td>
<td>21.5 ± 0.4</td>
<td>20.6 ± 2.1</td>
</tr>
<tr>
<td>R1</td>
<td>4.1 ± 0.5</td>
<td>3.9 ± 0.0</td>
</tr>
<tr>
<td>P21</td>
<td>None^c</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>P16</td>
<td>None^c</td>
<td>&lt;0.5</td>
</tr>
</tbody>
</table>

^a Relative to uninoculated controls, which contained 22.9 ± 0.3 \( \mu M \) PYRdHD.

Data are means and standard deviations of triplicate measurements.

^b Residual PYRdHD was not significantly different from amount obtained from controls.

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**TABLE 2. Concentration dependence of PYRQ formation from PYRdHD by Mycobacterium PYR-1 after 3 days**

<table>
<thead>
<tr>
<th>PYRdHD added (( \mu M ))</th>
<th>PYRQ detected (( \mu M ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>2.0 ± 1.1</td>
</tr>
<tr>
<td>100</td>
<td>18 ± 13</td>
</tr>
<tr>
<td>200</td>
<td>205 ± 37</td>
</tr>
</tbody>
</table>

^a All of the added PYRdHD (98.7% ± 6.8%) was recovered in uninoculated vials. No PYRdHD was recovered in any of the sample vials. Data are means and standard deviations of triplicate measurements.
Effects of pyrene metabolites on mineralization of BaP. Both PYRdHD and PYRQ at concentrations near their aqueous saturation concentrations strongly inhibited the mineralization of BaP by strains P15 and R1 throughout a 48-h incubation (Table 4). Radiochromatograms of culture fluids from incubation of either organism with 1.6 μM PYRQ indicated that no metabolites accumulated and less BaP was removed than in positive controls incubated without PYRQ (not shown).

### DISCUSSION

The ability of PAH-degrading organisms to transform PAH that are incapable of mineralizing has been noted previously (1, 20, 31, 47), but the products of such incomplete metabolism have not been characterized. Partial transformation of pyrene may be particularly relevant because of an apparently limited diversity in the ability of PAH-degrading bacteria to mineralize pyrene. The ability to grow on or mineralize pyrene is primarily associated with actinomycetes (25, 26), whereas a diverse group of bacteria isolated from PAH-contaminated soils by enrichment with phenanthrene can remove pyrene without mineralizing it (1).

Pyrene transformation by *P. stutzeri* P16 and *B. cereus* P21 during active growth on phenanthrene leads to the formation of significant amounts of PYRdHD as an apparently terminal, or “dead-end,” metabolite. PYRdHD has been identified as an intermediate in the degradation of pyrene by a number of strains that can grow on or mineralize pyrene (13, 24, 33, 35, 49). It also has been found in pyrene-contaminated marine sediments (30) and has accumulated when pyrene was added to enrichment cultures derived from the sediment (29). The formation of a cis-dihydriodiol from pyrene is consistent with its oxidation via a PAH dioxygenase, an enzyme class for which examples from several bacteria are known to have broad substrate ranges (14, 15, 18, 27, 28, 34, 37, 46).

When grown on phenanthrene in the presence of pyrene, *S. yanoikuyae* R1 accumulated significant amounts of PYRdHD and PYRQ in the medium, although only PYRQ was detected when [14C]pyrene was incubated with resting cells (Fig. 2d). Formation of PYRQ probably proceeds by subsequent metabolism of PYRdHD, as both strains R1 and P15 converted PYRdHD essentially stoichiometrically to PYRQ (Table 1). PYRQ has not been observed previously as an intermediate in the degradation of pyrene, but o-quinones such as PYRQ can arise from the autoxidation of an o-dihydroxy intermediate (7, 12, 32). We were not able to isolate 4,5-dihydroxyxylene, nor has it been isolated in previous work with pyrene-degrading organisms. Since 4,5-dihdroxyxylene is rapidly oxidized to PYRQ in air at ambient conditions (41), PYRQ presumably results from the autoxidation of 4,5-dihdroxyxylene.

*P. saccharophila* P15 can convert PYRdHD to PYRQ, but neither metabolite was identified in either growing- or resting-cell incubations of this organism with pyrene. The radiochromatograms from resting cell incubations of strains P15 and R1 (Fig. 2) indicate that other nonmineral products were formed by these organisms. We do not believe that these other products are metabolites further down the known pyrene degradation pathway, such as phenanthrene-dicarboxylic acid or phenanthrene-carboxylic acid. When the HPLC conditions used to analyze culture fluids were optimized for the fluorescence detection of the phenanthrene aromatic skeleton, no such peaks were detected in culture fluids from any of the strains. In addition, degradation beyond phenanthrene-

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**TABLE 3. Effects of pyrene metabolites on apparent rates of phenanthrene degradation**

<table>
<thead>
<tr>
<th>Strain</th>
<th>None</th>
<th>PYRdHD</th>
<th>PYRQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>P15</td>
<td>0.10 ± 0.032</td>
<td>0.005 ± 0.0005</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>P16</td>
<td>0.14 ± 0.04</td>
<td>0.12 ± 0.03</td>
<td>0.13 ± 0.04</td>
</tr>
<tr>
<td>P21</td>
<td>0.23 ± 0.03</td>
<td>0.15 ± 0.03</td>
<td>0.16 ± 0.03</td>
</tr>
<tr>
<td>R1</td>
<td>0.14 ± 0.07</td>
<td>0.02 ± 0.02</td>
<td>0.01 ± 0.01</td>
</tr>
</tbody>
</table>

* Best-fit first-order degradation coefficients and the associated 95% confidence interval.

**TABLE 4. Effect of PYRQ concentration on the mineralization of BaP by strains P15 and R1**

<table>
<thead>
<tr>
<th>PYRQ added (μM)</th>
<th>P15</th>
<th>R1</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>52 ± 2.1</td>
<td>25 ± 1.8</td>
</tr>
<tr>
<td>0.16</td>
<td>45 ± 1.4</td>
<td>3.7 ± 0.1</td>
</tr>
<tr>
<td>0.8</td>
<td>19 ± 2.6</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>1.6</td>
<td>8.0 ± 1.7</td>
<td>1.0 ± 0.1</td>
</tr>
</tbody>
</table>

Uninoculated control (1.6 μM) 0.4 ± 0.2 0.1 ± 0.1

* Data are means and standard deviations of triplicate measurements. Total recoveries of 14C ranged from 83 to 90% for strain P15 and from 88 to 98% for strain R1. Total recoveries in the uninoculated controls were 93 and 98%, respectively.

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FIG. 3. Mineralization of BaP by *P. saccharophila* P15 (a) and *S. yanoikuyae* R1 (b). Each strain was incubated alone (●) or in the presence of 155 μM PYRdHD (●) or 1.6 μM PYRQ (○). Data are means and standard deviations of triplicate measurements at each time point. Unobservable error bars are within the size of the symbol. Total recoveries of 14C ranged from 79 to 99% for strain P15 and from 95 to 99% for strain R1.
dicarboxylic acid should result in the formation of $^{14}\text{C}$CO$_2$, since the $^{14}$Cpyrene used in these experiments is labeled at the 4,5 position.

When isotope trapping with PYRdHD was used to examine the transformation of pyrene by strain P15, a single product accumulated that elutes at the same retention time as PYRQ (Fig. 2a). This result, and the relatively facile oxidation of PYRdHD to PYRQ by this organism, suggests that pyrene metabolism by strain P15 probably does proceed via oxidation at the 4,5 bond. When incubated with a large excess of PYRdHD, intracellular concentrations of 4,5-dihydroxypyrene are likely to be far greater than during the metabolism of pyrene alone. Under these conditions, the autoxidation of 4,5-dihydroxypyrene could be favored over other reactions in which this intermediate may be involved.

The unidentified products from pyrene transformation by strain P15 (and possibly strain R1) most likely represent adducts from the reaction of one or more pyrene metabolites with cellular constituents. The formation of such adducts is consistent with the presence of an insoluble residue when both strains were incubated with radiolabeled pyrene. Based on the accumulation of PYRQ in the isotope-trapping experiment, the reactive metabolite may be pyrene-4,5-dihydrodiol or a reactive intermediate derived from its intracellular oxidation. We rule out covalent reaction of PYRQ with cellular or buffer constituents, even though o-quinones derived from naphthalene and BaP can form conjugates in phosphate and glycine buffers and can also form adducts with nucleophiles such as cysteine and glutathione (32). We recovered 100% of the added PYRQ incubated with strains P15 or R1 for 3 days (not shown), and PYRQ also accumulated stoichiometrically with the removal of PYRdHD by these organisms.

*Mycobacterium* PYR-1 surprisingly formed significant amounts of PYRQ when incubated with a high concentration of PYRdHD, although it was not formed when strain PYR-1 was grown on pyrene. Similar to the isotope-trapping experiment with strain P15, it is likely that the rate of formation of 4,5-dihydroxypyrene at high concentrations of PYRdHD exceeds the subsequent rate of metabolism, resulting in the accumulation of 4,5-dihydroxypyrene and its consequent autoxidation to PYRQ. A similar phenomenon has been noted before in the bacterial degradation of naphthalene, for which the accumulation of naphthoquinone has been suggested to increase as the rate of naphthalene availability increased (2, 19). Strain PYR-1 is able to consume PYRQ as a growth substrate, which suggests that it would only accumulate transiently if formed by this organism or by other bacteria that might also be transforming pyrene in a complex system. It would be of interest to know if other organisms capable of growing on or mineralizing pyrene are able to degrade PYRQ, as our work suggests that PYRQ has the potential to accumulate in PAH-contaminated systems that contain organisms incapable of more extensive pyrene metabolism.

**Effects of pyrene metabolites on the degradation of other PAH.**

The present study clearly demonstrated that extracellular metabolites from the partial transformation of one PAH substrate can adversely affect the metabolism of other PAH. Both PYRdHD and PYRQ led to decreased rates of phenanthrene degradation by at least one of the organisms tested (Table 3). These effects did not appear to result from irreversible reactions of the metabolites or reactive species derived from them, since neither metabolite inhibited the growth of any strain when phenanthrene was present as a sole carbon source in excess of its aqueous solubility.

The ability of PYRdHD and PYRQ to virtually block BaP mineralization by both strains P15 and R1 (Fig. 3) is important, as BaP appears to be particularly recalcitrant in contaminated soil systems (5, 11, 21). Cornelissen et al. (11) have suggested that the recalcitrance of BaP and other high-molecular-weight PAH in contaminated soils is due to unexplained biological factors rather than to limitations in the bioavailability of these compounds. Combined with the possibility that BaP degradation can be much slower than that of other PAH (8), the accumulation of inhibitory metabolites could lead to long-term persistence in PAH-contaminated systems.

The mechanisms of inhibition by pyrene metabolites observed in this study are not apparent. It is possible that PYRdHD could competitively inhibit a dihydrodiol dehydrogenase utilized in the degradation of BaP or phenanthrene by strains P15 and R1, as it clearly is a substrate for these organisms. Alternatively, PYRQ could inhibit BaP mineralization via the formation of PYRQ, which also inhibited BaP mineralization by strains P15 and R1. As discussed above, PYRQ does not appear to react covalently with cellular constituents, although it is possible that only a small concentration of PYRQ is required to react with a critical enzyme or other constituent. However, in this case we would expect inhibition to be independent of PYRQ concentration, since PYRQ would be present in large excess at all concentrations. This was clearly not the case in the mineralization of BaP (Table 4). We also rule out competitive effects of PYRQ as an explanation for the inhibition of BaP oxidation; we would have expected such competition to lead to the accumulation of BaP metabolites, but this was not observed with radiochromatography. A possible explanation for the inhibitory effects of PYRQ is its potential to mediate futile redox reactions that could alter the balance of vital redox cofactors, as has been observed for other PAH o-quinones (32).

**Other considerations.** The potential fates of extracellular metabolites such as PYRdHD and PYRQ in a contaminated soil or sediment system are important to consider. Microbial consortia can lead to more extensive degradation of PAH than can be achieved with pure cultures (6, 42), and in some cases such consortia can degrade metabolites that otherwise would accumulate (6, 29). If pyrene-mineralizing organisms are not present in systems in which pyrene metabolites accumulate to inhibitory levels, it may be possible to enhance overall PAH degradation by inoculating the system with organisms capable of degrading the metabolites.

Abiotic reactions of pyrene metabolites with soil constituents, particularly natural organic matter (NOM), may also be important. Radiolabel originating from $^{14}$Cpyrene has been shown to interact with NOM at long incubation times in soil (22), and quinones are known to undergo oxidation-reduction reactions with NOM (36). Furthermore, the aqueous solubility of PYRQ (0.37 mg/l) is 2 orders of magnitude lower than that of PYRdHD; thus, PYRQ has the potential to precipitate in some systems, which might influence its subsequent degradation. The potential accumulation of o-quinones derived from PAH in contaminated systems also needs to be evaluated from a human risk assessment standpoint, since a variety of such o-quinones have been shown to be cytotoxic and mutagenic and to form DNA adducts in mammalian cells (32). Transient increases in mutagenicity and toxicity have been observed in PAH-contaminated soils undergoing active bioremediation (3, 16), which might be explained by the presence of PAH metabolites such as those described here.

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REFERENCES


ERRATA

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Comparison of Cryptosporidium parvum Viability and Infectivity Assays following Ozone Treatment of Oocysts

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Clancy Environmental Consultants, Inc., St. Albans, Vermont; University of Arizona, Tucson, Arizona; Thames Water Utilities, Reading, and Scottish Parasite Diagnostic Laboratory, Glasgow, United Kingdom; and Technology Planning and Management Corporation, Scituate, Massachusetts

Volume 66, no. 7, p. 2972–2980, 2000. Page 2978, column 2, last line: “(3, 16)” should read “(3, 15).” Page 2979, column 1, line 6: “(16)” should read “(15).”