

## A Study on the Degradation of PAH in Organic and Aqueous Phases by Ozone

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### 다환방향족탄화수소에 대한 오존처리의 방법에 관한 연구

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#### Abstract

Pyrene of natural and anthropogenic sources is one of the toxic, mutagenic polycyclic aromatic hydrocarbons (PAHs) listed as priority pollutants. The objectives of this research are to break down pyrene by using ozonation, identify the intermediates and byproducts of pyrene, and test the biodegradability of intermediates and byproducts of pyrene in the aqueous phase. Since pyrene is non-polar, hexane was chosen as a solvent to effectively dissolve pyrene. Pyrene solutions were treated with ozone, as it has high oxidation capacity and electrophilic characteristic. After different ozonation pretreatment times (2, 3, and 10 minutes), intermediates of pyrene in the form of yellowish solid were collected from the hexane solution using a centrifuge. They were identified by gas chromatography/mass spectrometer (GC/MS). BOD<sub>5</sub>, COD, and *E-coli* toxicity tests have been performed to assess the ozonation products.

**keywords** : Biological, Chemical, Ozone, PAHs, Pyrene, Treatment

### 1. Introduction

PAHs are generally formed during incomplete combustion or pyrolysis of organic matter containing carbon and hydrogen. PAH-contaminated sites are associated with processing, combustion, and/or disposal of fossil fuels. In order to reduce the risk of high PAHs levels in drinking water, several methods of water treatment are practiced, such as chlorination and ozonation. Although chlorination may reduce PAH levels, the formation of chlorinated PAH derivatives is likely (Oyler et al., 1982). However a series of chlorinations of some PAHs were accomplished and the chlorinated PAHs were tested for mutagenicity. Ozone is another strong disinfectant that has being widely used as an alternative to chlorine. Due to its high oxidant capacity and electrophilic character, ozone is the most appropriate oxidant to degrade PAHs (Beltran et al., 1995).

Previous studies of pyrene degradation by ozone using different solutions were accomplished. The different

solutions were methanol (Danheux et al., 1963), t-butyl alcohol (Sturrock et al., 1968), two-phase fluoro-carbon-water (Stich et al., 1987), two-phase inert fluoroinated hydrocarbon-water (Freshour et al., 1996), and oil/water-emulsions (Kormmueller et al., 1997). Beltran et al. (1995) studied three PAH compounds, fluorene, phenanthrene, and acenaphthene, in aqueous conditions subjected to ozonation at three pH levels. Ozonation in water at neutral pH is the most effective treatment. Hong et al. (2004) recently reported the use of a polar-nonpolar solvents system involving equal portions of acetic acid and heptane as the medium for ozonation of pyrene. They reported an advantage of the medium being able to maintain all parent and daughter compounds in solution throughout the treatment, hence maximizing the compounds' exposure to ozone through the entire course of treatment.

Stich et al. (1987) made use of the nature and characteristics of ozone as an oxidant. They studied an innovative two-phase ozonation system for the destruction of organics. They found that this two-phase ozonation process provided higher efficiency of organic destruction compared to a single-phase oxidation, or wet-air oxidation process.

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The ozonation results indicate that the process was indeed feasible. The results showed that a reduction of 29% in total organic compound occurred after 30 min. The ozone solubility in the fluorocarbon (FC 77) solvent is approximately 12 to 14 times more than in water. They pointed out that future work should include further experiments on selective oxidation of hazardous organics from mixed solute systems, and that a continuous two-phase ozonation system should be tested with model compounds as well as actual hazardous wastewater.

A combination of chemical and biological processes is potentially effective for the destruction of many organic contaminants in wastewater. Initial chemical oxidation can initiate destruction of compounds that would not otherwise be degraded by biological processes. Once the chemical process has concluded, the biological process for contaminants begin. The chemical process of organic degradation is intended to form intermediates that are amenable to further degradation by microorganisms (Zeng et al., 1999; Haapea et al., 2006). The degradation of PAHs is relative to the molecular weight of the compound. Although low-molecular-weight (LMW) PAHs can be degraded, high-molecular-weight (HMW) PAHs are not extensively biodegraded in soil and sediment media (Field et al., 1992; Heitkamp et al., 1989).

This study examines the breakdown of pyrene by ozone first in hexane, followed by further degradation of the intermediates by ozone in the aqueous phase. The purpose of this research is to identify the byproducts and intermediates of pyrene formed during ozonation, and demonstrates the biodegradability of these compounds.

## 2. Materials and Methods

### 2.1. Chemicals

Pyrene (99%, Aldrich Co.) was used. Indigo stock solution was prepared by potassium indigo trisulfonate,  $C_{16}H_7N_2O_{11}S_3K_3$  (Aldrich Co) per Standard Methods (APHA et al., 1992a). Polyseed (Hach Co.) was provided in diluted water for biochemical oxygen demand (BOD) measurements per Standard Methods (APHA et al., 1992b). COD digestion solutions (0-1,500 mg/L range, Hach Co.) were prepared for chemical oxygen demand (COD) measurement. Inoculum, ToxTrak™ accelerator solution, and ToxTrak™ reagent powder pillows (Hach Co.) were prepared for the toxicity test, and used according to the manufactures' instructions. In all procedures requiring water, distilled deionized (DD) water that contains low-organic (<15 ppb as TOC), low-ion (resistivity > 18  $M\Omega$ -cm) and nonpyrogenic (up to 4 -log reduction with

reverse osmosis pretreatment) was used (4 stage Mill-Q plus system, Millipore Co.). Cellulose nitrate membrane filter papers of 0.45- $\mu$ m (GelmanSciences) were used. Hexane (Fisher Scientific) of HPLC grade was used for dissolving pyrene. Methanol (Fisher Scientific) of HPLC grade was used for dissolving intermediates of pyrene.

### 2.2. Reactors

Ozonation of pyrene in hexane solution was done with a batch reactor. The experiments were carried out in 125 mL erlenmeyer flasks with a magnetic bar controlled by a magnetic stirrer. A high-voltage discharge ozonator (Model T-816 polymetrics Corp.) was used to produce ozone from air. The air was supplied to the ozone generator continuously at a flow rate of 2 L/min. The air/ozone mixture was introduced through a glass sparger. A total of 0.1 g of pyrene was dissolved in 50 mL of hexane. The ambient temperature was at 21°C.

Ozonation experiments in the aqueous phase were also done with a batch reactor. The experiments were carried out in a 300 mL beaker with a magnetic stir bar controlled by a magnetic stirrer. A variable speed pump controlled by a pH controller was used to deliver a 0.1 M of NaOH for pH adjustment. All experimental conditions were identical for all procedures. The ozonation products contained in both phases were identified by GC/MS.

### 2.3. Experiment Procedures

Prior to do ozonation pyrene was dissolved in hexane. Ozone was introduced to the solutions to begin reaction with pyrene for various durations. A yellowish solid was formed in the solution. This yellowish solids were collected using a centrifuge. The solid was removed and dried out at room temperature (21°C) for approximately 48 hours. The liquid (a solution in hexane solvent) from the centrifuge was used for identification purposes. A portion of the liquid was injected into a Gas Chromatograph/Flame Ionization Detector (GC/FID) to detect intermediate compounds. Another portion of the solution was injected into a Gas Chromatograph/Mass Spectrometer (GC/MS) to identify the types of intermediates and byproducts in the solution. Identification of the dried yellowish solid was also performed. The solid was completely dissolved in 50 mL of methanol. This solution was injected into a GC/FID and a GC/MS for identification.

An attempt to dissolve the solid in 300 mL distilled-deionized (DD) water failed. The solid dissolved in water of higher pH. A 0.1 M of NaOH was added to the solution in order to increase the pH to 11.4. The container of suspension was placed inside an ultrasound for 10 min

in order to assist in dissolving the solid. When the solution was removed from the ultrasound bath, it was poured through a filter funnel in order to separate the remaining solids from the solution.

The filtered solution was introduced to ozone at 10, 30, and 60 min periods. During ozonation, the pH level of the solution was automatically maintained around 7 with the use of a pH controller. This prepared the solution for the BOD<sub>5</sub>, COD, and toxicity tests.

Each sample containing pyrene and intermediates of pyrene were analyzed using a gas chromatograph (GC) (HP 6890, Hewlett Packard Co.) equipped with a capillary column (DB-1 nonpolar column, 60 m × 250 mm × 0.25 μm, J & W Co.), and a mass spectrometry detector (MS) (HP6890, Hewlett Packard Co.) interfaced and programmed with the HP Chemstation software (Hewlett Packard Co.). The test was set up using a split ratio of 5:1, solvent delay at 7 min, and scan range from m/z 15 to m/z 550 at 1.44 scan/sec. The oven temperature was set from 35°C (1 min) to 300°C (30 min) at 5°C/min ramp. The flow rate of He gas in column was 1.7 mL/min. The volume of injecting each sample was 1 mL.

To identify the species, the mass spectra were interpreted and the HP Chemstation library (Hewlett Packard Co.) was used. The pyrene and reaction products were analyzed qualitatively and quantitatively with GC/MS. Quantification of pyrene and intermediates of pyrene was based on pyrene calibration curve and peaks integration.

### 3. Results and Discussion

By using the Indigo Blue Method (APHA, 1992), the concentrations of ozone in hexane solution were calculated. Concentration of ozone in the aquatic batch systems were determined by samples absorbance at 600 nm using a 1-cm quartz cell with a HP-8452 Spectrophotometer (HP-8452 UV-V is Spectrophotometer, Hewlett Packard Co.) according to the Indigo Blue Method (APHA, 1992). Table 1 and Table 2 show concentrations of dissolved ozone in hexane solutions and aquatic solutions respectively. This clearly indicates that hexane is a much better solvent for pyrene.

After 2, 3, and 10-minute periods' ozonation in 2000 mg/L hexane pyrene solutions, the yellowish solids were coming out of the solution. So as to identify the inter-

**Table 1.** Ozone concentrations in hexane phase

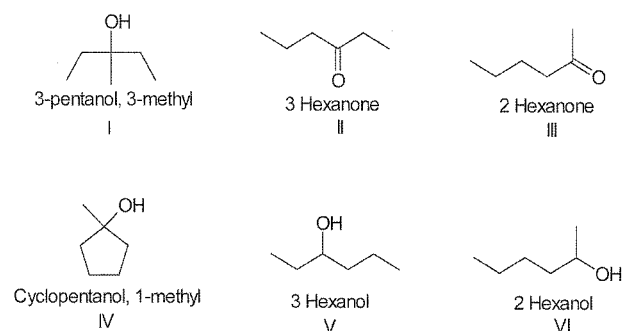
Time (min)	Ozone concentration (mM)
2	0.18
3	0.20
10	0.31

**Table 2.** Ozone concentrations in aquatic phase

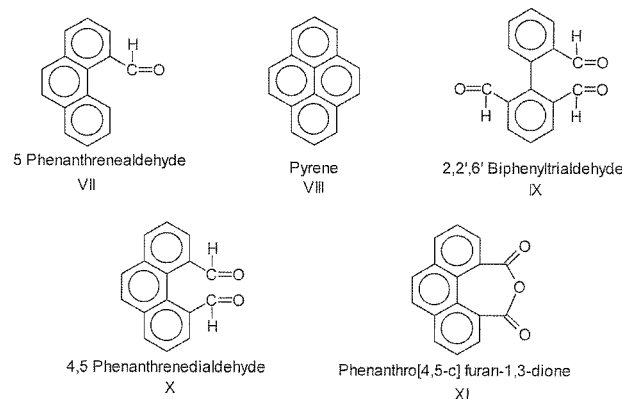
Time (min)	Ozone concentration (mM)		
	Pure water	Water with solids (after 2 min Ozone)	Water with solids (after 10 min Ozone)
10	0.038	0.020	0.021
30	0.065	0.045	0.043
60	0.101	0.079	0.079

mediates of pyrene, the yellowish solids and hexane solvent were separated with 2, 3, and 10-minute periods' ozonation in 2000 mg/L hexane pyrene solutions. After the yellowish solids were separated by using a centrifuge, and the solids were analyzed by a pyrolysis gas chromatography. The solids consist of 66% of Carbon, 4% of Hydrogen, and 27% of Oxygen. The solids were dissolved in 50 mL of methanol in order to inject GC/MS directly. 2, 3, and 10-minute periods' ozonation hexane solutions were injected in GC/MS.

In the hexane phase, two types of ozonation products were formed that ozone reacted with hexane and pyrene. Six compounds which were 3-Pentanol; 3-methyl; 3-Hexanone; 2-Hexanone; Cyclopentanol; 1-Methyl, 3-Hexanol and 2-Hexanol were found in the hexane-type products (Fig. 1). Major product of ozonation of hexane was 3-Hexanol. As the concentration of ozone increased, the concentration of hexane-type products also increased.



**Fig. 1.** Byproducts formed during ozonation of hexane itself.



**Fig. 2.** Compounds that remain dissolved in hexane after the first-stage ozonation in hexane.

When ozone reacted with pyrene in hexane phase, three phenanthrene-types which were 5 Phenanthrenealdehyde; 4,5 Phenanthrene and Phenanthro [4,5-c] furan-1,3-dione, and one bipheny-type that was 2,2',6'-Biphenyltrialdehyde was formed. Fig. 2 shows compounds remaining in the hexane solvent following the first-stage ozonation.

In the yellow solids, nine compounds which were Pyrene (VIII), 5-Methoxy phenanthrene-4-carbaldehyde (XII), 4,5-Phenanthrene (X), Phenanthrene-4,5- dicarboxylic acid (XIII), 9,10 dihydroxypyren-4,5-dione (XIV), 5-formylphenanthrene - 4 carboxylic acid (XV), 2-[6-(Butoxycarbonyl)-2-ethyl phenyl] benzoic acid (XVI), Ethyl 2-[2-(butoxycarbonyl) phenyl] bezonate (XVII), and 2-[6-Butyl- 2 - (ethoxycarbonyl) phenyl] benzoic acid (XVIII) were identified (Fig. 3).

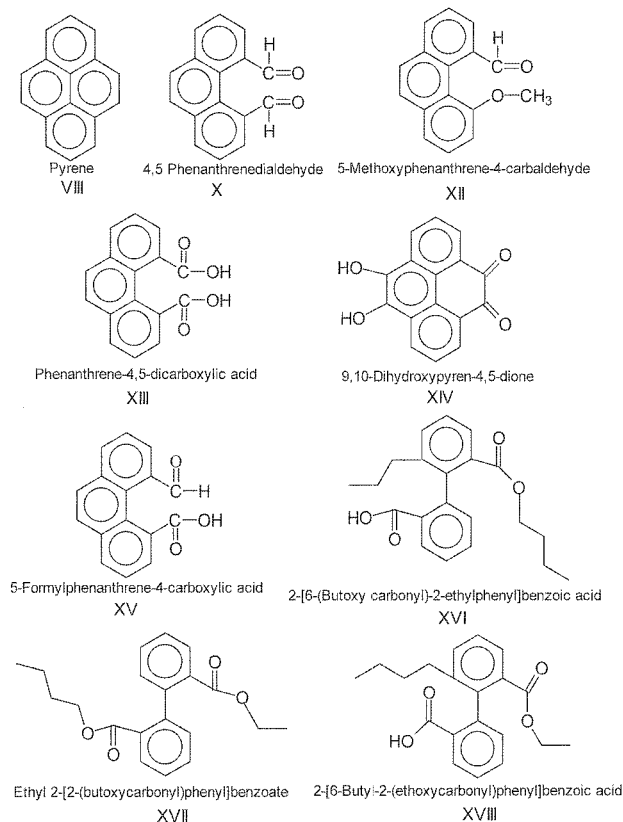


Fig. 3. Intermediate compounds in the yellowish solids.

Since structures of these compounds are more polar than structures of compounds in hexane phase, these yellowish solids were in hexane phase. XVI, XVII and XVIII are constituted isomers. Major compounds in the yellowish solids were X and XIII. The yellowish solids that were not dissolved in high pH water were separated using filtration. The mass of the remaining solids was 13% of the total mass. The solids were placed on a filter paper and dissolved in methanol, and then they were injected in GC/MS. The compounds that were not dissolved in higher pH water were VIII, XII, and Unknown. The compounds

that dissolved in an aqueous phase were XIII, XIV, XV, XVI, XVII and XVIII. A structure of most compounds contains an alcohol functional group. Therefore, these compounds could be dissolved well in an aqueous phase with respect to their structures. Since structures of these compounds are more polar than structures of compounds in hexane phase, these yellowish compounds were in hexane phase.

Ring opening of pyrene molecule occurred at the 4,5-bonds by ozone. By attacking 4,5-bond of pyrene under ozone, dialdehyde was formed at the 4,5 position. Upon further ozonation, the dialdehyde group could close ring with ketone group, and phenanthrene-type products became biphenyl-type product by second ring cleavage at the 9, 10 position (Yao et al., 1998). The bonds between fused angular rings, as 4,5- and 9,10- bonds show double-bond character and are more reactive than other bonds. The preferential attack of  $O_3$  on 4,5- and 9,10-bonds of the pyrene molecule could be given an account of in terms of less delocalization energy that makes the site first activated reactive site for oxidation most reactive (Bailey, 1982). At the initial stage of this reaction, pyrene molecule reacted with ozone to form an aldehyde and/or dialdehyde. 4,5-Phenanthrenedialdehyde was both in the hexane phase and the yellowish solids. In 4,5-Phenanthrenedialdehyde molecular ion peaks, a parent peak is  $m/z$  234. According to the loss of aldehydes group ( $CHO-$ ), other fragments have  $m/z$  205 and  $m/z$  176. A phenanthrene fragment was corresponded to  $m/z$  176. In 5-Methoxy phenanthrene-4-carbaldehyde molecular ion peaks, a parent peak is  $m/z$  236. According to the loss of methyl ether group ( $CH_3O-$ ) and aldehydes group ( $CHO-$ ), other fragments have  $m/z$  205 and  $m/z$  176.

Fig. 4 also showed a proposed mechanism with identified intermediates and byproducts to illustrate the degradation pathway of pyrene in the hexane phase with the sequence of their appearance. The bonds between fused angular rings, as 4,5- and 9,10- bonds, show double-bond character and are more reactive than other bonds.

Hong et al. (2004) accomplished cosolvent system consisting of heptane and acetic acid (1:1 v/v) for the ozonation treatment of pyrene. Initially 600 ppm pyrene (60 mg of pyrene/100 mL of solution) was ozonated over time (130 minutes). The only nonpolar compound remained in the nonpolar heptane solution while all other intermediates remained in the polar acetic acid solution. Six intermediates of pyrene were identified in the research. The identified intermediates were mainly oxygenated intermediates were similar to those found by our research.

Compounds dissolved in high pH water were XIII, XIV,

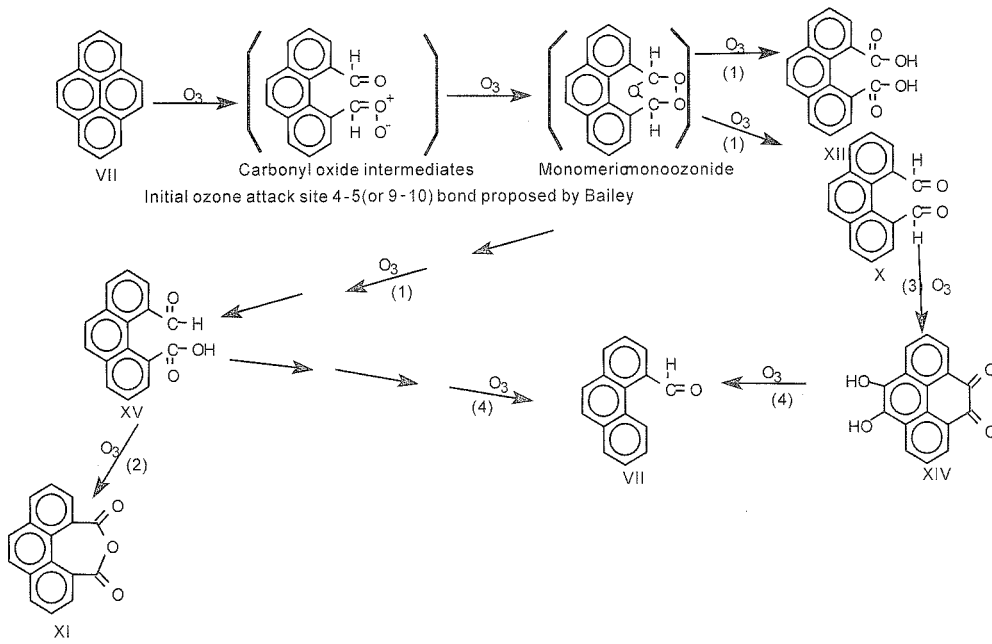


Fig. 4. Proposed Pathway for degradation of pyrene during ozonation, 1, 2, 3 (Baily, 1982) and 4 (Yao et al., 1998).

XV, XVI, XVII, and XVIII. Table 3 and Table 4 show the changes in BOD<sub>5</sub> and COD contents in the samples after first- and second-stage ozonation in hexane and aqueous phases. In the first-stage ozonation, there is a general increase of COD but little change in BOD<sub>5</sub> corresponding to the increase of ozonation periods from 2 to 10 min. In the second-stage ozonation, there is a decrease in COD contents, but slightly increasing BOD<sub>5</sub>, corresponding to the increasing duration of in the aqueous phase. These changes have resulted in an increasing BOD<sub>5</sub>/COD ratio according to the increasing second-stage ozonation. Such increasing ratios generally reflect the increasing biodegradability in the samples. At the end of 60 min of ozonation, the ratio has approached 70%, which approximates those of typical domestic wastewater (e.g., 68% of ultimate BOD as BOD<sub>5</sub>) that are routinely treated by the activated sludge process. Fig. 5 corroborates the reduced *E-coli* toxicity after second-stage ozonation in the aqueous phase. This method of toxicity test is based on the reduction of resazurin, a redox-active dye, by bacterial respiration. When it is reduced, resazurin changes color from blue to pink. Toxic substances can inhibit the rate of resazurin reduction. A chemical accelerant was added to shorten the reaction time. It should be noted that for the toxicity test (Hach; Toxicity Method 10017) an inhibition value between -10 and +10% is generally within the nontoxic range, while an inhibition value outside of this range indicates toxicity. The results suggest that toxicity is apparent in the samples having been subjected to first-stage ozonation but not the second-stage ozonation. The results also show that a second-stage ozonation of 10 min

Table 3. BOD<sub>5</sub> and COD of byproducts and intermediates of pyrene after 2 min of ozonation pretreatment

Solutions	BOD <sub>5</sub> (mg/L)	COD (mg/L)	BOD <sub>5</sub> /COD ratio
Reozonation 0 min of byproducts & intermediates of pyrene water solution	30	194	0.16
Reozonation 10 min of byproducts & intermediates of pyrene water solution	35	119	0.29
Reozonation 30 min of byproducts & intermediates of pyrene water solution	50	85	0.59
Reozonation 60 min of byproducts & intermediates of pyrene water solution	55	84	0.66

Table 4. BOD<sub>5</sub> and COD of byproducts and intermediates of pyrene after 10 min of ozonation pretreatment

Solutions	BOD <sub>5</sub> (mg/L)	COD (mg/L)	BOD <sub>5</sub> /COD ratio
Reozonation 0 min of byproducts & intermediates of pyrene water solution	40	328	0.12
Reozonation 10 min of byproducts & intermediates of pyrene water solution	45	239	0.19
Reozonation 30 min of byproducts & intermediates of pyrene water solution	55	126	0.44
Reozonation 60 min of byproducts & intermediates of pyrene water solution	60	91	0.66

would be sufficient to render the samples nontoxic, and that the duration of first-stage ozonation (2 or 10 min) exhibits little effect in the reduction of toxicity.

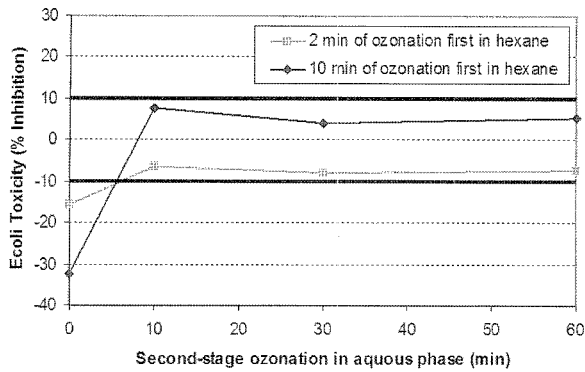


Fig. 5. *E. coli* toxicity changes after subjecting pyrene to sequential first- and second-stage ozonation durations in hexane and aqueous phases.

#### 4. Conclusions

The intermediates of pyrene can be clearly identified using a gas chromatography/mass spectrometer. One major intermediate is 4,5-Phenanthrene dialdehyde, which suggests that pyrene is attacked by  $O_3$  via ring cleavage at the 4,5- or 9,10-bonds, the bond of lowest bond-localization energy. The pyrene destruction intermediates could be brought to a state where they could be tested for  $BOD_5$ ,  $COD_{Cr}$  and toxicity tests in the aquatic phase. The biodegradability was tested on intermediates using the  $BOD_5$  and  $COD_{Cr}$  tests. The toxicity test showed that the intermediates were nontoxic.

Selection solvent is the most important requirement for an efficient ozonation when the ozone reacts with polycyclic aromatic hydrocarbons. This research shows there is an advantage of a higher solubility of PAH and ozone concentration in hexane in comparison to water. Dissolving and ozonating pyrene in hexane first make higher ozone solubility, that is, lower ozone generation requirements. By using the advantage of the higher ozone solubility, the insoluble recalcitrant pyrene as HMW PAHs could be easily attacked by biological process. The design of integrated chemical and biological systems are the more effective and efficient than an individual system. Since ozone reaction with pyrene produces a ring-cleavage and hydroxylated byproducts and intermediates such as aldehydes and carboxyl acid that become more soluble in water. This makes a further ozonation and could be subsequently biodegraded in the aqueous solution.

The application of this process is easily attained through engineering design. It is possible to collect PAHs from the air, water, and soil using different methods: an electrostatic precipitator, a spray tower, and soil collection. Dirty air contains PAHs, which is collected using an electrostatic precipitator. Dirty water is collected using a spray tower. Soil contaminants can also be collected and treated. The

PAHs in the air, water and soil can be separated using an extraction method with hexane solvent prior to ozone treatments.

#### 국문요약

대부분의 polycyclic aromatic hydrocarbons (PAHs) 은 최우선 오염물질로 간주되어지고 있는 매우 유독한 물질이다. Pyrene은 PAHs들 중에서도 그 유독성은 가히 심각하다. 그리고 Pyrene과 다른 PAHs화합물들은 물에 잘 용해가 되지 않는 소수성 성질을 가지고 있어 화학적 또는 생물학적 분해가 용이하지 않다. 이러한 성질을 극복하기 위하여 본 연구에서는 Pyrene을 대표 물질로 하여 2 단계 오존처리를 하였다. 첫 단계에서 Pyrene을 무극성인 헥산 용매에 대량(2000 mg/L) 으로 녹여 오존처리를 하였다. 이때 Pyrene은 극성 분자들, 즉 알콜과 알데하이드 그리고 에시드 기능기를 가지는 물질들로 변화되었으며 이 변화된 물질들을 다시 극성 용매, 물에 녹여 두 번째 오존처리를 하였다. 두 번째 오존 처리된 Pyrene의 부산물들과 중간생성물들은 생물학적 처리로 가능한지 연구되어지기 위해  $BOD_5$ 와  $COD$  그리고 *E. coli* toxicity tests가 이루어졌다. 그 결과 오존 처리된 Pyrene은 유독하지도 않았고 Pyrene의 부산물들과 중간생성물들은 생물학적 처리가 용이하여졌다. Gas chromatograph (GC) 분석을 통해 Pyrene의 부산물들과 중간생성물들을 밝혀내었다. 이 연구를 토대로 소수성 성질을 가지는 많은 방향족 물질들을 처리하기가 매우 용이해졌다.

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