Laboratory Investigations Into the Fate of Aromatic Pollutants in Natural Waters

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Abstract

Condensed phases in the atmosphere, such as cloud droplets and aerosols, often contain both water and organic matter (OM). Reactivity can differ significantly between aqueous and organic phases. We have measured photolysis kinetics of the polycyclic aromatic hydrocarbons (PAHs) anthracene and pyrene in several organic solvents and in water, in miscible and phase-separated aqueous-organic mixtures, in the presence of halide salts (NaCl, NaBr, and NaI), and in simulated seawater. Moreover, the roles of singlet oxygen and pH were investigated on the kinetics at atmospherically-relevant wavelengths. Photolysis rate constants generally increased with increasing solvent polarity. Our results suggest that OM could greatly affect the photochemical lifetimes of PAHs in atmospheric condensed phases such as aerosols, even if the OM does not itself absorb photons. Our results also indicate that the fate of PAHs in saline waters, such as oceans and atmospheric particulate matter, could be significantly different than those predicted by kinetics measured in deionized water.

Dark Fenton chemistry is an important source of hydroxyl radicals (OH) in natural waters in the absence of sunlight. We have investigated the effects of the iron-reducing bacteria *Shewanella oneidensis* (SO) on OH production rates from Fenton chemistry. We also demonstrate that bacteria-assisted Fenton chemistry can result in rapid degradation of aromatic pollutants such as anthracene. Our results suggest that iron-reducing bacteria such as SO may be important contributors to radical formation in dark natural waters.
Laboratory Investigations Into the Fate of Aromatic Pollutants in Natural Waters

By

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Dissertation

Submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in Chemistry.

Syracuse University

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Chapter One:

Introduction
1.1 Pollutants in the Environment

1.1.1 Production/Emission

PAHs are common pollutants that are produced from anthropogenic processes such as combustion for heat and power, the steel industry, coal mining, wood burning, incineration practices, forest fires, metal processing, and others.\textsuperscript{1-5} According to the Environmental Protection Agency (EPA), the average car produces approximately 30 pounds of polluting VOCs (Volatile Organic Compounds) alone annually, most of which are PAHs.\textsuperscript{6} There are approximately 30,000 tons of PAHs emitted per year in the USA.\textsuperscript{6,7} Half a million tons are emitted per year globally.\textsuperscript{7} PAHs are also produced naturally through vegetative decay and forest fires.\textsuperscript{2,4} It is also debated that PAHs may be naturally synthesized by bacteria, yeasts, some plant life in aquatic environments.\textsuperscript{2,4,8} For the anthropogenic emissions, approximately 20\% of total annual PAH emission is due to transportation sources using standard gasoline.\textsuperscript{1,7,9} Gasoline, being a mobile PAH source, has the potential to distribute PAHs over wide areas; stationary sources such as residential heating and industrial processes also have the potential to distribute PAHs due to long range transportation processes.\textsuperscript{1,4,10,11}

1.1.2 Physical Fate/Transportation/Partitioning

PAHs are produced through incomplete combustion processes and therefore are often found in the air or associated with particles.\textsuperscript{12-15} The higher molecular weight PAHs with numerous rings have low vapor phase concentrations and exist mostly as particle bound species and partitioning from air to condensed phases is common for PAHs.\textsuperscript{12-14,16}
Henry’s law constants of PAHs span a wide range and predict favorable partitioning of some PAHs to water over air. Several studies have detected PAHs in environmental waters, thus PAHs can be expected to be found in and adversely affect water sheds.\textsuperscript{18-22} PAHs have high octanol-water partition coefficients, with Log $K_{OW}$ ranging from 3 to 7 depending on the PAH. They are also non-polar and hydrophobic. When in the presence of mixed organic and aqueous phases, PAHs may primarily associate with the organic phase. Therefore, PAHs are expected to be found primarily associated with organics in condensed phases due to their $K_{OW}$. However, there is often very little organic matter present in natural waters, so $K_{OW}$ may be largely irrelevant allowing PAHs to associate with the aqueous phase in environmental media, shown by multiple field studies that find them in aqueous environmental media.\textsuperscript{23-27}

PAHs are often found in surface waters. Many of these pollutants end up in water bodies through several processes such as sewage effluent, runoff, atmospheric deposition (specifically wet deposition), and petroleum product spills.\textsuperscript{2, 4, 13, 15-17, 26} PAH presence in coral reefs has been found to be significantly higher than in the ambient sediment in seawater.\textsuperscript{2, 26, 28} The presence of PAHs in corals has been attributed to land run-off and from petroleum byproducts.\textsuperscript{4, 26, 29} In-cloud scavenging (PAHs being taken up by cloud droplets) is common for PAHs adsorbed to aerosols in the environment.\textsuperscript{2, 4, 26, 30-32} Most of the larger PAHs found in the particle phase are adsorbed to aerosols, which can be washed out through wet deposition and these PAHs can then interact with other chemical species if present in water bodies.\textsuperscript{2, 33} PAHs can also be deposited on land via aerosols and dry deposition and are often found in soil in these areas.\textsuperscript{2, 4, 6, 13, 15, 25}

PAHs are detected in remote regions of the planet due to the fact that they can be transported in the atmosphere. Larger PAHs have been shown to be so tightly adsorbed to soot particles that they can become part of the particle structure or hydrogen bond with the particle
Adsorption of PAHs onto organic matter is also common, which can lead to association with particles.\textsuperscript{17} Gas phase and particle phase transport are both possible for PAHs.\textsuperscript{17, 35}

Other methods of transport that do not involve aerosols or atmospheric particles are also common. In polar regions or in wintry conditions, PAHs can be incorporated into or scavenged by snowflakes leading to their presence in snowpacks and ice. The PAHs can also travel with the snowmelt into ocean water, ultimately leading to entrapment in sea ice and/or ocean transport.\textsuperscript{33, 36-39} This allows for long-range transport of the PAH molecules to other environments or areas where the PAHs were not produced.\textsuperscript{2, 13, 15}

\textit{1.1.3 Chemical Fate of PAHs in the Environment}

\textit{1.1.3.1 Photochemistry}

Common chemical sinks for PAHs include photochemical pathways, reactions with high energy species such as radicals, and biotic degradation.\textsuperscript{2, 4, 22, 40-47} PAHs can react both in the gas phase and in condensed phases.\textsuperscript{48} PAHs often react through photochemistry when present in water or aerosols. PAH reaction kinetics vary across the environment, as reaction kinetics are often slow in the gas phase, but faster in water.\textsuperscript{2, 4, 48} Photochemical reactions, or photolysis, of PAHs in the environment can often produce oxygenated products such as quinones, nitrated products, and other toxic compounds.\textsuperscript{2, 4, 49, 50} Reaction mechanisms can vary between condensed phases; for example, different photolysis products have been reported in aqueous and organic phases.\textsuperscript{2, 41, 44, 48, 51}
1.1.3.2 Reactions of PAHs in Natural Waters in the Absence of Sunlight

1.1.3.2.1 Radicals

PAHs react quickly in gas and condensed phases with hydroxyl radicals (OH). In condensed phases these are usually produced by the photo-Fenton reaction and photolysis of OH precursors such as nitrite, nitrate, hydrogen peroxide, and dissolved organic matter. These processes do not occur in the dark. Not all chemistry in the environment occurs from interactions with light. Important chemical processes occur at night or under limited light. This dark chemistry is a key component in environmental processes as many chemical species are produced under these conditions that cannot be produced during the day due to competing reactions with sunlight. Radicals are a propagator of atmospheric chemistry during the day. In the absence of light, dark Fenton chemistry is a primary radical source. Fenton chemistry proceeds by producing hydroxyl radicals through oxidation of Fe(II) to Fe(III) with hydrogen peroxide, then regenerating Fe(II) again through the reduction of Fe(III) by hydrogen peroxide. This is one of the few radical producing reactions that occurs in the absence of light. However, this chemistry is negligible at pH’s above 4, which is below the pH of most natural waters. The slower rate of the Fenton reaction at near-neutral pH is due to reduced iron solubility at higher pHs as well as the reduction of iron being rate limiting and also slow at higher pHs in the Fenton reaction. Therefore any enhancement in radical production at night will significantly affect oxidizing capacity of natural waters and total nighttime radical loads. These radical species are then free to interact with aromatic pollutants present in the media that persist due to weak absorbance of sunlight or to the absence of light.
1.1.3.2.2 Ozone

Ozone has also been implicated in the degradation of PAHs in the absence of light.\textsuperscript{22, 68} Ozonation of PAHs at the air-aqueous interface has been shown to proceed at a much faster rate than that of gas-phase reactions.\textsuperscript{69, 70} This process may be important in the environment as a degradation pathway for aromatic pollutants in the absence of light.

1.1.3.2.3 Bacteria

Bacteria can oxidize organic species, including pollutants such as PAHs.\textsuperscript{71, 72} This is an important process in natural waters and sediments as these bacteria oxidize species that are not readily degraded through other processes, such as aromatic pollutants.\textsuperscript{73, 74} Common environmental bacteria genera can reduce metals.\textsuperscript{75, 76} Some metal-reducing bacteria thrive in aerobic and anaerobic environments and are able to reduce metals without the need for light or acidic conditions.\textsuperscript{77-79} This type of bacteria has recently been detected in atmospheric aerosols near a heavily contaminated creek.\textsuperscript{80} These bacteria can also lead to the formation of secondary aerosols via the oxidation of dissolved organic species.\textsuperscript{81-83} The effects of bacteria on heterogeneous and multiphase chemistry in atmospheric aerosols and cloud droplets is an area of growing interest.\textsuperscript{2, 80, 84}

Bacteria have also been used in the waste remediation field to break down pollutants in techniques such as biostimulation and bioaugmentation.\textsuperscript{85-88} These bacteria may affect the oxidant load in dark waters through production of highly reactive chemical species such as hydroxyl radicals and reactions with pollutants themselves, although OH production rates from processes involving bacteria have not been reported.
1.1.4 Health and Ecosystem Effects

PAHs are dangerous pollutants in that they have many routes of environmental exposure and have detrimental human health effects. Mutagenicity testing show that PAHs cause DNA mutations at a frequency 10-100 times higher than their parent petroleum products.\(^8\) Reaction products of PAHs are often even more toxic than their parent PAHs as well.\(^2,\ 4,\ 90\) Reactions of PAHs in the environment can produce quinones, nitrated products, and other toxic compounds that have been shown to adversely affect human health.\(^2,\ 4,\ 49,\ 50\) These products can interfere with metabolic processes as well as exhibit mutagenic, carcinogenic, and genotoxic effects in humans.\(^49,\ 50,\ 90\) The lower molecular weight PAHs with fewer aromatic rings exist almost exclusively in the vapor phase in the atmosphere, which can lead to detrimental human health effects due to ease of inhalation.\(^2,\ 4,\ 13,\ 14,\ 16,\ 17\) Because of health concerns, PAHs are of interest to the EPA and other health organizations.

Studies have shown that PAH-DNA adducts can be created through metabolic processes in the liver leading to detrimental genotoxic effects in the human body when PAHs are ingested.\(^91,\ 92\) PAH reaction intermediates and excited states have also proven to be quite toxic in their own regard without the need to enter the body directly; studies have shown that certain PAHs in their excited states can form covalently bonded PAH-DNA adducts.\(^93-97\) These adducts can form both in the body and on the body in skin cells leading to genotoxicity. PAHs also exhibit other genotoxic effects, such as strand cleavage, leading to destruction of genetic material in mammalian cells.\(^98,\ 99\)

PAHs do not only affect mammalian organisms. PAHs have been shown to have a significant impact on aquatic life such as zooplankton abundance and health.\(^100,\ 101\) This can then
in turn cause detrimental differences in the biotic community of aquatic ecosystems. PAHs have also been implicated in coral bleaching and detrimental effects on aquatic plant life as well.\textsuperscript{29,102}

Terrestrial plant life is also susceptible to being affected by PAHs. Plant species such as trees have been shown to respond adversely to elevated PAH concentrations. For example, the spruce tree develops a yellowing of the leaves when exposed to PAHs as well as growth inhibition in new saplings and seeds.\textsuperscript{104}

1.2 Water in the Environment

Surface waters and atmospheric condensed phases are important chemical reactors in the environment.\textsuperscript{48,105-107} Most of the PAHs in these environmental media are degraded through oxidative and photochemical processes.\textsuperscript{23,27,40,108,109} Local physicochemical properties can be altered by the presence of different constituents of surface waters and different compositions of condensed phases can lead to significant changes in reaction kinetics.\textsuperscript{27,43,110-113}

Water in the environment is complex. In natural waters, there can exist many constituents such as ions, organics, and other species.\textsuperscript{106,107,114} These constituents can affect the photochemical mechanisms and reaction kinetics of chemical species present in the medium.\textsuperscript{27,47,113,115-121} For example, seawater contains halides such as chloride, bromide, and iodide. Chloride is found at molar concentrations in seawater, while bromide is found on the order of micromolar, and iodide on the order of nanomolar.\textsuperscript{106,107,122} Photolysis kinetics of some aromatic species have been reported to decrease in the presence of halide salts,\textsuperscript{116,118,119,123} and other species have been reported to increase.\textsuperscript{121,124-128} Halides also exist in aerosols in much higher local
concentrations than in water. Larger halides, such as iodide and bromide, have been shown to exhibit enhanced surface activity in water and aqueous aerosols, where they may further alter the local chemistry of PAHs.

Seawater and some freshwaters contain many different naturally occurring metals that can also affect reactivity of chemical species. Even at low concentrations metals can affect reaction kinetics and mechanisms of pollutant degradation through energy transfer and production of radical species. Sulfates and nitrates also exist in seawater and freshwater. Nitrate is able to affect PAH reaction kinetics by photolyzing and forming OH. Naturally occurring organic fractions in seawater (and some freshwaters), such as sea surface microlayers (SSM), can have high local surface concentrations and can greatly alter the local chemistry and reaction kinetics. Other common mechanisms that can play a role in mediating PAH fate are metal coordination chemistry, reactions with ozone, and biological processes, as well as indirect photochemistry such as photocatalysis and photosensitization.

Water is also centric to types of chemical reactions other than photochemistry. Reactive oxygen species can play an important role in chemical reactions within water. Radicals such as hydroxyl radicals can interact and degrade photochemically active and inactive species and create oxygenated products. Singlet oxygen is also present in water bodies and can interact with aromatic compounds and at high enough concentrations of singlet oxygen, enhance degradation of these compounds. This chemistry plays a large role in the breakdown of organic molecules that are not readily degraded by sunlight, such as aromatic species that don’t strongly absorb light at wavelengths longer than 290 nm.
1.3 Non-Chromophoric Organic Matter in the Environment

Organic matter is common in natural waters.\textsuperscript{48, 107, 132, 146} Reaction kinetics of PAHs and other pollutants can be affected by natural organic matter.\textsuperscript{27, 41, 112, 121, 147} Organic matter can be miscible with aqueous media or it can form distinct phases such as organic aerosols and organic films at the surfaces of lakes, aqueous aerosols, and fog and rain droplets. PAHs are often associated with these organic media due to their hydrophobicity and octanol-water partitioning coefficients.\textsuperscript{20, 21, 148} These organic coatings or films have been shown to affect the chemistry of adsorbed molecules such as PAHs.\textsuperscript{20-22} The effects of thin organic films at the surface of water on PAH photolysis kinetics has yet to be explored. Chromophoric dissolved organic matter (cDOM), or organic matter that absorbs sunlight, can affect PAH fate through participation in photochemistry. Many studies have investigated the effects of cDOM, but few have investigated the effects of non-chromophoric organic matter. Some studies suggest strong polarity dependences; however, this phenomenon has not been investigated thoroughly.

Not all aqueous and organic media is miscible.\textsuperscript{105, 149-153} Some of these phases can form heterogeneous mixed-phase aerosols, while some will form immiscible layers or films on water such as sea surface microlayers.\textsuperscript{18, 19, 153-158} Non-miscible mixed phase aerosols can have structure motifs such as core-shell morphologies as seen in Figure 1.1.\textsuperscript{150-152, 159} The location of PAHs in each phase-separated environment has implications on the reactivity of the PAH itself, as discussed in section 1.1.3.
Figure 1.1 Depictions of pure aqueous and pure organic aerosols and two different morphologies of mixed phase aerosols: miscible on the left and immiscible on the right (core-shell).

1.4 Laboratory Studies of PAH Photochemistry

Photochemical reactions are important environmental transformations. However, the environment can often be too complex to elucidate reaction mechanisms. Controlled experimental conditions, such as those present in a laboratory, allow for quantification of kinetic data such as rate constants and quantum yields. These values are used to model the reactions that
occur within the environment. A number of different light sources are used to mimic natural sunlight in the laboratory. Common light sources include mercury and xenon arc lamps, and lasers. These lights sources have defined spectral irradiance and researchers can control this irradiance during laboratory studies. Light at wavelengths shorter than approximately 290 nm does not appreciably reach Earth’s surface due to absorption by stratospheric ozone.\textsuperscript{48, 133}

Many studies have used light sources that do not accurately represent irradiation in the natural environment. Unfiltered xenon arc lamps that emit light below 290 nm, lasers at short wavelengths that do not reach earth’s surface, and mercury lamps, which emit light at 254 nm, are sometimes used to study the photochemistry of PAHs.\textsuperscript{160-162} These studies are not atmospherically or environmentally relevant as sunlight at wavelengths shorter than 290 nm will be filtered out by the ozone in the stratosphere and will not affect PAHs in the troposphere or on the Earth’s surface. These shorter wavelengths effects photolysis on different absorption bands of PAHs, leading to alternate photolysis mechanisms.\textsuperscript{40, 42, 43, 45, 160, 163}

Many models extrapolate the rate constants in pure water to predict kinetics in organic phases and other environmental media. However, components in non-pure water can change reaction kinetics. Even in DI water without reactive species, rate constants may not be accurate predictors for other environmental media such as organic phases.\textsuperscript{27, 41, 112, 121} In order to understand the fate and chemistry of PAHs in the environment, one must explore the key components, such as organics and halides, in these environments as they may affect the photochemical fate of the PAHs in that environment.
1.5 Goal of Research

Most research into the fate of PAHs has been done using simplified conditions in laboratory studies with deionized water. However, deionized water is not a good proxy for the environment in which PAHs react. Understanding the reaction kinetics of PAHs is complicated as there are many components in natural waters and it is difficult to isolate the effects of each species. In order to elucidate each constituent’s effects on the photolysis mechanism of PAHs one must separate out all other variables. The purpose of the work reported in this thesis was to understand the effects of constituents in water that may greatly alter reactivity in order to predict reaction kinetics of PAHs in environmental condensed phases.

The primary research focus of this work is to develop tools to predict the fate of aromatic pollutants in the natural environment. In order to do this we have performed experiments under controlled conditions in order to obtain kinetic parameters. We have introduced complexities to these controlled conditions so that the results can be used as predictors of PAH fate in the environment. Investigating these complexities in a controlled manner can improve PAH reactivity predictions in a range of environmental media including fresh water, seawater, cloud droplets, and organic aerosols. More specifically, we have looked at the fate of PAHs in the natural environment and how the constituents of natural waters and different environmental media can affect reaction kinetics. Chapter two of this work reports the effects of organic matter on PAH photolysis kinetics. Our results indicate that non-chromophoric organic matter can affect PAH reactivity by altering the local polarity. Chapter three of this work reports the effects of ionic species, seawater, and singlet oxygen on PAH photolysis kinetics. Our results show that halides can have a large effect on photolysis kinetics of PAHs and indicate that singlet oxygen may play a role in PAH reactivity in the presence of halides. Chapter four of this work reports
the effects of a naturally occurring bacterium on the oxidizing capacity of natural waters in the absence of light and the effects of the bacterium on PAH degradation. Our results show that hydroxyl radicals are generated at levels much higher than previously considered in the presence of the bacterium and that PAH degradation is rapid under these conditions.

The determined rate constants of these reactions can be used to better predict the fate of these pollutants in atmospheric and environmental models as well. The results from this work can be used to predict the rate constants of PAHs in waters of varying polarity and salt concentrations as well as in the presence of radical species when there is no sunlight. This research can also be incorporated into models and help us improve predictions of PAH fate and human health effects.

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Chapter Two:

**Anthracene and Pyrene Photolysis Kinetics in Aqueous, Organic, and Mixed Aqueous-Organic Phases**

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2.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are toxic molecules. Chemical processing in the environment often increases their toxicity, so accurate reaction kinetics are required in order to correlate PAH emissions to health effects.\(^1\) Polycyclic aromatic hydrocarbons are often associated with (and can react in) atmospheric condensed phases including fog and cloud droplets as well as aqueous and organic aerosols.\(^2 - 5\) Quantifying PAH reaction kinetics in atmospheric condensed phases is difficult due to the wide range of physical environments presented. Fog and cloud droplets are composed primarily of water, with low concentrations of inorganic and organic solutes, while aerosols have much higher solute concentrations – some do not contain liquid water at all.\(^2, 3, 6\)

Polycyclic aromatic hydrocarbons are hydrophobic, and partition readily to organic phases in the environment. The presence of organic solutes may therefore greatly affect the chemical fate of particle-bound PAHs. A number of studies have reported PAH photolysis kinetics in aqueous solution, and the role of organic matter (OM) on kinetics has also received significant attention. The vast majority of these studies have focused on the effects of chromophoric OM – that is, OM that can absorb sunlight. Chromophoric OM can reduce pollutant photolysis rates by competitively absorbing photons, or it can increase rates by acting as a photosensitizer or by generating reactive species such as singlet oxygen and hydroxyl radicals (OH).\(^7\) Chromophoric OM in oil spills, organic aerosols, and natural waters can affect PAH photolysis kinetics in complex ways.\(^8 - 15\)

A significant fraction of OM in aerosols and liquid droplets in the atmosphere consists of molecules that do not absorb sunlight (“non-chromophoric” OM).\(^3, 16\) While these compounds
will not act as photosensitizers or undergo photolysis themselves, they may alter PAH reactivity by changing the local environment. For example, the photolysis of some PAHs has been reported to be markedly slower in non-chromophoric organic solvents than in aqueous solution, and different PAH photolysis products have been reported in aqueous and organic solvents. The mechanisms by which non-chromophoric OM affects PAH photolysis remain unclear. Although literature exists on this topic, it is often not applicable to PAH photolysis in the environment, since photolysis in laboratory studies is often effected at wavelengths much shorter than those present at Earth’s surface. Photolysis at these short wavelengths can occur via different mechanisms than in the environment; for example, anthracene is directly ionized by 254 nm radiation, but reacts primarily via an excited triplet state under irradiation at longer wavelengths relevant to sunlight at Earth’s surface. It is currently thought that PAH photolysis is slower in organic solvents than in aqueous solution due to polarity effects, but this has not been rigorously investigated at environmentally-relevant wavelengths.

Further complicating quantification of the effects of OM on photolysis kinetics is the fact that atmospheric particulate matter rarely consists of a pure aqueous phase or a pure organic phase. Homogeneous aqueous phases containing OM are common (for example in fog and cloud droplets). To our knowledge, only one study has measured PAH photolysis kinetics in miscible aqueous-organic mixtures at environmentally-relevant irradiation wavelengths: lower anthracene and benzo[a]anthracene photolysis quantum yields were reported in aqueous solutions containing 50% acetonitrile than in solutions containing 30% acetonitrile. This result needs to be expanded upon to enable predictions of PAH photolysis kinetics in aqueous-organic particles. Specifically, measurements performed over a range of organic volume fractions would enable predictions of rate constants based on organic content.
Phase-separated organic-aqueous mixtures also abound in environmental particulate matter. Many organic compounds are surface-active, and aqueous particulate matter is often coated with monolayers or multilayers of OM.\textsuperscript{22-25} These coatings may provide distinct microenvironments to which PAHs can partition, and in which reactivity may differ from that in the dominant phase. Organic monolayers enhance the surface activity of PAHs in aqueous solution, and heterogeneous ozonation kinetics of PAHs at organic-coated water surfaces can be quite different from those at pristine water surfaces.\textsuperscript{24-27} The potential effects of these organic coatings on PAH photolysis kinetics have not been reported.

Larger organic fractions in atmospheric particulate matter can lead to the formation of two distinct bulk phases within an aerosol. Some common morphologies of phase-separated aerosols include core-shell structures and lenses. In core-shell structures, the inner phase is completely surrounded by a largely uniform layer of the outer phase (as in the yolk and white of a hard-boiled egg). In a lens structure, the outer layer is not uniform, but resides almost entirely on one side of the inner phase (somewhat like a hat perched on top of a head).\textsuperscript{28-32} Both structures present two distinct bulk phases. Little is known about PAH photolysis kinetics in phase-separated particulate matter. Work in our group has demonstrated that even very small concentrations (< 3 mM) of the water-immiscible organic solvents octanol and decanol reduce PAH photolysis rates in ice and at ice surfaces, likely due to the formation of a distinct organic phase to which the PAHs partition.\textsuperscript{33} To our knowledge this is the only study to report photochemical kinetics in phase-separated aqueous-organic mixtures, and we are not aware of any studies reporting kinetics in liquid immiscible aqueous-organic mixtures.

We measured anthracene and pyrene photolysis kinetics in aqueous solution and in several organic solvents, as well as in miscible and immiscible aqueous-organic mixtures. The
The aims of this work are as follows: (1) To elucidate which factors (e.g. polarity and singlet oxygen concentration) most strongly affect kinetics; (2) to determine whether photolysis kinetics in homogeneous organic-aqueous solutions (such as fog and cloud droplets) can be accurately predicted by OM content or some other variable (such as polarity); and (3) to determine the effects of organic monolayers and macroscopic organic phases on PAH photolysis in atmospheric particulate matter such as aqueous-organic aerosols with core-shell morphology. The implications of this work extend beyond atmospheric aerosols to other environments in which aqueous-organic mixtures exist, such as surface waters containing high OM loadings, and ocean surfaces in the presence of organic coatings such as sea-surface microlayers or oil slicks.

2. Materials and Methods

2.1 Materials

Solutions containing anthracene (Acros Organics, 99%) or pyrene (Alfa Aesar, 98%) in 18.2 MΩ·cm deionized water, methanol (MeOH, Sigma-Aldrich, ≥99.8%), dimethyl sulfoxide (DMSO, Sigma-Aldrich, ≥99.5%), isopropanol (2-prop, Sigma-Aldrich, ≥99.5%), acetonitrile (ACN, Alfa Aesar, ≥99.5%), octanol (Acros Organics, 99%), or decanol (Alfa Aesar, 98%) were newly prepared every week. Solutions containing hydrogen peroxide (H₂O₂, Fluka, ≥30%), benzoic acid (BA, Sigma-Aldrich, ≥99.5%), salicylic acid (SA, Alfa Aesar, 99%), 3-hydroxybenzoic acid (3-HBA, Sigma-Aldrich, 99%), 4-hydroxybenzoic acid (4-HBA, Sigma-Aldrich, ≥99%), furfuryl alcohol (FFA, Acros Organics, 98%), and water with 0.1% trifluoroacetic acid (“TFA water”, Fluka) were prepared immediately prior to use.
2.2.2 Photophysics

Absorption spectra of anthracene and pyrene were acquired in each organic solvent using a Cary 50 Bio UV-Vis spectrometer and a 1 cm path length quartz cuvette. Absorption spectra of the PAHs in 18.2 MΩ·cm deionized water and methanol were also acquired using a 10 cm path length quartz cuvette. Molar absorptivities in water and methanol were determined from the slope of the best-fit line to a plot of absorbance vs. PAH concentration, as shown in the Appendix. Steady-state fluorescence spectra of anthracene and pyrene were acquired in a 1 cm quartz cuvette using a Photon Technology International QuantaMaster 40 fluorimeter.

2.2.3 Photolysis

Photolysis was performed in a sealed air-saturated 1 cm path length quartz cuvette unless otherwise noted. Samples were irradiated with the output of a 150 W xenon arc lamp that was reflected off a cold mirror for heat dispersion and passed through a 295 nm long-pass cutoff filter to cut out wavelengths that are filtered out by Earth’s atmosphere. The distance between the lamp and the sample was ~65 cm. Fluorescence spectra of anthracene and pyrene were acquired prior to photolysis and after multiple known irradiation times until intensity no longer varied with photolysis time. The excitation wavelength was 272 nm for pyrene and 356 nm for anthracene, and wavelength-resolved emission of both species was detected between 360 and 460 nm. Rate constants were determined by monitoring the decrease in PAH fluorescence intensity over time. Pyrene fluorescence was monitored at 392 nm and anthracene fluorescence at 405 nm. First- and second-order rate constants were determined from the slope of the best-fit line to the data plotted as ln(I/I₀) or 1/I respectively, as shown in Equations 1 and 2:
\[
\ln \frac{I}{I_0} = -kt \quad (1^{\text{st}} \text{ order})
\]

\[
\frac{1}{I} = \frac{1}{I_0} + kt \quad (2^{\text{nd}} \text{ order})
\]

Where \( I \) is fluorescence intensity at time \( t \), \( I_0 \) is initial fluorescence intensity, and \( k \) is the rate constant. Anthracene and pyrene concentrations in solutions were \( 1.5 \times 10^{-7} \text{ M} \) and \( 3.0 \times 10^{-7} \text{ M} \) respectively for most experiments, although anthracene photolysis was also performed at concentrations of \( 7.5 \times 10^{-8} \text{ M} \). Photolysis of both PAHs was also performed at a concentration of \( 3.0 \times 10^{-5} \text{ M} \) in methanol.

For one set of experiments, octanol or decanol was mixed with 50 mL of an aqueous solution containing anthracene in a 6.5 cm diameter by 1 cm deep glass dish to form a 2.5 or 7.5 mM aqueous solution of the organic solvent, and the output of the lamp was directed onto the sample from above after the sample had been allowed to sit for at least 15 minutes to enable the formation of a monolayer or multilayers of the organic solvent at the water surface. Photolysis kinetics were analyzed by extracting 2 mL aliquots of the sample with a pipette after known time periods and transferring them to a quartz cuvette for fluorescence analysis. Samples were either extracted after mixing the solution or by carefully pipetting from the bottom of the solution to reduce disturbance of the organic phase. The extraction method did not affect the observed rate constants. For another set of experiments, water was added to anthracene solution in octanol to form a phase-separated aqueous-organic mixture containing between 2 M and 42 M (approximately 10\% to 75\% by volume) water in a small quartz bowl (17 mm diameter and 15 mm depth, with a volume of approximately 3 mL). After a known time period of irradiation (with the lamp output directed onto the sample from above), the solution was transferred to a quartz cuvette for fluorescence analysis, then replaced in the quartz bowl to undergo further
photolysis. Experiments were done with either constant stirring or stirring during analysis only (samples stirred for analysis only were allowed to equilibrate in the quartz reaction vessel before undergoing further photolysis). Photolysis was affected and analyzed as described for the individual solvents.

2.2.4 Chemical Actinometry

Chemical actinometry was performed to determine the photon flux reaching the sample. Full details are provided in the Appendix. The rate constant of hydroxyl radical (OH) production from H₂O₂ photolysis was measured, using benzoic acid as an OH trap. We determined the total photon flux in the actinic region reaching our samples to be \((1.47 \pm 0.04) \times 10^{14}\) photons cm\(^{-2}\) s\(^{-1}\). For comparison, actinic sunlight reaching Earth’s surface on a clear summer day at noon is on the order of \(10^{16}\) photons cm\(^{-2}\) s\(^{-1}\).

2.3 Results and Discussion

2.3.1 Photolysis Kinetics in Aqueous and Organic Solvents

2.3.1.1 Role of Polarity on Photolysis Kinetics

Log-normalized anthracene fluorescence intensity as a function of irradiation time in water and in methanol are shown in Figure 1.1. The slope of the linear fit to the data provides the first-order photolysis rate constant, as described by Equation 1. Equation 1 described the kinetics well in all solvents, and rate constants measured at concentrations spanning two orders of magnitude in methanol were independent of concentration. These results suggest that anthracene
and pyrene photolysis is first order in these solvents (and by inference in aqueous and organic atmospheric condensed phases).

![Figure 2.1. Anthracene fluorescence intensity as a function of irradiation time in water and in methanol. The solid traces are linear fits to the data.](image)

As shown in Figure 2.1, anthracene photolysis is faster in water than in methanol. Previous studies have reported faster PAH photolysis in water than in organic solvents, with the differences generally ascribed to polarity effects. To test this hypothesis, we measured anthracene and pyrene photolysis kinetics in five organic solvents and in water. None of the solvents employed absorb photons at the wavelengths emitted by the lamp used in our study, so the solvents will not participate directly in photochemistry (e.g. by acting as photosensitizers or by undergoing photolysis and producing reactive intermediates). Anthracene and pyrene photolysis rate constants in the different solvents are shown in Figure 2.2.
Figure 2.2. First-order photolysis rate constants of (a) $1.5 \times 10^{-7}$ M anthracene and (b) $3.0 \times 10^{-7}$ M pyrene in various solvents shown as a function of solvent polarity as measured by the pyrene polarity scale. Anthracene photolysis kinetics in decanol and pyrene photolysis kinetics in octanol and DMSO were below our detection limit, which we estimate to be approximately $1 \times 10^{-5}$ s$^{-1}$ based on the smallest rate constant we have successfully measured. Rate constants in the aprotic solvents (ACN and DMSO) are represented by open symbols. Error bars represent the standard deviation about the mean of at least three trials.
Polarity in these studies was determined by the pyrene polarity scale. \(^{35}\) Briefly, pyrene fluorescence intensity at 372 nm increases relative to that at 382 nm with increasing polarity. The ratio of these peaks is referred to as the 1:3 ratio, and is used as a measure of local polarity. Table S1 in the appendix shows our measured 1:3 ratios along with literature 1:3 ratios and dielectric constants for each solvent. Our measured ratios are in excellent agreement with literature values for all solvents. The pyrene polarity scale and dielectric constant indicate similar relative polarities for each solvent except for the aprotic solvents acetonitrile and DMSO. Polarity as measured by the pyrene scale is greater for these solvents than that predicted by dielectric constant because dielectric constant is sensitive to hydrogen bonding interactions. \(^{35,37}\)

Anthracene and pyrene photolysis rate constants increased with increasing pyrene 1:3 ratios, with the exception of the aprotic solvents acetonitrile and DMSO, in which pyrene photolysis was slower than expected based on polarity. These results suggest that local polarity will greatly affect PAH photolysis kinetics, and that photolysis in organic aerosols may proceed much more slowly than in aqueous aerosols. Pyrene’s suppressed reactivity in acetonitrile and DMSO may indicate that the hydrogen bonding nature of the local environment will affect pyrene photolysis rates in condensed phases. Although polarity describes most differences in anthracene and pyrene photolysis rate constants observed in the different solvents, we investigated several other factors that could affect reaction kinetics, including PAH molar absorptivity, self-association, and molecular oxygen content (ground state and excited singlet state).

2.3.1.2 Role of Molar Absorptivity on Photolysis Kinetics
We measured anthracene and pyrene molar absorptivities at wavelengths at which they absorb sunlight strongly (355 and 335 nm respectively) in water and methanol to determine whether the differences in measured photolysis rates could be due to changes in molar absorptivity in aqueous and organic solvents at atmospherically-relevant wavelengths. Molar absorptivity calculations are shown in the Appendix. Anthracene’s molar absorptivity at 355 nm was \((6.4 \pm 0.1) \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}\) in methanol, and was too small to measure in water. Pyrene’s molar absorptivity at 335 nm was \((1.65 \pm 0.09) \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}\) in methanol, and \((1.94 \pm 0.09) \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}\) in water. We also acquired absorbance spectra of \(3.0 \times 10^{-5} \text{ M}\) pyrene and anthracene in each solvent (except water due to solubility constraints). These spectra are shown in Figures S5 and S6 in the Appendix. Figure 2.3 shows total pyrene and anthracene absorbance (between 300 and 400 nm) in each solvent. Pyrene absorbance in water at this concentration was calculated by scaling the measured absorbance at \(3.0 \times 10^{-7} \text{ M}\) to \(3.0 \times 10^{-5} \text{ M}\) using the measured absorption cross-section at 335 nm. Since anthracene absorbance in water was too low to measure at any concentration, it is not included.
**Figure 2.3.** Absorbance of $3.0 \times 10^{-5}$ M pyrene and anthracene between 300 and 400 nm in each solvent. The absorbance of pyrene in water (denoted with *) was calculated as described in the text.

Pyrene and anthracene molar absorptivities were much larger in organic solvents than in water. Absorbance was similar in the different organic solvents, with the exception of DMSO, in which anthracene and pyrene absorbance were 65% and 140% greater, respectively, than the average absorbance in the other solvents. Based on these measurements, we conclude that the different photolysis kinetics of anthracene and pyrene measured in different solvents is not due to changes in molar absorptivity.

2.3.1.3 Role of PAH Self-Association on Photolysis Kinetics

At high concentrations, PAHs can self-associate and form excimers, which can have different absorption spectra and photolysis mechanisms than the monomers. Anthracene excimer emission is characterized by a reduction in the 380 nm emission band relative to the 405 nm band, and pyrene excimer formation is characterized by a broad emission band centered around 460 nm. We analyzed anthracene and pyrene emission spectra in each solvent and observed no evidence of excimer formation at the concentrations used in our experiments. Therefore we attribute the measured kinetics to photolysis of the PAH monomers.

2.3.1.4 Role of Oxygen on Photolysis Kinetics
Table 2.1 shows molecular oxygen solubilities in some solvents used in this study, as well as anthracene and pyrene photolysis rate constants in these solvents. Molecular oxygen is more soluble in organic solvents than in water, but photolysis was fastest in water.\textsuperscript{38-40} Since anthracene and pyrene photolysis kinetics have been shown to increase with increasing oxygen concentrations, we conclude that molecular oxygen content in the different solvents does not account for the slower photolysis observed in non-polar organic solvents.\textsuperscript{20}

Table 2.1. Molecular oxygen solubilities and anthracene and pyrene photolysis rate constants in aqueous solution in the presence and absence of furfuryl alcohol. Experiments were performed in an unsealed air-saturated cuvette.

<table>
<thead>
<tr>
<th>Molecular Oxygen Solubility (mM)\textsuperscript{a}</th>
<th>Rate Constant ($\times 10^{-5}$ s\textsuperscript{-1})\textsuperscript{b}</th>
<th>Anthracene</th>
<th>Pyrene</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH 2.15</td>
<td>2.3 ± 1.6 (3.4 ± 0.4)</td>
<td>4.1 ± 2.0 (4.2 ± 0.4)</td>
<td></td>
</tr>
<tr>
<td>ACN 2.42</td>
<td>17 ± 5 (12 ± 2)</td>
<td>1.7 ± 0.5 (1.5 ± 0.5)</td>
<td></td>
</tr>
<tr>
<td>H\textsubscript{2}O 0.26</td>
<td>23 ± 2 (22 ± 4)</td>
<td>18 ± 4 (18 ± 5)</td>
<td></td>
</tr>
</tbody>
</table>

a) From Ref 40

b) Numbers in parentheses represent the rate constant measured in the presence of 10 mM furfuryl alcohol.

It is possible that steady-state singlet oxygen concentrations, rather than total molecular oxygen concentrations, vary between solvents and affect the observed photolysis kinetics. Since singlet oxygen is thought to participate in PAH photolysis, this could explain some of the observed variations in PAH reactivity in different solvents.\textsuperscript{19-21} We measured anthracene and pyrene photolysis rate constants in solutions containing 10 mM furfuryl alcohol to investigate the role of singlet oxygen on the observed photolysis kinetics. Furfuryl alcohol is an effective singlet
oxygen trap, so if singlet oxygen plays a significant role in anthracene or pyrene photolysis, we expect measured rate constants to decrease in its presence. As shown in Table 2.1, anthracene and pyrene rate constants were the same within experimental uncertainty in the presence and absence of furfuryl alcohol in each solvent. Previous experiments and calculations suggest that although singlet oxygen is reactive toward PAHs, its concentration will be too low to noticeably affect PAH photolysis kinetics even in natural waters that contain significant singlet oxygen sources such as chromophoric OM. We stress that this does not mean that singlet oxygen is unreactive toward PAHs; our results simply imply that it does not affect photolysis kinetics under our experimental conditions, and that different singlet oxygen concentrations and lifetimes in different organic and aqueous solvents can not explain the different photolysis kinetics measured in the different solvents.

2.3.2 Photolysis in Miscible Aqueous-Organic Mixtures

We measured anthracene and pyrene photolysis kinetics in aqueous solutions containing various volume fractions of methanol or DMSO to investigate the effects of mixed organic-aqueous phases, which are common in the environment, on PAH photolysis kinetics. Figure 2.4 shows the effects of methanol on anthracene and pyrene photolysis kinetics. A decrease in reaction rate was observed with increasing methanol concentration: Anthracene’s measured rate constant decreased by $1.8 \times 10^{-5}$ s$^{-1}$ for every ten percent volume increase of methanol, and pyrene’s photolysis rate constant decreased by $1.6 \times 10^{-5}$ s$^{-1}$ for each additional ten percent volume of methanol. Figure S7 in the Appendix shows the effects of DMSO on the photolysis
kinetics: Anthracene photolysis rates did not vary significantly with DMSO concentration, but pyrene photolysis was immeasurably slow at DMSO concentrations as low as 25% by volume.

Figure 2.4. Effect of methanol on photolysis kinetics in aqueous solution of (a) anthracene and (b) pyrene. Error bars represent the standard deviation about the mean of three trials.

Our results show that PAH photolysis may be impeded by small organic molecules that do not absorb sunlight. Such small molecules (such as methanol and propanol) make up significant fractions of organic mass in fog and cloud droplets,\(^3,16\) although reduced photolysis
rates are only likely to be observed in the presence of high OM loadings, such as are found in organic and aqueous-organic aerosols (\textit{vide infra}). The results also indicate that the common laboratory practice of using organic solvents to increase PAH solubility in water could introduce artifacts in reported photolysis rate constants, since the presence of organic solvents will alter measured photolysis kinetics compared to those in pure water. We note that larger organic molecules such as those in chromophoric OM may provide very different local environments to PAHs and may not affect photolysis in the same way as the smaller organic molecules investigated in this work.

\textit{2.3.3 Photolysis in Immiscible Aqueous-Organic Mixtures}

The aqueous-organic mixtures discussed above were homogeneous solutions. However, many types of OM are not miscible with water, and as discussed in the Introduction, water in the environment is often coated by organic films.\textsuperscript{24,25} Polycyclic aromatics partition to water surfaces; this surface activity is enhanced in the presence of organic coatings.\textsuperscript{22-27} We measured anthracene and pyrene photolysis kinetics in aqueous solutions containing one to several monolayers of octanol or decanol at the water surface. The photolysis kinetics in samples containing up to 7.5 mM octanol or decanol did not differ significantly from those measured in pure water, despite the fact that photolysis in these organic solvents is at least an order of magnitude slower than in water.

In a separate set of experiments, we added water to octanol solutions containing 1.5 \times 10^{-7} M anthracene below and above the saturation limit of water in octanol (2.6 M). The average rate constant \((1.6 \pm 0.6) \times 10^{-5} \text{ s}^{-1}\) was indistinguishable from that in octanol \((1.4 \pm 0.5) \times 10^{-5}\)
s\(^{-1}\)), even at water concentrations as high as 10 M (~18 volume %). The results of these two sets of experiments suggest that PAH photolysis kinetics in aerosols and other environments containing immiscible aqueous and organic components will be well-described by kinetics in the dominant phase even in the presence of a few monolayers of a separate phase, be it organic or aqueous. Photolysis kinetics of the PAH within the minor phase may be quite different from those in the dominant phase, but the overall measured kinetics will likely reflect those predicted by the rate constant in the dominant phase.

Aerosols in the environment often contain distinct aqueous and organic *bulk* phases in which PAHs could react.\(^{28-30, 43}\) We measured anthracene photolysis kinetics in solutions containing significant fractions of both octanol and water. Figure 2.5 shows anthracene rate constants measured at various water/octanol ratios under stagnant (unstirred) and turbulent (stirred) conditions. When the solution was continuously stirred during irradiation, the photolysis rate constant increased with increasing water percentage, approaching that measured in pure water by 25% water by volume (~14 M water). Conversely, when samples were not stirred during photolysis, the observed rate constant increased only slightly with increasing water percentage; at 25% water by volume, kinetics were the same within the experimental uncertainty as those in pure octanol. More substantial increases in the rate constant were observed in stagnant solutions containing greater than 50% water by volume.
Figure 2.5. Effect of water on anthracene photolysis kinetics in octanol solution with constant stirring (solid black circles), and stirring during analysis only (open red squares). Error bars represent the standard deviation about the mean of three trials.

Although anthracene is primarily in the organic phase in these experiments, a small amount is in the aqueous phase, so photolysis can occur in both phases. If phase partitioning ceased after the initial equilibrium was established, we would not expect the observed rate constant to be measurably larger than that in pure octanol until water fractions exceeded 99%. However, anthracene concentrations in the aqueous fraction are depleted by photolysis more rapidly than in the organic fraction (by approximately a factor of eight), so anthracene will continually partition from the organic to the aqueous phase throughout the experiment to reestablish equilibrium concentrations. Increases in the measured photolysis rate constant were observed at water fractions well below 99% under both stagnant and turbulent conditions, likely due to equilibrium concentrations being reestablished throughout the course of the experiment. We hypothesize that stirring the sample increases the rate at which equilibrium is reestablished, leading to the greater observed dependence of the kinetics on water fraction under turbulent compared to stagnant conditions.
2.4 Atmospheric Implications

The local environment experienced by PAHs significantly affects the rate at which they are transformed (often to more toxic compounds) in atmospheric condensed phases. Variations in local polarity can change anthracene and pyrene photolysis kinetics by more than an order of magnitude. Thus, we predict that PAH photolysis kinetics will be significantly slower in organic aerosols than in aqueous aerosols (in the absence of species that participate in photochemistry). Photochemical reactivity in homogeneous mixed organic-aqueous particulate matter appears to be well-described by the relative volume fractions of organic and aqueous molecules. Our results suggest that thin organic coatings on aqueous aerosols, cloud and fog droplets, or surface water will not significantly affect the photochemical fate of anthracene and pyrene, and total concentrations of dissolved organic species in fog and cloud droplets are well below those at which we observed suppression of photolysis in our experiments.\textsuperscript{2, 3, 16} However, thicker organic coatings (such as exist in sea-surface microlayers, oil slicks coating surface waters, and aqueous-organic aerosols with core-shell morphology) may have an important effect on PAH photolysis kinetics. In these environments the dependence of photolysis kinetics on the composition is not straightforward, and is complicated by factors such as turbulence and mass transport. The photolysis products formed may also depend on solvent polarity. Anthracene photolysis in cyclohexane has been reported to form products that are not detected in aerated aqueous solutions, such as a photodimer.\textsuperscript{19}

We have demonstrated that solutes do not have to absorb solar photons in order to affect PAH photolysis kinetics in atmospheric condensed phases. While we have shown the large effects local polarity can have on photolysis kinetics, other physical properties such as viscosity
are likely also important. Predicting the fate of aromatic pollutants in atmospheric condensed phases therefore requires knowledge not only of the reactive species present, but also of the physical properties of the local environment.

2.5 References


Chapter Three:

Anthracene and Pyrene Photolysis Kinetics in Freshwater and Saltwater Environments
3.1 Introduction

Polycyclic aromatic hydrocarbons (PAHs) are toxic molecules. These pollutants are ubiquitous in environmental and atmospheric aqueous phases including surface waters and particulate matter.\textsuperscript{1-5} Photolysis is a common fate of PAHs in environmental waters; products are often even more toxic than the parent compounds.\textsuperscript{2} Accurate photolysis kinetics are therefore needed to predict the health effects of PAHs. These have been widely reported in aqueous solution, but most experiments do not account for the complex composition of natural waters, and may therefore not be applicable to photolysis in the environment.\textsuperscript{3, 6-12}

Halides are common components of surface waters and aerosols. A number of studies have investigated the effects of halides on the photolysis kinetics of aromatic species, with varying results. Photolysis rates of some aromatic species have been reported to increase in the presence of halide salts;\textsuperscript{10, 13-17} some have been reported to decrease;\textsuperscript{12, 18-20} and some have exhibited little or no dependence on halide concentrations.\textsuperscript{21, 22} Mechanisms by which halides affect photolysis rates are largely unknown; the few mechanistic studies in existence suggest complex and variable pathways. For example, halides have been reported to transfer electrons to photochemically-generated chlorophyll cations, forming reactive halogen atoms.\textsuperscript{23} Carbonyl compounds have been reported to photosensitize halides, also resulting in the formation of halogen atoms.\textsuperscript{24, 25} Irradiation of some aromatic-halide solutions has been reported to produce hydroxyl radicals via an unspecified mechanism.\textsuperscript{26} Finally, halides have been reported to enhance intersystem crossing of some aromatic species such as DOM via the heavy atom effect, resulting in enhanced singlet oxygen (\textsuperscript{1}O\textsubscript{2}) production.\textsuperscript{27} To further complicate matters, many studies have been performed under conditions not relevant to the environment; specifically, samples are often irradiated by light sources with output at wavelengths shorter than 290 nm.
which do not reach the Earth’s surface. This can lead to photolysis mechanisms that do not occur in the environment, especially given the fact that Cl-, Br-, and I- do not absorb sunlight.\textsuperscript{28, 29}

In this work, we quantify the effects of the halide salts NaCl, NaBr, and NaI on the photolysis rate constants of the PAHs anthracene and pyrene, and investigate the mechanisms by which halides affect PAH photolysis. We investigate factors such as ionic strength, pH, heavy atom effects, and the production of reactive species. Our results provide a better understanding of the effects of halide salts on PAH photolysis kinetics in natural waters, and may significantly improve chemical fate models of PAHs in saline environments.

3.2 Materials and Methods

3.2.1 Materials

Solutions containing anthracene (Acros Organics, 99%) or pyrene (Alfa Aesar, 98%) in 18.2 M\(\text{\Omega}\)-cm deionized water were newly prepared every week. Aqueous solutions containing sodium chloride (NaCl, Sigma-Aldrich, \(\geq 99.5\%\)), sodium bromide (NaBr, Sigma-Aldrich, \(\geq 99\%\)), or sodium iodide (NaI, Fisher Scientific, 99.9%) were newly prepared every week and combined with PAH solutions immediately prior to each experiment. Solutions containing sodium sulfate (Na\(_2\)SO\(_4\), Sigma-Aldrich, \(\geq 99.0\%\)), benzoic acid (Sigma-Aldrich, \(\geq 99.5\%\)), sodium hydroxide (NaOH, Sigma-Aldrich, \(\geq 97\%\)), hydrochloric acid (HCl, EM Science, 36.5 – 38\%), and 6-hydroxy-2,3-dihydro-6H-pyrano-3-one (pyranone, Oakwood Chemical, pure) were newly prepared every week. Instant Ocean\textsuperscript{©} (United Pet Group) was dissolved in deionized water for a final concentration of 58.5 g/L; these solutions were prepared each week. Solutions containing furfuryl alcohol (FFA, Acros Organics, 98%) and rose bengal (RB, Sigma Aldrich,
95%) were prepared immediately prior to use. Solvents on the HPLC system were methanol (MeOH, Fisher Scientific, 99.9%) and HPLC Water (Ricca). Nitrogen gas (Airgas UN1066) was used to purge some samples.

3.2.2 Photolysis

The total photon flux in the actinic region reaching our samples was \((1.47 \pm 0.04) \times 10^{14}\) photons cm\(^{-2}\) s\(^{-1}\) as determined by chemical actinometry.\(^{30}\) Photolysis was performed as described in a previous publication by our group.\(^{30}\) The light source was a 150W xenon arc lamp with a cold mirror and 295 nm long pass cutoff filter. PAHs were contained in a quartz cuvette, and photolysis kinetics were measured by fluorescence spectroscopy.\(^{30}\) Anthracene was excited at 252 nm and pyrene at 272 nm. Emission spectra were monitored at 405 nm for anthracene and 392 nm for pyrene.

For one set of experiments, benzoic acid was added to solutions containing anthracene and chloride or iodide. Hydroxyl radical production was assessed by monitoring the formation of salicylic acid, which is a major reaction product of OH and benzoic acid, via fluorescence spectroscopy.\(^{30, 31}\)

3.2.3 Singlet Oxygen

Samples containing FFA, anthracene or pyrene, and halides were irradiated with the xenon arc lamp and 0.5 mL aliquots were taken at selected time intervals and analyzed by HPLC in order to monitor production of pyranone, which is the reaction product of singlet oxygen and FFA.\(^{32, 33}\) The mobile phase used was 50/50 MeOH/water on a C18 column with a flow rate of 1 mL/min and a column oven temperature of 40° Celsius. Absorbance was detected at 219 nm. The yield of pyranone from the reaction between FFA and \(^{1}O_{2}\) is 85%.\(^{32}\) Based on the rate constants
of $^{1}$O$_2$ with FFA and water, 89% of the singlet oxygen will react with FFA, while 11% will be deactivated by water. The $^{1}$O$_2$ production rates presented in the manuscript are therefore determined from the measured pyranone concentrations based on Equation 1.

$$\frac{d[O_2]}{dt} = \frac{d[pyranone]}{dt} \times (1.33) \quad [1]$$

For experiments involving rose bengal (absorbance maximum ~550 nm), a 480 nm long pass cut off filter was placed in front of the lamp so that the PAHs did not absorb light and the only important reactions would be with singlet oxygen produced through the excitation of rose bengal or photosensitization by rose bengal.

For one set of experiments, the solution pH was adjusted with HCl or NaOH. pH was measured with a Vernier Instruments pH probe that was calibrated daily. Ionic strength was kept constant at 0.01 M for experiments involving pyrene. Chloride concentration was kept constant at 0.1 M in experiments involving anthracene. Ionic strength and Cl$^-$ concentrations were adjusted using NaCl. We purged solutions with nitrogen for 30 minutes via bubbling into a sealed cuvette at 0.01 SLPM to remove dissolved oxygen,

3.3 Results and Discussion

Table 3.1 shows pyrene and anthracene photolysis kinetics in DI water, Instant Ocean, aqueous solutions containing one of Cl$^-$, Br$^-$, or I$^-$ at sea water concentrations (0.56 M, $1.1 \times 10^{-3}$ M, and $3.0 \times 10^{-7}$ M respectively), and in aqueous solutions containing a mixture of the 3 halide salts at sea water concentrations; this mixture shall hereafter be referred to as the “3 salt solution”.$^{34,35}$ The pyrene photolysis rate constant measured in both Instant Ocean and the 3 salt solution was 31% that measured in DI water. Conversely, anthracene photolyzed more rapidly in
Instant Ocean and in the 3 salt solution than in DI water (by factors of approximately 5.4 and 2.4 respectively).

### Table 3.1. Effects of halides on pyrene and anthracene photolysis rate constants.

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Observed Rate Constant (10^{-4} s^{-1})</th>
<th>Pyrene</th>
<th>Anthracene</th>
</tr>
</thead>
<tbody>
<tr>
<td>DI Water</td>
<td></td>
<td>1.9 ± 0.5</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>Instant Ocean</td>
<td></td>
<td>0.6 ± 0.2</td>
<td>12 ± 4.6</td>
</tr>
<tr>
<td>3 Salt Solution</td>
<td></td>
<td>0.6 ± 0.2</td>
<td>5.3 ± 0.6</td>
</tr>
<tr>
<td>NaCl (0.56 M)</td>
<td></td>
<td>0.7 ± 0.2</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>NaBr (1.1 × 10^{-3} M)</td>
<td></td>
<td>1.3 ± 0.2</td>
<td>5.0 ± 0.3</td>
</tr>
<tr>
<td>NaI (3.0 × 10^{-7} M)</td>
<td></td>
<td>2.1 ± 0.4</td>
<td>2.4 ± 0.4</td>
</tr>
</tbody>
</table>

#### 3.3.1 Effects of Halides on Pyrene Photolysis

As shown in Table 3.1, pyrene’s photolysis rate constant in aqueous solution containing the Cl\(^-\) concentration expected in seawater was the same as that in the 3 salt solution and Instant Ocean, while rate constants in the presence of seawater concentrations of Br\(^-\) and I\(^-\) were the same as those in deionized water within our experimental uncertainty. We measured pyrene photolysis rate constants over a range of concentrations of individual sodium halide salts and other seawater components (CaCl\(_2\) and Na\(_2\)SO\(_4\)) to investigate the mechanism by which pyrene photolysis is suppressed in seawater. These results are shown in Figure 3.1. Each halide affected pyrene similarly. Rate constants decreased as ionic strength increased to approximately 0.1 M. At higher ionic strengths, rate constants remained stable. At high NaCl, NaBr, and NaI concentrations, the observed rate constants were approximately 35%, 49%, and 20% of that measured in pure water. The slight but reproducible variation in the rate constant in the presence
of the three different halides at high ionic strengths may be due to a currently undefined specific ion effect. While the rate constants measured at high ionic strengths vary by approximately a factor of 3 in the presence of the different halides, the rate constants measured in the 3 salt solution and in Instant Ocean agree most closely with that measured in aqueous NaCl at high ionic strengths, likely because Cl\(^-\) concentrations are orders of magnitude greater than other halides in seawater.

We also measured pyrene photolysis kinetics in the presence of CaCl\(_2\) and Na\(_2\)SO\(_4\). As shown in Figure 3.1, observed pyrene photolysis rate constants in the presence of these species were similar to those measured in the presence of sodium halide salts at the same ionic strengths. This supports our conclusion that halide salts affect pyrene photolysis kinetics by increasing ionic strength rather than via specific ion effects.

**Figure 3.1.** Effects of sodium halide salts and other ionic species on pyrene photolysis kinetics in aqueous solution.

Our observation that halide salts suppress pyrene photolysis are in agreement with a previous report that pyrene photolyzed more slowly in water collected from the Atlantic Ocean than in deionized water.\(^{28}\) However, two other studies reported accelerated pyrene photolysis in
the presence of NaCl up to concentrations of 0.1 M and 1.2 M respectively.\textsuperscript{10,36} Pyrene solutions in the latter two studies were irradiated with lamps with significant emission at wavelengths shorter than 280 nm. Photolysis initiated by excitation of absorption bands at shorter wavelengths proceeds via direct ionization of pyrene; direct ionization does not occur at wavelengths longer than 290 nm that are relevant to the Earth’s surface.\textsuperscript{37,38} Therefore, we expect the rate constant for pyrene photolysis initiated by sunlight in natural salty waters to be slower than that in deionized water, and to be determined by total ionic strength rather than by the identity of ionic species.

\textit{3.3.2 Effects of Halides on Anthracene Photolysis}

As shown in Table 3.1, anthracene photolyzed more quickly in Instant Ocean and in 3 salt solution than in DI water. The rate constant in Instant Ocean was approximately a factor of five greater than that in DI water, while that in the 3 salt solution was greater by a factor of 2.4. This indicates that effects of halide salts account for approximately half of anthracene’s enhanced reactivity in seawater. Figure 3.2 shows the effects of individual halide salts on anthracene photolysis kinetics, and Table 3.2 lists the maximum rate constants measured, as well as rate constants measured at seawater concentrations of the individual halide salts. As seen from Figure 3.2 and Table 3.2, at low halide concentrations the observed photolysis rate constant increased rapidly with increasing concentration, then gradually decreased at higher halide concentrations. Maximum rate constants, as well as the concentrations at which they were observed, followed the trend $\Gamma^- < \text{Br}^- < \text{Cl}^-$. The maximum rate constants were greater than those in DI water by factors ranging from 1.5 to 3.4, while the concentrations at which the fastest photolysis was observed spanned 7 orders of magnitude. This variability strongly suggests a
specific ion effect, rather than an ionic strength effect as appears to be responsible for variations in pyrene photolysis kinetics.
Figure 3.2. Effects of (a) NaCl, (b) NaBr, and (c) NaI on observed anthracene photolysis rate constants in aqueous solution. The dashed horizontal lines in (b) and (c) show the observed photolysis rate constant of anthracene in pure water. The insets of (b) and (c) show rate constants at low halide concentrations. The x-axes of the insets of (b) and (c) are in units of $10^{-6}$ M and $10^{-8}$ M respectively.
Table 3.2. Anthracene photolysis rate constants in the presence of halides.

<table>
<thead>
<tr>
<th>Halide Concentration (M)</th>
<th>Observed Rate Constant (10^-4 s^-1)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximum</td>
</tr>
<tr>
<td>Cl^-</td>
<td>0.2</td>
</tr>
<tr>
<td>Br^-</td>
<td>1 × 10^-5</td>
</tr>
<tr>
<td>I^-</td>
<td>1 × 10^-8</td>
</tr>
</tbody>
</table>

a) “Maximum” refers to the halide concentration at which the largest rate constant was measured, and “seawater” refers to the halide concentration in seawater.

b) “Maximum” refers to the largest rate constant measured, and “seawater” refers to the rate constant measured at the seawater concentration of the halide.

3.3.3 Role of Singlet Oxygen

Oxygen is present in air-saturated water (20° Celsius) at a concentration of ~0.6 mM. It is implicated in the photolysis mechanisms of several PAHs. To determine whether it is involved in the enhanced anthracene photolysis observed in the presence of halide salts, we measured anthracene photolysis kinetics in solutions containing 0.2 M NaCl or 1 × 10^-8 M NaI that had been purged with nitrogen. Rate constants of (2.1 ± 0.3) and (2.0 ± 0.2) × 10^-4 s^-1 were measured in these experiments; these are slower than the rate constants measured in the presence of the halides in air-saturated water, but similar to the rate constant of (1.8 ± 0.3) × 10^-4 s^-1 measured for anthracene in air-saturated water in the absence of halides. This indicates that oxygen is responsible for the enhanced reactivity observed in the presence of halides.

We measured anthracene photolysis kinetics in solutions containing 10 mM furfuryl alcohol (FFA), an electron and singlet oxygen scavenger. Table 3.3 shows anthracene
photolysis rate constants measured in the presence and absence of FFA and halide salts. In the absence of halides, FFA did not affect anthracene photolysis kinetics, but in the presence of Cl⁻ and I⁻ FFA reduced the observed rate constant to that measured in the absence of the halides. These observations suggest the involvement of singlet oxygen (rather than ground state) in the observed rate enhancement in the presence of halides. To confirm and quantify this effect, we determined ¹O₂ formation rates under our experimental conditions by measuring the production rate of 6-hydroxy-2,3-dihydro-6H-pyrano-3-one (pyranone), which is the product of reactions between singlet oxygen and FFA.³² Figure 3.3 shows ¹O₂ formation rates in aqueous anthracene solutions as a function of Cl⁻ and I⁻ concentrations. The dependence of ¹O₂ production on halide concentration follows the same trend as anthracene’s observed photolysis rate constant, which strongly suggests that anthracene’s enhanced reactivity in the presence of halide salts is due primarily to the production of singlet oxygen.

**Table 3.3. Anthracene photolysis rate constants in the presence of halides and FFA.**

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Observed Rate Constant (10⁻⁴ s⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No FFA</td>
</tr>
<tr>
<td>DI Water</td>
<td>2.2 ± 0.7</td>
</tr>
<tr>
<td>NaCl (0.2 M)</td>
<td>7.4 ± 1.4</td>
</tr>
<tr>
<td>NaI (1 × 10⁻⁸ M)</td>
<td>3.3 ± 0.7</td>
</tr>
<tr>
<td>NaI (0.01 M)</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>NaI (0.1 M)</td>
<td>1.2 ± 0.2</td>
</tr>
</tbody>
</table>
Figure 3.3. Singlet oxygen production rates (closed black circles, left y-axis) and observed anthracene photolysis rate constants (open red squares, right y-axis) in aqueous solutions containing $1.5 \times 10^{-7}$ M anthracene in the presence of (a) NaCl, and (b) NaI. Experiments measuring singlet oxygen production rates were performed in the presence of 10 mM FFA. No FFA was present in experiments measuring anthracene photolysis kinetics.

Singlet oxygen is generally produced in natural waters from energy transfer between electronically-excited organic molecules (most commonly the T$_1$ state of DOM) and molecular oxygen. Energy transfer from the T$_1$ state of aromatic hydrocarbons such as benzene and naphthalene to molecular oxygen has been reported to generate singlet oxygen in organic phases when the aromatics are present at very high concentrations ($> 10^{-3}$ M).$^{39,40}$ We did not observe any singlet oxygen production when aqueous anthracene or pyrene solutions (with concentrations of $\sim 10^{-7}$ M) were irradiated in deionized water.

Halide salts have been reported to increase $^1O_2$ production rates in irradiated aqueous DOM solutions by increasing $^3$DOM* yields via the heavy atom effect.$^{27}$ This is consistent with
the positive dependence of the $^1\text{O}_2$ production rate on halide concentrations in solutions containing anthracene shown in Figure 3.3. Singlet oxygen quantum yields from DOM irradiation have been reported to plateau at high halide concentrations; this was ascribed to scavenging of $^1\text{O}_2$ by halide ions. The rate constant for the reaction of singlet oxygen with iodide is approximately 4 orders of magnitude greater than that with chloride; this is in reasonable agreement with the relative concentrations at which singlet oxygen production rates switch from a positive to a negative dependence on halide concentration in our experiments. However, while scavenging of $^1\text{O}_2$ by halides may contribute to the negative concentration dependences of $^1\text{O}_2$ production and anthracene degradation kinetics at higher halide concentrations shown in Figures 3.2 and 3.3, it can not fully explain them, since anthracene photolysis rate constants at high bromide and iodide concentrations are lower than those in DI water, where no singlet oxygen formation was observed. This complex behavior may be due to halide salts promoting a $T_1$ to $S_0$ transition in anthracene (quenching excited anthracene). This heavy atom effect has been reported to be responsible for similar concentration dependences of photodimerization of acenaphthylene in solutions containing organohalides. To determine whether suppressed anthracene photolysis at high halide concentrations were due to scavenging of singlet oxygen or to a heavy atom effect, we measured anthracene photolysis kinetics in the presence and absence of FFA at 0.01 and 0.1 M I$. As shown in Figure 3.2, anthracene photolyzed more slowly at these I$ concentrations than in the absence of halides. The rate constants measured at these concentrations, listed in Table 3.3, were the same within experimental uncertainty in the presence and absence of FFA. This finding supports our hypothesis that deactivation of the $T_1$ state of anthracene via a heavy atom effect, rather than
scavenging of $^1$O$_2$ by halides, is primarily responsible for the observed suppression of anthracene photolysis at high halide concentrations.

We also observed singlet oxygen production in irradiated solutions containing pyrene and halides. Figure 3.4 shows singlet oxygen production in solutions containing pyrene and NaCl. The production rate increased with increasing Cl$^-$ concentration until approximately 0.1 M, at which point no further change in production rate was observed up to Cl$^-$ concentrations of at least 2 M. The maximum production rate was $(5.0 \pm 0.5) \times 10^{-8}$ M s$^{-1}$; a maximum rate of $(5.1 \pm 0.6) \times 10^{-8}$ M s$^{-1}$ was observed for NaI concentrations between $10^{-8}$ and $10^{-5}$ M. These production rates are approximately 50% and 36% of the maximum rates observed in irradiated solutions containing anthracene and NaCl or NaI.

![Figure 3.4](image)

**Figure 3.4.** Singlet oxygen production rates in aqueous solutions containing $3.0 \times 10^{-7}$ M pyrene in the presence of NaCl. Experiments were performed in the presence of 10 mM FFA.

Table 3.4 details the effects of FFA on pyrene photolysis rate constants in aqueous solutions containing NaCl. Despite significant $^1$O$_2$ production, FFA did not suppress pyrene photolysis in the presence of halides; measured photolysis rate constants at 0.3 and 0.01 M Cl$^-$
were $(3.8 \pm 1.5) \times 10^{-5} \text{ s}^{-1}$ and $(19 \pm 9.8) \times 10^{-5} \text{ s}^{-1}$, which agree with those measured in the absence of FFA at the same concentrations within our experimental uncertainty. This suggests that although $^{1}\text{O}_2$ was produced, pyrene did not react with it to an appreciable extent under our experimental conditions. We investigated the reactivity of anthracene and pyrene toward singlet oxygen by irradiating solutions containing the PAHs and rose bengal, which is a singlet oxygen generator. These samples were irradiated with visible light to ensure that any PAH loss was due to reactions with singlet oxygen generated by rose bengal rather than by direct photolysis. Under our experimental conditions, singlet oxygen was generated by rose bengal photolysis on the order of $10^{-6} \text{ M s}^{-1}$. Anthracene’s observed rate constant in the presence of rose bengal was $(5.2 \pm 0.7) \times 10^{-4} \text{ s}^{-1}$, while pyrene’s was an order of magnitude lower ($(5.6 \pm 0.6) \times 10^{-5} \text{ s}^{-1}$). Singlet oxygen was generated by halides much less rapidly than by rose bengal; in the presence of halides, we expect a maximum pyrene degradation rate constant due to reaction with singlet oxygen of approximately $2 \times 10^{-6} \text{ s}^{-1}$. This is orders of magnitude smaller than the direct photolysis rate constant, and would be undetectable in our experiments. Although we do not expect pyrene photolysis rates to increase in the presence of halides, pyrene should still be considered as a potential source of $^{1}\text{O}_2$ in illuminated waters containing halides.

Table 3.4. Pyrene photolysis rate constants in the presence of NaCl and FFA.

<table>
<thead>
<tr>
<th>Experimental Conditions</th>
<th>Observed Rate Constant $(10^{-5} \text{ s}^{-1})$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No FFA</td>
</tr>
<tr>
<td>DI Water</td>
<td>19 ± 5.0</td>
</tr>
<tr>
<td>NaCl (0.01 M)</td>
<td>20 ± 9.9</td>
</tr>
<tr>
<td>NaCl (0.1 M)</td>
<td>4.1 ± 1.4</td>
</tr>
<tr>
<td>NaCl (0.3 M)</td>
<td>4.0 ± 1.3</td>
</tr>
</tbody>
</table>
Singlet oxygen concentrations in natural surface waters are thought to be too low have an effect on PAH reaction kinetics, even in the presence of known singlet oxygen generators such as dissolved organic matter (DOM), and singlet oxygen has not been shown to affect PAH photolysis kinetics under environmentally relevant conditions.\textsuperscript{3, 36, 45} However, our results suggest that PAHs may themselves be singlet oxygen sources in the presence of halides; in saline waters singlet oxygen may affect the lifetimes of some PAHs (such as anthracene). To determine the importance of PAHs as singlet oxygen sources in saline waters, we investigated the dependence of \( ^1\text{O}_2 \) production on anthracene concentration. The \( ^1\text{O}_2 \) production rate was linear across the range of anthracene concentrations used. The dependence on anthracene concentration (for a chloride concentration of 0.1 M) is given by Eq. 2:

\[
\frac{d[\text{O}_2]}{dt} = [\text{anthracene}] \times (0.43) + (6 \times 10^{-9}) \tag{2}
\]

PAHs can be found in seawater at concentrations ranging from less than nM up to \( \mu \text{M} \) in more polluted waters.\textsuperscript{46-48} From Eq. 2, we predict \( ^1\text{O}_2 \) production rates in the presence of 0.1 M Cl\(^-\) to range from \( 6.4 \times 10^{-9} \text{ M s}^{-1} \) to \( 4.4 \times 10^{-7} \text{ M s}^{-1} \).

Halides can enhance degradation rates of organic species via mechanisms other than singlet oxygen production. Oxidized organics have been reported to photosensitize halide species under environmentally-relevant conditions; irradiation of aqueous solutions containing halide salts and species such as chlorophyll, benzophenone, and imidazole-2-carboxaldehyde has been reported to generate reactive halogen atoms.\textsuperscript{23-25} This photosensitization is thought to occur via electron transfer from the halide anion to carbocations formed from ionization of the organic species during irradiation. This pathway is likely unimportant for anthracene and pyrene, which do not form carbocations to an appreciable extent during photolysis under environmentally-
relevant conditions. Halide salts have been reported to enhance the photolysis rates of some PAHs by forming hydroxyl radicals (OH). We did not observe any OH when aqueous solutions containing anthracene and I or Cl were irradiated, and conclude that OH is not produced to an appreciable extent from PAH-halide interactions under our experimental conditions.

3.3.4 Photolysis in Instant Ocean

The effect of halides on pyrene photolysis kinetics are well explained by ionic strength, and the complex dependence of anthracene photolysis kinetics on halide concentrations can be explained by \(^1\)O\(_2\) production and heavy atom effects. The greater enhancement of anthracene photolysis in Instant Ocean than in 3 salt solution remains unexplained, however. We performed several experiments to investigate this difference. To determine whether components of Instant Ocean other than halides formed \(^1\)O\(_2\) to an appreciable extent, we measured \(^1\)O\(_2\) production rates in irradiated Instant Ocean solutions containing anthracene. The measured rate was \((1.0 \pm 0.5) \times 10^{-7} \text{ M s}^{-1}\), which is the same as that measured in 3 salt solution \((1.2 \pm 0.4) \times 10^{-7} \text{ M s}^{-1}\) within experimental uncertainty. This suggests that NaCl, NaBr, and NaI are responsible for essentially all singlet oxygen production in Instant Ocean under our experimental conditions. We also irradiated Instant Ocean solutions containing benzoic acid to determine whether trace compounds formed OH; no OH production was observed. This suggests that despite the presence of metals in Instant Ocean, photo-Fenton type reactions (in which hydrogen peroxide reacts with trace metals to form OH) are not responsible for anthracene’s enhanced reactivity.

Differences in the pH of Instant Ocean and 3 salt solution could account for different PAH reactivities. We measured anthracene and pyrene photolysis kinetics between pH 4 and 11. Pyrene solutions were kept at constant ionic strength, and anthracene solutions were kept at
constant chloride concentrations. As shown in Table 3.5, anthracene photolysis kinetics were independent of pH over the range studied, while a slight increase in pyrene photolysis kinetics was observed at high pH. However, this increase was within our experimental uncertainty. From these results we conclude that there is no significant pH effect on anthracene or pyrene photolysis kinetics in the pH range studied. This is in agreement with previous work, although short wavelength irradiation ($\lambda < 290$ nm) was used in those studies. Factors other than singlet oxygen production and pH must be responsible for anthracene’s greater reactivity in Instant Ocean than in 3 salt solution.

![Absorption Spectrum of Instant Ocean](image)

**Figure 3.5.** Absorption spectrum of Instant Ocean in water.

Figure 3.5 shows an absorption spectrum of Instant Ocean. Featureless absorbance extends well into the visible. Similar spectra have been reported in seawater samples. Seawater and seawater substitutes have a high turbidity and contain many minerals, leading to greater light scattering than in pure water. This is reflected in the non-zero absorbance well into the visible region in Figure 3.5. Major components in Instant Ocean are salts and nitrates; these may enhance anthracene photolysis via photosensitization or by producing reactive species other than OH and $^{1}$O$_{2}$. Copper and iron species are also present in trace concentrations in seawater.
Some trace metals absorb sunlight and some can also form organic complexes with other molecular species, causing them to become much more photoreactive.\textsuperscript{51} These complexes may then aid in the destruction of certain contaminants and pollutants present in the water.\textsuperscript{52}

**Table 3.5. Effect of solution pH on anthracene and pyrene photolysis kinetics.**

<table>
<thead>
<tr>
<th>pH</th>
<th>Anthracene (^{a})</th>
<th>Pyrene (^{b})</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>3.1 ± 0.1</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>7</td>
<td>3.2 ± 0.2</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>10</td>
<td>3.2 ± 0.3</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>11</td>
<td>3.2 ± 0.4</td>
<td>1.9 ± 0.2</td>
</tr>
</tbody>
</table>

\(^{a}\)Each sample contained 0.1 M chloride

\(^{b}\)Each sample had an ionic strength of 0.01 M

**3.4 Conclusions**

Photolysis rate constants under environmentally-relevant conditions are needed for accurate predictions of PAH fate in natural waters. We have demonstrated that PAH photolysis may proceed at very different rates in seawater and freshwater, largely due to effects of halide salts. Different PAHs are not affected uniformly by halides; we observe suppression of pyrene photolysis due to ionic strength effects, but enhancement of anthracene photolysis due to singlet oxygen production induced by the heavy atom effect. Our results demonstrate the importance of taking the composition of the local environment into account when predicting PAH photolysis kinetics in natural waters.

Our observation of singlet oxygen production in irradiated aqueous solutions containing PAHs and halide salts has important implications for our understanding of the oxidizing capacity
of natural waters. Based on our results, we predict $^1$O$_2$ production rates to be greater than currently expected in saline waters containing PAHs. Aromatic species other than PAHs may also produce $^1$O$_2$ in the presence of halide salts. Different anthracene reactivity in Instant Ocean and the 3 salt solution emphasize that reactivity in complex environments may not be well described by kinetics measured in simple models.

3.5 References

Chapter Four:

Hydroxyl Radical Formation from Bacteria-Assisted Fenton Chemistry at Neutral pH Under Environmentally-Relevant Conditions

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4.1 Introduction

Hydroxyl radicals (OH) are key oxidants in the atmosphere and in natural waters. They react rapidly (often at near-diffusion limited rates) with many organic and inorganic species.\textsuperscript{1-3} Some examples of their importance in atmospheric and environmental aqueous phases (such as surface waters, aqueous aerosols, and cloud and fog droplets) include decreasing local pH by oxidizing SO\textsubscript{2} to ultimately form H\textsubscript{2}SO\textsubscript{4}, and oxidizing dimethyl sulfide (DMS) and other small organic molecules to form higher molecular-weight species that lead to secondary aerosol formation.\textsuperscript{3-9} Hydroxyl radicals also react rapidly with numerous organic and inorganic pollutants that are resistant to other common degradation mechanisms in the environment such as photolysis and ozonation.\textsuperscript{1, 10-14}

Hydroxyl radicals in environmental waters are primarily formed through sunlight-initiated photolysis of OH precursors such as nitrite, nitrate, hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), and dissolved organic matter.\textsuperscript{15-21} Fenton chemistry (Equations 1 and 2) can also be an important OH source in waters that contain iron and H\textsubscript{2}O\textsubscript{2}. Iron is found in surface waters at concentrations as high as 300 µM, and H\textsubscript{2}O\textsubscript{2} has been detected in natural waters at concentrations around 30 µM and as high as 100 µM in rainwater.\textsuperscript{22-25}

\begin{equation}
H_{2}O_{2} + Fe(II) \rightarrow Fe(III) + OH + OH^{-} \tag{1}
\end{equation}

\begin{equation}
H_{2}O_{2} + Fe(III) \rightarrow Fe(II) + HO_{2}/O_{2}^{-} + H^{+} \tag{2}
\end{equation}

Sunlight rapidly reduces Fe(III) to Fe(II). This photo-Fenton chemistry is catalytic, and generates OH rapidly. It is an important source of OH in sunlit natural waters, and is often employed in water purification and remediation.\textsuperscript{26, 27} Fenton chemistry in the absence of sunlight is much slower. The reduction of Fe(III) via Reaction 2 is rate-limiting, with a rate constant of
approximately $2 \times 10^{-3}$ M$^{-1}$s$^{-1}$ (compared to a rate constant of approximately 55 M$^{-1}$s$^{-1}$ for Reaction 1) at pH 2.5. Further, Fe(III) is only soluble at low pH; Fe(III) saturated concentration at pH 4 is 500 nM. Despite the fact that dark Fenton chemistry is very slow and only occurs over a limited pH range, it is one of the most important sources of OH at nighttime, since there are very few dark radical sources in environmental waters. Given the paucity of nighttime radical sources in the environment, even small increases in radical production from existing mechanisms (such as Fenton chemistry) could significantly increase the oxidizing capacity of natural waters at nighttime.

Several naturally-occurring species have been shown to accelerate dark Fenton chemistry in the environment. Quinones can increase Fe(III) reduction rates through electron transfer, and iron chelators such as ascorbic acid and hydroxylamine can increase Fe(III) solubility, thereby increasing its reactivity at higher pH. Small acids and components of dissolved organic matter such as humic acid have also been shown to increase Fenton reaction kinetics under some conditions, likely through complex formation, radical production, and promoting faster Fe(II)/Fe(III) cycling. However, studies reporting these results have been restricted to low pH (below 4) or have used reagent concentrations orders of magnitude greater than those found in natural waters.

Another process that could promote Fenton chemistry in the environment is the reduction of Fe(III) by bacteria. Some common environmental genera such as Geobacter and Shewanella reduce metals, including iron, during metabolism. Shewanella oneidensis (SO) thrives in both aerobic and anaerobic environments, and is often found in natural waters such as lakes – especially those contaminated by heavy metals. Bacteria-assisted Fenton chemistry has been explored in the context of improving wastewater treatment techniques. At high iron
concentrations, significant degradation of the organic pollutants 1,4-dioxane and pentachlorophenol was observed in the presence of SO; the enhancement was attributed to faster Fenton chemistry due to reduction of Fe(III) by SO\textsuperscript{41,42}. These studies were performed under conditions relevant to water treatment facilities and focused only on organic pollutant degradation rates. To our knowledge, OH production rates from bacteria-enhanced Fenton chemistry have not been measured, and the effects of iron-reducing bacteria on dark Fenton chemistry have not been investigated under conditions relevant to natural waters (as opposed to in water treatment facilities).

The aim of this study was to determine whether an iron-reducing bacterium would significantly increase OH production rates from dark Fenton chemistry at near-neutral pH. We measured OH production rates at environmentally-relevant iron and H\textsubscript{2}O\textsubscript{2} concentrations in the presence and absence of SO. Our results suggest that bacteria-assisted Fenton chemistry could be an important dark radical source in natural waters.

4.2 Experimental

4.2.1 Materials

Solutions containing hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}, Fluka, 31.5%, standardized by KMnO\textsubscript{4} titration) in 18.2 MΩ·cm deionized water were newly prepared the day before experiments were performed and stored away from light. Solutions containing H\textsubscript{2}O\textsubscript{2}, iron(II) sulfate heptahydrate (Fe(II), Acros Organics, 99.5%), iron(III) sulfate pentahydrate (Fe(III), Acros Organics, 97%), hydrochloric acid (HCl, EM Science, 36.5-38%), sodium hydroxide (NaOH, Sigma-Aldrich, \textgeq97%), and benzoic acid (Sigma-Aldrich, \textgeq99.5%) were prepared immediately prior to use.
Solutions containing anthracene (Acros Organics, 99%) in 18.2 MΩ·cm deionized water were newly prepared every week. Solutions containing luminol (Sigma-Aldrich, 97%) in 0.1 M aqueous ammonium hydroxide solution (NH₄OH, Fluka, 5.0 M) were newly prepared every day prior to use.

*Shewanella oneidensis* MR-1 was cultured aerobically in a minimal growth medium containing lactate as an electron donor. The composition of the medium is detailed in the Appendix. The strain was cultured from −80 °C stocks by 48 hour incubation in growth medium at 37 °C or 30 °C. Cultured cells were at stationary growth phase. Stationary phase was chosen for these experiments to minimize artifacts caused by the rapid growth of SO, which has a doubling time of approximately 40 minutes in minimal media at room temperature. Reaction samples were made by filtering aliquots of SO stock solution through 2 inches of tightly packed sterile gauze pads in a column in order to separate out clumps, and then adding a known volume of the filtered solution to sterile medium.

### 4.2.2 Hydroxyl Radical Trap and OH Production Rates

Hydroxyl radical formation rates were measured using benzoic acid as an OH radical trap. Briefly, benzoic acid was incorporated into reaction solutions in excess, and salicylic acid formed from the reaction between benzoic acid and OH was detected by fluorescence spectroscopy. Details of the use of benzoic acid as an OH trap are provided in the Appendix. All experiments were performed with 7.5 × 10⁻⁴ M benzoic acid and 100 µM of the respective Fe compound, which will hereafter be referred to as “benchmark conditions”. Hydrogen peroxide concentrations and SO cell densities ranged between 5.0 × 10⁻⁶ and 2.0 × 10⁻³ M and 2.8 × 10⁵
and $7.0 \times 10^6$ cells mL$^{-1}$ respectively. The pH of samples in water was adjusted using HCl or NaOH, and sample pH was measured using a commercial pH meter (Vernier Software and Technology pH Sensor) that was calibrated using a three point calibration. Sample pH remained constant during experimental runs. The minimal medium used for our experiments is buffered at pH 7 by sodium phosphate, so all experiments in this medium (including all experiments involving SO) were performed at neutral pH.

Solutions containing all reagents except H$_2$O$_2$ were prepared, and a fluorescence spectrum was acquired between 375 and 425 nm with excitation at 300 nm using a Photon Technology International QuantaMaster 40 fluorometer. Hydrogen peroxide was introduced to the sample, and a fluorescence spectrum was immediately acquired. Samples were stored in the dark and fluorescence spectra were taken over set time intervals (up to 30 minutes total time) in order to monitor formation of salicylic acid. A rate of OH production was then quantified using the method described in the Appendix.

4.2.3 Anthracene Degradation Kinetics

Saturated anthracene solutions were prepared by stirring a few mg of solid anthracene into deionized water overnight. An aliquot of the stock solution was removed prior to each experiment and diluted to a final anthracene concentration of $7.5 \times 10^{-8}$ M with buffered medium. Changes in anthracene concentration were measured by fluorescence spectroscopy: Anthracene was excited at 356 nm and fluorescence intensity was monitored at 402 nm. A calibration curve relating anthracene fluorescence intensity at 402 nm to concentration in buffered medium was acquired between $5 \times 10^{-9}$ and $3 \times 10^{-7}$ M to ensure that fluorescence
intensity depends linearly on concentration over this range. Anthracene fluorescence spectra were acquired at set time intervals for up to 30 minutes. Experiments were performed in sterile medium with no added reagents, in the presence of H₂O₂, Fe(III), and SO, and for each possible combination of two out of three of the aforementioned reagents.

4.2.4 Detection of Fe(II) by Luminol Phosphorescence

An aqueous solution containing 0.5 mM luminol and 0.1 M NH₄OH was prepared and allowed to equilibrate for three hours. The concentration of Fe(II) was determined by luminol phosphorescence, which was monitored at 430 nm with excitation at 302 nm. Figure S8 in the Appendix shows the calibration curve relating phosphorescence intensity to Fe(II) concentration. In one set of experiments, Fe(II) and the luminol solution were combined with buffered medium, and the loss of Fe(II) due to oxidation was monitored over a 30 minute period. In another set of experiments, Fe(III) and the luminol solution were combined with buffered medium containing SO, and the formation of Fe(II) was monitored over a period of 10 minutes.

4.2.5 Flow Cytometry

Experiments were performed to determine the effects of SO cell density on OH production rates. For these experiments, filtered SO suspensions were diluted with medium by a factor ranging from 5- to 1000-fold. Half of the sample was mixed with iron, H₂O₂, and benzoic acid to measure OH production rates, and the other half of the sample was prepared for flow cytometry. Flow cytometry measurements were run concurrently with Fenton reactions for each
sample. Some Fenton experiments and flow cytometry measurements were also performed on unfiltered SO samples.

To prepare samples for flow cytometry, cells were stained using a solution consisting of 0.05 g acridine orange (Sigma-Aldrich, 75%) and 0.85 g sodium chloride (Sigma-Aldrich, ≥99.5%) in 100 mL of water. This solution was combined with the medium containing SO in a 1:1 (v/v) ratio and mixed by hand for two minutes. Flow cytometry was done using a BD Accuri C6.

4.3 Results and Discussion

Figure 4.1 shows OH concentrations as a function of reaction time in aqueous solution under benchmark conditions containing $1.0 \times 10^{-3}$ M H$_2$O$_2$ in the absence of SO at pH 7. At these short reaction times, a linear increase in OH concentration with reaction time is observed. Initial rates (calculated at short reaction times) were used to ensure that secondary reactions between OH and salicylic acid did not influence the calculated rate: This would result in a non-linear dependence of OH concentration on reaction time. Hydroxyl radical production rates were much larger in the presence of Fe(II) than Fe(III), as expected based on the larger rate constant for Reaction 1 compared to Reaction 2 (note the different scales of the y-axes).
Figure 4.1. Hydroxyl radical concentrations during a representative Fenton experiment in a sample containing $1.0 \times 10^{-3}$ M H$_2$O$_2$ and Fe(II) (black circles, left y-axis) or Fe(III) (red triangles, right y-axis) in deionized water at pH 7 under benchmark conditions in the absence of SO. The solid traces are linear fits to the data.

Figure 4.2 shows measured OH production rates at a range of pH levels in the presence of $1.0 \times 10^{-3}$ M H$_2$O$_2$ in the absence of SO. In the presence of either form of iron, OH production rates decreased with increasing pH. This is due primarily to increased Fe(II) oxidation rates and decreased Fe(III) solubility at higher pH. In the absence of H$_2$O$_2$, some OH production was observed, but at rates below our limit of detection (LOD) of $\sim 6 \times 10^{-12}$ M s$^{-1}$. The LOD was determined based on the smallest salicylic acid formation rate we could reproducibly measure. Some OH production was observed in the absence of iron at an H$_2$O$_2$ concentration of $1.0 \times 10^{-3}$ M. This rate, of $2.2 \times 10^{-11}$ M s$^{-1}$, was measured at all pH levels investigated, and is likely due to H$_2$O$_2$ photolysis in the fluorimeter or from room lights. This background rate contributed to the slowest OH production rates measured from dark Fenton chemistry, which were observed at pH 6 and 7 in the presence of Fe(III) in deionized water ($8.5 \times 10^{-11}$ M s$^{-1}$ and $4.2 \times 10^{-11}$ M s$^{-1}$ respectively). However, this OH production in the absence of iron did not affect experiments.
investigating the effects of SO on Fenton chemistry, since reactions in sterile medium were generally performed at \( \text{H}_2\text{O}_2 \) concentrations of \( 2.0 \times 10^{-4} \text{ M} \); at this lower concentration no OH was measured in the absence of iron.

**Figure 4.2.** Effect of pH on OH production rates from dark Fenton chemistry with \( 1.0 \times 10^{-3} \text{ M} \text{H}_2\text{O}_2 \) in deionized water under benchmark conditions in the absence of SO. Error bars represent the standard deviation about the mean of three trials. Error bars for Fe(III) are too small to be visible in this plot. The average percent error for Fe(III) was similar to that for Fe(II).

We performed several experiments in the presence of SO to determine its effects on OH production rates at neutral pH. These experiments were all performed under benchmark conditions in sterile medium. Figure 4.3 shows OH concentrations as a function of reaction time in the presence and absence of SO in solutions containing Fe(II) and Fe(III). Hydroxyl radicals are clearly produced more quickly in the presence of SO.
Figure 4.3. Hydroxyl radical concentrations during representative Fenton experiments in samples containing (a) Fe(II) and (b) Fe(III) in the presence of $7.0 \times 10^6$ cells mL$^{-1}$ SO (closed black circles) and in the absence of SO (open red squares). All experiments were performed under benchmark conditions with $2.0 \times 10^{-4}$ M H$_2$O$_2$ at pH 7.

Figure 4.4 shows measured OH production rates at pH 7 in the presence and absence of SO in buffered media containing $2.0 \times 10^{-4}$ M H$_2$O$_2$ and iron under benchmark conditions. The presence of SO increased OH production rates by factors of approximately 1.5 and 6 when iron was in the form of Fe(II) and Fe(III) respectively. These observations are in agreement with the
hypothesis that SO will increase dark Fenton rates by reducing Fe(III). To confirm this, we measured the formation of Fe(II) over time in a sample containing Fe(III) and SO in buffered medium. The results are shown in Figure 4.5. When no SO is present, all of the iron remains in the form of Fe(III) over the course of the experiment. However, in the presence of SO, all of the iron is converted to Fe(II) within 2 minutes. We determined a Fe(III) reduction rate of $(7.5 \pm 0.5) \times 10^{-7}$ M s$^{-1}$ in the presence of $7 \times 10^6$ cells mL$^{-1}$ SO. Given that only a small fraction of Fe(III) in our pH 7 samples is dissolved, our results suggest that SO is reducing both dissolved and solid phase iron. SO, other Shewanella, and Geobacter species have been shown to reduce solid-phase iron.$^{45-48}$ It is also possible, although less likely, that the rapid conversion of Fe(III) to Fe(II) in the presence of SO is due to SO dramatically increasing Fe(III) solubility at high pH. These results indicate that SO increases measured OH production rates via Fenton-like chemistry at neutral pH primarily by accelerating Fe(III) reduction, which is the rate-limiting step of the dark Fenton cycle.

**Figure 4.4.** Hydroxyl radical production rates in sterile medium containing $2.0 \times 10^{-4}$ M H$_2$O$_2$ under benchmark conditions in the presence (red) and absence (grey) of SO. Error bars represent the standard deviation about the mean of three trials.
Figure 4.5. Fe(II) generation from the reduction of ~100 µM Fe(III) by SO over time (black circles). Error bars represent the standard deviation about the mean of three trials. The solid trace is a linear fit to the data over the time period in which [Fe(II)] increased; the slope of the fit is $(7.5 \pm 0.5) \times 10^{-7} \text{M s}^{-1}$ with an $R^2$ value of 0.98. The solid red square shows [Fe(II)] after 10 minutes in buffered medium in the absence of SO.

The increased OH production rate at pH 7 in the presence of SO and Fe(III) is well-explained by the rapid reduction of Fe(III) by SO, as discussed above. However, as shown in Figure 4.4, we also observed a small increase in the OH production rate when iron was in the form of Fe(II). This is likely because at pH 7, Fe(II) oxidizes rapidly to Fe(III); in the absence of SO a mixture of Fe(II) and Fe(III) is present. We measured Fe(II) oxidation kinetics in sterile medium at a pH of 7 in the absence of SO to determine whether Fe(II) oxidation could account for the different OH production rates measured in the presence and absence of SO when iron was in the form of Fe(II). Figure S9 in the Appendix shows Fe(II) oxidation over time. The measured oxidation rate was $(2.2 \pm 0.2) \times 10^{-8} \text{M s}^{-1}$. Over the course of a 30 minute experiment, approximately 50% of the Fe(II) would be oxidized to Fe(III) in the absence of SO. These numbers are in agreement with modeling studies done in natural water conditions.\textsuperscript{49} This
oxidation explains the enhanced OH production observed from Fe(II) and H$_2$O$_2$ in the presence of SO, and also explains why the measured OH production rate was the same for both Fe(II) and Fe(III) in the presence of SO; as shown in Figure 4.5, in the presence of SO, all of the iron will be in the form of Fe(II), regardless of its original speciation.

Table 4.1 shows the effects of SO on OH production rates in the presence of H$_2$O$_2$ and iron. Rates were adjusted to those expected at $2.0 \times 10^{-4}$ M H$_2$O$_2$, which is the concentration used for experiments in the presence of SO. Rates were adjusted by multiplying the measured rate by the H$_2$O$_2$ concentration in the experiment divided by a concentration of $2.0 \times 10^{-4}$ M. This approach is valid because OH production depends linearly on H$_2$O$_2$ concentration in the absence of SO, as shown in Figure S10 in the Appendix. Production rates were faster in sterile medium than in water at the same pH. Hydroxyl radical production rates in sterile medium in the absence of added iron were approximately 60% of the rates in sterile medium with Fe(III) added due to metals such as iron and copper in the medium participating in Fenton or Fenton-like chemistry, which corresponded to a rate of $2.1 \times 10^{-11}$ M s$^{-1}$. We therefore normalized rates in the medium to those expected in water in the absence of metals by subtracting this background rate. Our results show that SO will have a small but measurable effect on OH production rates in the presence of Fe(II). Enhancement of OH production in the presence of Fe(III) is much more pronounced, with a predicted rate in aqueous solution in the presence of Fe(III) and SO at pH 7 of $2.0 \times 10^{-10}$ M s$^{-1}$, which is above rates at pH 2 of $1.6 \times 10^{-10}$ M s$^{-1}$ under the same conditions in the absence of the bacteria.

**Table 4.1.** Hydroxyl radical production rates normalized to $2.0 \times 10^{-4}$ M H$_2$O$_2$ at several different pHs under benchmark conditions in the presence and absence of $7.0 \times 10^6$ cells mL$^{-1}$ SO.
Experimental Conditions | OH Production rate ($10^{-11}$ M s$^{-1}$) | Fe(II) | Fe(III) \\
|--------------------------|-----------------------------------------|--------|--------
| pH 2, water              | 80 ± 10                                 | 16 ± 2 |
| pH 4, water              | 45 ± 10                                 | 13 ± 1 |
| pH 6, water              | 18 ± 7                                  | 1.7 ± 0.7 |
| pH 7, water              | 9 ± 3                                   | 0.84 ± 0.02 |
| pH 7, sterile medium$^a$ | 13 ± 1 [11 ± 0.6]                       | 3.6 ± 0.6 [1.5 ± 0.36] |
| pH 7, sterile medium + SO$^a$ | 20 ± 5 [18 ± 3]  | 22 ± 2 [20 ± 1.2] |

$^a$) The numbers in brackets have been adjusted to the rate expected in water based on the rates measured in deionized water and medium at pH 7 in the absence of SO.

To determine whether some of the effects of SO could be due to factors other than its reduction of Fe(III), we measured an OH production rate in the presence of heat-inactivated SO of $(3.7 ± 1) \times 10^{-11}$ M s$^{-1}$, and in the presence of spent SO (that had been stored for four months prior to use) of $(3.8 ± 1) \times 10^{-11}$ M s$^{-1}$. These rates are the same within error as the rate measured in sterile medium in the absence of SO ($(3.6 ± 0.6) \times 10^{-11}$ M s$^{-1}$). This indicates that the rate-enhancing effects of SO are due to the live bacteria themselves, rather than to exudates of the cells or heterogeneous reactions at the (active or inactive) cell surface.

Several experiments were performed to determine whether complexation between Fe(III) and components of the sterile medium affected the reported rates. In aqueous solutions containing 1 mM lactic acid under benchmark conditions in the absence of SO, the OH production rate was $(8.7 ± 1.0) \times 10^{-12}$ M s$^{-1}$, which is the same within error as that measured in the absence of lactic acid ($(8.4 ± 0.2) \times 10^{-12}$ M s$^{-1}$). Some iron solutions in sterile medium were
allowed to sit for up to a day before experiments were run to determine whether Fe(III) reactivity increased over time due to complexation with components of the reaction solution such as lactate or benzoic acid. When Fe(III) was allowed to equilibrate in sterile medium for 24 hours prior to adding H$_2$O$_2$ in the absence of SO, OH production rates were the same within our experimental uncertainty as those measured when experiments were performed immediately after preparing the solution. These results suggest that Fe(III) complexation to lactate or benzoic acid in the sterile medium is not responsible for the rate enhancement observed in the presence of SO. We also performed a set of experiments to ensure that the phosphate buffer used in this work did not interfere with OH measurements: Hydroxyl radicals were generated by H$_2$O$_2$ photolysis (as described in the Appendix) in aqueous solution in the presence and absence of 0.05 M phosphate buffer, which is the concentration in the sterile medium. Hydroxyl radical production rates measured by the benzoic acid OH-trap were $(1.44 \pm 0.02) \times 10^{-8}$ M s$^{-1}$ and $(1.45 \pm 0.04) \times 10^{-8}$ M s$^{-1}$ in the presence and absence of buffer, respectively. This demonstrates that the buffer did not introduce artifacts in our studies.

The H$_2$O$_2$ concentrations used in these experiments span a range of environmentally-relevant values. As noted in the Introduction, H$_2$O$_2$ concentrations of 30 µM have been detected in natural waters, with even higher concentrations reported in rainwater and wastewater (around 100 µM and 29 mM respectively).$^{22-25,50}$ The concentrations used in our study span those reported in natural waters and in rainwater. We are unaware of reported cell densities of SO in environmental surface waters, but cell densities of other iron-reducing bacteria have been reported: Shewanella putrefaciens is commonly found in the environment at cell densities ranging from $10^5$ to $10^8$ cells mL$^{-1}$, and cell densities of Geobacter species of similar magnitude
have been reported in natural waters.\textsuperscript{51,52} This gives us confidence that the cell densities used in our experiments span environmentally-relevant values.

We measured OH production rates at H\textsubscript{2}O\textsubscript{2} concentrations ranging from $5 \times 10^{-6}$ to $2 \times 10^{-3}$ M, and at SO cell densities ranging from $2.8 \times 10^{5}$ to $7.0 \times 10^{6}$ cells mL\textsuperscript{-1}. Each sample tested had a maximum SO cell density (as measured by flow cytometry) of $7.0 \times 10^{6}$ cells mL\textsuperscript{-1}. This value was obtained for both filtered and unfiltered SO samples, which suggests that this is the maximum cell density obtainable in the minimal medium used in these experiments. To our knowledge, this is the first report of the effects of bacteria cell density on OH production rates in aqueous solution, either in the context of natural waters or water treatment facilities. The effects of H\textsubscript{2}O\textsubscript{2} concentrations and SO cell density are shown in Figure 4.6. Linear dependences were observed in both cases. Since the data in Figures 4.6a and 4.6b were acquired at a single SO cell density and H\textsubscript{2}O\textsubscript{2} concentration, respectively, it is possible that interactions between SO and H\textsubscript{2}O\textsubscript{2} will result in a different OH formation rate dependence at different cell densities or concentrations. To determine whether this was the case, we used the slopes of the best-fit lines to the data in Figures 4.6a and 4.6b to make a rate expression for OH production from bacteria-assisted Fenton chemistry under our experimental conditions.

$$\frac{d[OH]}{dt} = 1.04 \times 10^{8} \text{M}^{-1} \text{s}^{-1}[H_{2}O_{2}][SO] + 1.8 \times 10^{-7} \text{s}^{-1}[H_{2}O_{2}]$$

[3]

Here, SO cell density is expressed in terms of molarity (i.e. 1 mol = 6.022 $\times$ 10\textsuperscript{23} cells), and the second term on the right hand side of the equation describes OH production from Fenton chemistry in the absence of SO. This unit conversion allows us to express OH production rates in standard units of M s\textsuperscript{-1}. We note that the rate constants used in this expression correspond to
kinetics measured in the sterile medium used in our experiments. In deionized water this expression would become:

\[
\frac{d[OH]}{dt} = 2.43 \times 10^7 \, M^{-1}s^{-1} [H_2O_2][SO] + 4.2 \times 10^{-8} \, s^{-1} [H_2O_2] \tag{5}
\]

Figure 4.6. The dependence of OH production rates on (a) H$_2$O$_2$ concentration and (b) SO cell density in the presence of Fe(III). Error bars represent the standard deviation about the mean of three trials. The solid traces are linear fits to the data. The slopes are (a) $(1.4 \pm 0.1) \times 10^{-6}$ s$^{-1}$ and (b) $(2.8 \pm 0.2) \times 10^{-20}$ mol cell$^{-1}$ s$^{-1}$ with $R^2$ values of 0.99 and 0.98 respectively. All experiments
were performed in sterile medium under benchmark conditions. All experiments in (a) were performed with $7.0 \times 10^6$ cells mL$^{-1}$ SO. Experiments in (b) were performed in the presence of $2.0 \times 10^{-4}$ M H$_2$O$_2$, except for the points represented by open circles, in which $2.0 \times 10^{-3}$ M H$_2$O$_2$ was used and measured rates were normalized to $2.0 \times 10^{-4}$ M. These points were not used to calculate the fit to the data.

Table 4.2 shows the OH production rates predicted by Equation 1 at three different SO cell counts in the presence of $2.0 \times 10^{-3}$ M H$_2$O$_2$, as well as rates measured under these conditions. Agreement was excellent at all SO cell counts (measured and predicted rates agreed within 5% in all cases). These rates (normalized to an H$_2$O$_2$ concentration of $2.0 \times 10^{-4}$ M) are plotted as open circles in Figure 4.6b. Equation 2 provides a first step toward quantitative predictions of OH production rates in dark circumneutral waters from bacteria-enhanced Fenton chemistry. To be truly quantitative, the dependence of OH production rates on several other variables, including pH, iron concentration, oxygen content, and organic matter concentration and identity need to be established.

**Table 4.2.** Hydroxyl radical production rates at SO cell densities and H$_2$O$_2$ concentrations that were not used to calculate the rate constant for SO-assisted Fenton chemistry, along with OH production rates predicted by Equation 1.
<table>
<thead>
<tr>
<th>SO cell density (cells mL$^{-1}$)</th>
<th>OH production rate ( (10^{-11} \text{ M s}^{-1}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predicted</td>
<td>Measured</td>
</tr>
<tr>
<td>2.8 \times 10^5</td>
<td>40</td>
</tr>
<tr>
<td>5.1 \times 10^5</td>
<td>48</td>
</tr>
<tr>
<td>7.0 \times 10^6</td>
<td>272</td>
</tr>
</tbody>
</table>

The H$_2$O$_2$ concentrations and SO cell densities used in our experiments span environmentally-relevant levels, but do not encompass the entire expected ranges. Both H$_2$O$_2$ and SO will exist in natural waters at concentrations and cell densities respectively that are lower than those used in our study. Extending measurements to lower concentrations and cell densities was limited by instrument sensitivity; specifically, we could not measure OH formation rates below our detection limit of \( \sim 6 \times 10^{-12} \text{ M s}^{-1} \) (vide supra). However, the linear dependences of OH formation rates on H$_2$O$_2$ concentrations and SO cell densities spanning over an order of magnitude give us confidence that OH production rates in the presence of SO will also depend linearly on H$_2$O$_2$ and SO at lower concentrations and cell densities, and that Equations 3 and 4 can be extrapolated to lower concentrations and cell densities than were used in our experiments.

Higher H$_2$O$_2$ concentrations than those used in our experiments are not common in natural waters, although higher concentrations (on the order of 0.03 M) have been reported in wastewaters.\textsuperscript{50} Hydrogen peroxide has been reported to impede SO growth at concentrations greater than 0.1 M H$_2$O$_2$, and it is possible that high H$_2$O$_2$ concentrations will also suppress SO’s ability to reduce iron, leading to a nonlinear dependence of OH formation on H$_2$O$_2$ at higher concentrations.
concentrations. Studies investigating the use of iron-reducing bacteria such as SO for waste-water treatment should consider this possibility, but it is not likely important in natural waters, which are the focus of the current study. Further, the linear dependence of OH formation rates on \( \text{H}_2\text{O}_2 \) concentration shown in Figure 4.6a gives us confidence that the \( \text{H}_2\text{O}_2 \) concentrations used in this study did not affect the ability of SO to reduce Fe(III).

All of the experiments discussed above were performed with SO that had been filtered to remove aggregates. We also performed some experiments with unfiltered SO samples to determine whether aggregation can alter the role of SO in bacteria-assisted Fenton chemistry. Hydroxyl radical formation rates in unfiltered samples were the same at all dilutions, and were the same as the maximum rate measured in filtered samples under benchmark conditions in the presence of \( 2.0 \times 10^{-4} \text{ M} \ \text{H}_2\text{O}_2 \) (both \( (2.2 \pm 0.2) \times 10^{-10} \text{ M s}^{-1} \)). The insensitivity of the rate to dilution is likely due to the fact that SO is a clumping bacteria. When unfiltered samples are diluted, clumps break up and repopulate the solution with individual cells, increasing the cell density. Figure 4.7 shows the effects of diluting stock solutions of filtered and unfiltered SO on cell density (as measured by flow cytometry). No decrease in cell density is observed for the unfiltered samples, while a nonlinear decrease is observed for the filtered samples. This nonlinear decrease is likely due to incomplete removal of clumps during filtration. Since flow cytometry does not include clumps in its cell count (due to the clumps being much larger than the individual cells being monitored), these results support our hypothesis that the insensitivity of OH production rate to dilution of unfiltered samples is due to an equilibrium being established between clumps and non-aggregated cells. We note that the extent of clumping was the same in samples grown at 30 °C and 37 °C. The extent of clumping may change in different growth media and in natural waters, and may depend on the stage of growth of the bacteria. These
results highlight the importance of measuring cell density by methods (such as flow cytometry) that do not include clumps in the cell count, since our results indicate that iron-reducing activity (and enhancement of OH production rates) depends only on the density of individual (i.e. non-aggregated) SO cells.

Figure 4.7. Cell Density as measured by flow cytometry in filtered (closed black circles) and unfiltered (open red circles) SO samples.

Factors other than H$_2$O$_2$ concentration and SO cell density and aggregation will likely affect bacteria-assisted OH production rates. For example, the identity and concentration of organic matter present in solution may affect SO reactivity. Lactate, which was used as an electron donor in our study (at a concentration of 90 mg L$^{-1}$), is not present in high concentrations in natural waters. Average total organic loadings in natural waters do not typically exceed 60 mg L$^{-1}$, but local concentrations can be much higher. For example, hydrophobic organic matter can form organic microenvironments within natural waters, organic films on the order of tens of micrometers often exist at the surface of natural bodies of water, and organic loadings in sediment can be very high.$^{55-58}$ *Shewanella oneidensis* is known to oxidize a wide range of organic molecules, although few studies have investigated its ability to use
environmentally-relevant organic molecules as electron donors.\textsuperscript{38-40} Formate, which is often an important component of the organic fraction of surface waters, aqueous aerosols, and fog and cloud droplets, has been reported to be as effective an electron donor to SO as lactate in the context of SO-mediated manganese reduction.\textsuperscript{59} Complex organic macromolecules collectively referred to as dissolved organic matter (DOM) or humic-like substances (HULIS) are common constituents of surface waters and atmospheric aerosols respectively. These molecules could affect reactivity in several ways: They could act as electron donors or acceptors, solubilize Fe(III), transport Fe(III) to SO cells, and scavenge dissolved Fe(II).\textsuperscript{48, 60-64} It has recently been reported that HULIS accelerates Fe(III) reduction by SO at low concentrations, but impedes it at higher concentrations.\textsuperscript{48} As noted above, other environmental factors such as temperature, pH, and molecular oxygen concentration will also likely affect SO-assisted Fenton chemistry. Further investigation into the effects of electron donor identity and concentration and environmental conditions (such as pH and temperature) are encouraged.

In a final set of experiments, we investigated the effects of SO on the degradation of the pollutant anthracene. Anthracene, along with other polycyclic aromatic hydrocarbons (PAHs), is an ubiquitous pollutant in natural waters, and is an EPA priority pollutant.\textsuperscript{65, 66} Anthracene degradation kinetics were measured in buffered media containing $2 \times 10^{-4}$ M $\text{H}_2\text{O}_2$, $7.5 \times 10^{-8}$ M anthracene, and 100 $\mu$M Fe(III). Figure 4.8 shows anthracene degradation rates as a function of SO cell density. In the absence of either $\text{H}_2\text{O}_2$ or Fe(III), no anthracene degradation was observed. Some anthracene loss was observed in the presence of $\text{H}_2\text{O}_2$ and Fe(III) in the absence of SO, but this loss was not significant within our experimental uncertainty. In the presence of $\text{H}_2\text{O}_2$, Fe(III), and SO, anthracene degradation rates increased linearly with increasing SO cell density, reaching a maximum of $(2.5 \pm 0.2) \times 10^{-10}$ M s$^{-1}$ at a cell density of $7 \times 10^6$ cells mL$^{-1}$. 
This rate is similar to the OH production rate we measured at that SO cell density of $2.2 \times 10^{-10}$ M s$^{-1}$, which suggests that OH produced from bacteria-enhanced Fenton chemistry is primarily responsible for anthracene’s degradation in these experiments. It also indicates that if OH production rates from bacteria-assisted Fenton chemistry are known, lifetimes of other aqueous species (both organic and inorganic) can be accurately predicted.

![Figure 4.8](image)

**Figure 4.8.** The dependence of anthracene degradation rate on SO cell density in the presence of Fe(III). Error bars represent the standard deviation about the mean of three trials. The slope is $(3.2 \pm 0.1) \times 10^{-20}$ mol cell$^{-1}$ s$^{-1}$ with an $R^2$ value of 0.99.

In the presence of high SO cell densities, bacteria-assisted Fenton chemistry could be an important fate for pollutants; under some circumstances, dark degradation rates could be similar to those during the day. For example, we have previously measured anthracene photolysis kinetics in aqueous solution; based on the rate constants reported in those studies, we expect $1.5 \times 10^{-7}$ M anthracene to photolyze at a rate on the order of approximately a factor of 6 lower than the degradation rate measured in this study due to bacteria-enhanced Fenton chemistry.$^{67,68}$ Direct photolysis is not the only degradation route available to pollutants such as anthracene in
sunlit waters, but this comparison serves to show that radical chemistry may not be negligible in
dark circumneutral waters, as has previously been assumed.

Our results demonstrate a previously-unconsidered mechanism for the production of radicals in dark natural waters. *Shewanella oneidensis* can increase OH production rates at near-neutral pH by up to a factor of six in the dark. Given that dark Fenton chemistry is currently only considered to be a significant source of radicals in acidic natural waters due to the insolubility of Fe(III) at near-neutral pH, bacteria-assisted Fenton chemistry could greatly increase the overall importance of Fenton chemistry, since many bodies of water have pH levels greater than 3. Enhancements in dark radical production rates even greater than those reported in this work may be observed in natural waters containing nutrient levels higher than those in the minimal medium used in our experiments (potentially leading to higher cell densities), or in the presence of additional metals that participate in Fenton-like chemistry. Other iron-reducing bacteria found in natural waters, such as *Geobacter* species, may have similar enhancing effects on dark Fenton chemistry.

The environmental relevance of bacteria-assisted Fenton chemistry may extend beyond surface waters. The effects of bacteria on heterogeneous and multiphase chemistry in atmospheric aerosols and cloud droplets is an area of growing interest, although existing studies have primarily focused on direct degradation of organic species by bacteria. SO has recently been detected in atmospheric aerosols near a heavily contaminated creek. Our results suggest that if SO or other iron-reducing bacteria are common in atmospheric fog and water droplets, they could significantly alter the oxidizing capacity of these droplets. This could have effects such as decreasing droplet pH by enhancing $\text{H}_2\text{SO}_4$ formation and contributing to the formation of secondary aerosols via the oxidation of dissolved organic species.
4.4 References

66. USEPA, Agency for Toxic Substances and Disease, Anthracene.
Appendix

A.1 Chapter Two Supporting Information

Figure S1  Absorption spectrum of \(3.0 \times 10^{-7}\) M anthracene in methanol acquired between 300 and 400 nm at 0.5 nm increments.

Figure S2  Absorption spectra of \(3.0 \times 10^{-7}\) M pyrene in water and methanol acquired between 280 and 400 nm at 0.5 nm increments.
Figure S3. Beer-Lambert plots for (a) anthracene in methanol; (b) pyrene in methanol; and (c) pyrene in water. The molar absorptivities calculated from the slopes of the best-fit lines to the data are (a) \((6.4 \pm 0.1) \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}\); (b) \((1.65 \pm 0.09) \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}\); and (c) \((1.94 \pm 0.09) \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}\). Error bars represent the standard deviation about the mean of three trials.

A.1.1 Actinometry and HPLC Methodology

Materials

Solutions containing hydrogen peroxide \((\text{H}_2\text{O}_2, \text{Fluka, 31.5%}, \text{standardized by KMnO}_4\text{ titration})\) in 18.2 M\(\Omega\)-cm deionized water were newly prepared the day before experiments and stored away from light. Solutions containing benzoic acid (Sigma-Aldrich, >99.5%), salicylic acid (SA, Alfa Aesar, 99+%), 3-hydroxybenzoic acid (3-HBA, Sigma-Aldrich, 99%), 4-hydroxybenzoic acid (4-HBA, Sigma-Aldrich, >99%), and water with 0.1% trifluoroacetic acid (“TFA water”, Fluka) were prepared immediately prior to use.

Method

Samples containing \(\text{H}_2\text{O}_2\) and benzoic acid were irradiated with the xenon arc lamp used in the experiments described in the manuscript. Hydrogen peroxide photolyzes to form hydroxyl radicals \((\text{OH})\); this reaction has been previously used to measure photon fluxes relevant to sunlight at Earth’s surface.\(^1\) Benzoic acid was used as a hydroxyl radical trap in these experiments; it reacts with \(\text{OH}\) at near-diffusion limited rates to produce SA, 3-HBA, and 4-HBA, and has been used as an \(\text{OH}\) trap in environmentally-relevant chemical actinometry.\(^2,3\) Salicylic acid fluoresces at 405 nm, while benzoic acid, 3-HBA, and 4-HBA do not fluoresce. Fluorescence intensity of SA can therefore be related to \(\text{OH}\) concentration.

We measured fluorescence intensity of SA (with excitation at 300 nm and emission monitored at 405 nm) before samples were irradiated and at multiple time intervals during irradiation. Salicylic acid intensity was plotted as a function of irradiation time, and the slope of the best fit line to the data provided the production rate of SA. Experiments were performed with a range of initial benzoic acid concentrations to ensure that \(\text{OH}\) radicals were completely scavenged under our experimental conditions. Only initial rates (where SA fluorescence intensity increased linearly with time) were used in our calculations. At longer time periods, when SA concentrations increase, SA can react with \(\text{OH}\); considering only short time periods (on the order of 10-20 minutes for our experiments) reduces the possibility of artifacts caused by secondary reactions.

As noted above, reactions between benzoic acid and \(\text{OH}\) form products other than SA (3-HBA and 4-HBA). These products must be considered in order to quantify \(\text{OH}\) production. We used High-Pressure Liquid Chromatography (HPLC) of irradiated benzoic acid and \(\text{H}_2\text{O}_2\) solutions to
determine the branching ratio of the three products listed above. HPLC was performed with a mobile phase consisting of 45% MeOH and 55% TFA water. All mobile phase components were de-gassed for 20 minutes prior to running through the instrument to prepare the column.

Figure S4 shows a representative chromatogram of an irradiated benzoic acid / \( \text{H}_2\text{O}_2 \) solution. We achieved good peak separation between all benzoic acid derivatives. Product peaks were assigned by comparing peak retention times to standards. Our measured branching ratio was SA:3-HBA:4-HBA = 1:2:1 with an uncertainty of 10%. We observed the same branching ratio for solutions that had been irradiated for 13 minutes and 20 minutes, suggesting that the ratio remains constant over the time period of our experiments. Since SA only accounts for 25% of the products formed from the reaction of benzoic acid with OH, we multiplied our measured SA formation rate by a factor of 4 to obtain the OH formation rate from \( \text{H}_2\text{O}_2 \) photolysis in our experiments.

![Figure S4](image)

**Figure S4.** HPLC trace of a sample of \( 6.25 \times 10^{-4} \) M benzoic acid and \( 9.9 \times 10^{-4} \) M hydrogen peroxide after 20 minutes of irradiation.

A limit of detection (LOD) for OH formation rates of \( 6.1 \times 10^{-12} \) M s\(^{-1} \) was determined based on the lowest SA fluorescence intensity we could accurately measure after 30 minutes of irradiation. Plots of OH formation rate as a function of \( \text{H}_2\text{O}_2 \) concentration were used to determine a photolysis rate constant for \( \text{H}_2\text{O}_2 \left( J_{\text{H}_2\text{O}_2} \right) \) of \( 7.17 \times 10^{-6} \) s\(^{-1} \). We then used \( J_{\text{H}_2\text{O}_2} \) to calculate the photon flux of our xenon arc lamp using Equation S1:

\[
J_{\text{H}_2\text{O}_2} = \int_{\lambda_o}^{\lambda} \sigma(\lambda) \Phi(\lambda) F_{\lambda} d\lambda \quad \text{[S1]}
\]

where \( \sigma(\lambda) \) is the absorption cross-section of hydrogen peroxide at a given wavelength, \( \Phi(\lambda) \) is the \( \text{H}_2\text{O}_2 \) photolysis quantum yield, and \( F_{\lambda} \) is the photon flux of our light source. We used absorption cross sections and photolysis quantum yields from Ref\(^4 \). The photon flux of the arc lamp was
then calculated from Equation S1. The total photon flux of the lamp (from 290-380 nm) was calculated to be \((1.47 \pm 0.04) \times 10^{14}\) photons cm\(^{-2}\) s\(^{-1}\). This compared well to the manufacturer’s stated photon flux of \((1.35 \pm 0.65) \times 10^{14}\) photons cm\(^{-2}\) s\(^{-1}\) in the same spectral region.

**Table S1.** Dielectric constants and pyrene 1:3 peak ratios of solvents used in this work.\(^5,6\)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Dielectric Constant(^5,6)</th>
<th>1:3 Ratio (this work)</th>
<th>Literature 1:3 Ratio(^7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decanol</td>
<td>8.1</td>
<td>0.86</td>
<td>n/a</td>
</tr>
<tr>
<td>Octanol</td>
<td>9.9</td>
<td>0.92</td>
<td>0.93</td>
</tr>
<tr>
<td>2-propanol</td>
<td>19.2</td>
<td>1.06</td>
<td>1.09</td>
</tr>
<tr>
<td>Methanol</td>
<td>32.7</td>
<td>1.32</td>
<td>1.35</td>
</tr>
<tr>
<td>Acetonitrile</td>
<td>36</td>
<td>1.89</td>
<td>1.79</td>
</tr>
<tr>
<td>Dimethylsulfoxide</td>
<td>46.5</td>
<td>1.92</td>
<td>1.96</td>
</tr>
<tr>
<td>Water</td>
<td>78.4</td>
<td>1.89</td>
<td>1.87</td>
</tr>
</tbody>
</table>

**Figure S5.** Absorption spectra of \(3.0 \times 10^{-5}\) M pyrene in organic solvents acquired at 0.5 nm increments.
Figure S6. Absorption spectra of $3.0 \times 10^{-5}$ M anthracene in organic solvents acquired at 0.5 nm increments.

Figure S7. Effect of DMSO on anthracene and pyrene photolysis kinetics in aqueous solution. Error bars represent the standard deviation about the mean of three trials. The solid traces are best-fit lines to the data, and are meant to guide the eye.
A.1.2 References

A.2 Chapter Four Supporting Information

A.2.1 Growth Medium Preparation

Medium components were dissolved in deionized water and placed in an autoclavable container and autoclaved for 15 to 20 minutes at 121°C. The medium was then transferred to sterile screw-cap tubes and stored in a refrigerator.

Growth Medium Composition

In deionized water:
- 1 mM lactic acid
- 2.45 g/L NaH_2PO_4 \cdot H_2O
- 4.58 g/L Na_2HPO_4
- 0.5 g/L NH_4Cl
- 0.15 g/L KCl
- 0.075 g/L CaCl_2 \cdot H_2O
- 1 mL/100 mL Wolfe’s trace minerals solution (In de-ionized water: 0.5 g/L EDTA, 3.0 g/L MgSO_4 \cdot 7H_2O, 0.5 g/L MnSO_4 \cdot H_2O, 1.0 g/L NaCl, 0.1 g/L FeSO_4 \cdot 7H_2O, 0.1 g/L Co(NO_3)_2 \cdot 6H_2O, 0.1 g/L CaCl_2 (anhydrous), 0.1 g/L ZnSO_4 \cdot 7H_2O, 0.01 g/L CuSO_4 \cdot 5H_2O, 0.01 g/L AlK(SO_4)_2 (anhydrous), 0.01 g/L H_3BO_3, 0.01 g/L Na_2MoO_4 \cdot 2H_2O, 0.001 g/L Na_2SeO_3 (anhydrous), 0.01 g/L Na_2WO_4 \cdot 2H_2O, 0.02 g/L NiCl_2 \cdot 6H_2O)

A.2.2 OH Quantification

Hydroxyl radicals (OH) react with benzoic acid at near-diffusion limited rates to produce salicylic acid (SA), 3-hydroxybenzoic acid (3-HBA), and 4-hydroxybenzoic acid (4-HBA).\(^1\) We have measured a product branching ratio of SA:3-HBA:4-HBA = 1:2:1.\(^2\) Salicylic acid can be measured by fluorescence spectroscopy; 3-HBA and 4-HBA do not fluoresce.

Hydroxyl radical production rates were measured by taking fluorescence readings over set time intervals and monitoring SA emission intensity at 407 nm (with 300 nm excitation). To obtain OH production rates, we multiplied the measured SA formation rate by 4 to account for all three reactions OH undergoes with BA (since 3-HBA and 4-HBA are not detected by our fluorescence measurements).\(^2\)

We used BA concentrations of \(7.5 \times 10^{-4}\) M in all Fenton experiments in this study. At this concentration, observed OH production rates are insensitive to BA concentration when OH
is generated from H$_2$O$_2$ photolysis, which is much faster than OH generation via Fenton chemistry under our experimental conditions. As further confirmation that SA production is insensitive to BA independent of concentration under our experimental conditions, we measured OH production rates from Fenton chemistry in the presence of 100 µM Fe(III), 2 × 10$^{-4}$ M H$_2$O$_2$, and 2.5 × 10$^{-3}$ M BA. At this higher BA concentration, the observed OH production rate was (8.6 ± 0.4) × 10$^{-12}$ M s$^{-1}$, which is the same within error as the rate measured in experiments using 7.5 × 10$^{-4}$ M BA ((8.4 ± 0.2) × 10$^{-12}$ M s$^{-1}$).

Under our experimental conditions, lactic acid concentrations are higher than BA concentrations (1 × 10$^{-3}$ and 7.5 × 10$^{-4}$ M respectively). Benzoic acid is much more reactive toward OH than is lactic acid (with rate constants of 5.9 × 10$^{9}$ M$^{-1}$ s$^{-1}$ and 2.0 × 10$^{6}$ M$^{-1}$ s$^{-1}$ respectively), so we expect OH to react almost quantitatively with BA even in the presence of lactic acid. To ensure that this was the case in our experiments, we measured OH production rates from dark Fenton chemistry (with iron in the form of Fe(III)) in aqueous solution in the presence and absence of 1 mM lactic acid. Measured rates were (8.7 ± 1.0) × 10$^{-12}$ M s$^{-1}$ and (8.4 ± 0.2) × 10$^{-12}$ M s$^{-1}$ respectively, which leads us to conclude that lactic acid is not a significant OH sink in our experiments.

**Figure S8.** Effect of Fe(II) concentration on the detector response to luminol phosphorescence in sterile medium. The slope is (10204 ± 251) counts µM$^{-1}$ with an $R^2$ value of 0.99.
Figure S9. Fe(II) oxidation over time in sterile medium at a pH 7 in the absence of hydrogen peroxide and SO.
Figure S10. Effect of H$_2$O$_2$ concentration on OH production rates from Fenton chemistry with iron in the form of Fe(III) under benchmark conditions in (a) water and (b) sterile medium. Error bars represent the standard deviation about the mean of three trials. Error bars for 0 M H$_2$O$_2$ indicate our limit of detection for OH production.

A.2.3 References

EDUCATION

2012-2016 Syracuse University G’16, Syracuse, NY
Ph.D., Chemistry

2008-2012 Drew University ’12, Madison, NJ
B.A., Chemistry
B.A., Environmental Science

RESEARCH/INDEPENDENT THINKING/TEAM WORK EXPERIENCE

EPA Analytical Chemistry Associate, US EPA: Fall 2016-Present
National Exposure Research Laboratory.

- Develop methods for measuring stressors (chemical, biological, physical, and psychosocial) in environmental and biological media
- Design and conduct measurement-based studies (field and laboratory) to identify sources (forensics), informing fate and transport processes, and estimating exposure and dose for ecological and human receptors
- Exposure classification in epidemiologic studies
- Exposure in the context of risk assessment
- Dose estimation for environmentally relevant toxicological studies

Research Assistant (PhD in Chemistry), Syracuse University: Fall 2012-Fall 2016
Department of Chemistry (Laboratory of T.F. Kahan). Created and conducted novel independent research that involved highly analytical thinking, problem solving, collaborative efforts, leadership, and statistical approaches as well as creative experimental design. Generated high impact research publications from results. Made purchasing decisions, built/repaired analytical instrumentation, managed multiple lab personnel, made decisions regarding research direction, wrote first author publications, and proofread grants.

Dissertation: Laboratory Investigations Into the Fate of Aromatic Pollutants in Natural Waters.

Undergraduate Research Assistant, Drew University: Spring 2011
Department of Chemistry (Laboratory of J.M. Liu). Developed and adapted an improved method for detection of a persistent organic pollutant molecule based off of previous methodology. Used and maintained analytical instrumentation as well as kept detailed notes and observation on use of instrumentation.

Summer Research Assistant, Drew University: Summer 2011
Department of Chemistry (Laboratory of J.M. Liu). *Used the aforementioned methodology to detect said toxic molecule with success and compare to a novel detection method that utilized bacteria.*

**Process Geomorphology Research Project, Drew University**  
*Independent research done across eastern coast on climate, temperature, and erosion. Analyzed results using GIS mapping software and statistical methods. Brought forth striking conclusions independently.*

**LEADERSHIP/TEACHING EXPERIENCE**

**Teaching Assistant, Syracuse University: Fall 2013 – December 2015**  
Department of Chemistry (General Chemistry). *Taught a 100 level course with a large student body, graded assignments, and helped students with academic and personal concerns in office hours.*

**Teaching Assistant, Syracuse University: Fall 2012 - Spring 2013**  
Department of Chemistry (Organic Chemistry). *Taught an advanced course with a large student body, graded assignments, and assisted students with life and career decisions in office hours.*

**National Science Foundation Scholar, American Chemical Society (ACS): 2012**  
Green Chemistry Scholar. *Attended national meeting and shared ideas and thoughts on green chemistry and chemistry in general. Led discussion on novel ideas for a paradigm shift in the reporting/measurement of reaction metrics.*

**Tutor, Drew University: Fall 2011- Spring 2012**  
Department of Chemistry (Organic Chemistry). *Tutored students in organic chemistry.*

**RESEARCH OUTREACH** (Full list available upon request)


**TECHNICAL SKILLS**

*(Extensive Use/Knowledge Underlined)*

**Instruments & Techniques**: GC/MS, Raman Microscopy, Fluorescence Spectroscopy, Fluorescence Lifetime Instruments, UV-Vis Spectroscopy, NMR (¹H, ¹³C Coupled, ¹³C Decoupled, 2D COSY), HPLC, FT-IR (KBr Pellet and ATR), ICP-OES, LC/MS, AAS, culturing bacteria, photolysis kinetics measurements, building instrumentation, repairing instrumentation, chemical actinometry, problem solving, field work, sampling, technical reports, time management, project management, personnel management, budget decisions, technical writing/proofreading, technical talks, technical education, conceptual chemistry expertise, and systems analysis.

**Software**: GIS, Excel, Gaussian, Chemstation, Waters, Chemdraw, Pymol, Igor, MathCad, PowerPoint, Adobe Photoshop, OpenLab CDS, SPSS, Word, endnote, and TecMag.

**ACTIVITIES/APPOINTMENTS/AWARDS**

*2012* National Science Foundation (NSF) Scholar

*2009-2012* Drew University Sustainability Committee

Drew University Chemistry Society (DUCS)

•Member 2010-2012
• Treasurer 2011-2012
  2009-2012 Chatham, NJ Green Initiatives Committee
  2008-2009 Drew University Sustainability Intern
  2008-2010 Drew University Head Eco-Rep

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