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A combined chemical and biological approach to transforming and mineralizing PAHs in runoff water

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Abstract

The water quality of lakes, rivers and streams associated with metropolitan areas is declining from increased inputs of urban runoff that contain polycyclic aromatic hydrocarbons (PAHs). Our objective was to transform and mineralize PAHs in runoff using a combined chemical and biological approach. Using ^{14}C-labeled phenanthrene, ^{14}C-benzo(a)pyrene and a mixture of 16 PAHs, we found that ozone transformed all PAHs in a H_2O matrix within minutes but complete mineralization to CO_2 took several weeks. When urban runoff water (7.6 mg C L^{-1}) replaced H_2O as the background matrix, some delays in degradation rates were observed but transforming a mixture of PAHs was still complete within 10 min. Comparing the biodegradability of the ozonated products to the parent structures in unsaturated soil microcosms showed that the 3-ring phenanthrene was more biodegradable (as evidence by $^{14}CO_2$ released) than its ozonated products but for the 5-ring benzo(a)pyrene, the products produced by ozone were much more biodegradable (22% vs. 3% mineralized). For phenanthrene, we identified diphenaldehyde as the initial degradation product produced from ozonation. By continuing to pump the ozonated products (^{14}C-labeled diphenaldehyde or ozone-treated benzo(a)pyrene) onto glass beads coated with microorganisms, we verified that biological mineralization could be achieved in a flow-through system and mineralization rates improved with acclimation of the microbial population (i.e., time and exposure to the substrate). These results support a combined ozone and biological approach to treating PAHs in urban runoff water.

Keywords: PAHS, Ozone, Bioremediation, Phenanthrene, Benzo(a)pyrene

1. Introduction

Industrial processes and human activities create a myriad of chemicals that are often generated and concentrated in urban cities. One class of chemicals associated with densely populated areas is polycyclic aromatic hydrocarbons (PAHs). PAHs occur naturally in oil, coal, and tar deposits and are present in the emissions of automobiles and power plants. PAHs are hydrophobic in nature and tend to adsorb to dust, debris, and sediments. When rain and snow fall on impervious surfaces (e.g., roads, pavements, parking lots, sidewalks), these waters ultimately dissolve, accumulate, and transport both dissolved and precipitated PAHs to storm water lines, drainage channels, and streams. For this reason, the PAH concentrations of many urban lakes and streams have been increasing and reversing that trend represents a significant environmental challenge worldwide (Hoffman et al., 1984; Krein and Schorer, 2000; Ngabe et al., 2000; Van Metre et al., 2000).

Because of the carcinogenic and mutagenic potential of PAHs

(Slaga and DiGiovanni, 1984; Durant et al., 1996), the United States Environmental Protection Agency has listed PAHs as Priority Chemicals (USEPA, 2008). As such, remediating PAH-contaminated water is necessary to prevent exposure. Although various storm water filtration systems have been developed, reducing PAHs from urban runoff is still challenging. One treatment that has been effective in removing organic contaminants from wastewater and drinking water is the use of ozone (Guinvarc'h, 1959; Rice et al., 1981; Stover et al., 1982; Peyton, 1988).

Ozone is a highly reactive oxidant that is particularly well suited for remediation purposes. Twelve times more soluble in water than oxygen (Langlais et al., 1989), ozone can be represented as a hybrid of four resonance structures that present negative and positively charged oxygen atoms (Beltrán, 2003). This electron configuration allows ozone to react directly and indirectly with target contaminants. Direct ozonation occurs though electrophillic substitution while indirect ozonation results from the formation of hydroxyl radicals via catalytic decomposition in water (Masten and Davies, 1997). Ozone can transform organic compounds to oxygenated intermediates that are more soluble in water and oftentimes more biodegradable than the parent compounds (Nam and Kukor, 2000). One disadvantage of using ozone is the high operational cost (electricity), especially if oxidation to CO_2 is desired (i.e., mineralization) (Choi et al., 2001). Bioremediation on the other hand, is less expensive and degradation of low-molecular weight PAHs by bacteria and fungi has been well documented (Cerniglia, 1984; Cerniglia, 1992; Cerniglia, 1997). Its drawbacks however, include long treatment times and the fact that higher molecular weight PAHs (>3-rings) are generally resistant to biodegradation (Cerniglia, 1992; Wilson and Jones, 1993). For this reason, many researchers have attempted to couple chemical treatments with biological treatments as a means of transforming hydrophobic contaminants to more hydrophilic products that can then be biodegraded (Zeng et al., 1999; Nam and Kukor, 2000; Zenaitis et al., 2002; Yerushalmi et al., 2006; Rauscher et al., 2012; Wang et al., 2013).

Our objective was to determine if a combined chemical-biological approach could be used to degrade and mineralize PAHs. This was accomplished by determining the efficacy of ozone to transform phenanthrene and benzo(a)pyrene as well as mixture of 16 PAHs and then quantify the ability of ozone-treated PAHs to be mineralized by microorganisms in unsaturated soil microcosms and in a recirculating bioreactor.

2. Materials and methods

2.1. Chemicals

Chemicals were purchased from a variety of vendors and used as received. These chemicals included: phenanthrene (Acros Organics, Fair Lawn, NJ); diphenaldehyde (TCI, Tokyo, Japan); diphenaldehydric acid (Maybridge, Cornwall, England); diphenic acid (Acros Organics, Fair Lawn, NJ); HPLC-grade methanol and acetonitrile (EMD Chemicals, Gibbstown, NJ); 16-PAH calibration mix (Supelco, Bellefonte, PA); ring-labeled ^{14}C-phenanthrene (52 mCi mmol^{-1}; Moravek Biochemicals and Radiochemicals, Brea, CA) and ^{14}C-benzo(a)pyrene (25 mCi mmol^{-1}; American Radiolabeled Chemicals, St. Louis, MO); 35% (v/v) hydrogen peroxide (Sigma–Aldrich, St. Louis, MO); mercury(II)chloride (Sigma–Aldrich, St. Louis, MO); sodium sulfate (Fisher, Fair Lawn, NJ); 50% (v/v) hydrofluoric acid (Fisher, Fair Lawn, NJ).

Chemicals used in bioremediation experiments included: sodium dihydrogen phosphate dihydrate (Sigma–Aldrich, Milwaukee, WI); potassium dihydrogen phosphate dihydrate (Mallinckrodt, Paris, KY); sodium chloride (Fisher, Fair Lawn, NJ); magnesium sulfate heptahydrate (Fisher, Fair Lawn, NJ); calcium chloride (Fisher, Fair Lawn, NJ); copper(II)sulfate (Fisher, Fair Lawn, NJ); potassium iodide (Sigma–Aldrich, St. Louis, MO); manganese sulfate monohydrate (Mallinckrodt, Paris, KY); zinc sulfate heptahydrate (Mallinckrodt, Paris, KY); boric acid (Mallinckrodt, Paris, KY); iron(III)chloride (Sigma–Aldrich, St. Louis, MO); phosphoric acid (Fisher, Fair Lawn, NJ).

2.2. Chemical analysis

Temporal changes in phenanthrene and its degradation products (i.e., diphenaldehyde, diphenaldehydric acid, and diphenic acid) were quantified by high performance liquid chromatography (HPLC) using a photodiode array detector (Shimadzu Scientific Instruments, Columbia, MD). Phenanthrene and diphenaldehyde were analyzed with an isocratic mobile phase of 80:20 methanol–water and an injection volume of 20 µL. For analysis of diphenaldehydric and diphenic acid, we used an isocratic mobile phase of 50:50 methanol–water, which was adjusted to pH 2.5 with phosphoric acid (Zeinali et al., 2008). Using a flow rate of 1 mL min^{-1}, samples were separated by a guard column that was coupled to a 4.6- by 250-mm Shimadzu Premier C18 column (Kyoto, Japan). Sample peaks were quantified at 254 nm by an external calibration curve. Matrix blanks were analyzed with samples to ensure the absence of background peaks. Sample peaks were also confirmed by comparing UV spectrum scans with the spectrum scans of standards.

When the higher molecular weight PAHs were analyzed (i.e., benzo(a)pyrene and 16-PAH mixture) a second HPLC method was used to account for differences in solubility and detector sensitivity. This analytical method was a modification of EPA Method 610 (USEPA, 2007), which is used for PAH quantification in municipal and industrial wastewater. Using a flow rate of 1 mL min^{-1}, mobile phases of acetonitrile and H_2O were used with the following gradient: 70:30 for 25 min followed by 100:0 for 20 min and then 70:30 for 5 min. Samples (20 µL) were injected into a Hypersil Green PAH column (Thermo Scientific, MA) that was connected to a fluorescence and photodiode array detector. Fluorescence detection used an excitation wavelength of 220 nm and an emission wavelength of 400 nm. Peak areas from the fluorescence detector were compared to standard calibration curves generated for each PAH. The total run time was ~50 min per sample (Rauscher et al., 2012).

^{14}C-activity was determined by removing 1 mL subsamples from the batch reactors and mixing with 5 mL of Ultima Gold liquid scintillation cocktail (Packard, Meriden, CT). Samples were then mixed on a vortex mixer and allowed to settle for 24 h in the dark before analyzing on a Packard 1900TR liquid scintillation counter (LSC; Packard Instrument, Downers Grove, IL). A blank consisting of 5 mL Ultima Gold liquid scintillation cocktail was analyzed prior to running the samples and used to correct sample activity (dpms) values.

2.3. Ozone system

To quantify transformation and mineralization of the PAHs by ozone, we used a custom flow-through ozone system that simultaneously ozonated and trapped emitted gases from three round-bottom glass reactors (Figure 1) (Adam et al., 2006). All ozone batch experiments were run in triplicate. Teflon tubing (0.32 cm id; 0.48 cm od) and ozone-resistant Kynar fittings and adapters were used in construction. To quantify mineralization, the ozone/oxygen gas stream flowed from each experimental unit through FEP-lined flexible tubing (Tygon SE 200, 0.64 cm id; 0.79 cm od) into a series of two 25-mL glass midget bubblers (Ace Glass, Vineland, NJ) containing 15 mL of 0.5 M NaOH (Figure 1). Ozone was generated, measured, and destroyed (residual) via an OzoneLab generator (OL80W-R), analyzer (OLA/DLS), and catalytic destructor (Ozone Services, Burton, British Columbia, Canada). Gas flow was

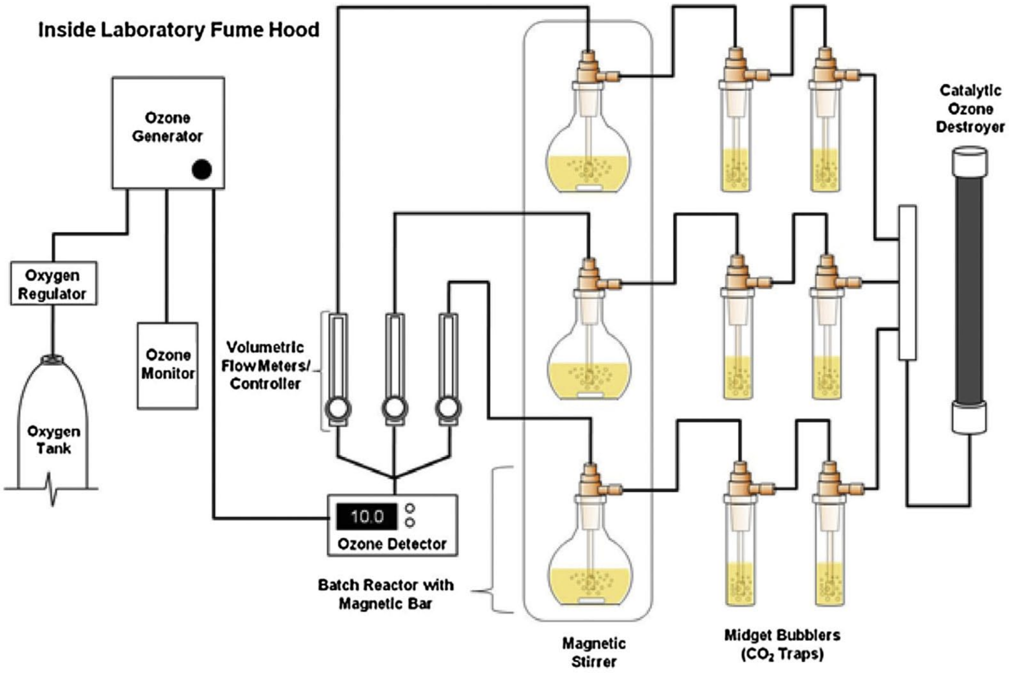

Figure 1. Diagram of ozone system.

forced through the system with pressurized oxygen (Industrial Oxygen, Matheson, Lincoln, NE). A stainless-steel oxygen regulator (Smith Equipment, Watertown, SD), and Teflon volumetric flow meters with sapphire floats (Cole-Parmer, Vernon Hills, IL) were used to control gas flow through the system.

2.4. Ozonation of phenanthrene and related degradation products

Initial batch experiments treated phenanthrene with the advance oxidation process treatment of ozone + H_2O_2. Experimental units were 250-mL round bottom flasks where each flask received 200 mL of 1 mg L^{-1} phenanthrene solution. The flasks were placed on a magnetic stirrer to provide continuous mixing. Initial batch experiments fixed the ozone flux at 1.5 mg min^{-1} and varied concentration of H_2O_2 between 0 to 1×10^{-1} M, while a subsequent batch experiment fixed the concentration of H_2O_2 at 1×10^{-2} M and varied ozone flux between 0.15 and 2.25 mg min^{-1}. The order in which the chemicals were added was phenanthrene, H_2O_2, and ozone gas.

Temporal samples were collected for HPLC analysis from 0 to 5 min after initiating treatment. To prevent phenanthrene from volatilizing and adsorbing to sample vials, the sampling protocol maintained a ratio of 50% aqueous sample to 50% methanol. At each sampling, 0.7 mL of sample was placed into a 1.5-mL HPLC vials that contained 0.7 mL methanol; vials were then immediately mixed and sealed for HPLC analysis.

Long-term ozonation experiments quantified mineralization rates of phenanthrene and benzo(a)pyrene for up to 21 d. These experiments treated 200 mL of ^{14}C-labeled phenanthrene and benzo(a)pyrene (~10,000 dpms mL^{-1} spiked into 1 mg L^{-1} unlabeled parent compounds) with 0.15 mg min^{-1} ozone. Released $^{14}CO_2$ was trapped in the glass midget bubblers (Figure 1). One mL of the trapping solution was collected and analyzed for ^{14}C-activity via LSC every day for up to 21 d and used to quantify cumulative mineralization.

Because diphenaldehyde, diphenaldehydric acid, and diphenic acid had previously been reported to be possible products of ozone-treated phenanthrene (Schmitt et al., 1955; O'Connor et al., 1957; Sturrock et al., 1963; Trapido et al., 1994), we conducted complementary batch experiments using these compounds as starting substrates (1 mg L^{-1}); these compounds were treated with 1.5 mg min^{-1} ozone. Samples were collected periodically for 24 h after applying ozone gas. At each sampling, 1.4 mL of sample was placed into a 1.5-mL HPLC vials containing 0.1 mL methanol and sealed for HPLC analysis.

2.5. Treatment of PAHs in H_2O and urban runoff

To expand upon the experiments with phenanthrene, we determined the efficacy of treating multiple PAHs with ozone. This was accomplished by treating a mixed solution of 16 PAHs spiked in water and runoff water with ozone. Experimental units were 250-mL round bottom flasks where each flask received 200 mL of the mixed 16 PAHs solution (25 μg L^{-1}). The flasks were placed on a magnetic stirrer to provide continuous mixing. Initial batch experiments fixed the ozone flux at 1.5 mg min^{-1}.

Twelve temporal samples were collected within the first 4 min after initiating treatment. To prevent PAHs from volatilizing and adsorbing to sample vials, sample collection protocol maintained a ratio of 50% aqueous sample to 50% acetronitrile.

To compare PAH degradation by ozone in H_2O versus runoff water, we collected urban runoff from an automated monitoring station located in Lincoln, NE (USA). Chemical characteristics of the runoff water were performed by standard methods (see Supplementary Material (SM), Table SM-1). The urban runoff collected consisted primarily of water, with some suspended solids and dissolved organic material. To this matrix, we spiked in the 16 PAH mixture at an initial concentration of ~5.0 μg L^{-1}. Temporal samples were collected during the first 5 min for HPLC analysis after initiating ozone treatment. At each sampling, 0.7 mL of sample was placed into a 1.5-mL centrifuge vial contained 0.7 mL acetronitrile. The samples were centrifuged for 5 min at 14,000 rpm. The clear supernatant was placed in an HPLC vial and sealed for HPLC analysis. The commercially purchased PAHs mixture included: acenaphthene, acenaphthylene, anthracene, benzo(a)anthracene, benzo(a)pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, benzo(k)fluoranthene, chrysene, dibenzo(a,h)anthracene, fluoranthene, fluorene, indenol(1,2,3-cd)pyrene, naphthalene, phenanthrene, and pyrene.

2.6. Bioremediation experiments

2.6.1. Mineralization experiments in soil microcosms

Given that complete mineralization of phenanthrene and benzo(a)pyrene took several weeks to complete, we determined if the products initially transformed by ozonation could be degraded in soil microcosms. Differences in biodegradability between the parent PAH structures and ozonated products were quantified. This was accomplished by treating 200 mL-batches of ^{14}C-phenanthrene and ^{14}C-benzo(a)pyrene (1000 dpms mL^{-1} spiked into 1 mg L^{-1} unlabeled parent compounds) with ozone at 1.5 mg min^{-1} for 30 min. We then confirmed that the parent compounds were completely transformed (via HPLC), and the ^{14}C-activity remained unchanged (via LSC).

Equal activities (dpms) of ozone-treated ^{14}C-labeled phenanthrene and ^{14}C-benzo(a)pyrene were then added to 150 g of air-dried soil. Additional H_2O was added to the microcosm to bring the soil gravimetric water content to 22.8%. Controls included untreated ^{14}C-labeled phenanthrene and ^{14}C-labeled benzo(a)pyrene. Both ^{14}C-labeled phenanthrene and benzo(a)pyrene also contained unlabeled parent compounds (1 mg L^{-1}).

To quantify mineralization, open vials of 0.5 N NaOH (5 mL) were placed inside the sealed microcosm to trap released $^{14}CO_2$. Each week, the $^{14}CO_2$ traps were replaced and sample volumes of 1 mL were removed and analyzed by LSC. Cumulative mineralization was quantified for up to 42 d.

2.7. Biodegradation and mineralization of diphenaldehyde (ozone-treated phenanthrene) in recirculating bioreactor

2.7.1. Enrichment of phenanthrene-degrading microorganisms

The microorganisms used in this study originated from PAH-contaminated soil collected from outside a local automobile repair shop in Lincoln, NE (USA). Phenanthrene-degrading microorganisms were enriched for 90 d using mineral salt solutions containing phenanthrene as the sole carbon and energy source. Specifically, 2 g of contaminated soil was added to 200 mL of mineral salt solution (~pH 6.8). The mineral salt solution was composed of 8.5 g L^{-1} sodium dihydrogen phosphate dehydrate, 3 g L^{-1} potassium dihydrogen phosphate dehydrate, 0.5 g L^{-1} sodium chloride, 0.5 g L^{-1} magnesium sulfate heptahydrate, 14.7 mg L^{-1} calcium chloride, 0.4 mg L^{-1} copper(II)sulfate, 1 mg L^{-1} potassium iodide, 4 mg L^{-1} manganese sulfate monohydrate, 4 mg L^{-1} zinc sulfate heptahydrate, 4 mg L^{-1} boric acid, 2 mg L^{-1} iron(III)chloride (Zhao et al., 2009), and 250 mg L^{-1} phenanthrene. The suspension was agitated on an orbital shaker in the dark at room temperature. Every 7 d, we transferred 10 mL of the suspension into 200 mL of freshly prepared mineral salt solution containing 250 mg L^{-1} phenanthrene (Zhao et al., 2009). This enrichment process was repeated five times. We then continued shaking the solution for up to 7 wk prior to using microorganisms in the column experiments.

2.7.2. Recirculating bioreactor

To determine if the ozonated products could be mineralized in a flow-through system, we constructed a recirculating bioreactor (Figure SM-1) that consisted of: (i) piston pump (QSY-2; FMI lab pump, Syosser, NY), (ii) Viton tubing (Coleparmer, Vernon Hills, IL), (iii) a custom-designed glass column (Ace Glass, Vineland, NJ), (iv) two 50 mm thread size Teflon adapter, (v) 5 mm cap with PTFE-faced septum. The column contained 618.3 g of 0.25% HF acid-etched glass beads coated with microorganisms (Choi et al., 2008). The microorganisms used were obtained from the enrichment work (Section 2.7.1) and from water containing activated sludge from a local waste water treatment plant. This column system had a volume of 243 cm^3 and a pore volume of 149.8 mL. Using a flow rate of 3 mL min^{-1}, the empty bed contact time was approximately 81 min (~17.2 cycles d^{-1}).

We generated ^{14}C-diphenaldehyde and ^{14}C-ozoned-treated benzo(a)pyrene by treating ^{14}C-phenanthrene and ^{14}C-benzo(a) pyrene (~1000 dpms mL^{-1} spiked into 1 mg L^{-1} unlabeled compound) with 1.5 mg min^{-1} ozone for 30 min. A 150 mL of ^{14}C-diphenaldehyde solution or ^{14}C-ozoned-treated benzo(a)pyrene was slowly pumped into the bioreactor and re-circulated for 14 d. Samples were collected every 24 h at the bottom of the column for HPLC and LSC analysis for 336 h (i.e., one cycle). When the first cycle was complete, the solution was drained and the process repeated with freshly prepared ^{14}C-diphenaldehyde or ^{14}C-ozoned-treated benzo(a)pyrene. This process was repeated three times (i.e., 3 cycles).

3. Results and discussion

3.1. Short and long-term ozonation of PAHs and related degradates

The ozonation system (Figure 1) was used to treat PAHs that were spiked into background matrices of H_2O and field-collected runoff water. A test of this ozone system verified that PAH loss from the reactors was from oxidation by ozone and not induced from the bubbling of oxygen into the reactors (i.e., volatilization, Figure SM-2). This ozone system was used to treat ^{14}C-labeled phenanthrene and benzo(a)pyrene for short durations (minutes) to induce transformations and longer times (days) to achieve mineralization (conversion to CO_2).

Experiments that varied ozone and H_2O_2 concentrations showed that short-term ozonation of phenanthrene resulted in near stoichiometric transformation to diphenaldehyde within 2 min (Figure 2). The ozonolysis of phenanthrene and its associated mechanisms have been carefully studied since the 1950s (O'Connor et al., 1957). The mechanism reported by Criegee et al. (1953) is now generally accepted (Kuczkowski, 1983). In brief, phenanthrene has been shown to pass through the molozonide intermediate to the Criegee intermediate, and then to a geminal hydroperoxide by reaction with water. Intramolecular reaction then gives a cyclic dihydroxy peroxide and then, by loss of hydrogen peroxide, diphenaldehyde (Figure SM-3a) (Sturrock et al., 1963). The intermediates formed in the process are difficult or impossible to isolate in water, but aldehydes are known to be resistant to ozonolysis (Story et al., 1971). For this reason, it is not surprising that fast oxidation reactions lead to diphenaldehyde and that this product, which is comparatively slow to react further under the present conditions (Figure SM-4), is easily detected.

In experiments that held the ozone rate constant but increased the concentrations of H_2O_2, the rate of phenanthrene removal from solution did not increase but rather decreased (Figure 2). Therefore, at the ozone rate applied (1.5 mg min^{-1}), H_2O_2 did not improve the transformation rate because it was reducing the available O_3 present in solution to generate hydroxyl radicals (Beltŕan et al., 1996a; Beltŕan et al., 1996b; Trapido et al., 1994; Trapido et al., 1995). Thus, it may be concluded that the phenanthrene destruction proceeds mostly by molecular ozone (direct reactions) (Beltŕan et al., 1996a; Beltŕan et al., 1996b; Trapido et al., 1994; Trapido et al., 1995). However, by holding the H_2O_2 concentration constant (0.01 M) and increasing the ozone rate (0.15–2.25 mg min^{-1}), a steady increase in phenanthrene degradation was observed (Figure 2). These results confirm (along with additional results presented below), that ozone alone was an effective oxidant for transforming PAHs in solution.

Past research has shown that in addition to diphenaldehyde, additional oxidation products of phenanthrene include diphenaldehydric acid and diphenic acid either by biological degradation (Bezalel

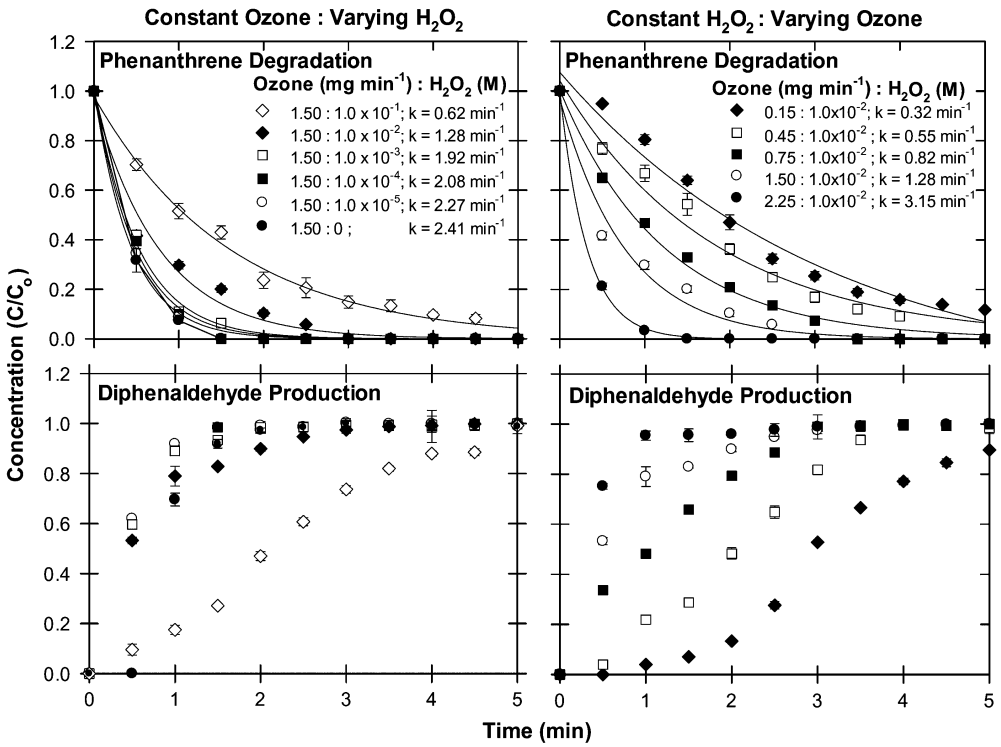

Figure 2. Phenanthrene degradation and diphenaldehyde generation following treatment with varying ozone or H_2O_2 concentrations.

et al., 1996; Seo et al., 2009) or treatment with ozone and $KMnO_4$ (Schmitt et al., 1955). Using ozone (1.5 mg min^{-1}), we observed that these three degradates were more resistant than the parent phenanthrene to ozone but were ultimately transformed within 2–4 d (Figure SM-4). While the transformation of phenanthrene to diphenaldehyde by ozone occurred within minutes, and further transformation of diphenaldehyde within days (Figs. SM-2 and SM-4), complete conversion to CO_2 was a much longer process (Figure SM-5). Removal of ^{14}C-phenanthrene activity from solution took approximately 14 d while benzo(a)pyrene took 21 d (Figure SM-5).

Of the 16 PAHs treated with the ozone system, we were able to identify 10 of the 16 PAHs in the mixture based on the initial concentration of the PAHs (25 µg L^{-1}) and the detection limits of our HPLC (with fluorescence detection). Once ozonation began, phenanthrene, anthracene, and pyrene were immediately transformed while the remaining seven were recalcitrant enough to allow for temporal changes in individual concentrations to be quantified. The seven that could be quantified included: benzo(a)anthracene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)prylene, chrysene, benzo(b)fluoranthrene, and benzo(k)fluoranthrene. Results showed that these 7 PAHs were transformed within 4 min, with benzo(a)anthracene and benzo(a)pyrene being transformed the fastest and benzo(k)fluoranthrene and benzo(b)fluoranthrene being the most resistant. Decreases in PAH concentrations did not follow first-order kinetics as observed with phenanthrene (Figure 2 vs. Figure 3). This deviation from a first-order rate can likely be explained by the fact that the batch test was performed using a mixture of 16 PAHs, not individual compounds, and competition for ozone likely occurring among multiple PAHs and their degradation products. Moreover, at the concentration tested, some PAHs were present as both dissolved and precipitated species, which would be indicative of runoff water.

While biodegradation rates of PAHs can generally be related to number of rings (and solubility), Forsey et al. (2010) pointed out that predicting reaction rates based on ring number is a generalization because reaction rates can be affected by steric interactions, reaction conditions, and the connectivity of the aromatic rings. The Clar (1972) model, which predicts chemical reactivity of PAHs based on the localization of aromatic sextets, indicates that PAH reactivity depends upon three elements. These include: (1) true carbon–carbon double bonds, (2) stabilizing sextets, and (3) shared carbon–carbon double bonds. As a result, the most reactive PAH possess a high number of true double bonds and shared double bonds and a low number of stabilizing sextets. Based on the initial rates in which the PAHs were removed from solution within approximately the first 60 s, we found the order of reactivity was: benzo(a)pyrene ≈ benzo(a)anthracene > dibenzo(a,h)anthracene ≈ benzo(g,h,i) prylene ≈ benzo(b)fluoranthrene ≈ chrysene ≈ benzo(k)fluoranthrene (Figure 3). This order is similar to what the Clar model would predict (Table SM-2) but again, given that a mixture of 16 PAHs was used and that different molar concentrations of each PAH were in solution (based on their solubilities), competition between PAHs and its degradates likely complicated the order in which the PAHs were degraded.

The ability of ozone to transform PAHs spiked into runoff water was investigated. Using starting solution concentrations of phenanthrene (1 mg L^{-1}) and benzo(a)pyrene (0.2 mg L^{-1}), we determined transformation rates of these PAHs by ozone when present in H_2O versus runoff water (Figure 4). Results showed that the transformation rate of phenanthrene was slowed ~15-fold when in a runoff matrix as compared to H_2O (2.5 min^{-1} vs. 0.17 min^{-1}). Likewise, transformation rates of benzo(a)pyrene also decreased (4.4 min^{-1} vs. 0.79 min^{-1}). Given that these two PAHs have different solubilities (phenanthrene = 1.3 mg L^{-1}; benzo(a)pyrene = 0.0038 mg L^{-1}; Mackay and Hoigne, 1977; Cerniglia, 1992; Trapido et al., 1995; Miller and Hawthorne, 1998) and that the benzo(a)pyrene would have been initially present in both dissolved and precipitated phases, it is noteworthy that the higher ring benzo(a)pyrene was transformed faster (Figure 4). This order however, does follow what would be predicted from the Clar structures (Table SM-2).

The effect of background matrix was also investigated using the PAH mixture. Based on the initial concentration of the PAHs

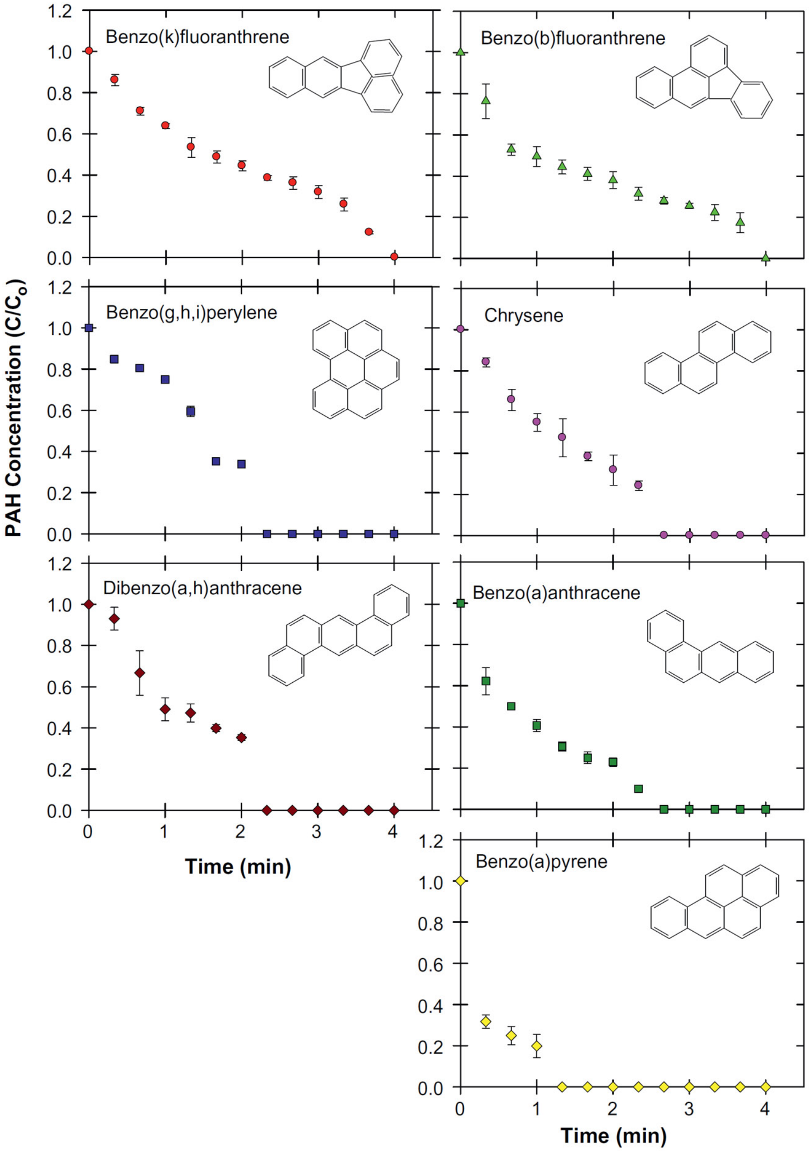

Figure 3. Degradation of individual PAHs following treatment of mixed PAHs in H_2O with ozone.

(0.005 mg L^{-1}) and the detection limit of the fluorescence detector, we were able to quantify 5 of the 16 PAHs in the mixture. These included: anthracene, pyrene, benzo(a)anthracene, benzo(k)fluoranthrene, and benzo(a)pyrene. The results showed that PAHs were removed within 2.5 min in the H_2O matrix and 10 min in a runoff matrix (Figure 5). Both individual tests of single PAHs (Figure 4) and combined treatment of mixed PAHs (Figure 5) confirm that the natural oxidant demand of the runoff water (7.6 mg C L^{-1}; Table SM-1) will slow transformation kinetics. While the oxidant demand of the runoff water will ultimately dictate treatment times, it appears that 10 min of ozonation should be sufficient to transform most PAHs.

3.2. *Biodegradation in soil microcosms*

If PAH-containing runoff water was ozonated and released back to the environment, the fate of ozone-transformed products would surely be questioned. To partially address this, we compared the biodegradability of the parent compounds to the O_3-treated products by measuring cumulative $^{14}CO_2$ emitted in soil microcosms. Results showed that the parent phenanthrene structure, which has a lower oxidation state, was easier to degrade in the soil microcosm than the O_3-treated products (e.g., diphenaldehyde. Figure 6A). Given that PAHs consisting of 3-rings or less are considered biodegradable (Tabak et al., 1981; Cerniglia, 1992; Wilson and Jones; 1993; O'Mahony et al., 2006), the difference in biodegradability of phenanthrene versus the ozone-transformed products can at least partially be explained by the higher oxidation states of the transformed products and resistance of the diphenaldehyde to further degradation. By contrast, benzo(a)pyrene was not biodegradable in the soil microcosm but its ozone-transformed products were (Figure 6B). This stark difference is likely due to very low solubility and high adsorption properties of the parent benzo(a)pyrene (0.0038 mg L^{-1}; Mackay and Hoigne, 1977; Cerniglia, 1992; Trapido et al., 1995; Miller and Hawthorne, 1998; log Koc = 6.7 L kg^{-1}; McCarthy and Jimenez, 1985; Torben et al., 1997) versus the more polar functional groups found on the degradation products. While individual degradation products were not identified in this study, past research has shown that ozonation of benzo(a)pyrene could include quinones, aromatic diols, and short chain alkanes (Moriconi et al., 1961; Huang et al., 2002; Brown et al., 2003; Koeber et al., 2006). In a detailed study by Zeng et al. (2000), they found that eight different

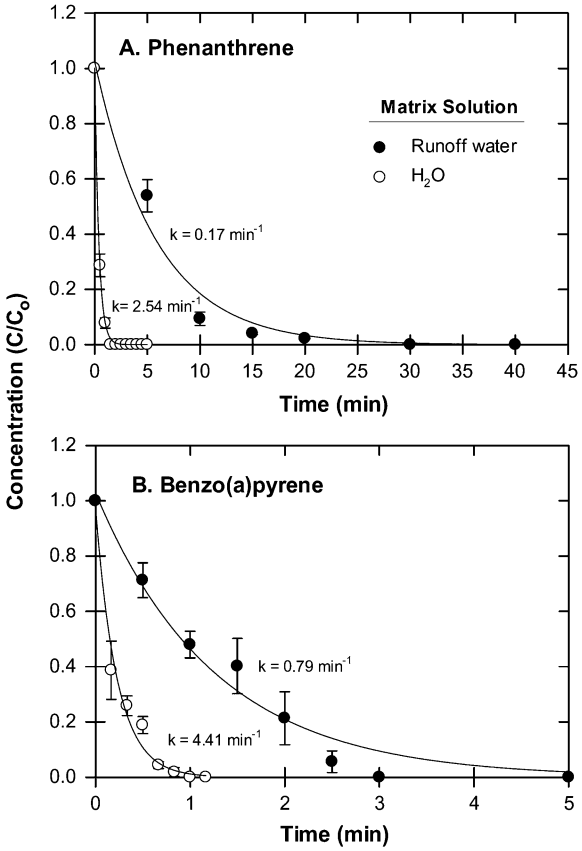

Figure 4. Comparison of phenanthrene and benzo(a)pyrene degradation in H_2O and runoff water matrices following ozonation.

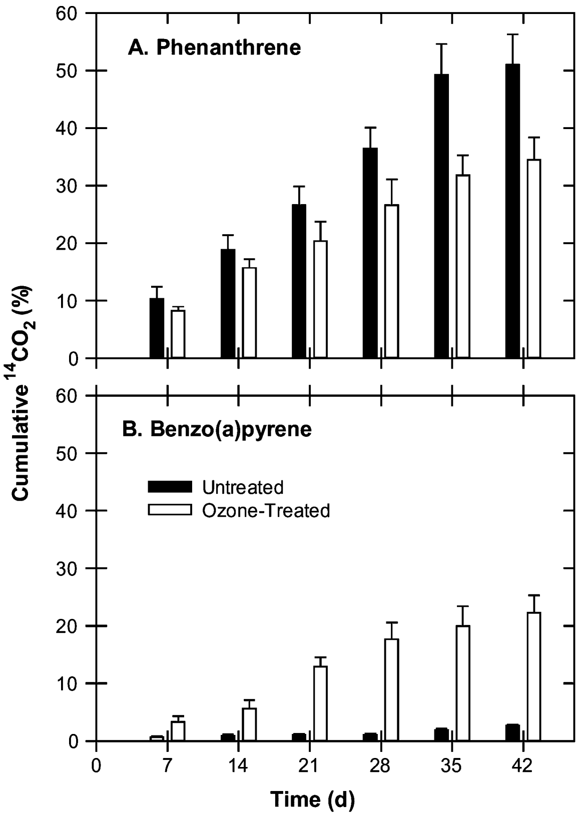

Figure 6. Cumulative $^{14}CO_2$ released from soil microcosms spiked with untreated and ozone-treated (A) ^{14}C-phenanthrene and (B) ^{14}C-benzo(a)pyrene.

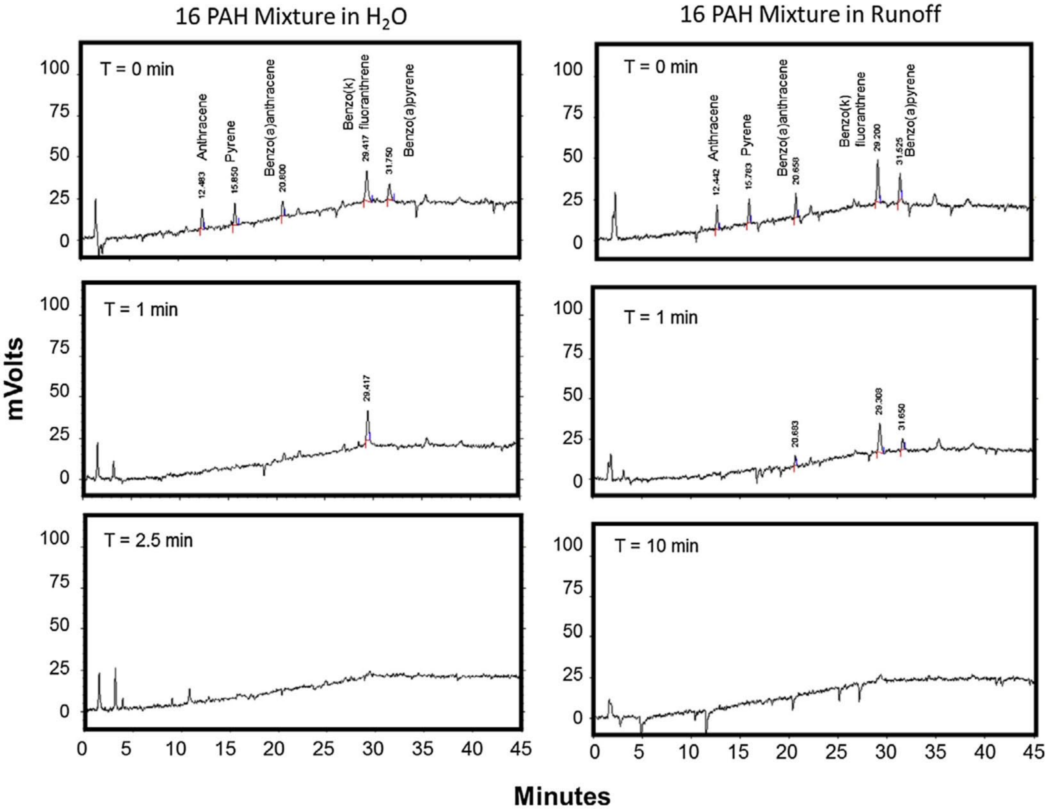

Figure 5. Comparison of mixed PAH degradation in H_2O and runoff water matrices following ozonation.

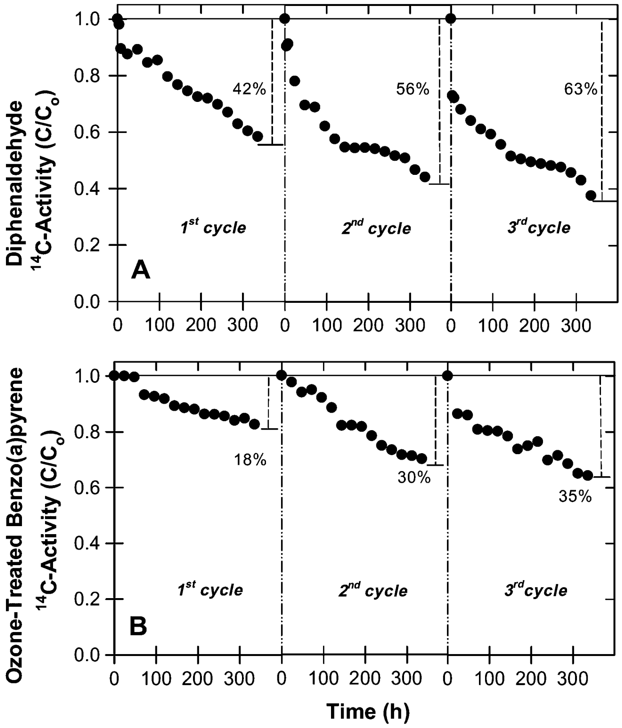

Figure 7. Mineralization of (A) ^{14}C-Diphenaldehyde and (B) ^{14}C-ozone-treated benzo(a)pyrene following three cycles of pumping fresh solutions through the column system.

compounds can form by three different pathways (Figure SM-3b) and that these eight compounds are then further degraded into other compounds in a sequence with at least five stages and with some sequences involving more than ten steps. Many of these products would be more soluble and thus more available for natural biotic mineralization than the original parent structure, which was evident from our mineralization results (Figure 6B). Nam and Kukor (2000); Rauscher et al. (2012) also found that oxidative treatment of PAHs resulted in more biological products.

3.3. Biodegradation and mineralization of diphenaldehyde and O_3-treated benzo(a)pyrene in recirculating bioreactor

Given that diphenaldehyde was the initial ozonation product of phenanthrene, we further determined the ability of a microbial community to degrade this compound in a recirculating bioreactor (Figure 7). By adding the ozonation product, ^{14}C-diphenyldehyde, into the recirculating bioreactor packed with biofilm-coated glass beads, we found that the transformation rate increased as the experiment was repeated (i.e., 2nd and 3rd cycles), likely due to the enrichment of PAH-degrading in the reactor (Figure SM-6). Tracking the ^{14}C-carbon backbone of both diphenylaldehyde and the ozone treated benzo(a)pyrene, the percentage of ^{14}C-diphenaldehyde mineralized increased from 42% to 63% and ozone-treated benzo(a)pyrene increased from 18% to 35% with repeated cycling (Figure 7). Given that adsorption tests confirmed that neither diphenaldehyde or the ozone-treated benzo(a)pyrene products were adsorbed by the glass beads or glass beads coated with microorganisms (Figure SM-7), the losses in ^{14}C recovered confirm that biological mineralization could be achieved in a flow-through system and mineralization rates improved with repeated cycles. Collectively, the results reported in this study, combined with previous chemical-biological approaches (Zeng et al., 1999; Zenaitis et al., 2002; Yerushalmi et al., 2006; Rauscher et al., 2012), support combining ozone with biological treatments for treating PAH-contaminated water.

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Appendix A. Supplementary material follows the References.

References

Adam, M., Comfort, S., Snow, D., Cassada, D., Morley, M., Clayton, W., 2006. Evaluating ozone as a remedial treatment for removing RDX from unsaturated soils. J. Environ. Eng. 132, 1580–1588.

Beltrán, F.J., Gonzàlez, M., Rivas, F.J., 1996a. Advanced oxidation of polynuclear aromatic hydrocarbons in natural waters. J. Environ. Sci. Health. 31, 2193–2210.

Beltrán, F.J., Ovejero, G., Rivas, F.J., 1996b. Oxidation of polynuclear aromatic hydrocarbons in water. 4. Ozone combined with hydrogen peroxide. Ind. Eng. Chem. Res. 35, 891–898.

Beltrán, F.J., 2003. Ozone-UV radiation-hydrogen peroxide oxidation technologies. In: Tarr, M.A. (Ed.), Chemical Degradation Methods for Wastes and Pollutants. Marcel Dekker, New York, pp. 1–76.

Bezalel, L., Hadar, Y., Fu, P.P., Freeman, J.P., Cerniglia, C.E., 1996. Metabolism of phenanthrene by the white rot fungus Pleurotus ostreatus. Appl. Environ. Microbiol. 62, 2457–2553.

Brown, G.S., Barton, L.L., Thomson, B.M., 2003. Permanganate oxidation of sorbed polycyclic aromatic hydrocarbons. Waste Manage. (Oxford) 23, 737–740.

Cerniglia, C.E., 1984. Microbial metabolism of polycyclic aromatic hydrocarbons. Adv. Appl. Microbiol. 30, 31–71.

Cerniglia, C.E., 1992. Biodegradation of polycyclic aromatic hydrocarbons. Biodegradation 3, 351–368.

Cerniglia, C.E., 1997. Fungal metabolism of polycyclic aromatic hydrocarbons: past, present, and future applications in bioremediation. J. Ind. Microbiol. Biotechnol. 19, 324–333.

Choi, H., Kim, Y.-Y., Lim, H., Cho, J., Kang, V., Kim, K.-S., 2001. Oxidation of polycyclic aromatic hydrocarbons by ozone in the presence of sand. Water Sci. Technol. 43 (5), 349–356.

Choi, Y.C., Li, X., Raskin, L., Morgenroth, E., 2008. Chemisorption of oxygen onto activated carbon can enhance the stability of biological perchlorate reduction in fixed bed biofilm reactors. Water Res. 42, 3425–3434.

Clar, E., 1972. The Aromatic Sextets. Wiley, London.

Criegee, R., Blust, G., Lohaus, G., 1953. Ozonization cleavage. III. 1. Ozonides from cyclopentene, cyciohexene, and their 1-methyl derivatives. Justus Liebigs Ann. Chem. 583, 2–6 (in German).

Durant, J.L., Busby, W.F.R., Lafleur, A.L., Penman, B.W., Crespi, C.L., 1996. Human cell mutagenicity of oxygenated, nitrated and unsubstituted aromatic hydrocarbons associated with urban aerosols. Mutation Res. 371, 123–127.

Forsey, S.P., Thomson, N.R., Barker, J.F., 2010. Oxidation kinetics of polycyclic aromatic hydrocarbons by permanganate. Chemosphere 79, 628–636.

Guinvarc'h, P., 1959. Three years of ozone sterilization of water in Paris. Ozone Chem. Technol. 21, 416–429.

Hoffman, E.J., Mills, G.L., Latimer, J.S., Latimer, J.G., 1984. Urban runoff as a source of polycyclic aromatic hydrocarbons to coastal waters. Environ. Sci. Technol. 18, 580–587.

Huang, K.C., Couttenye, R.A., Hoag, G.E., 2002. Kinetics of heat-assisted persulfate oxidation of methyl tert-butyl ether (MTBE). Chemosphere 49, 413–420.

Koeber, R., Bayona, J.M., Niessner, R., 2006. Analysis of ozonolysis products of benzo(a)pyrene with capillary gas chromatography mass spectrometry and liquid chromatography mass spectrometry. Int. J. Environ. Anal. Chem. 66, 313– 325.

Krein, A., Schorer, M., 2000. Road runoff pollution by polycyclic aromatic hydrocarbons and its contribution to river sediments. Water Res. 34, 4110– 4115.

Kuczkowski, R.L., 1983. Formation and structure of ozonides. Acc. Chem. Res. 16, 42–47.

Langlais, B., Cucurou, B., Aurelle, Y., Capdeville, B., Roques, H., 1989. Improvement of a biological treatment by prior ozonation. Ozone Sci. Eng. 11, 155–168.

Mackay, D., Hoigne, J., 1977. Aqueous solubility of polynuclear aromatic hydrocarbons. J. Chem. Eng. Data 22, 399–402.

Masten, S., Davies, H.R., 1997. Efficacy of in-situ ozonation for the remediation of PAH contaminated soils. J. Contam. Hydrol. 28, 327–335.

McCarthy, J.F., Jimenez, B.D., 1985. Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: binding and dissociation. Environ. Sci. Technol. 19, 1072–1076.

Miller, D.J., Hawthorne, S.B., 1998. Method for determining the solubilities of hydrophobic organics in subcritical water. Anal. Chem. 70, 1618–1621.

Moriconi, E.J., Rakoczy, B., O'Connor, W.F., 1961. Ozonolysis of polycyclic aromatics. VIII. Benzo(a)pyrene. J. Am. Chem. Soc. 83, 4618–4623.

Nam, K., Kukor, J.J., 2000. Combined ozonation and biodegradation for remediation of mixtures of polycyclic aromatic hydrocarbons in soil. Biodegradation 11, 1–9.

Ngabe, B., Bidleman, T.F., Scott, G.I., 2000. Polycyclic aromatic hydrocarbons in storm runoff from urban and coastal South Carolina. Sci. Total Environ. 255, 1– 9.

O'Connor, W.F., Schmitt, W.J., Moricomi, S.J., Moriconi, E.J., 1957. Ozone oxidation of phenanthrene to diphenic acid. Ind. Eng. Chem. 49, 1701–1702.

O'Mahony, M.M., Dobson, A., Barnes, J.D., Singleton, I., 2006. The use of ozone in the remediation of polycyclic aromatic hydrocarbon contaminated soil. Chemosphere 63, 307–314.

Peyton, G.R., 1988. Destruction of pollutants in water with ozone in combination with ultraviolet radiation. 3. Photolysis of aqueous ozone. Environ. Sci. Technol. 22, 761–767.

Rauscher, L., Sakulthaew, C., Comfort, S., 2012. Using slow-release permanganate candles to remediate PAH-contaminated water. J. Hazard. Mater. 241–242, 441–449.

Rice, R.G., Robson, C.M., Miller, G.W., Hill, A.G., 1981. Uses of ozone in drinking water treatment. J. Am. Water Works Assoc. 73 (1), 44–57.

Schmitt, W.J., Moriconi, E.J., O'Connor, W.F., 1955. The ozonolysis of phenanthrene. J. Am. Chem. Soc. 77, 5640–5642.

Seo, J.S., Keum, Y.S., Li, Q.X., 2009. Review Bacterial degradation of aromatic compounds. Int. J. Environ. Res. Public Health 6, 278–309.

Slaga, T.J., DiGiovanni, J., 1984. Inhibition of carcinogenesis. In: Searle, C.E. (Ed.), Chemical Carcinogens, second ed. ACS Monograph, Washington, DC, p. 182.

Story, P.R., Alford, J.A., Burgess, J.R., Ray, W.C., 1971. Mechanisms of ozonolysis. Reductive ozonolysis with aldehydes and ketones. J. Am. Chem. Soc. 93, 3042– 3044.

Stover, E.L., Wang, L.W., Medley, D.R., 1982. Ozone assisted biological treatment of industrial wastewaters containing biorefractory compounds. Ozone Sci. Eng. 4, 177–194.

Sturrock, M.G., Cline, E.L., Robinson, K.R., 1963. The ozonation of phenanthrene with water as participating solvent. J. Org. Chem. 28, 2340–2343.

Tabak, H.H., Quave, S.A., Mashni, C.I., Barth, E.E., 1981. Biodegradability studies with organic priority pollutant compounds. J. Water Pollut. Control Fed. 53, 1503– 1518.

Torben, N., Siigur, K., Helweg, C., Jorgensen, O., Hansen, P.E., Kirso, U., 1997. Sorption of polycyclic aromatic compounds to humic acid as studied by high performance liquid chromatography. Environ. Sci. Technol. 31, 1102–1108.

Trapido, M., Veressinina, J., Munter, R., 1994. Ozonation and AOP treatment of phenanthrene in aqueous solutions. Ozone Sci. Eng. 16, 475–485.

Trapido, M., Veressinina, J., Munter, R., 1995. Ozonation and advanced oxidation processes of polycyclic aromatic hydrocarbons in aqueous solutions – a kinetic study. Environ. Sci. Technol. 16, 729–740.

USEPA, 2007. Methods for Organic Chemical Analysis of Municipal and Industrial, Method 610-Polynuclear Aromatic hydro carbons, <http://water.epa.gov/ scitech/methods/cwa/organics/upload/ 2007_07_10_methodsmethod_organics_610.pdf>.

USEPA, 2008. Office of Solid Waste. Polycyclic Aromatic Hydrocarbons (PAHs). U.S. Environmental Protection Agency. <http://www.epa.gov/osw/hazard/ wastemin/minimize/factshts/pahs.pdf>.

Van Metre, P.C., Mahler, B.J., Furlong, E.T., 2000. Urban sprawl leaves its PAH signature. Environ. Sci. Technol. 34, 4064–4070.

Wang, J., Zhang, X., Li, G., 2013. Compositional changes of hydrocarbons of residual oil in contaminated soil during ozonation. Ozone Sci. Eng. 35, 366–374.

Wilson, S.C., Jones, K.C., 1993. Bioremediation of soil contaminated with polynuclear aromatic hydrocarbons (PAHs): a review. Environ. Pollut. 81, 229–249.

Yerushalmi, L., Nefil, S., Hausler, R., Guiot, S.R., 2006. Removal of pyrene and benzo(a)pyrene from contaminated water by sequential and simultaneous ozonation and biotreatment. Water Environ. Res. 78, 2286–2292.

Zeinali, M., Vossoughi, M., Ardestani, S.K., 2008. Degradation of phenanthrene and anthracene by Nocardia otitidiscaviarum strain TSH1, a moderately thermophilic bacterium. J. Appl. Microbiol. 105, 398–406.

Zenaitis, M., Sandhu, H., Duff, S.J.B., 2002. Combined biological and ozone treatment of log yard run-off. Water Res. 36, 2053–2061.

Zeng, Y., Hong, P.K.A., Wavrek, D.A., 1999. Chemical-biological treatment of pyrene. Water Res. 34, 1157–1172.

Zeng, Y., Hong, P.K.A., Wavrek, D.A., 2000. Integrated Chemical–biological treatment of benzo(a)pyrene. Environ. Sci. Technol. 34, 854–862.

Zhao, H.P., Wu, Q.S., Wang, L., Zhao, X.T., Gao, H.W., 2009. Degradation of phenanthrene by bacterial strain isolated from soil in oil refinery fields in Shanghai China. J. Hazard. Mater. 164, 863–869.

A combined chemical and biological approach to transforming and mineralizing PAHs in runoff water

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Supplementary Material

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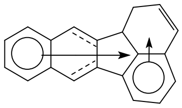
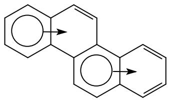
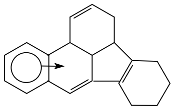
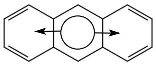
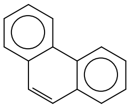
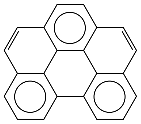
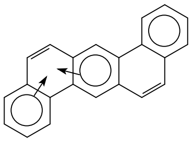
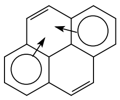
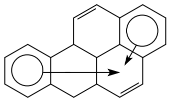
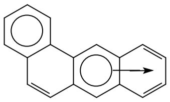
Table SM-1

Chemical characteristics of runoff water and analytical methods

Chemical parameter	**Units**	**Value**	**Method**	**Reference**
Ammonia(NH_3)	mg NH_3-N L^{-1}	0.22	EPA 350.1	USEPA, 2011
Nitrate plus nitrite	mg (NO_3^-+NO_2^--N) L^{-1}	0.84	EPA 353.2	USEPA, 2011
Total Nitrogen	mg N L^{-1}	2.74	4500-N_{org}	APHA, 1998
Total organic carbon (TOC)	mg C L^{-1}	7.62	5310	APHA, 1998
Conductivity	μS cm^{-1}	221	2510	APHA, 1998
pH	pH units	7.59	4500-H^+	APHA, 1998
Total suspended solids (TSS)	mg L^{-1}	22.70	2540D	APHA, 1998
Turbidity	NTU	33.58	2130	APHA, 1998
Soluble reactivity phosphorous	mg PO_4^{3-}P L^{-1}	0.53	Hach10209	Hach, 2008
Total phosphorous (TP)	mg PO_4^{3-}-P L^{-1}	1.26	Hach10210	Hach, 2008
Chemical oxygen demand (COD)	mg COD L^{-1}	35.00	Hach8000	Hach, 2008
Chloride (Cl^-)	mg Cl^- L^{-1}	14.50	Hach8113	Hach, 2008

Table SM-2

Clar structures showing number of true double bonds, stabilizing aromatic sextets, and shared double bonds for representative PAHs (PAHs listed in the table are arranged in increasing order of chemical reactivity according to the Clar model).

Order	PAH	Clar structure	True double bonds	Stabilizing sextets	Shared double bonds	Water solubility (μg L^{-1})
1	Benzo(k)fluoranthrene		0	2	1	0.7
2	Chrysene		0	2	3	2.0
3	Benzo(b)fluoranthrene		0	1	3	1.2
4	Anthracene		0	1	4	73
5	Phenanthrene		1	0	0	1300
6	Benzo(g,h,i)perylene		2	3	0	0.2
7	Dibenzo(a,h)anthracene		1	3	1	0.5
8	Pyrene		1	2	1	135
9	Benzo(a)pyrene		1	2	1	3.8
10	Benzo(a)anthracene		1	2	2	2.0

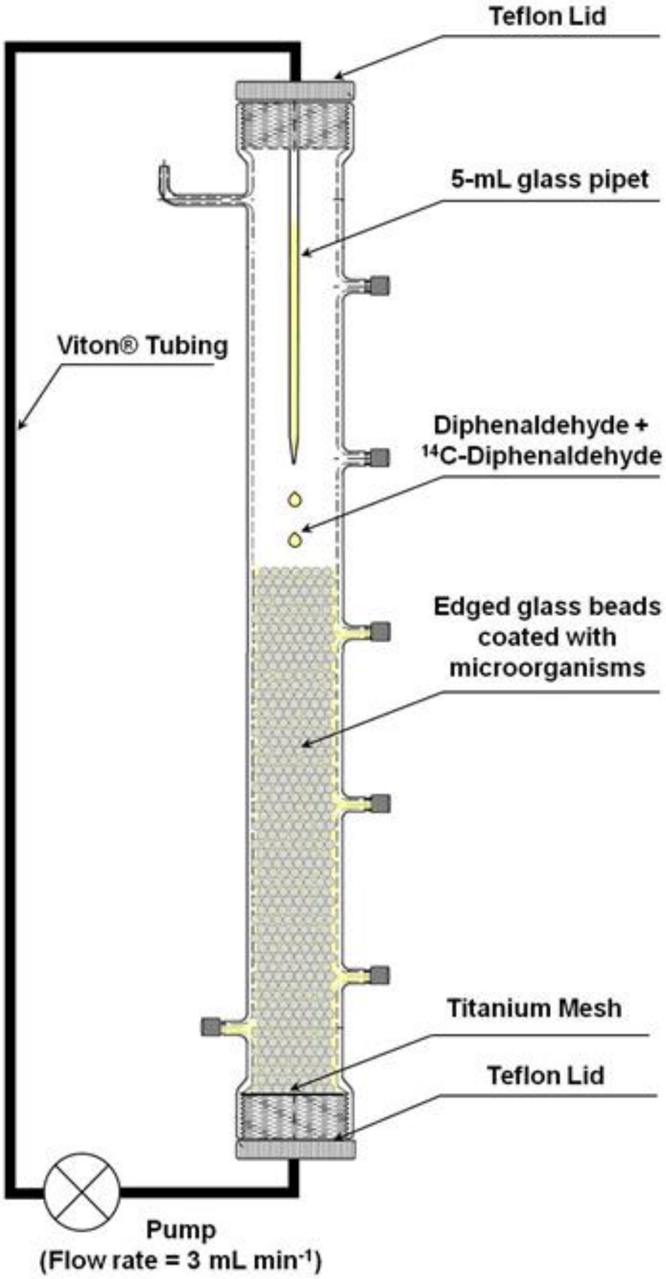


Figure SM-1. Diagram of the recirculating bioreactor system.

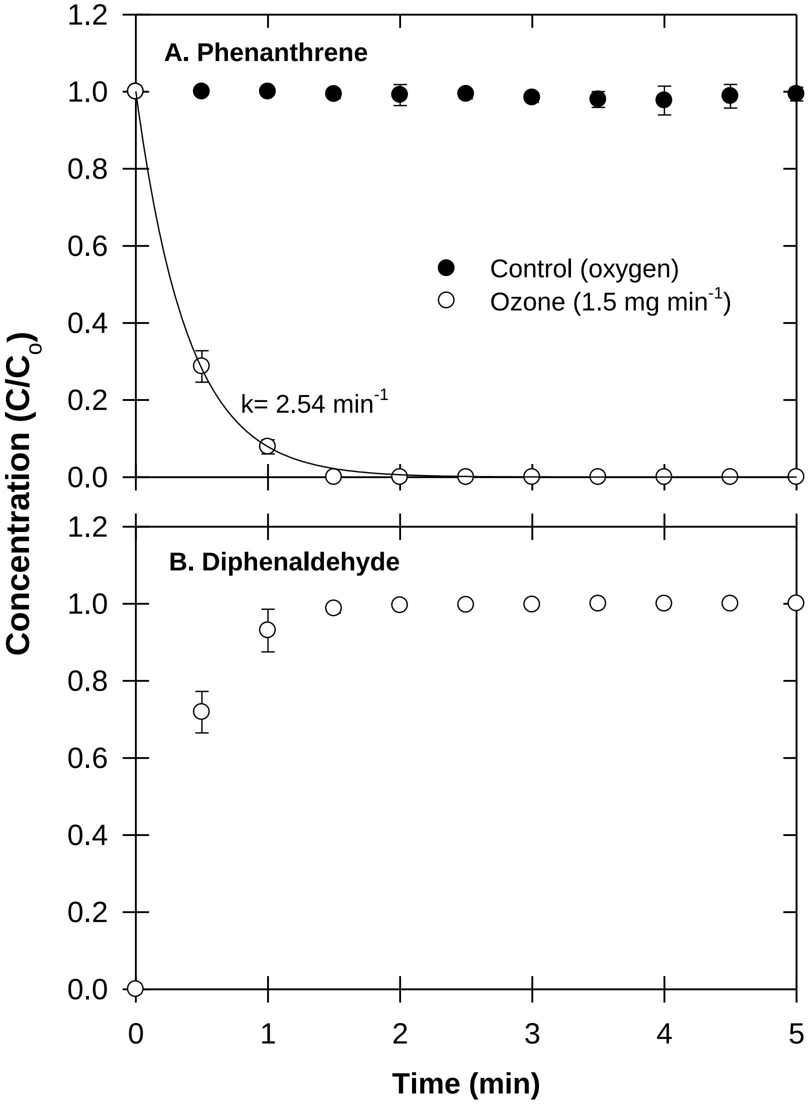
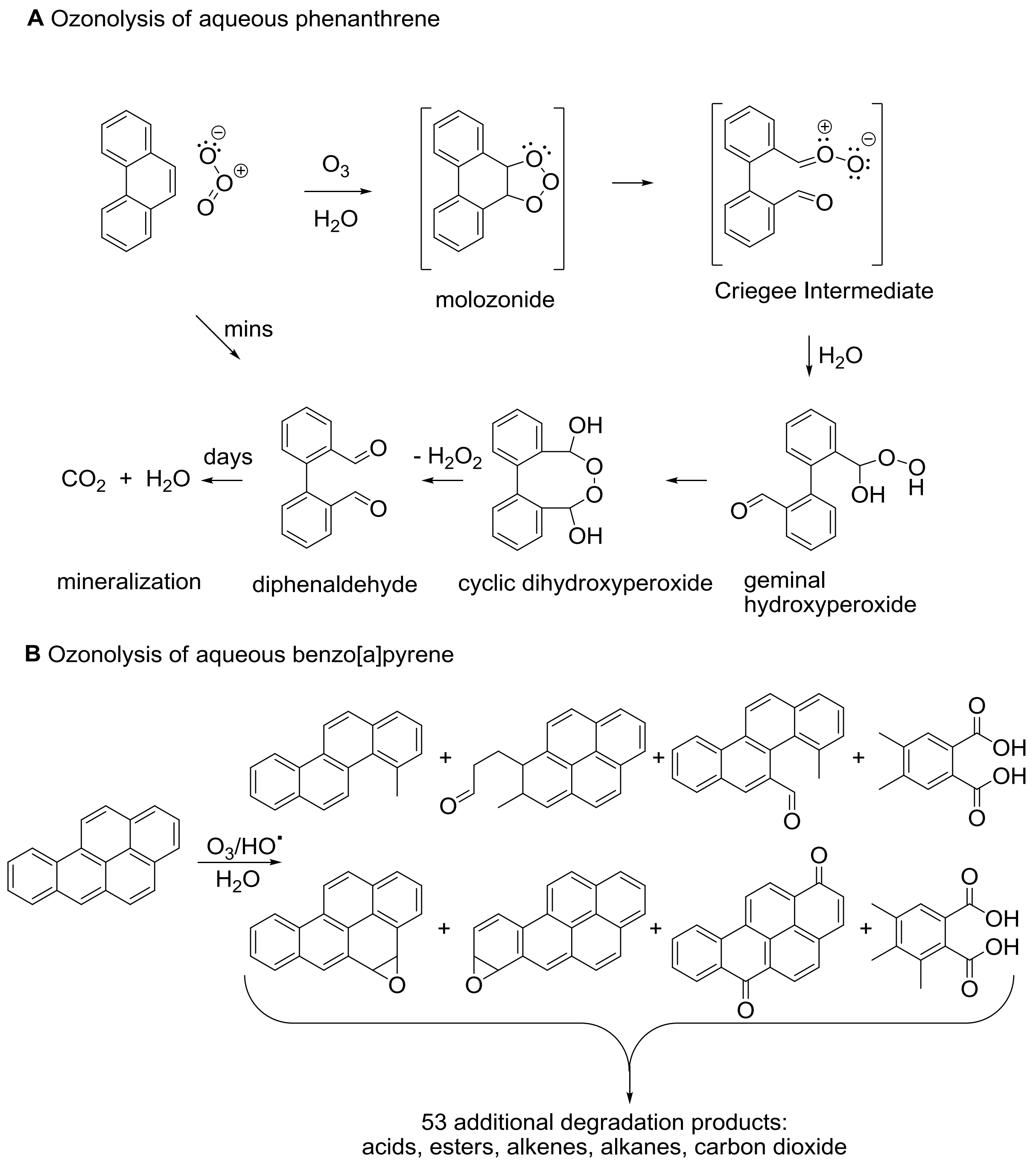


Figure SM-2. Phenanthrene degradation and diphenaldehyde generation following treatment with 1.50 mg min^{-1} ozone or oxygen gas (control) in water.


A Ozonolysis of aqueous phenanthrene

B Ozonolysis of aqueous benzo[a]pyrene

Figure SM-3. Phenanthrene and benzo(a)pyrene degradation schemes via ozonation.

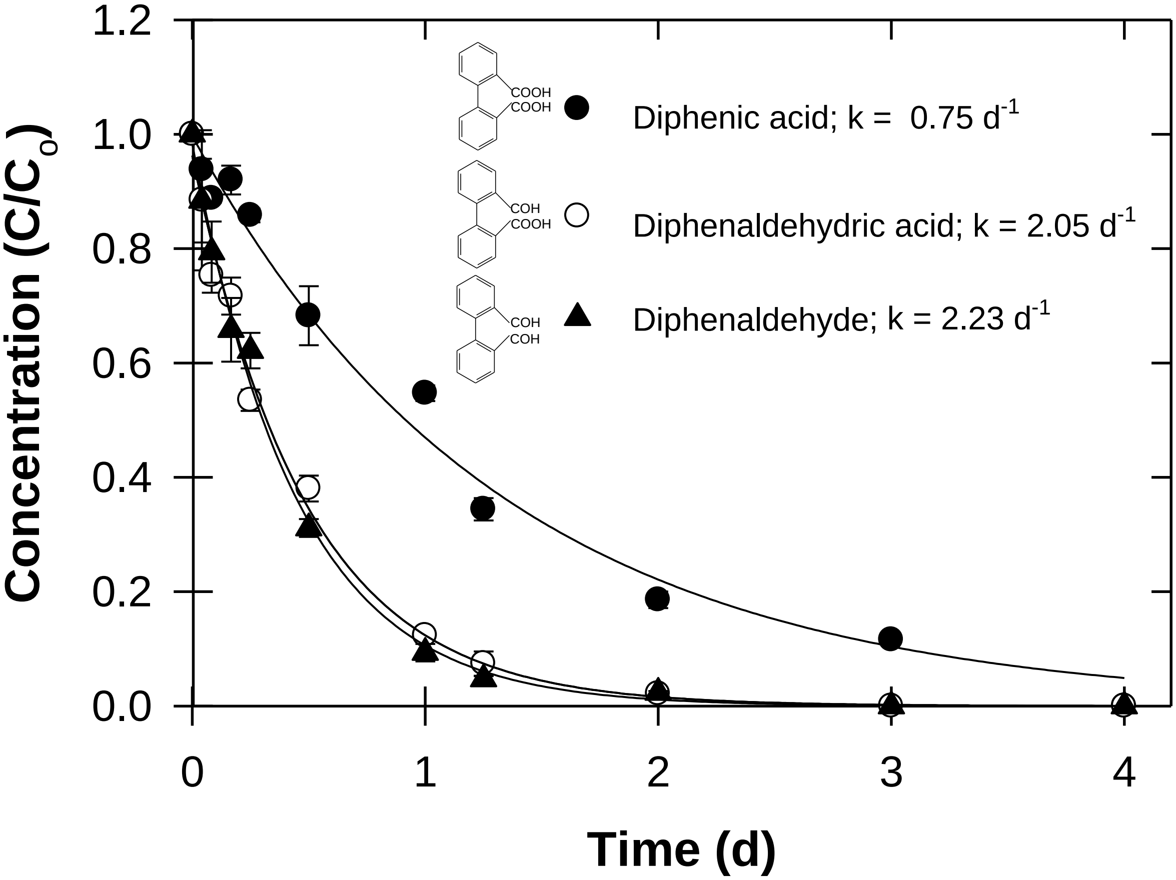


Figure SM-4. Degradation of phenanthrene degradation products following treatment with 1.50 mg min^{-1} ozone.

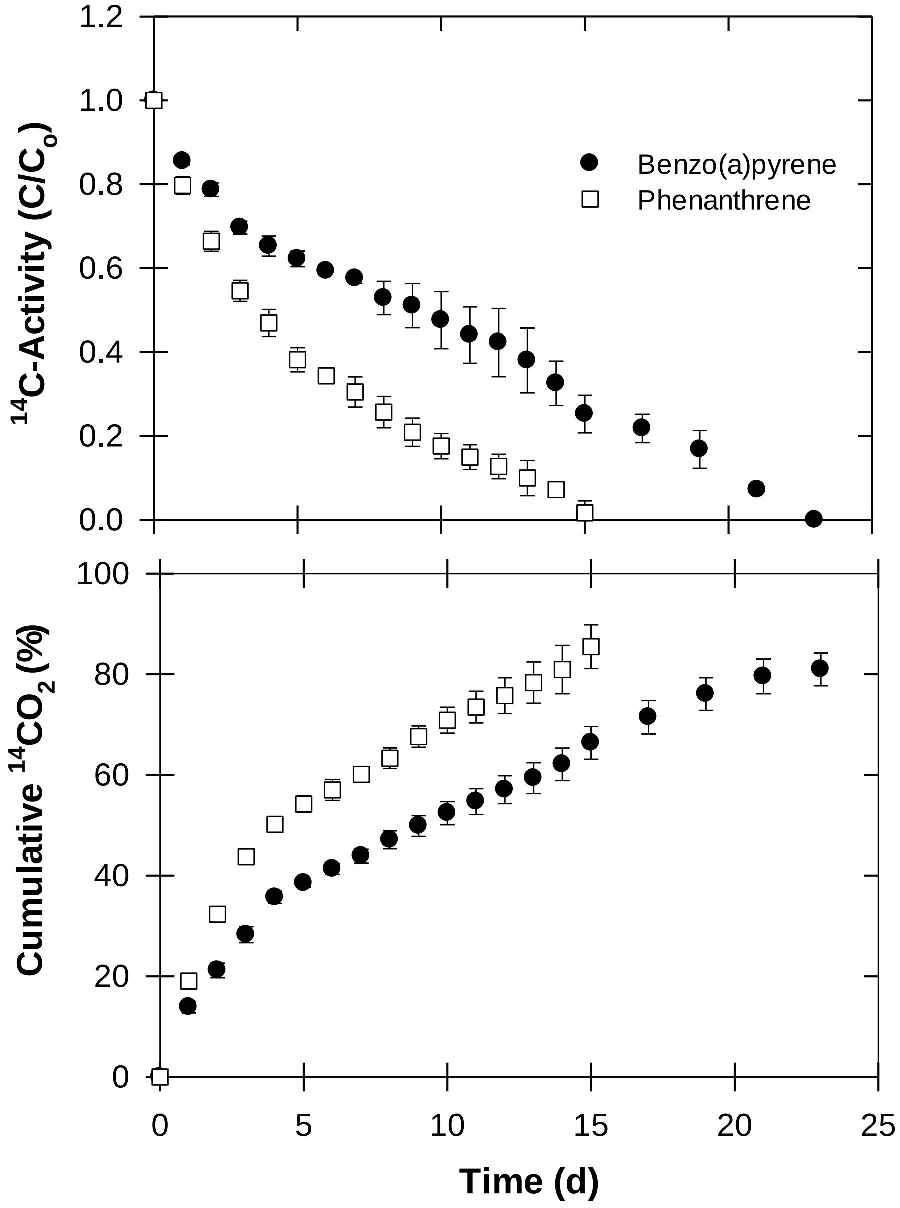


Figure SM-5. ^{14}C-phenanthrene and ^{14}C-benzo(a)pyrene degradation and cumulative^{14}CO$_2$ generation following ozonation.

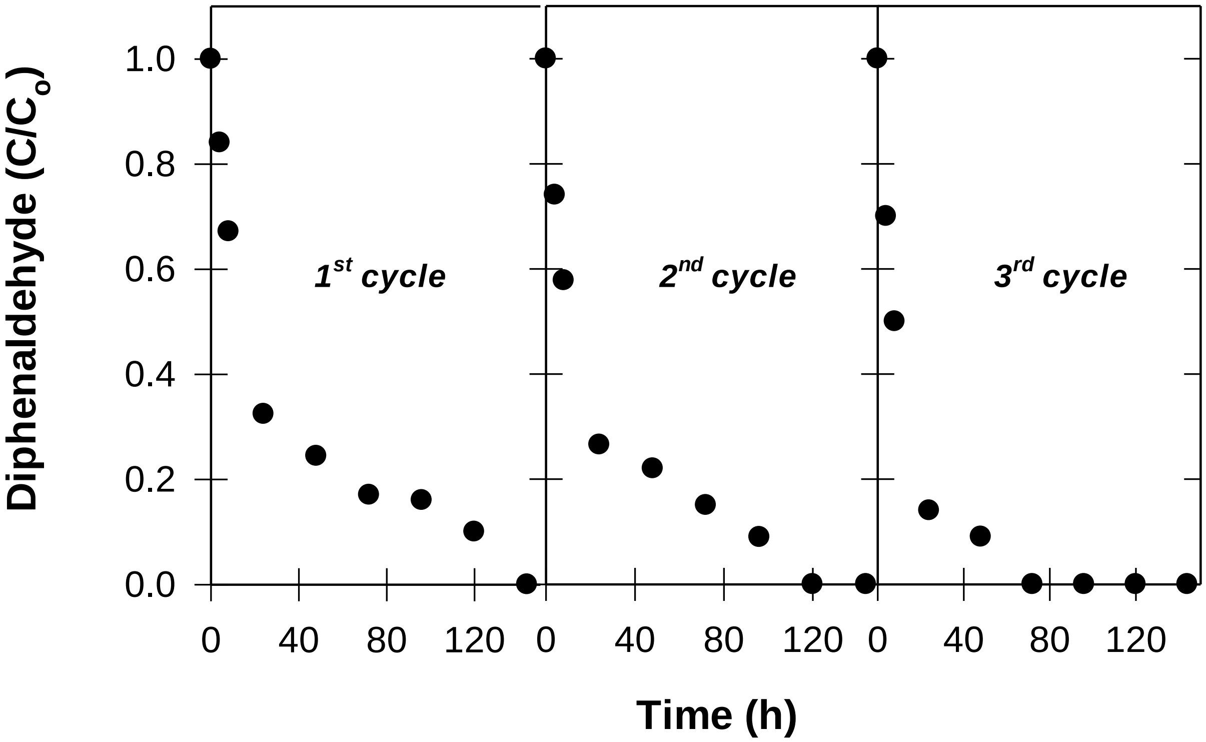


Figure SM-6. Transformation of diphenaldehyde following repeated cycling through recirculating bioreactor.

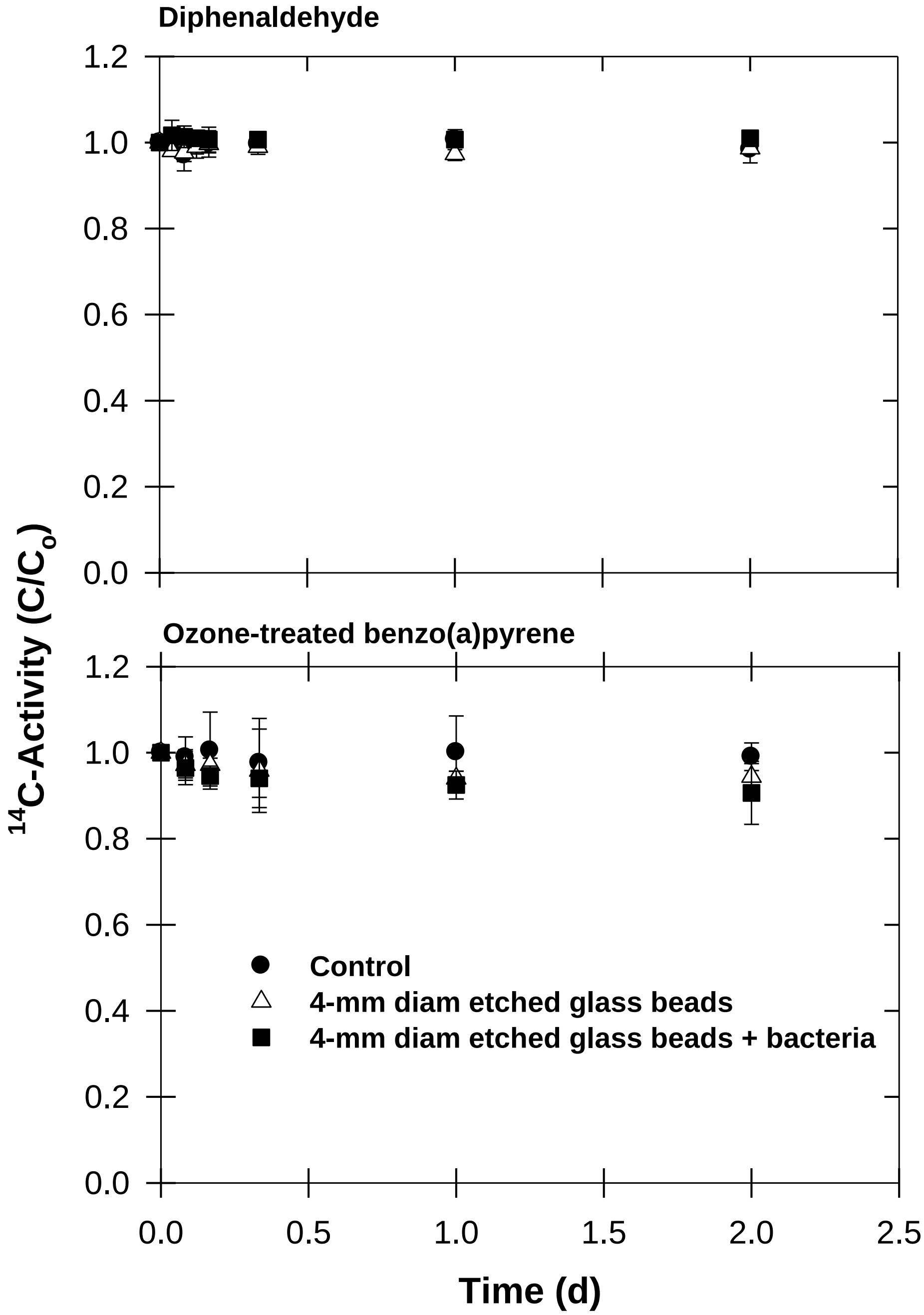


Figure SM-7. Adsorption of ozone-treated phenanthrene and benzo(a)pyrene to etched glass beads and etched glass beads coated with bacteria.

REFERENCES

APHA, AWWA, and WEF. 1998. Standard Methods for the Examination of Water and Wastewater (20th ed). American Public Health Association, American Water Works Association, Water Environment Federation, Washington, D.C.

Hach, D.R., 2800. Spectrophotometer: Procedures Manual, Hach Company,Germany.

USEPA, 2011. Approved General-Purpose Methods. U.S. Environmental Protection Agency. http://water.epa.gov/scitech/methods/cwa/methods_index.cfm.