

Degradation of Acenaphthene by Ozone¹

DI WU[#], YONG-JUN XU^Δ, AND JIAN-LONG WANG^{#, **}

[#]Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, China;

^ΔHarbin Institute of Technology, Harbin 150001, Heilongjiang, China

Objective To investigate the oxidation of acenaphthene (Ace), a polycyclic aromatic hydrocarbon (PAH) with a saturated C-C bond by ozone and to characterize the intermediate products of ozonation. **Methods** Ozone was generated from filtered dry oxygen by an ozone generator and continually bubbled into a reactor containing 1g/L Ace dissolved in an acetonitrile/water solvent mixture (90/10, v/v) at a rate of 0.5 mg/s. HPLC was used to analyze the Ace concentration. Total organic carbon (TOC) was used to measure the amount of water soluble organic compounds. GC-MS was used to identify the ozonized products. Oxygen uptake rate (OUR) of activated sludge was used to characterize the biodegradability of ozonized products. **Results** During the ozonation process, Ace was degraded, new organic compounds were produced and these intermediate products were difficult mineralize by ozone, with increasing TOC of soluble organics. The ozonized products were degraded by activated sludge more easily than Ace. **Conclusion** Ozonation decomposes the Ace and improves its biodegradability. The ozonation combined with biological treatment is probably an efficient and economical way to mineralize acenaphthene in wastewater.

Key word: Ozone; Acenaphthene; PAHs; Biodegradation; Persistent organic pollutant

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are a group of aromatic compounds containing two or more fused benzene rings in linear, angular, or cluster structure. Health concerns of PAHs arise from their toxicity, mutagenicity, and carcinogenicity^[1-2].

Ozonation has been found to be effective in degrading PAHs but complete mineralization is expensive^[3]. In addition, biological treatment technology is cost-effective. However, it is inefficient in degradation of PAHs because of their recalcitrant nature of low solubility and inhibition of metabolism by microbes^[4-5]. Therefore, taking the effect and efficiency into account, ozonation is presented as a potential pretreatment for biological degradation because it may improve solubility and bioavailability^[6-7].

Ace, one of the 16 PAHs listed by the US EPA as priority pollutants, is characterized by the molecular structure of saturated C-C bond. The objective of this study was to investigate the ozonation of Ace and characterize the ozonized products.

MATERIALS AND METHODS

Chemicals

Ozone was generated from filtered dry oxygen by an ozone generator (Sorbios, German). Polyvinyl alcohol (PVA), calcium chloride, and glucose were purchased from Beijing Chemical Reagents Company. Other commercially available analytical reagents were of laboratory grade.

Microorganisms

Activated sludge was collected from Beijing Gaobeidian Sewage Treatment Plant and cultivated under aerobic conditions at 30°C. The growth medium consisted of 1 mg CaCl₂·2H₂O, 19 mg MgSO₄·7H₂O, 4.4 mg K₂HPO₄, 26.8 mg NH₄HCO₃, and 0.1031 g C₆H₁₂O₆. The pH of the medium was maintained at 7.0±0.2.

Immobilization of Microbial Cells

Ten g of PVA (nominal degree of polymerization=1750, approx. molecular weight=75000-80000) was dissolved in 50 mL of distilled water and cooled to

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**Correspondence should be addressed to Dr. Jian-Long WANG, Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, China. Tel: +86-10-62784843. Fax: +86-10-62771150. E-mail: wangjl@tsinghua.edu.cn

Biographical note of the first author: Di WU, female, born in 1975, post doctor, majoring in the field of environmental engineering.

40°C, then mixed thoroughly with 50 mL of cell suspension at a concentration of ca. 4.0×10^7 cells/mL. The resulting mixture was dropped into saturated boric acid solution for 1 h to form spherical beads. The formed gel beads with an average diameter of 3 mm were then soaked in 0.5 mol/L sodium orthophosphate solution for 1 h. The particles were washed with physiological saline, then dried at 4°C for 24 h and stored at 4°C before use.

Ozonation of Ace

Ozonation was carried out as previously described^[6-7]. Ozone generated was continually bubbled into a reactor containing 1 g/L acenaphthrene (99.9% purity, Peking Chemical Co., Beijing, China) dissolved in an acetonitrile/water solvent mixture (90/10, v/v) at a rate of 0.5 mg/s. The pH of the solution was approximately 3.9.

Assay Methods

Quantity of Ace in ozonated products was detected using HPLC (HP1100) with column of C18 (Eclipse@XDB-C8) and indicator of DAD. Eluent was CH₃OH/H₂O (8:2, v/v) at a flow rate of 1 mL/min.

One mL of ozonated sample was initially dried under helium. The residue was dried over phosphorus pentoxide (P₂O₅) in a vacuum desiccator. Then, 50 mL of water was added to get a solution of intermediates. TOC of this solution was analyzed by Elemental Analysis High TOC.

GC/MS was performed using a PE Turbo mass coupled with a HP 5890J GC. A DB-5MS (a film, 30 m in length, *0.32 mm in diameter, *0.25 mm in thickness) fused silica capillary column (J & W Scientific, Rancho Cordova, CA) was employed for GC separation. A splitless injector was used and helium was used as the carrier gas, with a flow rate of 1 mL/min. The initial column temperature was held for 1 min at 70°C, increased to 300°C at 10°C/min. The mass spectrometer was operated in electron impact mode. Mass calibration of the spectrometer was performed by using perfluorokerosene.

RESULTS

Degradation of Ace

During Ace ozonation process, the amount of Ace decreased, the quantity of Ace was detected by HPLC. The variation of Ace with time is depicted in Fig. 1.

TOC in Soluble Ozonized Products

The total organic carbon in water soluble ozonized

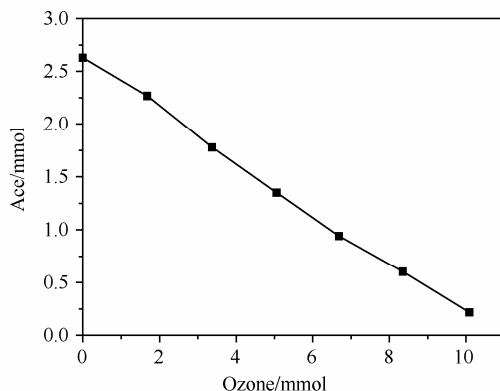


FIG. 1. Decrease of acenaphthrene.

products was measured with a TOC analyzer (Fig. 2).

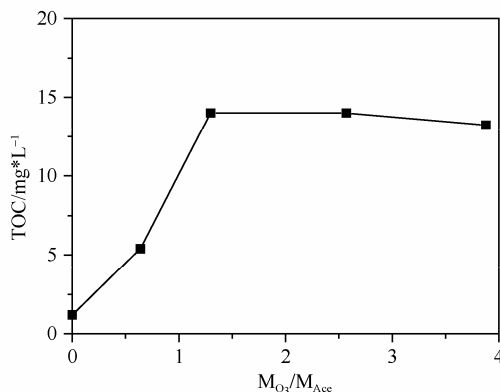


FIG. 2. TOC of soluble ozonized products.

Influence of Intermediate Products on OUR of Activated Sludge

The effect of intermediate ozonized products on OUR of activated sludge was detected using the intermediate products as carbon source for microbial respiration to investigate the possibility of degradation of ozonized products by activated sludge (Fig. 3).

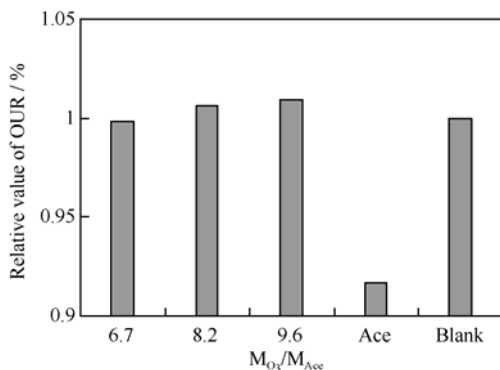


FIG. 3. Influence of intermediate products on OUR of activated sludge.

GC-MS Spectra of Ozonized Products

The ozonized products were determined by

using GC-MS (Fig. 4 and Table 1). Fifteen organic compounds were found in ozonized sample.

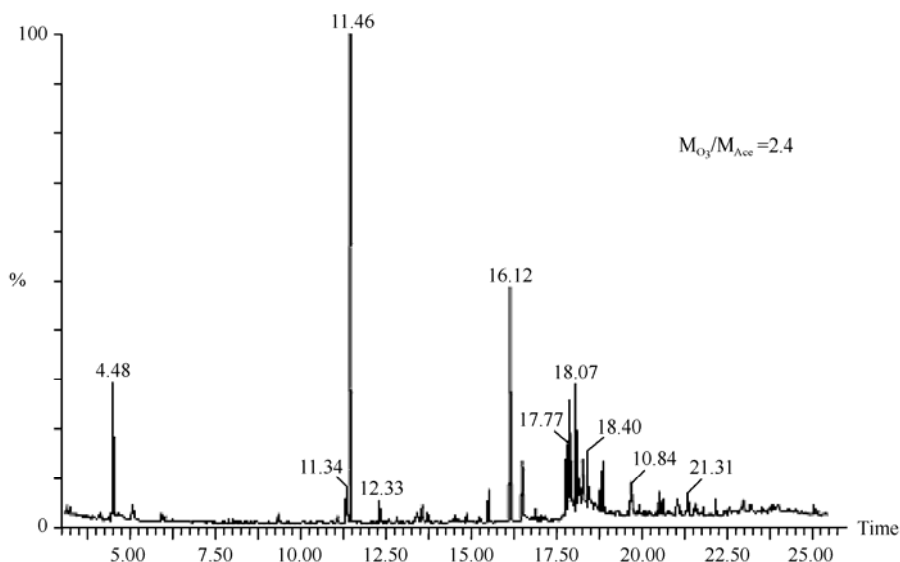


FIG. 4. GC-MS spectra of ozonized products.

TABLE 1

Intermediate Products of Ozonation of Ace

Retention Time (min)	M/z	Name and Molecule Formula of Compounds
4.5	38,39,40,65,66,67,94,95	Phenol C ₆ H ₆ O
5.1	39,41,57,70,83,98,105	1-Hexanol,2-ethyl C ₈ H ₁₈ O
11.34	35,51,63,76,77,126,151,152,153,154,155	Acenaphthene C ₁₂ H ₁₀
11.45	39,41,57,67,91,105,145,177,205,220,221	Butylated Hydroxytoluene C ₁₅ H ₂₄ O
15.48	39,41,57,76,104,105,149,150,167,223,224	Dibutyl Phthalate C ₁₆ H ₂₂ O ₄
16.1	39,41,43,55,74,87,97,129,143,185,227,270,271	Hexadecanoic Acid, Methyl Ester C ₁₇ H ₃₄ O ₂
16.47	38,39,41,43,60,73,83,85,129,149,157,171,213,223,256,257	Hexadecanoic Acid C ₁₆ H ₃₂ O ₂
17.77	39,41,55,67,81,95,96,110,135,164,263,294,295	9,12-Octadecadienoic Acid, Methyl Ester C ₁₉ H ₃₄ O ₂
17.83	39,41,43,55,57,69,74,83,98,123,137,180,222,264/265,	9-Octadecenoic Acid(Z)-, Methyl Ester C ₁₉ H ₃₆ O ₂
17.89	37,39,41,55,69,74,83,96,110,111,137,180,222,264,265,296	6-Octadecenoic Acid, Methyl Ester C ₁₉ H ₃₆ O ₂
18.07	39,41,43,55,74,87,97,129,143,185,199,213,255,298,299	Octadecanoic Acid, Methyl Ester C ₁₉ H ₃₈ O ₂
18.122	39,41,67,91,105,117,147,187,201,223,258,290	Heptanoic,7-(O-pentylphenyl)-, Methyl Ester C ₁₉ H ₃₀ O ₂
18.251	39,41,55,69,91,105,117,133,183,197,259,264,290	Benzeneoctanoic Acid, 2-butyl-, Methyl Ester C ₁₉ H ₃₀ O ₂
18.40	38,39,43,55,60,73,83,105,129,133,185,199,241,284,290	Octadecanoic Acid C ₁₈ H ₃₆ O ₂
18.82	39,41,43,55,57,83,97,111,126,152,181	Acetic Acid, Octadecyl Ester C ₂₀ H ₄₀ O ₂

DISCUSSION

As shown in Fig.1, ozonation could decrease Ace with excessive ozone. The quantity of consumed ozone was four times that of reduced Ace in mole. The TOC increased rapidly before specific ozone

ratio (M_{O_3}/M_{Ace}) reached to 1.5, suggesting that ozone cannot mineralize Ace in one step but produces intermediate products that are more soluble in water than Ace. Then, TOC is kept although more ozone is input and reacts with Ace to produce more intermediates. This may be ascribed to the limitation

of solubility of the intermediate products and small part of them is mineralized. In one word, ozonation improves solubility of organic compounds in ozonized sample and little of Ace is mineralized while Ace being degraded.

The OUR of Ace was the lowest while that of intermediate products of ozonized sample was much higher in this study, indicating that ozonation can obviously improve the biodegradability of Ace, and that the ozonized products can be degraded by activated sludge. Ozonation may be an alternative pretreatment method for recalcitrant PAHs like Ace.

Fifteen organic compounds were identified in ozonized sample in this study, including acid, ester and derivatives of phenol. All the intermediates have been reported in ozonation of other PAHs constructed only with benzene rings, although the Ace has obviously different saturated C-C bond in molecule. The similarity of intermediate products indicates that the reaction mechanisms of ozonation of PAHs are the same. In addition, the intermediates are less recalcitrant to microbes than Ace as previously reported^[8].

In conclusion, ozonation decomposes Ace and improves the biodegradability of Ace, suggesting that

ozonation in combination with biological treatment is probably an efficient and economical way to mineralize acenaphthene in wastewater.

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