Biodegradation **11:** 1–9, 2000.

Combined ozonation and biodegradation for remediation of mixtures of polycyclic aromatic hydrocarbons in soil

Kyoungphile Nam* & Jerome J. Kukor**
*Biotechnology Center for Agriculture and the Environment, Cook College Campus, Rutgers University, 59 Dudley Road, New Brunswick, NJ 08901-8520, USA (** author for correspondence)*

Accepted 29 March 2000

Key words: Biodegradation, ozonation, polycyclic aromatic hydrocarbons

Abstract

A study was conducted to investigate the feasibility of a combined treatment (i.e., ozonation and biodegradation) to overcome the inherent bacterial bioavailability limitation, and hence bioremediation limitation, of polycyclic aromatic hydrocarbons in soil. Ozonation was very efficient in the removal of naphthalene, fluorene, phenanthrene, and anthracene, but not for pyrene, chrysene, and benzo(a)pyrene from soil freshly spiked with the hydrocarbons. A similar result was obtained from coal tar-contaminated soil. Elimination of polycyclic aromatic hydrocarbons increased appreciably in sand containing 0.03% organic carbon, indicating the adverse effect of organic carbon on the efficiency of ozone treatment. In spiked and coal tar-contaminated soils, ozonation followed by biodegradation significantly increased the degradation of various polycyclic aromatic hydrocarbons including chrysene and benzo(a)pyrene which were not degraded by the test bacterial consortium alone. In particular, the effect of the combined treatment was more pronounced in coal tar-contaminated soil than in sterile soil spiked with hydrocarbons, probably due to the augmented biological activity of the introduced consortium. The results suggest that a combined treatment including ozonation and biodegradation may be a promising bioremediation technology in soil contaminated with mixtures of polycyclic aromatic hydrocarbons such as former manufactured gas plant sites.

Abbreviations: ANT, anthracene; BaP, benzo(a)pyrene; CHR, chrysene; FLU, fluorene; NAP, naphthalene; PAHs, polycyclic aromatic hydrocarbons; PHE, phenanthrene; PYR, pyrene

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a major class of environmental pollutants and the U.S. Environmental Protection Agency has listed several of these hydrocarbons as priority pollutants. They are of particular concern because of their potential hazards to human beings and to ecosystems. Biodegradation has been successfully employed for remediation of soils contaminated with low-molecular-weight PAHs. However, bioremediation strategies often have limited applicability when soils and aquifers are contaminated with mixtures of high-molecular-weight PAHs (Madsen 1991; Macdonald and Kavanaugh 1994), probably due to the tendency of these compounds to sorb strongly to natural soil organic matter (Means et al. 1980), the resonance energies and stabilities of their structures, and their low water solubilities (Klevens 1950).

Ozone is a very strong oxidant (E^0 = +2.07 V) that has been used successfully in wastewater treatment for the oxidation of organic contaminants (Stover et al. 1982; Peyton and Glaze 1988). Ozone oxidizes organic compounds either by direct oxidation or through the generation of hydroxyl radicals, or both. Organic compounds treated with ozone are transformed to oxygenated intermediates which are more soluble and

* Present address: School of Civil, Urban, and Geosystem Engineering, Seoul National University (Bldg 35, room 303), Seoul 151-742, Korea.

hence more biodegradable (Legube et al. 1981; Gilbert 1983; Gilbert 1987). Unlike for wastewater treatment, very few studies have been conducted using ozonation to degrade organic contaminants in soil. In their study on ozonation of phenanthrene, pyrene, and chrysene, Yao and Masten (1992) found that phenanthrene was the most sensitive to treatment in unsaturated soil, and the reactivity of ozone decreased as the number of fused benzene rings increased. Masten and Davies (1997) showed that gaseous ozone can be readily delivered through columns packed with soil, sand, or aquifer material, suggesting that *in situ* ozonation may be a feasible means for the remediation of contaminated soil.

The use of ozone is likely to facilitate the oxidation of high-molecular-weight PAHs, and thus enhance water solubility of the hydrocarbons. In addition, when used with biodegradation, the combined treatment might overcome the inherent bioavailability limitation of high-molecular-weight PAHs. A combined approach using ozone and biodegradation has been successfully demonstrated in the destruction of pesticides (Leeson et al. 1993; Hapeman et al. 1995) and chlorinated benzenes and phenols (Stockinger et al. 1995; Adams et al. 1997) in wastewater treatment. A recent field study suggested that *in situ* ozonation may be a promising bioremediation technology (Leahy et al. 1997). This study was thus conducted to investigate a means for enhancing the biodegradation of PAHs in soil by combining ozonation and biodegradation. A prairie soil to which seven PAHs (i.e., naphthalene, fluorene, phenanthrene, anthracene, pyrene, chrysene, and benzo(a)pyrene) were freshly added, as well as coal tar-contaminated soil collected from a former manufactured gas plant site were used as model systems.

Materials and methods

Soil samples and chemicals

Quakertown silt loam was collected from a depth of 0 to 15 cm below the surface at the Snyder farm of Rutgers University (Pittstown, NJ). The soil has never been exposed to anthropogenic input of PAH compounds. The soil was air-dried, passed through a 2-mm sieve, and sterilized by gamma irradiation (2.5 Mrad) from a ^{60}Co source (Ward laboratory, Cornell University, Ithaca, NY). The soil consisted of 36% sand, 54% silt, and 10% clay. It contained 2.94% organic carbon and had a pH of 5.9 in water (1 : 1). Sand (0.03% organic carbon) was purchased from Sigma Chemical Company (St. Louis, MO). It was suspended in 0.1 M HCl solution and autoclaved for 2 h to remove surface alkali. The acid-treated sand was then washed thoroughly with distilled water until the pH of the sand suspension reached about 6 to 7. Coal tar-contaminated soil was collected from a former manufactured gas plant site in Newark, NJ. The soil was classified as loamy sand, consisting of 78% sand, 11% silt, and 11% clay.

Table 1. Physical and chemical properties of PAHs used in this study[a]

Chemicals	Number of rings	Solubility in water (mg/l)[b]	Log K_{OW}[c]	Vapor pressure (at 20 °C)
Naphthalene	2	32	3.37	4.9×10^{-2}
Fluorene	3	1.9	4.18	1.3×10^{-2}
Phenanthrene	3	1.0	4.46	6.8×10^{-4}
Anthracene	3	0.07	4.45	1.9×10^{-4}
Pyrene	4	0.16	5.32	6.8×10^{-7}
Chrysene	4	0.006	5.61	6.3×10^{-7}
Benzo(a)pyrene	5	0.0038	6.04	5.0×10^{-7}

[a]Data from Sims and Overcash (1983) and Lee et al. (1992).
[b]Crystal solubility at 25 °C.
[c]Logarithm of the octanol:water partition coefficient.

Seven PAHs including naphthalene (NAP), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), pyrene (PYR), chrysene (CHR), benzo(a)pyrene (BaP), and radiolabeled [7-^{14}C]BaP (specific activity, 26.6 mCi/mmol; purity, >95%) were used for this study. All chemicals were purchased from Sigma Chemical Company. Ten milliliters of stock solution were made in an 20-ml amber vial with a Teflon-lined cap by dissolving 10 mg each of NAP, FLU, PHE, ANT, and PYR and 5 mg each of CHR and BaP in 1 ml of dichloromethane. For artificial contamination of PAHs, 10 g of Quakertown silt loam were placed in a 125-ml flask and spiked with 100 μl of the stock solution. The solvent was allowed to evaporate in a fume hood. This gave a total PAHs concentration of 600 μg gram^{-1} of soil. The physical and chemical properties of the PAHs used for this study are presented in Table 1.

Ozone treatment

Ozone was generated from ambient air by a portable ozone generator (Model 165; Thermo Environmental

Instruments Inc., Franklin, MA). Ambient air was passed through Drierite and activated carbon to remove moisture before entering the ozone generator. The concentration of ozone was determined by a UV photometric ozone analyzer (Model 49; Thermo Environmental Instruments Inc., Franklin, MA) and was 1500 ppb in air flow, which was equivalent to a delivery of approximately 12 mg of ozone day^{-1}. Air flow containing ozone was introduced into the bottom of a 125-ml flask through Teflon tubing. The flask contained either 10 g of PAHs-spiked soil or coal tar-contaminated soil and 20 ml of distilled water containing 0.02% of $HgCl_2$. To ensure good contact between the ozone and PAHs the flask was mixed on a rotary shaker (200 rpm), and the treatment was continued for up to four days at room temperature.

Inoculum and biodegradation

A consortium capable of degrading various PAHs was obtained from Dr. C. Peters (Princeton University, Princeton, NJ). The consortium was maintained and grown in 50 ml of inorganic salts solution (0.10 g $CaCl_2 \cdot 2H_2O$, 0.01 g $FeCl_3$, 0.10 g $MgSO_4 \cdot 7H_2O$, 0.10 g NH_4NO_3, 0.20 g KH_2PO_4, and 0.80 g K_2HPO_4 l^{-1} of dH_2O; pH 7.0) containing NAP, FLU, PHE, and PYR as carbon sources. One milligram each of the hydrocarbons was dissolved in 1 ml of methanol, and 500 μl of the PAHs-methanol solution were added to 50 ml of inorganic salts solution. After 7 days of incubation at 30 °C with shaking (200 rpm), the culture was centrifuged at 7600 $\times$ *g* for 10 min. The cells were washed twice with the inorganic salts solution and an inoculum of more than 10^8 cells was used for biodegradation. The number of viable cells was determined by plate counting on Trypticase Soy Agar (TSA).

For biodegradation of PAHs either before or after ozonation, 10 g each of PAHs-spiked or coal tar-contaminated soil were suspended in 20 ml of inorganic salts solution, inoculated with more than 10^8 cells $gram^{-1}$ of soil, and then incubated at room temperature with shaking (150 rpm) for 30 days. In one experiment, mineralization of ozonated BaP was determined in inorganic salts solution. BaP amended with 10^5 dpm of ^{14}C-labeled BaP (20 μg of BaP in total) in dichloromethane was applied to a 125-ml flask, and the solvent was allowed to evaporate in a fume hood. Inorganic salts solution (20 ml) was then added to the flask and shaken at 30 °C for one day. More than 10^8 cells were inoculated to the solution after treating it with ozone for 24 or 48 h at the concentration of 750 and 1500 ppb, respectively. The flask was sealed with a Teflon-wrapped silicone stopper through which was placed an 18-gauge hypodermic needle and a 16-gauge steel cannula. From the cannula was suspended a small vial containing 1.0 ml of 0.5 *N* NaOH to trap $^{14}CO_2$ released by mineralization. The flask was then incubated at 30 °C with shaking (150 rpm) and $^{14}CO_2$ formation was determined for 25 days by periodically removing the NaOH and replacing it with fresh solution. The radioactivity was measured by liquid scintillation counting (LS 5000 TD; Beckman Instruments, Inc., Fullerton, CA).

Extraction and determination of PAHs

The soil slurry used for either biodegradation or ozonation, or both, was transferred to a 50-ml Teflon centrifuge tube and centrifuged at 18,600 $\times$ *g* for 15 min. After removing the supernatant, 10 ml each of dichloromethane and acetone were added to the soil and the soil-solvent suspension was shaken (200 rpm) for 48 h at 30 °C for the extraction of PAHs. The tube was then centrifuged at 18,600 $\times$ *g* for 15 min and the solvent mixture was transferred to a 50-ml test tube. After removing excess water (upper layer; ca. 2 ml) by pipetting, 4 g of anhydrous sodium sulfate were mixed with the PAHs-containing solvent to remove water completely. The concentration of PAHs in the water layer was less than the detection limit of this study. The extract was then concentrated to 1 to 2 ml using an evaporator (Büchi Rotavapor; Buchler Instruments Inc., Fort Lee, NJ) for further analysis. By this procedure, 100% of the PAHs freshly spiked to Quakertown silt loam were recovered.

The extract was passed through a 0.45-μm PTFE syringe filter to remove any particulates present and analyzed by a gas chromatograph (GC) equipped with a flame ionization detector (Varian Star 3500; Varian Chromatograph Systems, Walnut, CA). The GC was installed with a Rtx-5 silica column crossbonded with 5% diphenyl and 95% dimethylpolysiloxane (30 m $\times$ 0.53 mm inner diameter; Restek Corporation, Bellefonte, PA). The oven temperature was programmed at 40 °C for 6 min, followed by a linear increase of 10 °C per minute to 300 °C, and then the temperature was held for 15 min. Injector and detector temperatures were maintained at 300 °C. Two microliters of the extract were injected and nitrogen was used as a carrier gas.

Data calculation

Precautions were taken to correct for differences in extractability and volatilization of PAHs that occurred during the four-week treatment intervals of our study. Since the extractability of organic compounds decreases as the residence of the compounds in soil increases (Hatzinger and Alexander 1995) we have determined the time dependency of the efficiency of the extraction method used for this study with PAHs-spiked soil samples. A decreased extractability was observed, especially with CHR and BaP. Extractability of the two hydrocarbons was about 70 to 80% of their initial concentrations after 7 days of incubation. To correct for this, separate sets of soil samples spiked with PAHs were made in parallel with soil samples for treatments, and run as controls during the incubation period of each treatment. Values presented in the tables and figures represent the percentages of the amounts of PAHs recovered from each control of corresponding incubation period. All values are the means of triplicate independent experiments unless explained otherwise.

Results and discussion

Mineralization of BaP after ozonation

To test whether ozone-treated hydrocarbons were more available to the microorganisms used for this study, BaP was treated with ozone and the amount of $^{14}CO_2$ evolved was determined using radiolabeled BaP (Figure 1). In samples which were not treated with ozone, less than 1% of BaP was mineralized during the 23 days of incubation, however about 3% of BaP was mineralized by the 24-h ozonation at 750 ppb (ca. 6 mg of ozone). An appreciable increase in the extent of mineralization was observed by an increased ozone dosage. After 48 h of ozonation at 1500 ppb (ca. 24 mg of ozone), more than 16% of BaP was mineralized during the same incubation period.

It has been previously shown that ozonation of organic compounds produces oxygenated intermediates that are more biodegradable (Gilbert 1983; Legube et al. 1981). In this study, increased mineralization of BaP was obvious after ozonation of the hydrocarbon, suggesting that ozonated BaP is more water-soluble and hence more biodegradable to the test microbial consortium. A few studies have identified ozonated byproducts of BaP including BaP quinones and

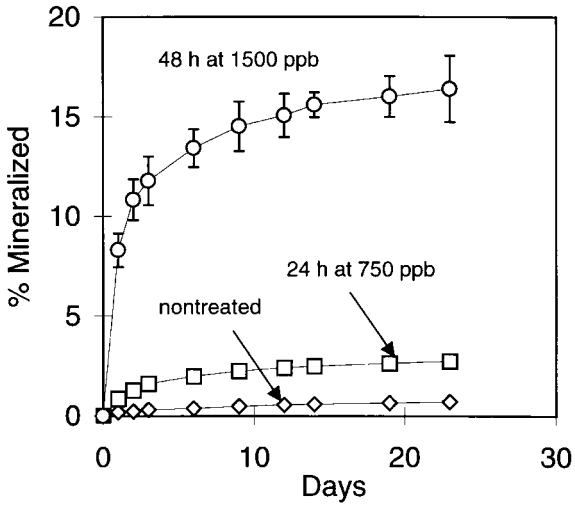

Figure 1. Biodegradation of ozone-treated and non-treated benzo(a)pyrene by consortium P in an inorganic salts solution. The error bars represent the standard deviations of triplicate independent experiments.

carboxylic acids (Koeber et al. 1997) and highly carcinogenic BaP-4,5-epoxide (Pitts et al. 1980). This is of toxicological significance because oxidation of aromatic hydrocarbons can result in hydroxylated and epoxidated intermediates, which are biologically active and more toxic than the parent compounds (Pelkonen and Nebert 1982; Aryton et al. 1990). Upham et al. (1994) showed that ozonation by-products of PYR and BaP inhibited intracellular communication of rat liver epithelial cells more than the parent compounds did. However, more extensive ozone treatment further removed the inhibition in PYR (Upham et al. 1995). It is also worthwhile mentioning that these inhibitory components, which are polar compounds, may be further degraded by microbial attack when the treatment process also includes biodegradation.

Ozone treatment of PAHs-spiked soil

Hydrocarbons such as NAP and FLU are volatile and thus they are also removed from soil during the treatment. In addition, since each PAH has a different vapor pressure the amount of each compound volatilized during the treatment needs to be accounted for separately. Thus, parallel samples were run as controls to correct for the amounts of PAHs volatilized during the treatment. Values in the Tables showing the effect of ozonation do not include the amount of each PAH removed by 'spontaneous' volatilization. However, since ozone is delivered through an air stream some

Table 2. Recovery of PAHs spiked to silt loam and sand after ozonation[a]

Chemicals	% of control remaining in soil				
	Silt loam				Sand
	1 day	2 day	3 day	4 day	2 day
Naphthalene	9.3	nd[b]	nd	nd	nd
Fluorene	45.8A	30.1B	14.3[c]C	4.9D	26.5B
Phenanthrene	80.4A	65.2B	31.3C	5.8D	30.6C
Anthracene	91.0A	90.8A	65.5B	40.3C	56.3B
Pyrene	92.7A	96.7A	81.2B	89.7AB	53.5C
Chrysene	95.8A	97.4A	86.9AB	83.2B	66.6C
Benzo(a)pyrene	92.1A	88.8ABC	82.0B	84.3BC	67.6D

[a]All values are the means of triplicate independent experiments. Values in a row followed by the same letter are not significantly different ($p < 0.05$).
[b]Not detected.
[c]Detected from only one sample.

volatilization is inevitable. These amounts could not be separated from the net effect of ozone molecules in the present study, and thus they were considered as an effect of ozonation.

The effect of ozonation on the removal of PAHs was determined following the treatment of PAHs-spiked Quakertown silt loam or sand with gaseous ozone at the concentration of 12 mg day^{-1} (Table 2). In silt loam containing 2.94% organic carbon, NAP was immediately removed from the soil and not detected after 2 days of treatment. Although three-ring compounds FLU and PHE did not disappear quickly, extensive degradation was observed with prolonged ozonation; only 4.9 and 5.8% were detected after 4 days of ozonation, respectively. Another three-ring hydrocarbon, ANT, was quite resistant and only about 10% was removed by 2 days of ozonation. However, more destruction was evident after 3 days and 40.3% remained in soil after a 4-day treatment. Four- and five-ring compounds including PYR, CHR, and BaP were very resistant to ozone treatment. Less than 10% was removed in 1 day and an additional 3 to 12% transformation was achieved after 4 days of ozonation.

To test whether the efficiency of ozonation was affected by the amount of organic carbon present in soil, sand containing 0.03% organic carbon was spiked with the seven PAHs at the equivalent concentrations (600 ppm in total), followed by treatment with ozone. After 2 days of treatment, significant increases were obtained in the removal of all PAHs tested in the sand compared to the silt loam. For PHE, ANT, PYR, CHR, and BaP, an additional 20 to 40% elimination was achieved for the same period of ozonation. In particular, the amounts of high-molecular-weight hydrocarbons (i.e., PYR, CHR, and BaP) remaining in the sand after a 2-day ozonation treatment were significantly lower than for the same hydrocarbons detected from the silt loam after a 4-day ozonation treatment.

The results described above suggest that ozonation can be an effective means to remove PAHs present as a mixture in soil, especially for two- and three-ring hydrocarbons. Given the fact that these compounds are more volatile than high-molecular-weight PAHs (Table 1), it is likely that the air stream delivering ozone volatilized some of the hydrocarbons. The efficiency of ozonation in removing high-molecular-weight PAHs was not significant. Likely explanations for this are that the amount of molecular ozone delivered was insufficient for appreciable elimination of the hydrocarbons, or that the concentration of PAHs (600 ppm) exceeded the oxidizing capacity of the ozone provided, or both. Yao and Masten (1992) observed that a mixture of PAHs required a greater than arithmetic sum increase in ozone dosage to achieve the same levels of removal as that obtained when hydrocarbons were present singly in soil. It is also possible that ozone reacted with soil organic carbon as well, which reduced the efficiency of ozonation. A decrease in the reaction rate between NAP and ozone was previously observed in the presence of humic materials (Yao and Masten 1992).

It is apparent that the reactivity of ozone decreases as the number of fused benzene rings increases. This is contrary to what would be expected based on consideration of bond localization energies of PAHs, inasmuch as ozone molecules more easily attack organic compounds having lower bond localization energy. For example, ANT and PYR are known to be more reactive to ozonation than NAP and PHE (Bailey 1982). Considering that the reactivities of PAHs were measured from experiments with an organic solvent containing a single target hydrocarbon, one possible explanation for the inconsistency observed here could relate to the sorption properties of these high-molecular-weight PAHs. Since they sorb to soil very strongly it is likely that a higher ozone dosage is required to destroy the compounds than the hydrocarbons with a reduced tendency to sorb, such as NAP, FLU, and PHE. Another reason could be attributed to the generation of hydroxyl radicals from ozone. Ozone is most stable in water at pH of about 3.5 to 4.0 and self-decomposition of ozone is slowest at this pH. At higher pH more ozone molecules spontaneously

Table 3. Degradation of PAHs spiked to silt loam by the combined treatment of biodegradation and ozonation[a]

Chemicals	% of control remaining in soil	
	Biode. → Ozone	Ozone → Biode.
Naphthalene	nd[b]	nd
Fluorene	nd	nd
Phenanthrene	53.5A	28.4B
Anthracene	76.8A	26.5B
Pyrene	89.5A	59.2B
Chrysene	79.5A	58.1B
Benzo(a)pyrene	82.9A	87.3A

[a]Biodegradation was performed for 4 weeks at room temperature and ozonation for 2 days. All values are the means of triplicate independent experiments. Values in a row followed by the same letter are not significantly different ($p < 0.05$).
[b]Not determined.

decompose to hydroxyl radicals. The pH of the ozonation system used in this study was about 6.0 to 7.0, which probably facilitated the generation of hydroxyl radicals from ozone. Based on this consideration, it is plausible that hydroxyl radicals would have been scavenged more preferentially by soil organic matter present in the system since hydroxyl radicals tend to be more nonselective than ozone.

Combined treatment in a silt loam

Combined treatments were performed to determine which combination of biodegradation and ozonation was more effective in the removal of PAHs in an artificially contaminated soil (Table 3). Biodegradation was performed for 4 weeks and ozonation for 2 days for the combined treatment. In the case of a sequential treatment in which ozonation followed biodegradation, NAP and FLU were completely removed. For the other hydrocarbons including PHE, ANT, PYR, CHR, and BaP, an additional decrease of 5.9 to 17.9% was obtained compared to a 2-day ozonation treatment alone. When ozonation was followed by biodegradation, all of NAP and FLU were eliminated and only 28.4 and 26.5% of PHE and ANT, respectively, remained in the soil. Moreover, a significant amount of reduction was observed for PYR and CHR; an additional 37.5 and 39.3% removal was achieved, respectively, compared to the other combination. However, the level of BaP was not significantly affected by the treatment sequence order.

The combined treatment appreciably increased the degradation of three- and four-ring hydrocarbons compared to ozonation alone. Also, when comparing the two possible sequences for the combined treatment, ozonation followed by biodegradation was more efficient in transformation of PAHs than was biodegradation followed by ozonation. These results can be explained by the fact that ozonation generates more water-soluble and oxygenated metabolites, which are more biodegradable. Although production of such oxygenated intermediates is advantageous from the perspective of ultimate biodegradation, there is potential concern in that oxygenated intermediates of some organic compounds are more toxic than the parent compound. Upham et al. (1994) reported that, in some instances, ozonation of PAHs resulted in the formation of intermediates which were more toxic than the parent compounds. This implies that simple transformation of parent compounds from a site does not necessarily mean that the site no longer poses hazards. In addition, it is still unclear whether ozonation mineralizes organic compounds completely or not. In this regard, the treatment sequence of ozonation followed by biodegradation seems to be beneficial since it provides more chances for the complete degradation of PAHs. Degradation of BaP hardly increased by either treatment, possibly due to its strong binding tendency to soil. This is further supported by the observation that the efficiency of ozonation alone, as well as the efficiency of ozonation followed by biotreatment, in the degradation of PAHs decreased as the number of benzene rings increased, and thus the octanol-water partitioning coefficient (K_{OW}) increased. An inverse relationship between the rates of biodegradation and the number of benzene rings present in PAHs has been reported previously (Heitkamp and Cerniglia 1987; Grosser et al. 1995). Our results suggest that a similar trend exits even after ozone treatment in soil.

For this study, we used a mixture of PAHs as soil contaminants since PAHs are commonly present as complex mixtures in the environment. Such complexity, in some instances, may alter the biodegradability of PAHs, compared to when they are present as single soil contaminants. Meuller et al. (1989) reported that four-ring hydrocarbons are utilized only after degradation of more labile hydrocarbons. In contrast, the degradation of five- and seven-ring PAHs has been shown to improve in the presence of low-molecular-weight hydrocarbons (Juhasz et al. 1996). Although a body of evidence exits that BaP can be degraded and mineralized via cometabolism (Grosser et al. 1991; Kanaly et al. 1997; Aitken et al. 1998) when low molecular weight PAHs are also present, in our experimental system the presence of low molecular weight

Table 4. Extraction of PAHs from a coal tar-contaminated soil[a]

Chemicals	Initial conc. (μg/g soil)	% of control remaining after 2-day ozonation
Naphthalene	1205 (103)	7.4 (2.1)
Fluorene	252 (24)	37.0 (3.5)
Phenanthrene	921 (85)	12.4 (1.7)
Pyrene	524 (22)	93.2 (4.8)
Chrysene	454 (57)	94.0 (3.9)
Benzo(a)pyrene	366 (49)	95.7 (9.6)

[a]Values are the means of triplicate independent experiments and values in the parentheses are standard deviations.

PAHs apeared not to improve the biodegradation of high molecular weight PAHs.

Combined treatment in a coal tar-contaminated soil

Organic contaminants were extracted from coal tar-contaminated soil by solvent extraction, and approximately 100 compounds were detected. The concentrations of PAHs including NAP, FLU, PHE, PYR, CHR, and BaP were determined by gas chromatography (Table 4). For this soil, ANT was not considered because the hydrocarbon was not always detected and the corresponding peak was not discernible. The soil was treated with ozone for two days, and decreases in the concentrations of the PAHs were determined. For NAP, only 7.4% remained after the treatment, which was consistent with the result found for spiked soil (above). PHE was more readily removed than FLU in coal tar-contaminated soil while FLU was more vulnerable than PHE in spiked soil. For four- and five-ring hydrocarbons, less than 10% was eliminated by a 2-day ozonation treatment.

The results from the spiked soil indicated that ozonation followed by biodegradation was more effective in the removal of PAHs from soil. Thus, this treatment sequence was used for the degradation of PAHs in coal tar-contaminated soil. More than 10^8 cells of the test consortium were inoculated into coal tar-contaminated soil to initiate biodegradation of PAHs. After four weeks of biodegradation, only about 5% each of NAP, FLU, and PHE were recovered from the soil whereas the same consortium did not significantly degrade PYR, CHR, and BaP, which is significantly different at $p < 0.05$ (Figure 2). However, when two days of ozonation preceded biodegradation, appreciable amounts of four-ring hydrocarbons were degraded, leaving about 35% each of PYR and CHR in the soil. Also, about 17% of BaP was removed by the same treatment (Figure 2). Combined ozonation and biodegradation successfully transformed PAHs containing two- to four-benzene rings, but the treatment was not as efficient for the degradation of BaP, a five-ring hydrocarbon. A previous field study indicated that up to 80% of BaP was removed by four days of ozonation in an aquifer (Leahy et al. 1997). In this study, ozone was generated from pure oxygen and its concentration was 5% of the oxygen stream, which was much higher than the concentration of ozone attained with our system. This difference in ozone delivery concentration probably accounts for the difference in the transformation of BaP between the two studies.

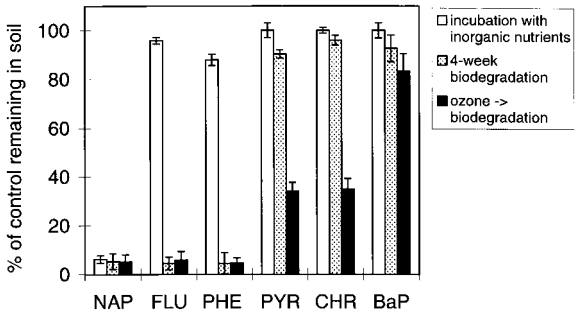

Figure 2. The effect of combined treatment on the transformation of PAHs in coal tar-contaminated soil. The unshaded bar represents soil incubated in an inorganic salts solution for 4 weeks. The speckled bar represents biodegradation for 4 weeks. The solid bar represents a 2-day ozonation followed by a 4-week biodegradation treatment. The error bars represent the standard deviations of triplicate independent experiments.

When coal tar-contaminated soil was incubated with an inorganic salts solution alone for four weeks, concentrations of NAP and PHE decreased by about 94 and 13%, respectively, when compared to the soil incubated with distilled water containing 0.02% $HgCl_2$ (Figure 2). Clearly, this did not result from volatilization of the compounds since the soil samples containing $HgCl_2$ which were used as controls to inhibit any microbial activity also allowed us to account for the amounts of volatilization of each PAH from the samples. When only distilled water was used, removal of PAHs was not significant (data not shown in Figure 2). The results suggest that the soil needed inorganic nutrients for successful biodegradation, and that the indigenous microorganisms in the soil were capable of degrading low-molecular-weight PAHs. A similar result has been reported for NAP and PHE in a former manufactured gas plant site (Madsen et al. 1991).

Although the concentration of PAHs was much higher in the coal tar-contaminated soil than in the sterile soil spiked with PAHs, transformation of the hydrocarbons was more appreciable in the coal tar-contaminated soil. This result suggests that the introduced consortium may have enhanced the total biological activity of the contaminated soil. This process, which has been termed "bioaugmentation", has also been demonstrated in aquifer materials (Munakata-Marr et al. 1996).

Conclusions

Ozone treatment alone was successful in the removal of PAHs containing two- and three-benzene rings, but was not as effective for four- and five-ring hydrocarbons. By use of a sequential treatment process consisting of ozonation and biodegradation, about 95% each of NAP, FLU, and PHE, and about 65% each of PYR and CHR were degraded in a former manufactured gas plant soil. Although transformation of BaP was not as pronounced as for the other PAHs in the soil, about 17% destruction was achieved through the combined treatment process. In addition, when BaP was treated with ozone, the test consortium, which was not capable of degrading the compound alone, readily mineralized the hydrocarbon. Our results suggest that a combined treatment protocol of chemical oxidation and biological degradation may be a successful technology in the remediation of soils contaminated with recalcitrant PAHs.

Acknowledgments

We would like to thank Wayne Wittman for helpful discussions. This research was supported by a grant (Project BICM-51) from the New Jersey Hazardous Substance Management Research Center. The technical assistance of Andrew Berger and Maria Savillo is gratefully acknowledged. We thank Mr. Ed Deger of the Snyder Experimental Farm for assistance in gathering soil samples, and Mr. Steve Szulecki for use of the ozone generator.

References

Adams CD, Cozzens RA & Kim BJ (1997) Effects of ozonation on the biodegradability of substituted phenols. Water Res. 31: 2655–2663

Aitken MD, Stringfellow WT, Nagel RD, Kazunga C & Chen S-H (1998) Characteristics of phenanthrene-degrading bacteria isolated from soils contaminated with polycyclic aromatic hydrocarbons. Can. J. Microbiol. 44: 743–752

Aryton AD, McFarlane M, Walker R, Neville S, Combs MM & Ioannides C (1990) Induction of the P-450 I family of proteins by polycyclic aromatic hydrocarbons: possible relationship to their carcinogenicity. Toxicology 60: 173–186

Bailey PS (1982) Ozonation in Organic Chemistry, Vol. 2, Nonolefinic Compounds. Academic Press, New York

Gilbert E (1983) Investigations on the changes of biological degradability of single substances induced by ozonation. Ozone Sci. Eng. 5: 137–149

Gilbert E (1987) Biodegradability of ozonation products as a function of COD and DOC elimination by example of substituted aromatic substances. Water Res. 21: 1273–1278

Grosser RJ, Warshawsky D & Vestal JR (1991) Indigenous and enhanced mineralization of pyrene, benzo(a)pyrene, and carbazole in soils. Appl. Environ. Microbiol. 57: 3462–3469

Grosser RJ, Warshawsky D & Vestal JR (1995) Mineralization of polycyclic and *N*-heterocyclic aromatic compounds in hydrocarbon-contaminated soils. Environ. Toxicol. Chem. 14: 375–382

Hapeman CJ, Karns JS & Shelton DR (1995) Total mineralization of aqueous atrazine in the presence of ammonium nitrate using ozone and *Klebsiella terragena* (strain DRS-I): mechanistic considerations for pilot scale disposal. J. Agric. Food Chem. 43: 1383–1391

Hatzinger PB & Alexander M (1995) Effect of aging of chemicals in soil on their biodegradability and extractability. Environ. Sci. Technol. 29: 537–545

Heitkamp MA & Cerniglia CE (1987) Effect of chemical structure and exposure on the microbial degradation of polycyclic aromatic hydrocarbons in freshwater and estuarine ecosystems. Environ. Toxicol. Chem. 6: 535–546

Juhasz AL, Britz ML & Stanley GA (1996) Degradation of high molecular weight polycyclic aromatics hydrocarbons by *Pseudomonas cepacia*. Biotechnol. Lett. 18: 577–582

Kanaly R, Bartha R, Fogel S & Findlay M (1997) Biodegradation of [14C]benzo(a)pyrene added in crude oil to uncontaminated soil. Appl. Environ. Microbiol. 63:4 511–4515

Klevens HB (1950) Solubilization of polycyclic hydrocarbons. J. Phys. Colloid Chem. 54: 283–298

Koeber R, Bayona JM & Niessner R (1997) Analysis of ozonolysis products of benzo(a)pyrene with capillary gas chromatography mass spectrometry and liquid chromatography mass spectrometry. Intern. J. Anal. Chem. 66: 313–325

Leahy MC, Nelson CH, Fiorentine AM & Schmitz RJ (1997) Ozonation as a polish technology for *in situ* bioremediation. In: Alleman BC & Leeson A (Eds), In Situ and On-Site Bioremediation, Vol. 3, pp. 479–483. Battelle Press, Columbus, Ohio

Lee LS, Rao PSC & Okuda I (1992) Equilibrium partitioning of polycyclic aromatic hydrocarbons from coal tar in water. Environ. Sci. Technol. 26: 2110–2115

Leeson A, Hapeman CJ & Shelton DR (1993) Biomineralization of atrazine ozonation products: application to the development of a pesticide waste disposal system. J. Agric. Food Chem. 41: 983–987

Legube B, Langlais B, Sohm B & Dore M (1981) Identification of ozonation products of aromatic hydrocarbon micropollutants: effect on chlorination and biological filtration. Ozone Sci. Eng. 3: 33–48

Macdonald JA & Kavanaugh MC (1994) Restoring contaminated groundwater: an achievable goal? Environ. Sci. Technol. 28: 362A–368A

Madsen EL, Sinclair JL & Ghiorse WC (1991) In situ biodegradation: microbial patterns in a contaminated aquifer. Science 252: 830–833

Madsen EL (1991) Determining in situ biodegradation: facts and challenges. Environ. Sci. Technol. 25: 1662–1673

Masten SJ & Davies HR (1997) Efficacy of in-situ ozonation for the remediation of PAH contaminated soils. J. Contam. Hydrol. 28: 327–335

Means JC, Ward SG, Hassett JJ & Banwart WL (1980) Sorption of polynuclear aromatic hydrocarbons by sediments and soils. Environ. Sci. Technol. 14: 1524–1528

Meuller JG, Chapman PJ & Pritchard PH (1989) Action of a fluoranthene-utilizing bacterial community on polycyclic aromatic hydrocarbon components of creosote. Appl. Environ. Microbiol. 55: 3085–3090

Pelkonen O & Nebert DW (1982) Metabolism of polycyclic aromatic hydrocarbons: etiologic role in carcinogenesis. Pharmacol. Rev. 34: 189–222

Peyton GR & Glaze WH (1988) Destruction of pollutants in water with ozone in combination with ultraviolet radiation. Environ. Sci. Technol. 22: 761–767

Pitts JN, Lokensgard DM, Ripley PS, vanCauwenberghe KA, van Vaeck L, Shaffer SD, Thill AJ & Belser WL (1980) Atmospheric epoxidation of benzo(a)pyrene by ozone: Formation of the metabolite benzo(a)pyrene-4,5-oxide. Science 210: 1347–1349

Sims RC & Overcash MR (1983) Fate of polynuclear aromatic compounds (PNAs) in soil-plant systems. Residues Rev. 88: 1–68

Stockinger H, Heinzle E & Kut OM (1995) Removal of chloro and nitro aromatic wastewater pollutants by ozonation and biotreatment. Environ. Sci. Technol. 29: 2016–2022

Stover EL, Wang L-W & Medley DR (1982) Ozone assisted biological treatment of industrial wastewaters containing biorefractory compounds. Ozone Sci. Eng. 4: 177–194

Upham BL, Masten SJ, Lockwood BR & Trosko JE (1994) Non-genotoxic effects of polycyclic aromatic hydrocarbons and their ozonation by-products on the intracelluar communication of rat liver epithelial cells. Fundam. Appl. Toxicol. 23: 470–475

Upham BL, Yao J-J, Trosko JE & Masten SJ (1995) Determination of the efficacy of ozone treatment systems using a gap junction intracellular communication bioasssays. Environ. Sci. Technol. 29: 2923–2928

Yao J-J & Masten SJ (1992) Use of ozone for the oxidation of PAHs in dry soil. In: Proceedings of the 24th Mid-Atlantic Industrial and Hazardous Waste Conference, July 14–17, pp. 642–651. Morgantown, West Virginia