ABSTRACT

BAEZA, ANA CAROLINA. Removal of Pharmaceutical and Endocrine Disrupting Chemicals by Sequential Photochemical and Biological Oxidation Processes. (Under the direction of Dr. Detlef Knappe).

The presence of biochemically active compounds (BACs) such as endocrine disrupting chemicals (EDCs) and antimicrobial compounds in the aquatic environment is an issue of great concern. For example, EDCs can cause gender bending in aquatic life, and antimicrobial compounds may lead to the evolution of antimicrobial resistant bacteria. The principal objective of this research was to quantify the effectiveness of combining UV/H_2O_2 and biological oxidation processes for the mineralization of BACs that commonly occur at trace levels in municipal wastewater and in drinking water sources.

Initially, the photolysis and UV/H_2O_2 photooxidation rates of six BACs [the antimicrobial compounds sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfadiazine (SDZ), and trimethoprim (TMP), the EDC bisphenol-A (BPA), and the analgesic diclofenac (DCL)] were determined. Experiments were conducted in ultrapure water, lake water (Lake Wheeler, NC) and wastewater treatment plant effluent (North Cary Water Reclamation Facility, Cary, NC). Photolysis and UV/H_2O_2 oxidation rates of BACs were quantified with a quasi-collimated beam (QCB)

apparatus equipped with low pressure UV lamps, and the effects of the following factors on the BAC oxidation rates were evaluated: (1) pH, (2) H_2O_2 concentration, and (3) presence/absence of background organic matter. With the QCB apparatus, parameters such as quantum yields and second order rate constants describing the reaction between hydroxyl radicals and BACs were determined. With these parameters the level of BAC transformation at different UV fluences and H_2O_2 concentrations was predicted. For example, at treatment conditions used at a full-scale UV/H_2O_2 plant in the Netherlands (UV fluence = 540 mJ cm^{-2}, H_2O_2 dose = 6 mg L^{-1}), the following BAC transformation percentages would be obtained in NC lake water: ~98% for DCL, ~79% for SMX, ~60% for SMZ, ~51% for SDZ, ~43% for TMP, and ~46% for BPA. In wastewater treatment plant effluent, predicted BAC transformation percentages were lower at the same treatment conditions because hydroxyl radical scavengers were present at higher concentrations.

Apart from determining parent compound removal rates in UV photolysis and UV/H_2O_2 photooxidation processes, antimicrobial activity removal was quantified by conducting growth inhibition assays. Using the Enterobacteriaceae organism *E. coli* ATCC® 25922, growth inhibition assay data showed that the antimicrobial activity in photochemically treated water samples was principally a result of the parent compound concentration that remained upon treatment. Therefore, no measurable antimicrobial activity was exerted by the photolysis and UV/H_2O_2 oxidation products

of the studied sulfonamides and TMP. These results were consistently obtained for the different background water matrices and solution pH values that were studied.

Finally, the mineralization potential of three ^{14}C-labeled BACs (sulfadiazine, bisphenol-A, and diclofenac) after UV/H_2O_2 treatment was examined with a consortium of lake water bacteria and with bacteria associated with lake sediments. Upon UV/H_2O_2 oxidation, mineralization of ^{14}C-labeled BAC oxidation intermediates by lake water bacteria was extremely slow (<1.1% for SDZ, <0.8% for BPA and <0.8% for DCL in 30 days). The use of lake sediments enhanced the biodegradation rate of sulfadiazine and its UV/H_2O_2 oxidation intermediates, but mineralization rates were still slow (1.1% for SDZ and 5.2% for SDZ UV/H_2O_2 oxidation intermediates after 30 days).

Overall, the results of this research suggest that the UV/H_2O_2 process is able to remove BAC parent compounds and, for antibiotics, antimicrobial activity; however, oxidation intermediates may be persistent in the environment. Additional studies should be performed to determine the effects of these intermediates on aquatic life and their toxicological importance in the context of direct or indirect potable water reuse.

Removal of Pharmaceutical and Endocrine Disrupting Chemicals by Sequential Photochemical and Biological Oxidation Processes.

by
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BIOGRAPHY

Ana Carolina Baeza received her Degree of Licentiate of Engineering Sciences in 1996 and graduated in July 1999 with a B.S. in Chemical Engineering from the University of Concepción, Chile. After graduating, Carolina's interest in environmental engineering led her to pursue a Postgraduate Diploma in Environmental Engineering (January 2000) and work on environmental issues as a research assistant at the University of Concepción for 2 years. In 2001, Carolina came to the United States and joined Master of Science program in Department of Civil, Construction and Environmental Engineering at North Carolina State University. After obtaining her M.S. degree in 2003, Carolina continued her graduate studies towards a Ph.D. degree in the Department of Civil, Construction and Environmental Engineering at North Carolina State University under the direction of Dr. Detlef Knappe. Upon graduation, Carolina will join the Environmental Science Center EULA at the University of Concepción, Chile.

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Chapter 1.

INTRODUCTION AND OBJECTIVES

1.1. Problem Statement

Exponential growth of the human population continues to increase demands for potable water supplies. As a result, alternative water sources are being explored, and water reuse will play a prominent role in this quest. In the US, recycled and reclaimed water use is growing at an estimated 15% per year (Miller, 2006). The removal of trace organic contaminants from drinking water sources or water intended for reuse application is a challenge for water utilities and regulatory agencies. Apart from more traditional organic pollutants such as pesticides, organic solvents, and fuel hydrocarbons, a wide range of emerging organic contaminants are present in wastewater and wastewater-impacted surface and ground water (Halling-Sorensen, *et al*., 1998, Hirsch *et al*, 1999, Lindsey *et al,* 2001, Heberer *et al.,* 2002, Kolpin *et al.,* 2002, Yang *et al.,* 2004, Zuccato, *et al.* 2006). Among the emerging organic contaminants, endocrine disrupting chemicals (EDCs) and pharmaceutically active compounds (PhACs) in aquatic environments are being studied intensively because (1) EDCs can alter the endocrine system of humans and wildlife at very low concentrations (Campbell, et al. 2006) and (2) the presence of antimicrobial compounds in water may accelerate the evolution of antimicrobial-resistant bacteria (Kummerer, 2001, Kummerer, 2004). In addition, the effects of chronic exposure to

mixtures of these compounds at sub-therapeutical doses are not yet known (Snyder *et al.*, 2005). Therefore, it is important to investigate treatment alternatives that effectively remove EDCs and PhACs from wastewater treatment plant discharges and drinking water sources.

Oxidation processes have been successfully utilized to transform organic contaminants in drinking water and wastewater. When treating trace organic contaminants with oxidants, removal of the parent compound is often readily achieved, but little is known about many of the oxidation intermediates that are formed in the process. Mineralization of trace organic compounds may therefore be a preferred goal when treating wastewater effluents for water reuse applications or drinking water from wastewater-impacted sources. One relatively energy-intensive and costly option would be to apply advanced oxidation technologies that are capable of mineralizing a large number of organic compounds. The mineralization of organic contaminants may be more economical, however, when sequential photochemical/biological oxidation processes are employed (Ollis, 2001). The latter option has been tested for concentrated waste streams, but not extensively for trace organic contaminants that coexist with background organic matter. Hence, the hypothesis that was tested in this research was that sequential photochemical/biological oxidation processes can efficiently mineralize trace levels of biochemically active compounds (BACs) that coexist with background organic matter in wastewater treatment plant effluents and

drinking water sources. The validity of the above hypothesis was tested with the ^{14}C-labeled BACs sulfadiazine, bisphenol A, and diclofenac.

1.2. Research Objectives

The ***principal objective*** of this research was to develop an advanced water/wastewater treatment strategy that provides a barrier against the entry of BACs into surface waters or drinking water distribution systems. The research described throughout this thesis evaluated UV photolysis and UV/H_2O_2 oxidation processes for BAC transformation and assesed the integration of sequential photochemical and biological oxidation processes for the mineralization of BACs and their photolysis/oxidation intermediates. ***Specific objectives*** included (1) characterization of UV photolysis and UV/H_2O_2 processes by determining BAC conversion kinetics and fundamental photochemical parameters, (2) assessment of antimicrobial activity exhibited by UV photolysis and UV/H_2O_2 oxidation products of antimicrobial compounds, and (3) determination of the mineralization rate of ^{14}C-labeled BAC and ^{14}C-labeled BAC photooxidation intermediates in batch bioreactors inoculated with either lake water bacteria or bacteria associated with lake sediments.

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Chapter 2.

LITERATURE REVIEW

2.1. Presence of biochemically active compounds in the aquatic environment

The presence of biochemically active compounds (BACs) in surface and ground water has been a growing concern over the last decade. Several studies have illustrated that prescription and non-prescription drugs are present at detectable levels in surface and ground waters, in drinking water sources, and in treated drinking water (Halling-Sorensen, *et al.,* 1998, Lindsey *et al.,* 2001, Heberer *et al.,* 2002a, Kolpin *et al.,* 2002, Boyd *et al.,* 2003, Yang *et al.,* 2004). Due to advances in analytical techniques, it is feasible to measure environmental concentrations of trace contaminants in the low ng L^{-1} levels. Pharmaceuticals that have been detected in water include antibiotics, analgesics, anti-inflammatory drugs, lipid regulators, beta-blockers, antiepileptic drugs, steroids, and hormones. In the United States, a study conducted by the US Geological survey detected antibiotics in about 22% of samples collected from 139 streams in 30 states (Kolpin *et al.* 2002). The most frequently detected antimicrobial compounds were triclosan (~60% of samples), trimethoprim (~27% of samples), lincomycin (~19% of samples), and sulfamethoxazole (~19% of samples). Concentrations of antibiotics in US surface waters are typically below 1 µg/L (Kolpin *et al.* 2002). Similar surveys have been done in the EU. Hirsch *et al.* (1999) detected

erythromycin (max. 1.7 μg/L), roxithromicyn (max. 0.56 μg/L), and sulfamethoxazole (max. 0.48 μg/L) in many German surface water samples. Zuccato *et al.* (2006) detected at least 14 pharmaceuticals in the Po river on Italy; lincomycin was the major pharmaceutical detected with a maximum concentration of 0.25 μg/L and a median concentration of 0.033 μg/L.

The presence of antimicrobial compounds in the environment is of concern because their presence can lead to the evolution of antimicrobial-resistant bacteria. Organisms can attain resistance by mutation or by acquisition of the genetic information that encodes the resistance (Kummerer *et al.,* 2004). It is thought that diverse ecological niches are formed throughout the environment, in which the gene transfer mechanisms (conjugation, transduction and transformation) can occur. As a result, water sources could be considered as a potential pool of resistant genes and a medium for their propagation (Davison *et al.* 1999, Jones *et al.* 2003, Schwartz *et al.* 2003). In addition, some studies have evaluated the effect of antibiotics on aquatic organisms; results of these studies suggest that higher concentrations than those present in the environment are required to produce any damaging effects (Snyder *et al.,* 2005). It is important to mention, however, that aquatic organisms are exposed to a diverse mixture of trace contaminants, and potential synergistic effects involving pharmaceuticals at sub-inhibitory concentrations are unknown (Flaherty and Dodson, 2005).

In the case of EDCs, these chemicals may cause intersexuality in fish (Vajda *et al.,* 2008) and their presence in WWTP effluents has been extensively reported; e.g., 17α-ethinylestradiol (EE2) was found at concentrations up to 3.4 ng L^{-1} (Baronti *et al.,* 2000, Williams *et al.,* 2003) and BPA concentrations in WWTP effluents range from <20 – 7625 ng L^{-1} (Fuerhacker, 2003, Aguayo *et al.,* 2004, Hohne and Puttmann, 2008).

Depending on utilization and disposal patterns, BACs will enter the environment by different pathways. Pharmaceuticals are administered in three ways: therapeutic (human and veterinary applications), prophylactic (as a preventive treatment – primarily in veterinary applications), and as growth promoters in livestock production (Willis, 2000). In 2001, the *Union of Concerned Scientists* estimated that 70 percent of the antibiotics produced in the US were utilized for non-therapeutic veterinary purposes (Mellon *et al.,* 2001). The *Federal Food, Drug, and Cosmetic Act* established that if an antibiotic is used or becomes important in human medicine, the drug will be prohibited for non-therapeutic use in animals unless the FDA establishes there is no risk to human health. In contrast to the U.S., the European Union banned the use of antibiotics for livestock growth promotion on January 1, 2006 (*Union of Concerned Scientists,* 2006).

Figure 1 summarizes possible pathways for BACs occurrence in the environment (Heberer *et al.* 2002b). BACs used in human and veterinary medicine are subject to different metabolic reactions and are released from the organism either unchanged or as metabolites via urine and feces. Most of the drugs used for veterinary purposes will be (1) directly discharged into water in aquaculture applications, (2) excreted directly on pastures or (3) spread onto agricultural land during land application of manure or sludge from lagoons containing animal waste. BACs that are present in land-applied manure/sludge or are disposed in landfills can eventually contaminate groundwater (Jorgensen *et al.* 2000). BACs residues are also released into the environment through the effluents from municipal wastewater treatment plant. Several studies have shown that the biodegradability for certain antibiotics is low during wastewater treatment and many antibiotics have been detected in waste water treatment plant (WWTP) effluents at concentrations as high as 6 μg/L (Hirsch *et al.,* 1999). Although there are no established regulatory limits on pharmaceuticals in WWTP effluents, the FDA requires environmental risk assessment for new pharmaceuticals with predicted environmental concentrations greater than 1 μg/L (Snyder *et al.,* 2005).

In this study three sulfonamides **(**SMX, SMZ, and SDZ) and one dihydrofolate reductase inhibitor (DHFR) TMP (Figure 1, Chapter 3) were selected as model antimicrobial compounds. In addition, the analgesic diclofenac (DCL) and the endocrine disruptor chemical (EDCs) bisphenol A (BPA) were studied.

Sulfonamides are a group of synthetic antimicrobials that interfere with bacterial folic acid synthesis. Sulfa drugs compete with a natural metabolic compound called para-aminobenzoic acid that is required by bacteria in the synthesis of the coenzyme tetrahydrofolic acid. This coenzyme is important in the synthesis of purines and some amino acids (Talaro and Talaro, 2002). Sulfonamides, specifically sulfamethoxazole, are often applied in a synergistic combination with trimethoprim (Figure 2). Trimethoprim interferes with the bacterial enzyme dihydrofolate reductase and inhibits the synthesis of tetrahydrofolic acid.

Sulfonamides have been detected in surface and ground waters worldwide. Sacher *et al.* (2001) reported the occurrence of sulfamethoxazole in ground water up to a concentration of 0.42 μg/L in Germany. Perret *et al.* (2006) found sulfonamide residues in Italian surface and mineral water with maximum concentrations of 0.24 and 0.08 μg/L, respectively. In the U.S., Lindsey *et al.* (2001) detected sulfonamides in surface and ground water at concentrations ranging from 0.07 to 15 μg/L. Kolpin *et al.* (2002) found sulfamethoxazole at concentrations up to of 1.9 μg/L in U.S. streams. Yang et al (2004) detected sulfamethoxazole downstream of a WWTP in a Colorado stream at concentrations up to 0.17 μg/L.

2.2. Removal of pharmaceutically active compounds during drinking water treatment

Conventional drinking water treatment plants include a sequence of the following processes: coagulation/flocculation, sedimentation, filtration, and disinfection. An ozonation stage has been included in a number of larger drinking water treatment plants over the last decades. Ozone is capable of oxidizing more persistent organic contaminants present in drinking water sources. Ozone can be added at several points in the treatment plant: pre-oxidation of raw water, settled water oxidation/disinfection or filtered water oxidation/disinfection. After an ozone oxidation stage, a biologically active filter is beneficial to remove biodegradable dissolved organic carbon that formed oxidation of natural organic matter (NOM). Additional technologies that are commonly employed in drinking water treatment include activated carbon adsorption, membrane filtration, and advanced oxidation processes (e.g. UV/H_2O_2, O_3/H_2O_2, O_3/UV). Table 1 summarizes important water treatment goals and commonly applied treatment processes used to achieve the goals.

Because the effects of chronic ingestion of subtherapeutical doses of pharmaceuticals via drinking water consumption are not known and because pharmaceuticals are ubiquitous in water sources, the removal of pharmaceuticals in drinking water production is important (Kummerer, 2001, Snyder *et al.* 2005). Stackelberg *et al.*

(2004) studied the potential presence of 106 wastewater-related trace organic contaminants in conventionally treated drinking water (without ozonation) and two streams that served as the main water sources. Sulfamethoxazole and trimethoprim were detected in the two source water streams at frequencies of 8% and 83%, but they were below the detection limit in the finished drinking water. Stackelberg *et al.* (2004) suggests that the absence of pharmaceuticals in finished water does not mean complete mineralization of the parent compound and that chemical transformations of the parent compound could be occurring within the drinking water treatment processes.

Additional investigations evaluated the removal of pharmaceuticals by simulating individual drinking water treatment processes at the bench scale. A summary of the findings follows below:

- Removal of pharmaceuticals by coagulation and flocculation is dependent on a compound's propensity to sorb to surfaces. Consequently hydrophobic compounds with high octanol-water partition coefficients (log K_{ow}) can potentially be removed by coagulation/flocculation. However, many pharmaceuticals are polar and have a smaller tendency to sorb to surfaces. For example, the log K_{ow} values for

sulfamethoxazole and sulfamethazine are 0.89 and 0.28 (Howard and Meylan, 1997), respectively, and their removal by coagulation is not significant (Adams *et al.,* 2002, Westerhoff *et al.*, 2005, Vieno *et al.,* 2006). Westerhoff *et al.* (2005) found that the removal of sulfamethoxazole by alum coagulation was statistically insignificant (less than 20 percent), but for pharmaceuticals with higher log K_{ow} values removal was significant, e.g. 33% removal was obtained for erythromycin-H_2O (log K_{ow} = 3.06) during alum coagulation. Similar results were found for lime softening processes (Adams *et al.,* 2002, Westerhoff *et al.,* 2005).

- Adsorption processes depend on the characteristic of the adsorbent and the target adsorbate. Hydrophobic characteristics of activated carbon result in the effective removal of non-polar and polar organic compounds. Westerhoff *et al.* (2005) observed that log K_{ow} values of pharmaceuticals are reasonable indicators of the removal effectiveness that can be achieved by powdered activated carbon (PAC) adsorption processes. Furthermore, removal percentages of pharmaceuticals were independent of the initial contaminant concentration. In 4-hour batch tests conducted with a PAC dose of 5 mg/L, Westerhoff *et al.* (2005) found a sulfamethoxazole removal of 36%. Adams *et al.* (2002) studied the adsorptive removal of sulfamethoxazole and sulfamethazine and found that after a contact time of 4 hours, both compounds were removed by 52% and 85% with PAC doses of 10 and 20 mg/L, respectively.

- For slow sand filtration, Rooklidge *et al.* (2005) observed <4% removal for both sulfamethazine and sulfamethoxazole with slow sand filtration after a study period of 14 days.

- Oxidation of pharmaceuticals by ozone is well documented. Huber *et al.* (2003) studied ozone and hydroxyl radical oxidation reactions for different pharmaceuticals and EDCs. Sulfamethoxazole is rapidly oxidized by ozone (Westerhoff *et al.,* 2005, Adams *et al.* 2002, Huber *et al.* 2003) with a second order rate constant of 5×10^4 to 5×10^5 M^{-1} s^{-1} between pH 3 and 7 (Dodd *et al.* 2006). Ozone reacts with sulfamethoxazole primarily by attacking the aniline (p-sulfonylaniline) moiety (Dodd *et al.* 2006). With hydroxyl radicals, sulfamethoxazole oxidation kinetics are more rapid with a second order rate constant of 5.5 (± 0.7) $\times 10^9$ M^{-1} s^{-1} at pH 7 (Huber *et al.* 2003). Boreen *et al.* (2004, 2005) obtained hydroxyl radical rate constants of 5.8 (± 0.2) $\times 10^9$ M^{-1} s^{-1} and 5.0 (± 0.3) $\times 10^9$ M^{-1} s^{-1} for sulfamethoxazole and sulfamethazine, respectively, at pH 3 using Fenton's reaction.

- Chlorine is used as an oxidant and disinfectant in water treatment. Chlorine is able to oxidize a great variety of endocrine disruptors and personal care products (Adams *et al.* 2002, Westerhoff *et al.*, 2005). For sulfamethazine 90% of the parent compound

was removed with free chlorine at a CT of 9.7 min-mg L^{-1} (Adams *et al.* 2002). Westerhoff *et al.* (2005) observed sulfamethoxazole removal greater than 85% with free chlorine and a CT ~ 90 min-mg L^{-1}. Compared with CT requirements for the removal of protozoa, both sulfonamides required low doses of free chlorine to be oxidized. Dodd and Huang (2004) found that sulfamethoxazole reacts with free available chlorine with specific second order rate constants of 1.1×10^3 M^{-1} s^{-1} and 2.4×10^3 M^{-1} s^{-1} for the neutral and anionic species, respectively. These authors reported that the half life of sulfamethoxazole was 23 s with free available chlorine at 1.4 mg L^{-1}, pH 7 and 25°C. With combined chlorine, the sulfamethoxazole half life was 38 h under similar conditions. Dodd and Huang (2004) suggest that a fraction of chlorinated sulfamethoxazole intermediates are capable of reacting back to the parent sulfamethoxazole when there is not enough chlorine added to initiate the rupture of the sulfamethoxazole molecule.

- Chlorine dioxide (ClO_2) is an oxidant and disinfectant that is sometimes utilized to treat ground water or higher quality surface waters. Huber *et al.* (2005) studied the oxidation of sulfonamides (sulfamethoxazole, sulfamethazine, sulfapyridine and sulfathiazole) in ground water with ClO_2. All compounds were oxidized more than 95% after a CT ~ 3 min-mg L^{-1} at pH 7.5. Sulfonamide oxidation was strongly influenced by the protonation state of the nitrogen in the sulfonamide group and ClO_2

preferred to attack the isoxazole moiety of sulfamethoxazole. A second-order rate constant of 6700 ± 700 M^{-1} s^{-1} was reported for sulfamethoxazole oxidation with ClO_2 (Huber *et al.*, 2005).

- Photolysis of some pharmaceuticals and endocrine disruptors by irradiation with ultraviolet (UV) light is possible, but requires higher UV dosages than those required for disinfection (~ 30 mJ cm^{-2}) (Adams *et al.*, 2002). Adams *et al.* (2002) observed that for UV doses of ~7,000 mJ cm^{-2}, a maximum removal of 80 % was achieved for sulfamethoxazole and sulfamethazine with low pressure 254 nm lamps. Further detail on BACs photolysis is summarized in chapter 3.

2.3. Advanced oxidation processes

To date, advanced oxidation processes (AOPs) are not commonly used in drinking water treatment. AOPs involve the combination of multiple oxidants or oxidants with UV photons and transition metal catalysts (Table 2). Ozone/H_2O_2 has been used in drinking water treatment plants by mixing H_2O_2 with the influent of the ozone reactor. For UV/H_2O_2, the plant in Andjik, Netherlands was the first large drinking water treatment plant that installed the UV/H_2O_2 AOP, which has been in operation since 2004. This unique plant applies UV light and hydrogen peroxide treatment for both

primary disinfection and organic contaminant control (Cunningham, 2004, Kruithof et al., 2004). AOPs rely on the hydroxyl radical as the dominant oxidant. The hydroxyl radical is a short-lived and extremely potent oxidizing agent (Table 3). The high oxidation potential of the hydroxyl radical makes it an effective option for the oxidation of natural organic matter and trace contaminants that react only slowly with other oxidants. The rate of reaction between the hydroxyl radical and an organic compound is described by a second-order rate expression. Figure 3 shows the second order rate constant of a wide range of organic compounds (Schwarzenbach *et al.* 2003). The reaction rates of highly reactive compounds are limited by the hydroxyl radical diffusion rate in water. As seen in Figure 3, aromatic compounds, including those with halogen substitutents, react rapidly with the hydroxyl radical at nearly diffusion-controlled rates. In general, $^{\bullet}OH$ reacts faster with double and triple carbon-carbon bonds and aromatic compounds with electron-donating substituents than with single carbon-carbon and carbon-hydrogen bonds (Haag and Yao, 1992, Westerhoff *et al.*, 1999, Schwarzenbach *et al.* 2003).

The presence of hydroxyl radical scavengers can negatively affect the effectiveness of AOPs. Scavengers include humic substances, oxidation by-products, inorganic compounds such as carbonate and bicarbonate and hydrogen peroxide at high doses. High alkalinity waters lower the hydroxyl radical concentration and thus the overall oxidation rate of the targeted contaminants. The second order oxidation rate constant

for bicarbonate ($K_{\bullet OH}$) is 8.5×10^6 M^{-1} s^{-1} (Buxton *et al.,* 1988), while the carbonate species is a stronger hydroxyl radical scavenger with a $K_{\bullet OH}$ value of 3.9×10^8 M^{-1} s^{-1} (Buxton *et al.,* 1988). In the presence of carbonate species, carbonate radicals are also forming. Carbonate radicals are less reactive oxidants than the hydroxyl radical, and second order reaction rate constants describing reactions between the carbonate radical and organic contaminants cover several orders of magnitude (10^5 – 10^9 M^{-1} s^{-1}). In natural water, carbonate radicals compete with hydroxyl radicals for organics, and the carbonate radical will enhance oxidation rates when the second order rate constant exceeds 5×10^6 M^{-1} s^{-1} (Canonica *et al.*, 2005).

2.4. UV/Hydrogen peroxide processes

Hydrogen peroxide generates hydroxyl radicals upon irradiation with ultraviolet light:

$$H_2O_2 \xrightarrow{h\nu} 2\ HO^{\bullet} \quad \text{(Initiation)}$$

The molar absorptivity of hydrogen peroxide at 253.7 nm is 18.6 M^{-1} cm^{-1} and the overall quantum yield is 0.98. The overall quantum yield is defined as the amount of reactant consumed or product formed per einstein of photons absorbed (Bolton 2001). The molar extinction coefficient of hydrogen peroxide at 254 is very low compared to ozone (3,300 M^{-1} cm^{-1}), therefore, higher hydrogen peroxide concentrations are required to obtain the same quantity of hydroxyl radical per incident photon (Table 4).

The photolysis of aqueous hydrogen peroxide is dependent on pH and is faster at alkaline conditions. The molar absorptivity of the peroxide ion (HO_2^-) at 253.7 nm is 240 M^{-1} cm^{-1} (Legrini *et al.,* 1993). In addition, Table 4 shows the theoretical amounts of oxidants and UV photons required per mol of hydroxyl radical formed (Glaze *et al.,* 1987).

The hydroxyl radical can be consumed by oxidizing organic compounds and by inorganic scavengers. In addition, it can recombine with other hydroxyl radicals to regenerate hydrogen peroxide or participate in a radical chain degradation that produces new hydroxyl radicals, perhydroxyl radicals ($HO_2^\bullet$) and superoxide anion radicals ($O_2^{\bullet-}$), as summarized in the following reactions (Bose *et al.,* 1998 , Tang, 2005 , Crittenden *et al.* 1999) (k: rate constants) :

Propagation: $H_2O_2/HO_2^- + HO^\bullet \rightarrow HO_2^\bullet / O_2^{\bullet-} + H_2O$ $\quad k = 2.7 \times 10^7\ M^{-1}\ s^{-1}$

$H_2O_2 + HO_2^\bullet \rightarrow HO^\bullet + H_2O + O_2$ $\quad k = 3.0\ M^{-1}\ s^{-1}$

Termination: $HO_2^\bullet + HO_2^\bullet \rightarrow H_2O_2 + O_2$ $\quad k = 8.3 \times 10^5\ M^{-1}\ s^{-1}$

$O_2^{\bullet-} + HO_2^\bullet \rightarrow HO_2^- + O_2$ $\quad k = 9.7 \times 10^7\ M^{-1}\ s^{-1}$

$HO^\bullet + HO_2^\bullet \rightarrow H_2O + O_2$ $\quad k = 6.6 \times 10^9\ M^{-1}\ s^{-1}$

. $HO^\bullet + HO^\bullet \rightarrow H_2O_2$ $\quad k = 5.5 \times 10^9\ M^{-1}\ s^{-1}$

Hydroxyl radicals oxidize organics by the following mechanisms (Legrini *et al.*, 1993, Tang, 2005)

1. Electrophilic addition: $HO^{\bullet} + PhX \rightarrow HOPhX^{\bullet}$

2. Hydrogen abstraction: $HO^{\bullet} + RH \rightarrow R^{\bullet} + H_2O$

3. Electron transfer: $HO^{\bullet} + RX \rightarrow RX^{\bullet +} + OH^{-}$

A fourth mechanism that should be taken into account is the radical-radical reaction. This reaction occurs when high concentrations of hydroxyl radical dimerize to hydrogen peroxide.

$$HO^{\bullet} + HO^{\bullet} \rightarrow H_2O_2$$

In addition, hydroxyl radicals will react with high concentrations of hydrogen peroxide to produce perhydroxyl radicals. These radicals have a lower oxidation potential and do not contribute significantly to the oxidation of organic contaminants.

$$HO^{\bullet} + H_2O_2 \rightarrow H_2O + HO_2^{\bullet}$$

Legrini *et al.* (1993) presented a reaction pathway of an organic compound oxidized by the hydroxyl radical (Figure 4). It is important to point out that when the reaction is occurring in the presence of oxygen, hydrogen peroxide will be regenerated.

Therefore, lower doses of hydrogen peroxide are required. This is an advantage when the dose of peroxide is being optimized to reduce treatment cost (Tang, 2005).

Compared with ozone-based AOPs, additional advantages of UV/H_2O_2 processes are: (1) relatively easy H_2O_2 storage and high thermal stability, (2) infinite H_2O_2 solubility with water and no gas-liquid mass transfer limitations, (3) reactions with organic compounds form peroxyl radicals that are also reactive, leading to succeeding oxidation reactions (von Sonntag *et al.,* 1997), and (4) rapid installation and simple operation (good for small water treatment plants). Disadvantages of UV based AOPs are the light filtering effect produced by highly colored waters or compounds with high light absorbance. Another issue is the lamp maintenance. The lamp should be cleaned on a regular basis due to the accumulation of deposits on the lamp sleeve and need to be replaced because of finite lamp lifetime. Finally, energy requirements of the UV/H_2O_2 process need to be considered, both in terms of energy consumption by the UV lamps and the embedded energy of the H_2O_2.

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Table 1. Conventional drinking water unit processes (Huber *et al.*, 2004)

Goal	Targeted Constituents	Unit Processes
Removal of suspended solids	Inorganic particles Organic particles Microorganisms	Coagulation/Flocculation Sedimentation Granular Media Filtration Ultrafiltration
Removal of dissolved organic compounds	Color, taste and odor compounds Trace organic contaminants Biodegradable organic compounds (BDOC and AOC) Disinfection byproducts precursors	Oxidation Adsorption Biological filtration Coagulation/Flocculation
Disinfection	Viruses Bacterias Protozoa	Chlorine-based disinfectants Ozone Ultraviolet radiation

Table 2. Common AOPs used in water and wastewater treatment (Tang, 2005, Legrini *et al.*, 1993).

		Oxidants		Catalyst		
				Metal oxides		Metal ions
		O_3	H_2O_2	TiO_2	Fe_2O_3	Fe^{2+}
Photolysis	**UV**	✓	✓	✓		✓ (+H_2O_2, photo Fenton)
Oxidants	O_3	-	✓			✓
	H_2O_2	✓	-	✓	✓	✓ (Fenton)
	O_2			✓	✓	✓

Table 3. Oxidation potential for different oxidant species (Legrini *et al.*, 1993).

species	oxidation potential (V)
fluorine	3.03
hydroxyl radical	2.80
atomic oxygen	2.42
ozone	2.07
hydrogen peroxide	1.78
perhydroxyl radical ($HO_2^{\bullet}$)	1.70
permanganate	1.68
hypobromous acid	1.59
chlorine dioxide	1.57
hypochlorous acid	1.49
hypoiodous acid	1.45
chlorine	1.36
bromine	1.09

Table 4. (a) Theoretical amounts of oxidants/UV required for hydroxyl radical formation and (b) theoretical formation of hydroxyl radicals from photolysis of ozone and hydrogen peroxide (Glaze *et al.*, 1987).

(a)	moles of oxidant required /mol of $^{\bullet}OH$ formed		
	UV[*]	O_3	H_2O_2
O_3/ UV	0.5	1.5	(0.5)[**]
H_2O_2/UV	0.5		0.5

[*]: moles of photons (Einsteins) required for each mol of $^{\bullet}OH$ formed
[**]: hydrogen peroxide created in situ

(b)	Molar absorptivity at 254 nm (M^{-1} cm^{-1})	$^{\bullet}OH$ formed per incident photon[***]
O_3	3,300	2.00
H_2O_2	20	0.09

[***]: Assumes a 10-cm path length; quantum yields as predicted from stoichiometry; O_3 and H_2O_2 concentration = 1×10^{-4} M

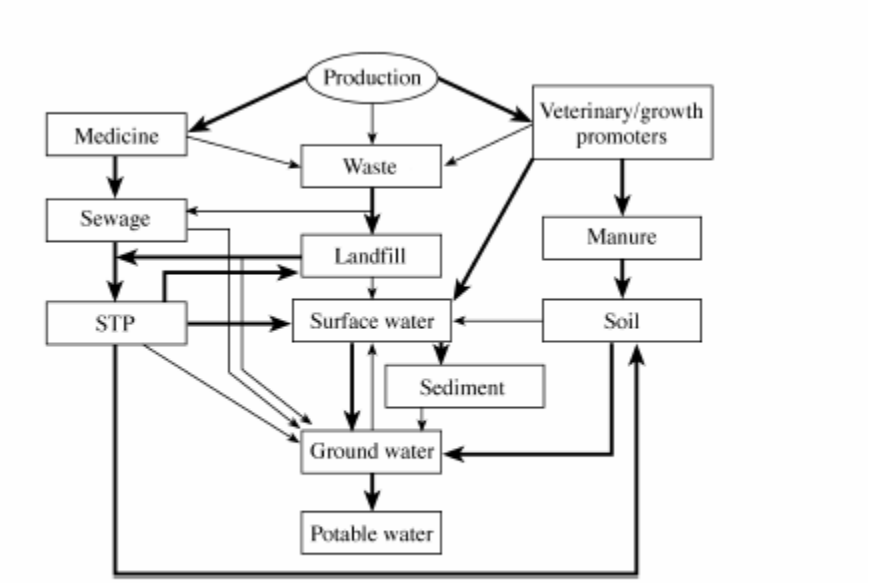

Figure 1: Possible sources and pathways for pharmaceutical residues in the environment (Kummerer, 2003).

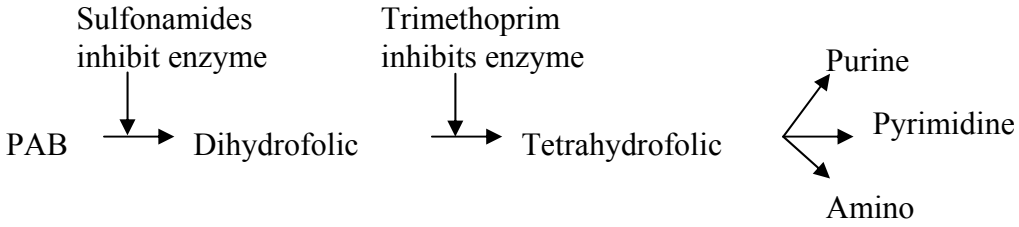

Figure 2. Mode of action of sulfonamide drugs and trimethoprim (Talaro and Talaro, 2002).

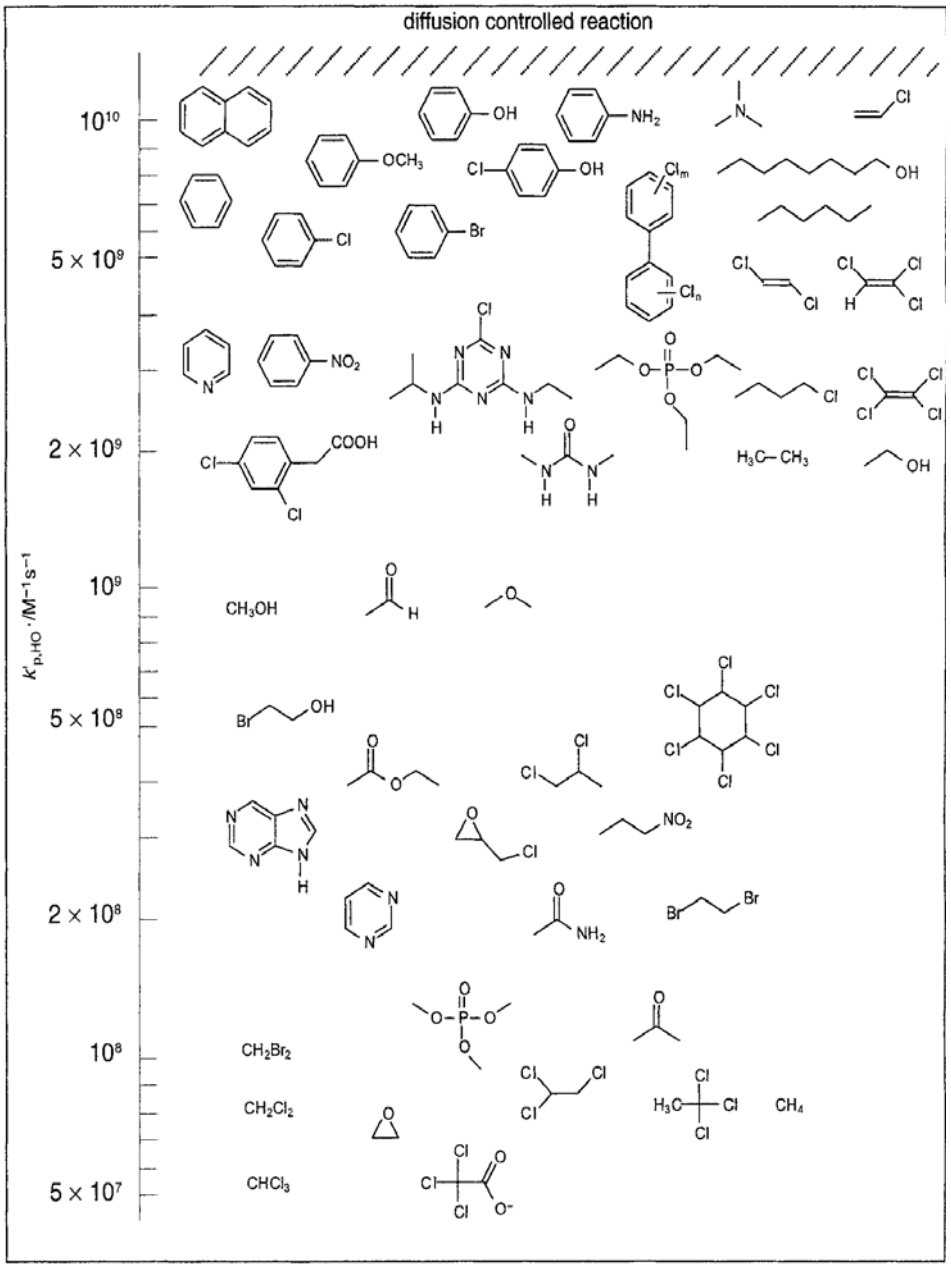

Figure 3. Second order rate constant of various organic compounds (Schwarzenbach *et al.* 2003).

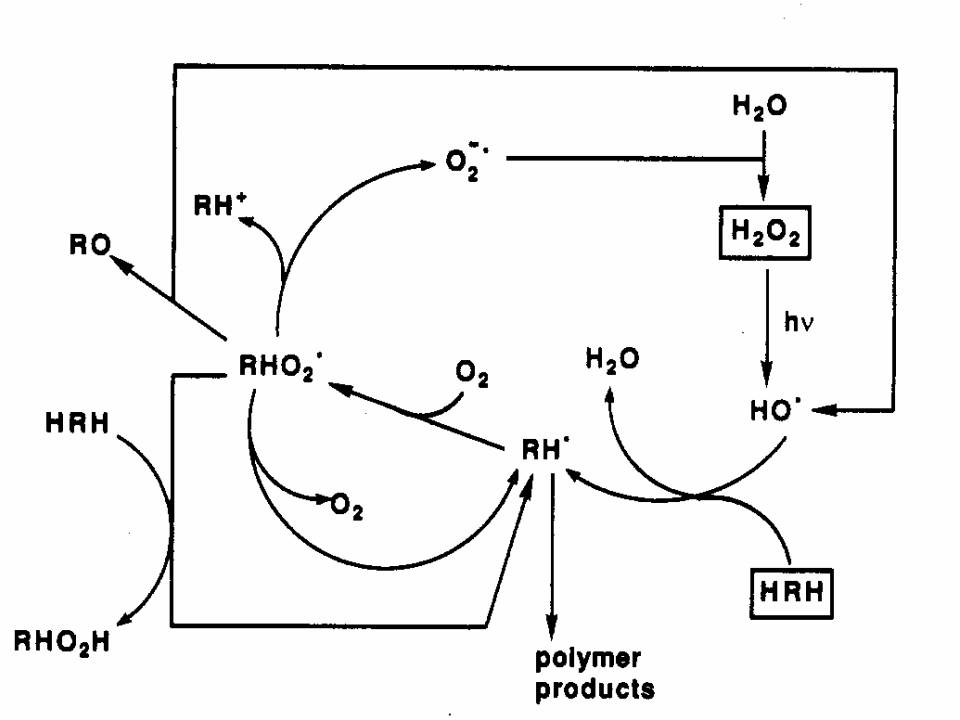

Figure 4. Oxidation pathway of an organic compound in UV/H_2O_2 processes (Legrini *et al.* 1993).

Chapter 3.

KINETICS OF BIOCHEMICALLY ACTIVE COMPOUND REMOVAL BY LOW-PRESSURE UV PHOTOLYSIS AND UV/H_2O_2 ADVANCED OXIDATION PROCESSES

3.1. Introduction

Biochemically active compounds (BACs) are commonly detected in surface and ground water, and concentrations of antibiotics and endocrine disrupting chemicals (EDCs) can reach low μg/L levels (Alexy and Kümmerer 2006, Petrovic *et al.*, 2004). While EDC concentrations in some surface water bodies are sufficiently high to cause gender bending in fish, it is still debated whether the evolution of antibiotic-resistant bacteria is facilitated at such concentrations. Also, the effects of chronic human exposure to different pharmaceuticals at trace levels in drinking water are not known (Snyder *et al.* 2005). Wastewater treatment plants (WWTPs) represent one important entry point for BAC into the environment (e.g. Gobel *et al.* 2005), and BACs are not effectively removed by conventional drinking water processes (Adams *et al.* 2002, Westerhoff *et al.* 2005).

The use of UV disinfection processes has increased dramatically in drinking water and wastewater treatment. While the transformation of organic compounds by direct

photolysis at disinfection doses is limited (Adams *et al.,* 2002, Canonica *et al.,*2008), advanced oxidation processes (AOPs) present an efficient alternative for micropollutant removal. Several studies evaluating UV/H_2O_2 processes for EDC and pharmaceutical removal have been conducted. E.g., Rosenfeldt and Linden (2004) showed that bisphenol A was degraded up to 99% in ultrapure water at a low pressure UV fluence of 1000 mJ cm^{-2} and an addition of 15 mg L^{-1} of H_2O_2. Pereira *et al.* (2007) studied the oxidation of carbamazepine, naproxen, clofibric acid, and iohexol in surface water at pH 7 with a low pressure UV lamp and a H_2O_2 dose of 10 mg L^{-1}, and found that a 99% transformation of carbamazepine, naproxen, and clofibric acid required UV fluence > 1500 mJ cm^{-2}, while iohexol required a UV fluence of 720 mJ cm^{-2}. In a pilot plant operating at a UV fluence of 540 mJ cm^{-2} and a H_2O_2 dose of 6 mg L^{-1}, pharmaceutical transformation in lake water ranged from 67 – 98%.(Kruithof *et al.,* 2007).

This research was conducted to provide a more detailed understanding of factors controlling BAC transformation rates in UV photolysis and UV/H_2O_2 processes. Specific objectives were to determine the effects of solution pH and the influence of lake water and wastewater treatment plant effluent matrices on BAC transformation rates. The BACs tested in this study were the antimicrobial compounds sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfadiazine (SDZ), and

trimethoprim (TMP), the EDC bisphenol-A (BPA) and the analgesic diclofenac (DCL) (Table 1).

3.2. Materials and methods

3.2.1. Experimental approach.

Batch photolysis and UV/H_2O_2 oxidation experiments were carried out in a bench scale quasi-collimated beam (QCB) apparatus (Bolton and Linden, 2003). The purpose of the QCB apparatus is to ensure that the UV rays reaching the sample are perpendicular to the water surface, which permits the accurate measurement of UV irradiance at the water surface and thus the accurate determination of the UV fluence (dose) delivered to the sample. The QCB was equipped with four low pressure (LP) UV lamps, and a UV radiometer (UVX Radiometer, Upland, CA, USA) was used to measure the UV irradiance at the surface of the sample. An iodide/iodate actinometer was used to calibrate the radiometer readings (Rahn et al., 2006). The delivered UV fluence to the sample was calculated with the method described by Bolton and Linden (2003).

Photolysis and UV/H_2O_2 oxidation experiments were conducted at initial BAC concentrations of 4 (±1) μM and the parent compound transformation was monitored as a function of UV dose (mJ cm^{-2}). Aqueous BAC concentrations were quantified by

HPLC. Samples collected in the presence of H_2O_2 were quenched with catalase (1% v/v of a 0.2 mg/L stock solution) and filtered with a 0.22 μm membrane prior to HPLC analysis. The effects of the following factors on the oxidation rates were evaluated: (1) pH, (2) H_2O_2 concentration, and (3) background water matrix composition (scavenging due to background organic matter, alkalinity and other inorganic constituents). To determine the pH-dependent quantum yield of SMX, SMZ, SDZ, and TMP, photolysis experiments were conducted in ultrapure water (UPW) buffered at pH values at which either the neutral or ionic form of the antimicrobial compounds was dominant (neutral form of sulfonamides at pH 3.6, neutral form of TMP at pH 9.7, anionic form of SMX at pH 7.85, anionic forms of SMZ and SDZ at pH 9.7, and cationic form of TMP at pH 3.6). For the UV/H_2O_2 degradation of SMX, SMZ, SDZ and TMP in UPW, only experiments at pH 3.6 and 7.85 were conducted to evaluate pH effects on the oxidation rates. At pH 9.7, the reaction between $^{\bullet}OH$ and the contaminant was affected by elevated carbonate concentrations due to enhanced dissolution of atmospheric CO_2. Solution pH effects were not evaluated for BPA and DCL, because their pKa values, 9.78 and 4.15, respectively, are distant from typical water treatment pHs; at the tested pH 7.85, the neutral form of BPA and the anionic form of DCL were dominant. To quantify BAC oxidation rates in the UV/H_2O_2 process, QCB experiments were conducted with H_2O_2 concentrations of 2, 6, and 10 mg L^{-1}. Experiments in presence of background organic matter were conducted at pH 7.85 in lake water (LW) collected from Lake Wheeler, NC, USA and wastewater

treatment plant effluent (WWTPE) collected from the North Cary Water Reclamation facility (Cary, NC, USA). Both LW and WWTPE were stored at 4ºC and filtered through a 0.45-µm nylon membrane (Magna-R, MSI, Westboro, MA) prior to use. Characteristics of LW and WWTPE are shown in Table 2.

Photolysis and oxidation rate data were evaluated using two approaches. First, a fluence-based *pseudo first order reaction rate* approach was used to evaluate the effects of pH, H_2O_2 concentration, and natural organic matter on BAC transformation rates. Furthermore, the quantum yield for each BAC was determined from the fluence-based pseudo first-order photolysis rate. Second, the *second order rate constant* describing BAC oxidation by the hydroxyl radical in ultrapure water at pH 7.85 was obtained through competition kinetics (Huber *et al.* 2003). In this study, p-chlorobenzoic acid (p-CBA) was selected as the reference compound because it is not measurably degraded by direct photolysis. Benitez *et al.* (2004) reported a quantum yield of 0.0030 mole Einstein^{-1} for p-CBA at pH 7 and a wavelength of 254 nm. The second order rate constant describing the oxidation of p-CBA by $^{\bullet}OH$ is well known and has a value of 5×10^9 M^{-1} s^{-1} in the pH range of 6 - 9.4 (Buxton *et al.*, 1988). Initial concentrations of the reference (R) and the target compound (TC) were equimolar. The data were evaluated according to the following expression:

$$\ln\left(\frac{[TC(t)]}{[TC(0)]}\right) = \ln\left(\frac{R(t)}{R(0)}\right) * \frac{k_{OH}(TC)}{k_{OH}(R)}$$

The second order rate constant of the target compound was obtained by plotting $\ln\left(\frac{[TC(t)]}{[TC(0)]}\right)$ versus $\ln\left(\frac{R(t)}{R(0)}\right)$, and the slope was multiplied by the second order rate constant for p-CBA. In addition, $k_{(\bullet OH)}$ of the target compound was corrected by the percentage of the pseudo-first order reaction rate that accounted for the direct photolytic degradation of each BAC.

3.2.2. Reagents

All BACs were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). SMX, SDZ, BPA and DCL, were stored at ambient temperature, while SMZ and TMP were stored at 4 °C. All compounds were stored in the dark to minimize photodegradation. Catalase was obtained from Sigma Chemical Corporation. Acetonitrile used for high-performance liquid chromatography (HPLC) analysis was HPLC grade (Fisher Scientific, Pittsburgh, PA, USA).

3.2.3. Analytical methods.

3.2.3.1. BAC concentration: BAC analyses were conducted with a HPLC system (Breeze, Waters, Milford, MA) equipped with a C18-AQ HPLC column (5 μm, 4.6 x 250 mm, Alltima HP, Grace) and a dual-wavelength UV detector. The mobile phase flow rate was 1.0 mL/min. For SMX and SMZ analyses, the mobile phase was composed of 24% v/v acetonitrile and 76% v/v 25 mM ammonium acetate buffer (pH 5). For SDZ and TMP, the mobile phase was 20% v/v acetonitrile and 80% v/v 25 mM ammonium acetate buffer (pH 5). For BPA and DCL, the mobile phase was 42% v/v acetonitrile and 58% v/v 25 mM ammonium acetate buffer (pH 5). The detector wavelength was set at 266 nm for SMX, SMZ and SDZ, 238 nm for TMP, 225 nm for BPA, and 220 nm for DCL.

3.2.3.2. Hydrogen peroxide*:* Hydrogen peroxide concentrations were quantified with the Ghormley method (Klassen *et al.,* 1994). This method is based on the spectrophotometric determination of I_3^- that is produced when hydrogen peroxide reacts with I^-.

3.2.3.3. Water matrix*:* Dissolved organic carbon (DOC) was quantified with a Total Organic Carbon Analyzer (Model TOC-5000A, Shimadzu, Columbia, MD).

Alkalinity was measured by *Standard Method* 2320A (AWWA, 1998). Chloride, nitrate, nitrite, sulfate and bromide were measured by ion chromatography (DIONEX ICS2500, Sunnyvale, CA). Table 2 shows the water quality parameters for LW and WWTPE

3.3. Results and discussion

3.3.1. Photolysis rate and quantum yields

Photochemical reactions occur when a photon is absorbed by a molecule, and the likelihood of a compound to absorb light at a specific wavelength is defined by the decadic molar absorption coefficient (Schwarzenbach *et al.,* 2003). Decadic molar absorptivities (ε) were derived from spectrophotometric data, and Figure 1 summarizes the decadic molar absorption spectra for the six studied BACs. For the antibiotics SMX, SMZ, SDZ and TMP, Figure 1 depicts spectra at pHs at which the neutral and ionic forms (anionic for the sulfonamides, cationic for TMP) of the antibiotics dominated. Values of ε at 254 nm for the six BACs are summarized in Table 3. At 254 nm, the three sulfonamides (SMX, SMZ, and SDZ) exhibited high molar absorptivities compared to TMP (at both pHs), BPA, and DCL. For BPA, the ε value was less than a 1,000 M^{-1} cm^{-1}, and the degradation of BPA by direct photolysis is therefore expected to be minor. In addition, quantum yields were calculated for each

BAC. The quantum yield (ϕ) determines the efficiency of photolysis and is defined as the moles of a compound that are transformed per mole of photons that were absorbed by the compound (Bolton, 2001). Bolton and Stefan (2002) derived an expression of the photolysis rate as a function of the average fluence rate; thus, the quantum yield can be determined directly from the fluence-based pseudo-first order rate constant describing the photolytic degradation of a compound; i.e.,

$$k_{p'254nm} = \frac{\phi_{254nm} \times \varepsilon_{254nm} \times \ln(10)}{10 \times U_{254nm}}$$

where $k_p'_{254nm}$ is the fluence-based pseudo-first order photolysis rate constant ($m^2\ J^{-1}$), ϕ_{254nm} is the quantum yield at 254 nm (mol $Einstein^{-1}$), ε_{254nm} is the decadic molar absorption coefficient ($M^{-1}\ cm^{-1}$), and U_{254nm} is the molar photon energy at 254 nm (4.72×10^5 J $Einstein^{-1}$). Data obtained for the determination of fluence-based pseudo-first order photolysis rate constants are summarized in Appendix A (H_2O_2 dose = 0 mg L^{-1})

Table 3 summarizes the fluence-based pseudo-first order photolysis rate constants (k_p', $cm^2\ mJ^{-1}$) and quantum yields (Φ) for each BAC in ultrapure water at different pHs. As shown in Table 3, the neutral form of SMX (pH 3.6) photolyzed more readily than the anionic form (pH 7.6), but the same was not observed for SDZ and SMZ. In

addition, the molar absorptivity (ε, 254 nm) of each compound was not related to the compound's photolysis rate; i.e. the molar absorptivity of SMX at pH 7.6 was higher than at pH 3.6, but the neutral form exhibited a higher quantum yield (Φ) and faster photolysis rate. Analogous results were obtained by Boreen *et al*. (2004) for solar photolysis conditions (λ>290 nm) and by Canonica *et al.* (2008). For SMZ and SDZ, the molar absorptivities of the anionic species were also higher than those of the neutral species, but quantum yields were similar for both the neutral and anionic forms of SDZ or slightly lower for the neutral form of SMZ. The net result was that the anionic forms of SMZ and SDZ photolyzed more rapidly than their neutral forms. The photolysis data obtained here at 254 nm suggest that the N-bond substituent on the sulfonamide moiety is responsible for the unrelated photochemical behavior of the sulfonamides. TMP and BPA had very low quantum yields, in addition to their low molar absorptivity; as a result, both TMP and BPA photolysis rates were very slow. Although DCL exhibited a lower molar absorptivity than the sulfonamides, it had a faster photolysis rate and hence a higher quantum yield.

3.3.2. Rate constants describing BAC oxidation by UV/H_2O_2.

This section describes results of (1) competition kinetics experiments conducted to determine second order rate constants describing the oxidation of BACs by the hydroxyl radical and (2) experiments conducted to quantify the effects of solution pH and background water matrix on fluence-based pseudo-first order rate constants

describing BAC transformation rates in the UV/H_2O_2 process. Data obtained for the determination of fluence-based pseudo-first order rate constants in UPW, LW and WWTPE are tabulated in Appendix A.

Second order rate constants. Competition kinetics experiments were conducted at pH 7.85 to determine second order rate constants ($k_{\bullet OH}$) that describe the oxidation of BACs by the hydroxyl radical. Values of $k_{\bullet OH}$ ranged from 5-10$\times 10^9$ M^{-1} s^{-1} (Table 4), and this range agrees with values reported previously for different pharmaceutical compounds (e.g., Dodd *et al.,* 2006 and Pereira *et al.,* 2007). Representative second order constants that were found in literature are shown in Table 4, for comparison.

The $k_{\bullet OH}$ value for each BAC was corrected by the percentage of the pseudo-first order reaction rate that accounted for direct photolytic degradation. SMX and DCL removal in the UV/H_2O_2 process was strongly influenced by direct photolysis; i.e., direct UV photolysis explained 39.1% and 55%, respectively, of the UV/H_2O_2 pseudo-first order reaction rate. In contrast, SMZ, SDZ, TMP and BPA removal in the UV/H_2O_2 process was dominated by $^{\bullet}$OH oxidation, and only 18.1%, 12.2%, 0.45% and 0.44 %, respectively, of the UV/H_2O_2 pseudo-first order reaction rate was explained by direct photolysis.

Effect of solution pH. Figure 2 shows that pseudo first-order oxidation rates for SMX, SMZ, SDZ and TMP at pH 3.6 and 7.85 increased linearly with the initially applied H_2O_2 concentration. SMX removal rates were higher when the compound was predominantly present in the neutral form (pH 3.6), while SMZ and SDZ removal rates increased when the fraction of the anionic form was increased (pH 7.85). The same pH effect for SMZ and SDZ was expected due to their structural similarity. Another interesting observation is the uniformity within the slopes of the lines for the sulfonamides at different pHs suggesting that the pH effect on the UV/H_2O_2 transformation rates of sulfonamides is mainly due to differences between the photolysis rates of the neutral and anionic species of SMX, SMZ and SDZ. In contrast, the hydroxyl radical appears to react at a similar rate of both the neutral and anionic forms of the three sulfonamides. The latter result differs from those obtained for the oxidation of sulfonamides by ozone; Dodd et al. (2006) suggested that ozone attacks primarily the p-sulfonylaniline moiety and reacts faster at pHs at which SMX is neutral. For TMP, the UV/H_2O_2 oxidation rate of the cationic form (pH 3.6) was faster. At both pH, 3.6 and 7.85, TMP photolysis was negligible, thus TMP degradation occurred primarily via hydroxyl radical oxidation. The different slopes obtained at pH 3.6 and 7.85 therefore suggest that the second order rate constant for the reaction between TMP and hydroxyl radical is pH dependent and that the protonated form reacts more readily than the neutral form. This trend also differs from that Dodd et al. (2006) found for TMP oxidation by ozone, which was faster at pHs at

which TMP is neutral. Additional competition kinetics experiments should be conducted with nitrobenzene at pHs at which TMP is cationic to determine the pH dependence of the TMP reaction with the hydroxyl radical.

Effect of background water matrix. Figure 3 depicts the dependence of the fluence-based pseudo-first order oxidation rate constants as a function of the applied hydrogen peroxide concentration for the six studied BACs at pH 7.85 in UPW, LW, and WWTPE. For all compounds, the rate constants increased linearly with the applied H_2O_2 concentration (R^2 ranged from 0.9768 to 0.9999). The regression equations relating the fluence-based pseudo-first order constants to the initial H_2O_2 concentration are shown in Appendix B. As seen in Figure 3, BAC transformation rates were slower in LW and WWTPE than in buffered UPW. For TMP, BPA and DCL, the photolysis rates in LW and WWTPE appear to be slightly higher than in UPW. Although the enhanced photolytic transformation due to presence of photosensitizers in LW and WWTPE cannot be completely ruled out (Canonica *et al*, 2008), the observed differences are likely due to experimental uncertainty. Even though the mean values of the photolysis rates of TMP, BPA, DCL in LW and WWTPE were higher than in UPW, they were not significantly different at the 95% confidence level; therefore no case can be made that photosensitizers in LW and WWTPE enhanced photolysis rates. For the remaining compounds (SMX, SMZ, and SDZ) the photolysis rates in LW and WWTPE were lower than in UPW. In general,

the water matrix affected less the photolysis rate than the hydroxyl radical oxidation rates, suggesting that the presence of hydroxyl radical scavengers in LW and WWTPE was the dominant factor that decreased BAC transformation rates. Hydroxyl radical scavengers include dissolved organic carbon (DOC), alkalinity, chloride, sulfate and nitrate. As shown in Table 2, concentrations of these scavengers in WWTPE were higher than in LW, which explains why BAC removal rates were lower in WWTPE than in LW. The percentage contribution of each water quality parameter to the hydroxyl radical scavenging was calculated by the reaction rate of each individual scavenger with $^{\bullet}OH$ and is described in detail below. It should be noted that SMX and DCL removal rates in WWTPE did not increase substantially with the addition of H_2O_2 (Figures 3a and 3f). SMX and DCL in WWTPE were removed mostly by photolysis, and the hydroxyl radical played a minor role in their transformation rates because of the strong scavenging effect of WWTPE. Therefore, SMX and DCL removal from WWTPE does not require the addition of H_2O_2.

Fluence-based pseudo-first order rate constants can be used to determine the required UV fluence to achieve a desired treatment goal. With the linear regression equations derived from Figure 3, the UV doses required to achieve a 90% BAC transformation with photolysis and UV/ H_2O_2 at H_2O_2 concentrations of 2, 6, and 10 mg L^{-1} were calculated (Figure 4). For SMX and DCL, UV doses ≤1000 mJ cm^{-2} were needed to transform 90% of the parent compound. In contrast, for SDZ, SMZ, TMP and BPA,

90% removal from LW and WWTPE required UV doses ≥1000 mJ cm^{-2}, even when the H_2O_2 dose was 10 mg L^{-1}. A UV dose of 1000 mJ cm^{-2} is high compared to those typically used in disinfection applications (40 mJ cm^{-2}). At disinfection UV doses (and no H_2O_2 addition), SMX and DCL, transformations percentages would be small (~8% and ~ 21%, respectively) in both LW and WWTPE, while SDZ, SMZ, TMP and BPA transformation would be negligible (≤3%). Another interesting reference point is a full scale UV/H_2O_2 water treatment plant that is operating in the Netherlands. This plant operates at a UV dose of 540 mJ cm^{-2} and a H_2O_2 concentration of 6 mg L^{-1}. At these conditions, the plant is able to achieve 80 % degradation of the herbicide atrazine (Kruithof *et al.,* 2007). Using the LW data obtained here, the following transformation percentages can be expected for the conditions used at the Dutch UV/H_2O_2 plant: ~98% for DCL, ~79% for SMX, ~60% for SMZ, ~51% for SDZ, ~43% for TMP and ~46% for BPA. Kruithof *et al.* (2007) found similar percentage values for SMX (~75%) and DCL (~97%) in Dutch lake water (Ijssel Lake, Netherlands) at a pilot plant at the same conditions as the Dutch UV/H_2O_2 plant.

Mathematical modeling. The parameters obtained in this study (quantum yields, molar absorptivities and second order rate constants) were incorporated into a mathematical model to calculate BAC transformation rates in UV/H_2O_2 processes (Sharpless *et al.,* 2003 , Rosenfeldt *et al.,* 2004, and Pereira *et al.,* 2007). Predicted

BAC transformation rates were then compared with experimentally determined fluence-based pseudo-first order rate constant for the six BACs in LW and WWTPE.

Contaminant degradation rates in a UV/H_2O_2 process are described by the sum of the direct photolysis rate (k_d') and the hydroxyl radical oxidation rate or indirect photolysis rate (k_i'):

$$\frac{d[BAC]}{dt} = -(k_d' + k_i')[BAC]$$

The direct photolysis fraction of the overall reaction rate is described by,

$$k_d' = K_{s,\ BAC\ at\ 254nm} \times \phi_{BAC\ at\ 254nm}$$

where $\phi_{BAC,\ at\ 254nm}$ is the quantum yield for the BAC at 254 nm (Table 3) and $K_{s,\ BAC\ at\ 254nm}$ is the specific rate of light absorption of the target compound and is calculated with the following expression:

$$K_{s,\ BAC\ at\ 254nm} = \frac{E^{o}_{254nm} \times \varepsilon_{BAC\ at\ 254nm} \times \left[1 - 10^{-a_{254nm} Z}\right]}{a_{254nm} Z}$$

where E^{o}_{254nm} is the incident photon irradiance, $\varepsilon_{BAC\ at\ 254nm}$ is the decadic molar absorption coefficient of the targeted BAC at 254 nm (M^{-1} cm^{-1}), z is the solution depth (equal to 3.51 cm for the experiments in this study) and a_{245nm} is equal to the

absorbance of the solution (comprised of the water background, H_2O_2 and the BAC), i.e.:

$$a_{254nm} = a_{\text{water background}} + \varepsilon_{H_2O_2 \text{ at } 254\text{ nm}}[H_2O_2] + \varepsilon_{\text{BAC at } 254\text{ nm}}[\text{BAC}]$$

The indirect photolysis fraction of the reaction rate is described by,

$$k_i' = k_{\bullet OH/BAC} \times [^{\bullet}OH]_{ss}$$

where $k_{\bullet OH/BAC}$ is the second order rate constant describing the reaction between $^{\bullet}OH$ and a given BAC (Table 2) and $[^{\bullet}OH]_{ss}$ is the steady state concentration of the hydroxyl radical in solution that is formed via H_2O_2 photolysis and is calculated from:

$$[^{\bullet}OH]_{ss} = \frac{K_{H_2O_2,254nm} \times \phi_{H_2O_2,254nm} \times [H_2O_2]}{\sum_i \left(k_{\bullet OH/Scavengers}[\text{Scavengers}]\right)} \text{ ; and}$$

$$K_{H_2O_2 \text{ at } 254nm} = \frac{E^o_{254nm} \times \varepsilon_{H_2O_2 \text{ at } 254nm} \times \left[1 - 10^{-a_{254nm}Z}\right]}{a_{254nm}Z}$$

where, E^o_{254nm}, ε_{254nm}, z, and a_{254nm}, are the same as defined previously.

To determine the scavenging factor $\sum_i \left(k_{\bullet OH/Scavengers}[\text{Scavengers}]\right)$, the water quality parameters of the LW and WWTPE shown in Table 1 were used in combination with

$k_{\bullet OH/Scavengers}$ values obtained from the literature. For the inorganic scavengers, the following values were used: 8.5×10^{6} M^{-1} s^{-1} for HCO_3^- (Buxton *et al.,* 1998), 3.9×10^{8} M^{-1} s^{-1} for CO_3^{2-} (Buxton *et al.,* 1998), 2.7×10^{7} M^{-1} s^{-1} for H_2O_2 (Buxton *et al.,* 1998), 1.5×10^{6} M^{-1} s^{-1} for SO_4^{2-} (Nakatani *et al.,* 2007), 2×10^{7} M^{-1} s^{-1} for Cl^- (Nakatani *et al.,* 2007) and 5×10^{5} M^{-1} s^{-1} for NO_3^- (Nakatani *et al.,* 2007). For the background organic matter in LW and WWTPE a second order rate constant of 1.9×10^{4} L $(mg\text{-}C)^{-1}$ s^{-1} (Westerhoff *et al.,* 2007) was used.

With the above model, a time-based pseudo-first order rate constant was obtained. This result was multiplied by the average fluence rate in a given collimated beam test to compare the model results with the experimentally determined fluence-based pseudo-first order rate constants (Figure 5). Good agreement between the model and the experimental values were obtained for SMX, SMZ, SDZ and DCL, while larger discrepancies were obtained with BPA and TMP (up to 41%), especially at higher H_2O_2 doses. Uncertainties in the second order rate constants for the BACs as well as the scavengers could have contributed to the differences between the model and the experimental values. The dominant contributors to the scavenging effect were the DOC, alkalinity and the added H_2O_2. For experiments at an H_2O_2 concentration of 10 mg L^{-1}, the DOC represented 86% and 71% of the scavenging effect in LW and WWTPE, respectively. The second order rate constant describing the reaction between

the dissolved organic carbon and the hydroxyl radical was 1.9×10^4 L $(mg\text{-}C)^{-1}$ s^{-1} (Westerhoff *et al.*, 2007), while a value of 2.5×10^4 L $(mg\text{-}C)^{-1}$ s^{-1} (Larson and Zepp *et al.,* 1998) has been used in many other studies. Dissolved organic matter characteristics vary among water sources, and Westerhoff *et al.* (2007) reported second order rate constants that ranged from 1.2×10^4 L $(mg\text{-}C)^{-1}$ s^{-1} to 3.8×10^4 L $(mg\text{-}C)^{-1}$ s^{-1} for different DOC fractions and water sources from which the DOC fractions were isolated. Thus, the actual rate constant will vary among water sources and therefore the DOC contribution to hydroxyl radical scavenging may be under or overestimated in the model.

Considering the ions sulfate, chloride and nitrate in WWTPE, the overall scavenging factor is ~18% higher than if only DOC, H_2O_2 and alkalinity are considered. For LW, sulfate, chloride and nitrate contributed just ~3% to the overall scavenging factor, thus the consideration of these scavengers is not crucial. In both waters, the major scavenger contribution among sulfate, chloride and nitrate ions was from the chloride ion. Even though the sulfate concentration in WWTPE was relatively high, the rate constant for sulfate (1.5×10^6 M^{-1} s^{-1}) is one order of magnitude smaller than that for chloride (2×10^7 M^{-1} s^{-1}). Hydroxyl radical scavenging due to sulfate accounted for <0.1% and <0.4% in LW and WWTPE, respectively.

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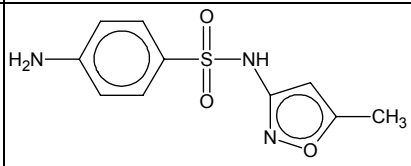
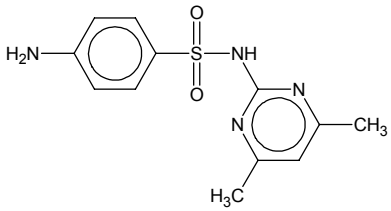
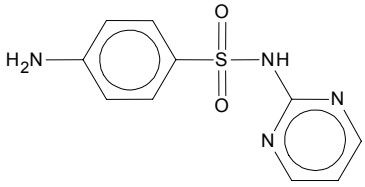
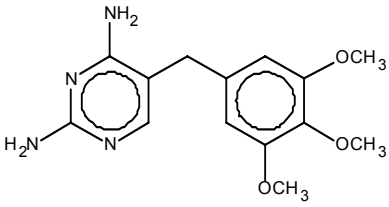
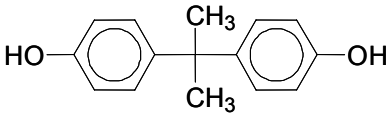
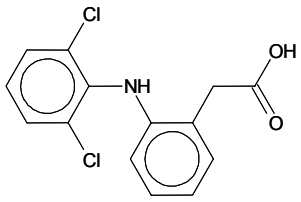
Table 1. Molecular structures of the studied BACs.

BAC	Chemical structure	
Sulfamethoxazole (SMX)		Antibiotic class: Sulfonamide molecular weight = 253.3 Da $pK_{a,1}$ = 1.74[1] and $pK_{a,2}$ = 5.65[2]
Sulfamethazine (SMZ)		Antibiotic class: Sulfonamide molecular weight = 278.3 Da $pK_{a,1}$ = 2.26[1] and $pK_{a,2}$ = 7.65[2]
Sulfadiazine (SDZ)		Antibiotic class: Sulfonamide molecular weight = 280.2 Da $pK_{a,1}$ = 2.02[1] and $pK_{a,2}$ = 6.43[2]
Trimethoprim (TMP)		Antibiotic class: DHFR Inhibitor molecular weight = 290.3 Da $pK_{a,1}$ = 3.23[3] and $pK_{a,2}$ = 6.76[3]
Bisphenol-A (BPA)		EDCs molecular weight = 228.3 Da $pK_{a,1}$ = 9.78[4] and $pK_{a,2}$ =10.52[4]
Diclofenac (DCL)		Analgesic compound molecular weight = 29.15 Da pK_a = 4.15[5]

[1]Lin *et al.* (1997a), [2] Lin *et al.* (1997b), [3]Qiang and Adams (2004), [4]Calculated from SPARCS v.4. [5]As shown in Database of experimental values in EPI SUITE V.3.20

Table 2: Representative water quality parameters: lake water (LW) and wastewater treatment plant effluent (WWTPE).

	LW	**WWTPE**
DOC (mg L^{-1})	5.1	7.3
A_{254} (cm^{-1})	0.130	0.146
Alkalinity (mg L^{-1}as $CaCO_3$)	24.4	74.3
Nitrate (mg L^{-1})	1.9	4.9
Nitrite (mg L^{-1})	<0.25	<0.25
Sulfate (mg L^{-1})	4.6	47.5
Chloride (mg L^{-1})	5.8	59.2
Bromide (mg L^{-1})	<0.25	<0.25

(filtered through a 0.45-µm membrane)

Table 3. Parameters describing the photochemical behavior of BACs.

	pH	3.6	7.85	9.7
SMX	ε (M^{-1} cm^{-1})	11,130	16,580	-
	Φ (mol $Einstein^{-1}$)*	0.180 (±0.0073)	0.0297 (±0.00086)	-
	k_p' (cm^2 mJ^{-1})*	0.00976	0.00240	-
SMZ	ε (M^{-1} cm^{-1})	16,196	18,525	20,538
	Φ (mol $Einstein^{-1}$)*	0.00282 (±0.00017)	0.00870 (±0.00022)	0.00849 (±0.00021)
	k_p' (cm^2 mJ^{-1})*	0.000223 (±0.000013)	0.000787 (±0.000020)	0.000851 (±0.000021)
SDZ	ε (M^{-1} cm^{-1})	13,590	20,150	20,660
	Φ (mol $Einstein^{-1}$)*	0.00430 (±0.00045)	0.00581 (±0.00054)	0.00378 (±0.000036)
	k_p' (cm^2 mJ^{-1})*	0.000284 (±0.000030)	0.000572 (±0.000054)	0.000382 (±0.0000036)
TMP	ε (M^{-1} cm^{-1})	4,956	2,942	2,635
	Φ (mol $Einstein^{-1}$)*	0.00059 (±0.00029)	0.00118 (±0.00011)	0.00149
	k_p' (cm^2 mJ^{-1})*	0.000014 (±0.000007)	0.000017 (±0.000002)	0.000019
BPA	ε (M^{-1} cm^{-1})	-	750	-
	Φ (mol $Einstein^{-1}$)*	-	0.00460 (±0.00043)	-
	k_p' (cm^2 mJ^{-1})*	-	0.000017 (±0.000002)	-
DCL	ε (M^{-1} cm^{-1})	-	5,202	-
	Φ (mol $Einstein^{-1}$)*	-	0.213 (±0.0047)	-
	k_p' (cm^2 mJ^{-1})*	-	0.00533 (±0.00013)	-

* Values in parentheses represent one standard deviation

Table 4. Second order rate constants ($k_{\bullet OH}$, M^{-1} s^{-1}) describing BAC oxidation by the hydroxyl radical.

	$k_{\bullet OH}$ (M^{-1} s^{-1}) this study at pH 7.85	$k_{\bullet OH}$ (M^{-1} s^{-1}) found in the literature
SMX	$5.56(\pm0.042) \times 10^9$	$5.50(\pm0.7) \times 10^9$ $M^{-1}s^{-1}$ at pH 7.0 Huber *et al.,* 2003
SMZ	$5.65(\pm0.047) \times 10^9$	$5.0(\pm0.3) \times 10^9$ $M^{-1}s^{-1}$ at pH 3.0 Boreen *et al.,* 2005
SDZ	$5.30(\pm0.086) \times 10^9$	$3.7(\pm0.3) \times 10^9$ $M^{-1}s^{-1}$ at pH 3.0 Boreen *et al.,* 2005
TMP	$5.70(\pm0.029) \times 10^9$	$6.9(\pm0.2) \times 10^9$ $M^{-1}s^{-1}$ at pH 7.0 Dodd *et al.,* 2006
BPA	$5.80(\pm0.079) \times 10^9$	$1.02(\pm0.06) \times 10^{10}$ $M^{-1}s^{-1}$ at pH 7.35 Rosenfeldt and Linden, 2004
DCL	$9.26(\pm0.260) \times 10^9$	$7.5 (\pm1.59) \times 10^9$ $M^{-1}s^{-1}$ at pH 7.0 Huber *et al.,* 2003.

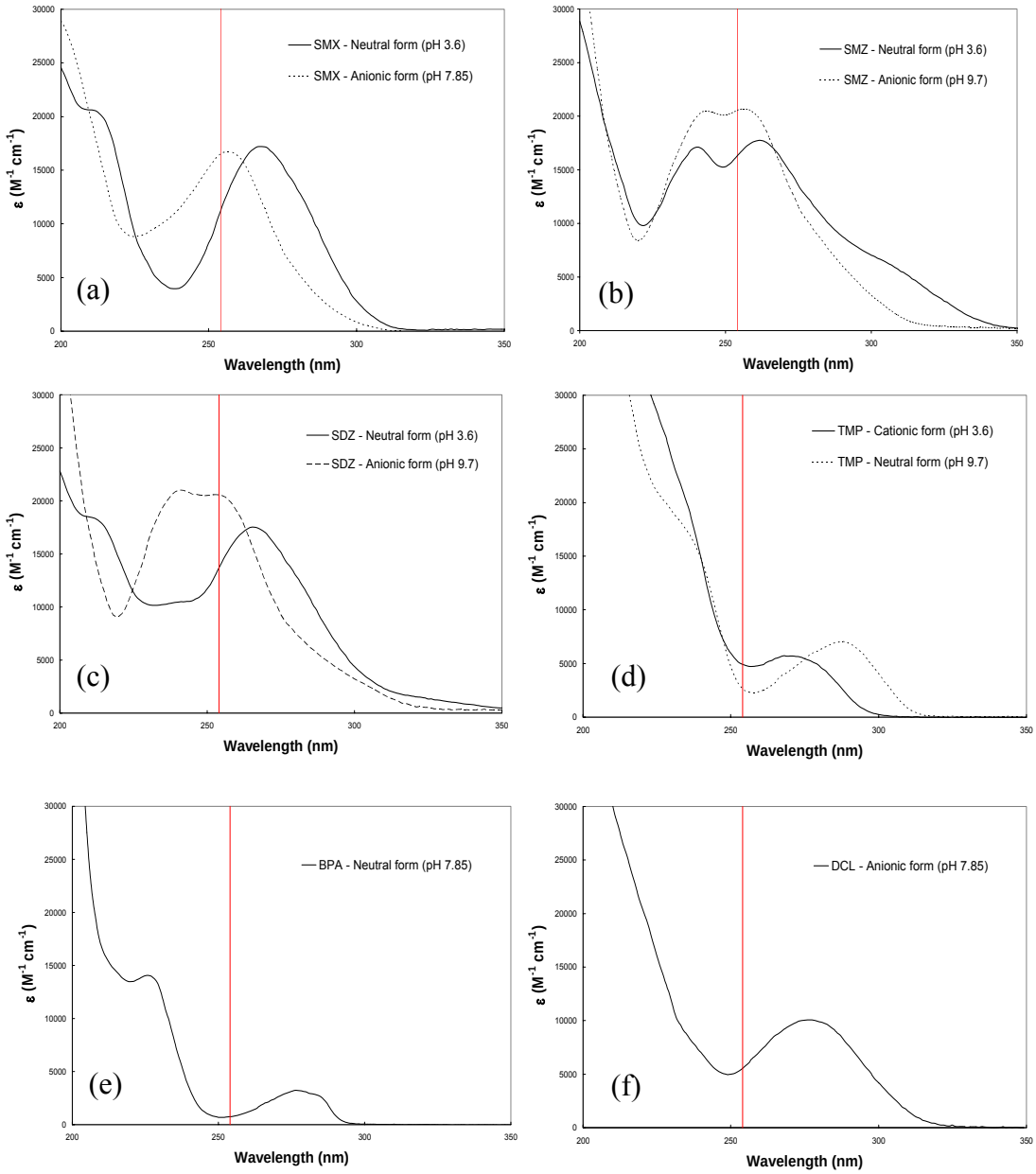


Figure 1. Decadic molar absorptivities ε (M^{-1} cm^{-1}) for (a) SMX, (b) SMZ, (c) SDZ, (d)TMP, (e) BPA and (f) DCL.

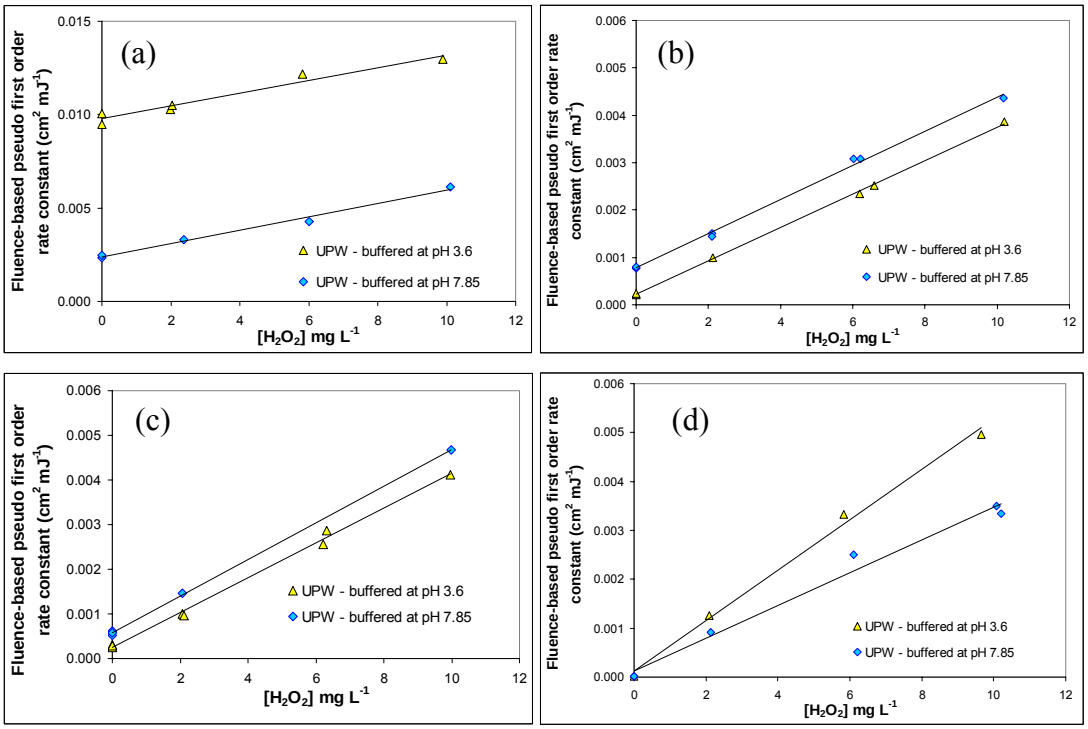


Figure 2. Effect of pH on the fluence-based pseudo first order rate constants for (a) SMX, (b) SMZ, (c) SDZ and (d)TMP. Note different y-axis scale for SMX.

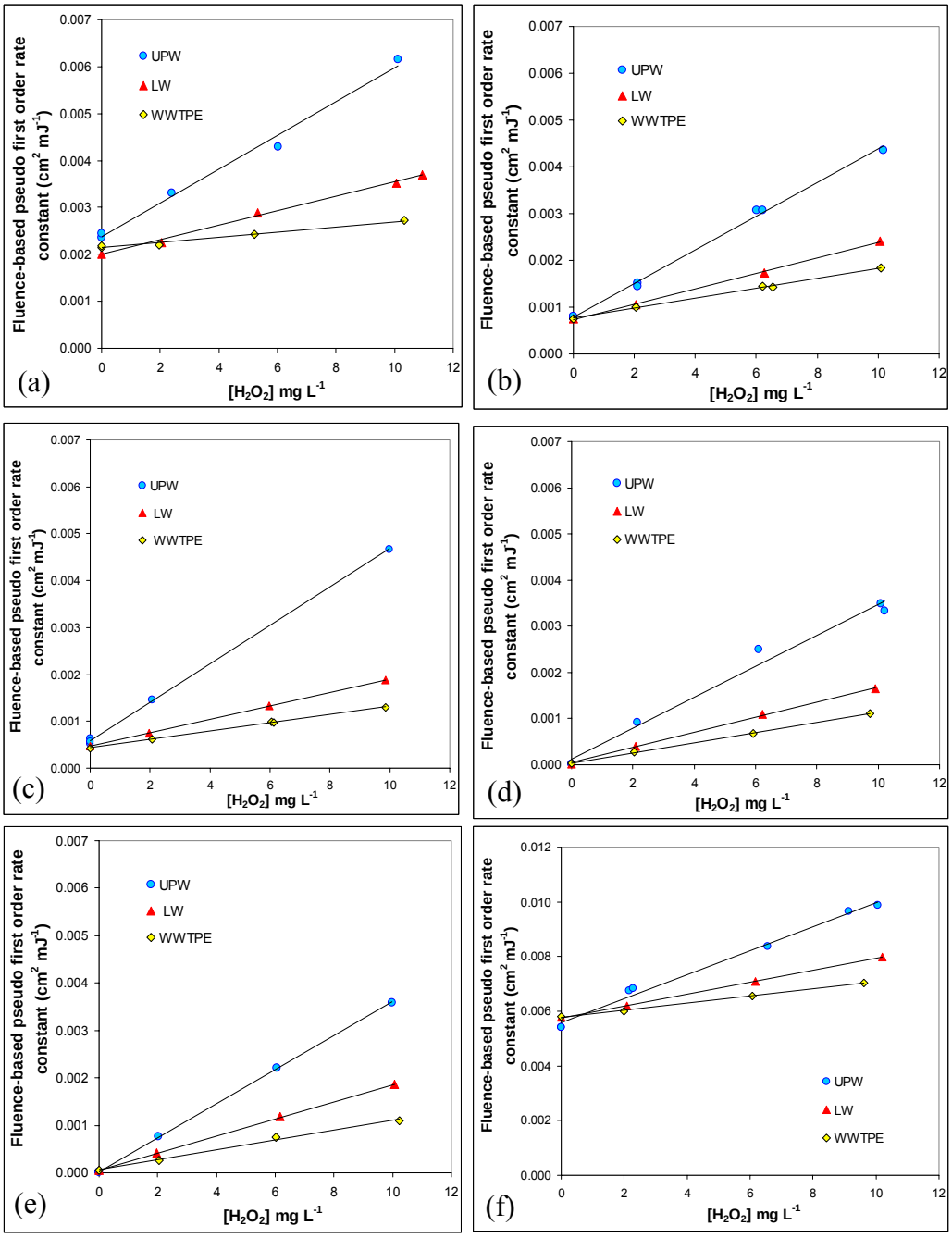


Figure 3. Effect of background water matrix on fluence-based pseudo first order rate constant for (a) SMX, (b) SMZ, (c) SDZ, (d)TMP, (e) BPA and (f) DCL. Note different y-axis scale for DCL.

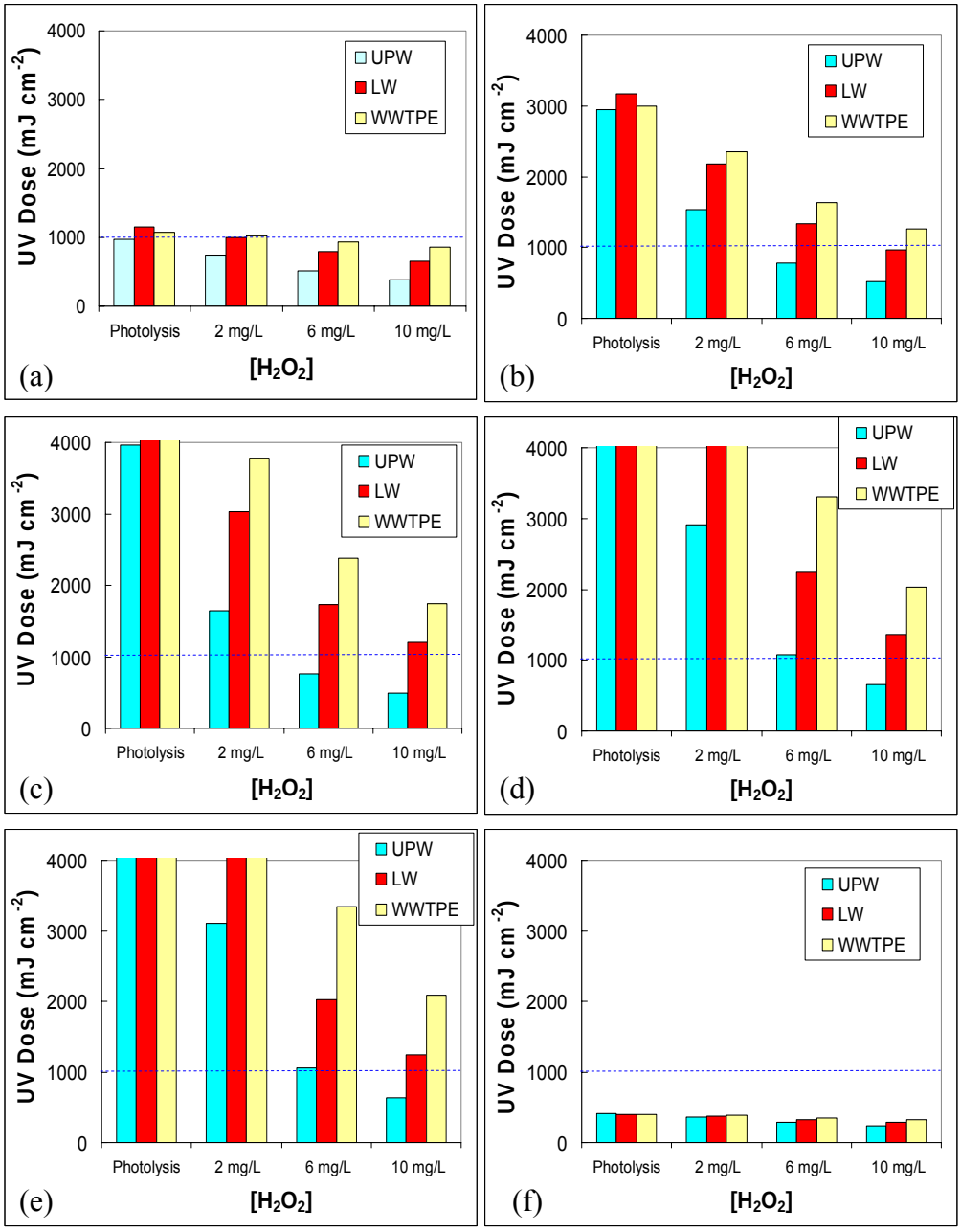


Figure 4. UV fluence (mJ cm^{-2}) required to achieve 90% removal of (a) SMX, (b) SMZ, (c) SDZ, (d)TMP, (e) BPA and (f) DCL in UPW, LW and WWTPE (pH 7.85).

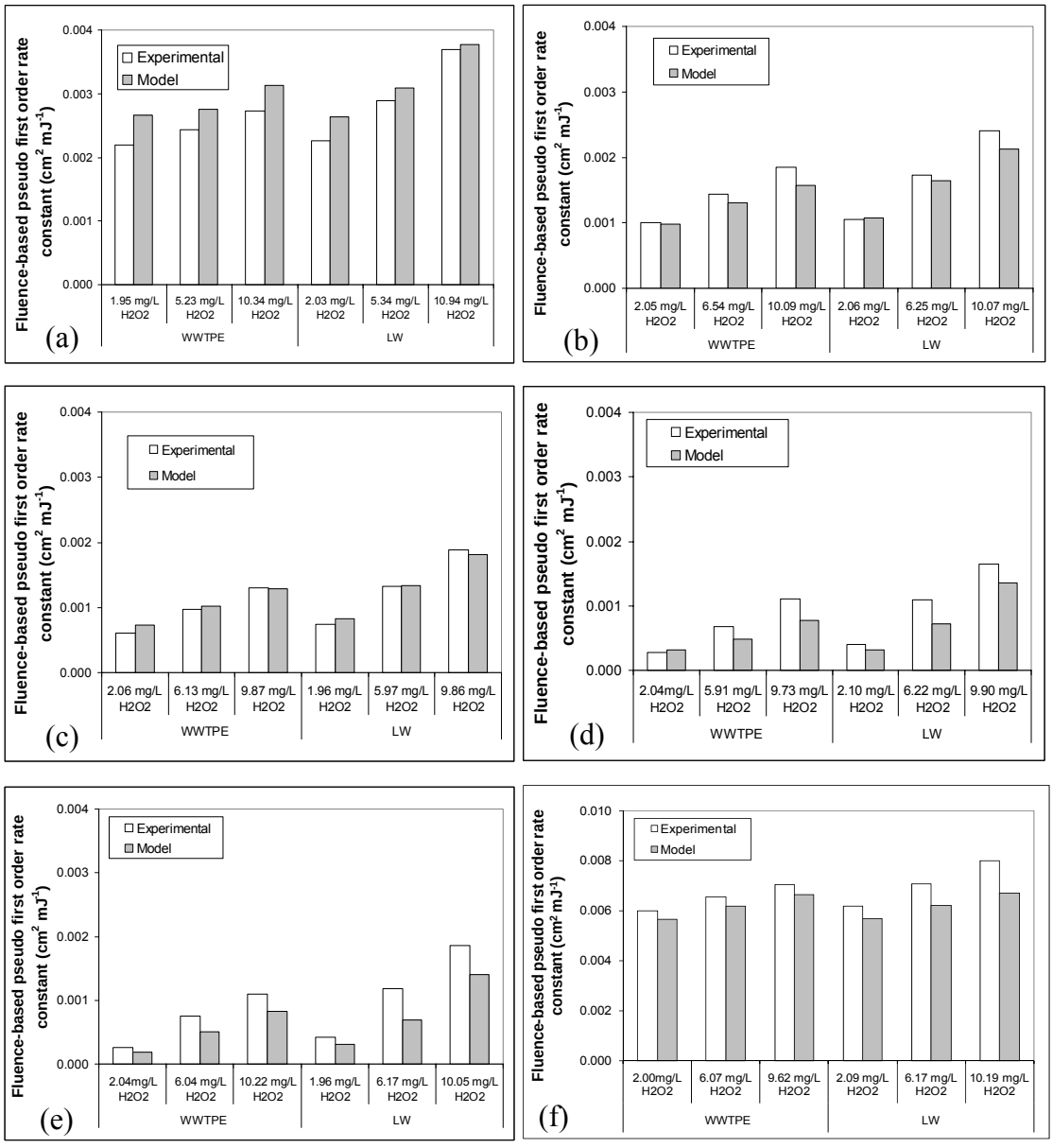


Figure 5. Comparison of the experimentally determined fluence-based pseudo first order rate constants with model predictions for (a) SMX, (b) SMZ, (c) SDZ, (d)TMP, (e) BPA and (f) DCL in UPW, LW and WWTPE (pH 7.85). Note different y-axis scale for DCL.

Chapter 4.

REMOVAL OF ANTIMICROBIAL COMPOUNDS AND THEIR ASSOCIATED BIOCHEMICAL ACTIVITY BY UV PHOTOLYSIS AND UV/H_2O_2 PROCESSES.

Abstract

The removal of four antimicrobial compounds [sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfadiazine (SDZ), and trimethoprim (TMP)] and their associated antimicrobial activity by photolysis and UV/H_2O_2 oxidation was evaluated with a flow-through annular photo-reactor equipped with a low-pressure UV lamp (λ=254 nm). Experiments were performed in ultrapure water at pH values at which the antimicrobial compounds were predominantly neutral or ionic. Additional experiments were conducted in lake water and wastewater treatment plant effluent. Transformation rates were well described by pseudo-first order models, and the concept of electric energy per order (EEO) was used to compare energy requirements for the removal of the four antibiotics. Results showed that SMX was easily degraded by photolysis and that the addition of H_2O_2 provided little benefit for SMX removal. The UV energy required for 90% SMX removal by photolysis was equal to 1 kWh per 1 m^3 of lake water. Because of slow photolysis rates, effective SMZ, SDZ and TMP transformation required the presence of

hydroxyl radicals, and EEO values were strongly influenced by the applied H_2O_2 dose. Using an EEO value of 2.6 kWh/m^3/order as an upper feasibility limit for UV/H_2O_2 oxidation process, 90% TMP removal from WWTPE would require a H_2O_2 dose of at least 6.8 mg L^{-1}. To test whether photooxidation intermediates exhibit antimicrobial activity, growth inhibition assays were conducted with *E. coli*. For all four antimicrobial compounds, the assays showed that photolysis and hydroxyl radical oxidation products did not exert any antimicrobial activity and that growth inhibition was due to remaining parent compound in the treated water. Neither solution pH nor background matter matrix affected this conclusion. These results show that both photolysis and hydroxyl radical oxidation processes degraded the biochemically active moieties of the studied antimicrobial compounds.

Keywords: pharmaceuticals, UV/H_2O_2, photolysis, hydroxyl radical, growth inhibition, antimicrobial activity.

4.1. Introduction

The presence of biochemically active compounds (BACs) such as antimicrobial compounds and endocrine disrupting chemicals (EDCs) in surface and ground

water has been a growing concern over the last decade. Several studies have illustrated that prescription and non-prescription drugs are present at detectable levels in surface and ground waters, in drinking water sources, and in treated drinking water (Halling-Sorensen, *et al.,* 1998, Lindsey *et al.,* 2001, Heberer *et al.,* 2002, Kolpin *et al.,* 2002, Yang *et al.,* 2004). The environmental presence of antimicrobial compounds at sub-inhibitory concentrations may lead to the evolution of antimicrobial-resistant bacteria, and the effects of chronic exposure to antibiotics at trace levels in drinking water are not known (Kümmerer, 2001, Snyder *et al*., 2005).

Antimicrobial compounds are not well removed by conventional drinking water treatment processes (Westerhoff *et al.,* 2005, Adams *et al.,* 2002). Advanced oxidation processes (AOPs) can be one effective method for the removal of antimicrobial compounds from drinking water. UV/H_2O_2 is an AOP that relies on the direct photolysis of hydrogen peroxide to generate highly reactive $^{\bullet}OH$ radicals.

In this study, four model antimicrobial compounds were selected (Figure 1): the sulfonamides sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfadiazine (SDZ); and the dihydrofolate reductase (DHFR) inhibitor trimethoprim (TMP). TMP is often applied in a synergistic combination with sulfonamides and is

frequently detected when sulfonamides are present in water (Stackelberg *et al.*, 2004 and Kolpin *et al.,* 2002). Sulfonamides are a class of antimicrobial compounds that have a common sulfonamide backbone and differ in the N-bond substituent on the sulfonamide bond. Sulfonamides are weak acids, and inhibit microbial growth by competing with a natural metabolic compound called para-aminobenzoic acid required in the folic acid synthesis process (Talaro and Talaro, 2002). TMP inhibits microbial growth by interfering with the action of other bacterial enzymes also required for folic acid synthesis (Talaro and Talaro, 2002). Sulfonamides have been detected at a maximum concentration of 15 μg/L in US surface water (Lindsey *et al.,* 2001) and TMP has been detected at concentrations up to 0.70 μg/L (Kolpin *et al.,* 2002).

Although the removal of the antimicrobial parent compound is readily achieved by oxidation processes, little is known about the antimicrobial activity of oxidation intermediates that are formed in the process. Boreen *et al.* (2004) found that sulfanilic acid was the predominant byproduct in the photolysis ($\lambda > 295$ nm) of sulfamethoxazole. In contrast, Zhou and Moore (1994) reported that the major identified product was 4-amino-N-(5-methyl-2-oxazolyl)benzenesulfonamide when SMX was exposed to UV light at $\lambda > 290$ nm. Other photolysis intermediates of sulfonamides include aniline and sulfanilamide (Boreen *et al.*, 2004, Zhou and Moore, 1994). For sulfamethazine, Boreen *et al.* (2005) proposed

that the photolysis (λ> 295 nm) intermediate 4-(2-imino-4,6-dimethylpyrimidin-1(2H)-yl)aniline is the most abundant result from SO_2 extrusion from the parent compound. The detected photolysis products contain the para-aminobenzoic acid backbone structure; therefore it is important to determine whether the mixture of the byproducts formed in the photooxidation process exhibits antimicrobial activity and whether such antimicrobial activity is higher or lower than for the parent compound.

To date few studies have addressed the change of antibacterial activity on the oxidation of antimicrobial compounds. Wammer *et al.* (2006) found no observable antimicrobial activity for the intermediates formed during simulated solar irradiation photolysis (λ>280 nm) of three sulfonamides (sulfathiazole, sulfamethoxazole and sulfachloropyridazine) and triclosan in buffered water. Suarez *et al.* (2007) showed that the antimicrobial activity of triclosan oxidation products formed by ozonation in wastewater effluents do not exhibit a measurable inhibitory activity.

The principal objective of this study was to determine whether low pressure UV photolysis (λ = 254 nm) and UV/H_2O_2 oxidation intermediates of SMX, SMZ, SDZ and TMP exhibit antimicrobial activity. Experiments were conducted in lake water and wastewater treatment plant effluent as well as in ultra pure water at

different pH values to assess whether the antimicrobial activity of photolysis and oxidation intermediates differs when the antimicrobial compounds were predominantly present in the neutral or ionic form. In addition, transformation rates of the antimicrobial compounds were determined and electrical energy per order (EEO) values were calculated for SMX, SMZ, SDZ and TMP.

4.2. Material and methods.

4.2.1. Photochemical reactor and experimental design

The effects of the following factors on SMX, SMZ, SDZ and TMP oxidation rates were evaluated: (1) pH, (2) H_2O_2 concentration, and (3) presence/absence of natural organic matter (NOM). Batch photolysis and UV/H_2O_2 oxidation experiments were conducted by recirculating 3L of water through an annular UV light reactor (Figure 2, Trojan Technologies, Model A, London, Ontario, Canada). The 25-W low pressure lamp had an emission wavelength of 254 nm. The total incident photon flux entering the reactor from the UV lamp was 5.74 $*10^{-4}$ Einstein min^{-1} as determined by potassium ferrioxalate actinometry (Kuhn *et al.,* 2004). The reactor had a bypass line that permitted pre-heating of the lamp prior to initiation of the oxidation reaction. Experiments were conducted in ultrapure water (UPW) buffered at pH values that were at least two pH units above and below the

pK_a of the antimicrobial compounds; thus, the degradation of the neutral and ionic forms of the antimicrobial compounds were evaluated. Phosphate and borate buffers were utilized to set the desired pH. Both inorganic buffers were selected because they did not measurably affect photolysis and oxidation rates (Buxton *et.al,* 1988). Kinetic tests were conducted at initial SMX, SMZ, SDZ and TMP concentrations of 4 (±1) μM and the degradation of the antimicrobial compounds was monitored as a function of time. Additional kinetic tests were conducted in the presence of NOM by spiking the antimicrobial compounds (4 μM) into Lake Wheeler water (Raleigh, NC, USA) and wastewater treatment plant effluent (Cary, NC, USA).

4.2.2. Reagents

All antimicrobial compounds were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). SMX and SDZ were stored at ambient temperature, while SMZ and TMP were stored at 4 °C. All antimicrobial compounds were stored in the dark to minimize photodegradation. Catalase was obtained from Sigma Chemical Corporation. Iso-Sensitest bacterial growth medium was purchased from Oxoid (Ogdensburg, NY, USA). Acetonitrile used for high-performance liquid chromatography (HPLC) analysis was HPLC grade (Fisher Scientific, Pittsburgh, PA, USA).

4.2.3. Analytical methods.

4.2.3.1. Antibiotics: Analyses were conducted with a HPLC system (Breeze, Waters, Milford, MA) equipped with a C18-AQ HPLC column (5 μm, 4.6 x 250 mm, Alltima HP, Grace) and a dual-wavelength UV detector. The mobile phase flow rate was 1.0 mL/min. For SMX and SMZ analyses, the mobile phase was composed of 24% v/v acetonitrile and 76% v/v 25 mM ammonium acetate buffer (pH 5). For SDZ and TMP, the mobile phase was 20% v/v acetonitrile and 80% v/v 25 mM ammonium acetate buffer (pH 5). The detector wavelength was set at 266 nm for SMX, SMZ and SDZ, and at 238 nm for TMP. Prior to HPLC analysis, all samples collected in the presence of H_2O_2 were quenched with catalase (1% v/v of a 0.2 mg/L stock solution).

4.2.3.2. Hydrogen peroxide: Hydrogen peroxide concentrations were quantified with the Ghormley method (Klassen *et al.* 1994). This method is based on the spectrophotometric determination of I_3^- that is produced when hydrogen peroxide reacts with I^-.

4.2.3.3. Water matrix*:* Apart from UPW, experiments were conducted in (1) Lake Wheeler (LW) water (Raleigh, North Carolina) and (2) wastewater treatment plant effluent (WWTPE, Cary, North Carolina). Both waters were stored at 4°C

and filtered through a 0.45-μm nylon membrane (Magna-R, MSI, Westboro, MA) prior to use. The pH values of LW and WWTPE was 7.6 and 7.85, respectively. Dissolved organic carbon (DOC) was quantified with a Total Organic Carbon Analyzer (Model TOC-5000A, Shimadzu, Columbia, MD) and DOC concentrations in LW and WWTPE were 5.1 mg L^{-1} and 7.3 mg L^{-1}, respectively. Alkalinity was quantified by *Standard Methods* 2320A (AWWA, 1998), and was 24.4 mg L^{-1} as $CaCO_3$ and 74.3 mg L^{-1} as $CaCO_3$ for LW and WWTPE, respectively.

4.2.4. Antimicrobial activity of photooxidation intermediates.

To test whether photooxidation intermediates exhibit antimicrobial activity, a growth inhibiton assay was implemented by adapting the macrodilution method described by Andrews (2001) and the EUCAST (2003). A similar protocol was described by Wammer *et al*. (2006) for assessing the antimicrobial activity of triclosan and sulfonamide photolysis products that are formed during simulated solar irradiation at wavelengths >280 nm. Modifications implemented in this study permitted a decrease in the initial antimicrobial compound concentration in the oxidation experiments; therefore the sample transmittance was higher and led to decreased reaction times. E.g. the initial SMX concentration could be decreased from 500 μM (127 mg L^{-1}, Wammer *et al*. 2006) to 80 μM (20 mg L^{-1}) in this study.

Figure 3 provides an overview of the methodology used to asses the antimicrobial activity of photooxidation intermediates. The Enterobacteriaceae organism *E. coli* ATCC® 25922 was acquired to conduct growth inhibition assays. *E. coli* cells were added to Iso-Sensitest broth to yield an initial concentration of $1x10^6$ cells mL^{-1} (Iso-Sensitest broth was prepared and autoclaved as indicated by manufacturer). One mL of this inoculum was added to 8-mL sterile glass tubes containing 1 mL of water spiked with different concentrations of the antimicrobial compound or 1 mL of sample after different photolysis or UV/H_2O_2 oxidation times. The water that contained the spiked antimicrobial compound was UPW, LW or WWTPE. Also, positive controls were prepared without the addition of antimicrobial agent. Positive controls contained the same water as the samples (phosphate buffer, borate buffer, LW or WWTPE plus catalase at the same concentration as the samples). It should be noted that upon mixing the samples with the inoculums, the pH was always close to 7 because of the strong buffer capacity of the Iso-Sensitest broth. The positive controls represented the maximum *E. coli* growth that could be reached. After incubating for 8 hours at 37°C, the optical density of each sample was measured at 600 nm. To obtain an absorbance <0.6, samples were diluted (2 mL sample + 5 mL broth). To assure the *E. coli* inoculation yielded $1x10^6$ cells mL^{-1}, plating experiments were performed on TCA plates and incubated at 30° C overnight. Additional plating was done with 100 μL

of Iso-Sensitest broth to guarantee there was no contamination. All plating was done in duplicate.

4.3. Results and discussion.

4.3.1. Kinetics

The goal of the kinetic study was to determine the effects of solution pH and background matrix on the photochemical fate of four antimicrobial compounds in a commercial photoreactor. Both photolysis and UV/H_2O_2 oxidation data were described by a simple pseudo-first order rate model and corresponding rate constants (k) for experiments conducted in UPW, LW and WWTPE are summarized in Table 1 and in Figure 4.

pH effect. As indicated by the k values in Table 1, the neutral form of SMX (pH 3.6) reacted more readily than the anionic form (pH 7.6). In contrast, the photolysis data for SMZ showed that the anionic form of SMZ (pH 9.7) reacted more readily than the neutral form (pH 3.6). For SDZ photolysis, the pH dependence of the k values was weaker than for SMZ, but the anionic form was again more photolyzable than the neutral form. The photolysis results suggest that the N-bond substituent on the sulfonamide group may be responsible for the unrelated photochemical behavior of the sulfonamides. The molar absorptivity (ε)

of each compound was not related to the compound's photolysis rate i.e. the calculated molar absorptivity of SMX at pH 7.6 (16,200 M^{-1} cm^{-1}, 254 nm) was higher than that at pH 3.6 (11,129 M^{-1} cm^{-1}, 254 nm), but the neutral form exhibited a faster photodegradation rate. For SMZ and SDZ, the molar absorptivity of the anionic species (20,540 M^{-1} cm^{-1} for SMZ and 20,660 M^{-1} cm^{-1} for SDZ at 254 nm) was also higher than the molar absorptivity of the neutral species (16,200 M^{-1} cm^{-1} for SMZ and 13,600 M^{-1} cm^{-1} for SDZ at 254 nm), but the anionic form exhibited a faster photodegradation rate. The similarity in pH effects on photolysis rates and molar absorptivity between SMZ and SDZ are likely due to the similarity of their molecular structure (both have a diaizine group as the N-bond substituent of the sulfonamide group). In contrast SMX has an isoxazole group as N-bond substituent of the sulfonamide group and exhibits a very different photolytic behavior when exposed to UV light at 254 nm. For sulfonamide solutions irradiated with natural sunlight (λ>290nm), Boreen *et al.*(2004) reported that quantum yields of the anionic and neutral forms of sulfonamides exhibit no uniform trend and that SMX degrades more rapidly at low pH values, at which the neutral form is dominant; the results obtained at 254 nm in this study support these observations Photolysis rates are related to the fraction of neutral and ionic forms of the pharmaceutical compounds in solution (Boreen *et al.* 2004 and Canonica *et al.* 2008); therefore, the overall photolysis rate at different pH values can be calculated from the rates of neutral and ionic forms as shown for SMX in Figure 5.

For the three sulfonamides, the addition of H_2O_2 increased the parent compound removal rate at all tested conditions. The incremental change in k-value resulting from the H_2O_2 addition did not show a strong pH dependence for the three sulfonamides. Therefore the overall removal rate of sulfonamides in the UV/H_2O_2 process is strongly affected by the photolysis rate of the sulfonamide at the treatment pH. For trimethoprim, the photolysis rate was negligible at both pH 3.6 (cationic form) and 9.7 (anionic form) (Table 1). Consequently, TMP removal during UV/H_2O_2 treatment was almost completely governed by hydroxyl radical oxidation.

SMX removal by photolysis is quite fast. While the addition of H_2O_2 has a little benefit in the SMX degradation, H_2O_2 addition should be considered when further transformation of photolysis intermediates or mineralization is desired; e.g. Lam and Mabury (2005) found that the photolysis of SMX by sunlight produced the intermediate, 3-amino-5-methylisoxazole, which was not removed by direct sunlight photolysis but was degraded in the presence of hydroxyl radical.

For SMZ, SDZ and TMP UV/H_2O_2 experiments that were completed at pH 9.7, the reaction between $^{\bullet}OH$ and the contaminant was hindered by elevated carbonate

concentrations due to enhanced dissolution of atmospheric CO_2. Carbonate is an important $^{\bullet}OH$ radical scavenger. Therefore, the reaction rate constants in the presence of H_2O_2 do not only reflect changes in reactivity that were related to the state of (de)protonation of the antimicrobial compound but also the lower $^{\bullet}OH$ concentration that was present at pH 9.7.

Background organic matter effect: Removal rates of all four antimicrobial compounds were slower in LW and WWTPE than in buffered ultrapure water (Table 1), a result that was due to (1) higher UV light absorbance by the background organic matter and (2) the presence of hydroxyl radical scavengers (e.g., HCO_3^-, CO_3^{2-}, NOM). Oxidation rates in the presence of background organic matter could be improved by increasing the hydrogen peroxide concentration. Figure 4 shows a linear relationship between the hydrogen peroxide concentration and the pseudo first-order oxidation rates in the range of the studied H_2O_2 concentrations for SMX, SMZ, SDZ and TMP in LW. Therefore, increasing the hydrogen peroxide concentration up to at least 0.2 mM (6.8 mg L^{-1}) is beneficial to promote higher oxidation rates.

Figure 4 also shows that the slopes of the lines for the three sulfonamide compounds were similar; suggesting that the hydroxyl radical reacts preferentially

with the sulfanilamide structure common to the sulfonamide compounds instead of the N-bond substituent on the sulfonamide bond.

To evaluate background matrix effects on UV/H_2O_2 oxidation rate additional experiments were conducted with SMX, SMZ, SDZ and TMP in WWTPE at an H_2O_2 dose of 0.2 mM. At an H_2O_2 dose of 0.2 mM, the rates of SMX, SMZ, SDZ and TMP oxidation in LW were 1.2, 1.2, 1.6 and 2.0 times faster than in WWTPE, results that can be explained by the higher DOC and alkalinity of WWTPE and thus the higher concentration of $^{\bullet}OH$ scavengers.

4.3.2. Electrical Energy per Order (EEO).

To compare parent compound removal efficiencies among different experimental conditions, the concept of EEO was employed (Bolton *et al.* 2001a). EEO is directly related to the electrical energy consumption during the removal of a contaminant; therefore EEO can be used to determine the feasibility of a certain treatment in terms of energy efficiency. This figure-of-merit allows the fair comparison between AOPs that utilize UV irradiation. EEO is defined as the electrical energy required (kWh) to lower the contaminant concentration by one order of magnitude (90% degradation) in 1 m^3 of water (Bolton *et al.* 2001a). The

EEO was calculated from the pseudo-first order rate constants (k) through the following expression (Bolton *et al.*, 2001a):

$$EEO = \frac{1000 \times P \times t}{60 \times V \times \log(Co/C)} = \frac{38.4 \times P}{k \times V}$$

where P is electrical power (kW), t is irradiation time (min), V is volume (L) and Co and C are the initial and final target compound concentrations, respectively.

Figure 6 displays the EEO for removal of (a) SMX, (b) SMZ, (c) SDZ, and (d) TMP with direct photolysis and UV/H_2O_2 oxidation. Data shown in Figure 6 also considers the energy required to produce the hydrogen peroxide consumed in a UV/H_2O_2 oxidation process. A hydrogen peroxide energy cost of 10.81 kWh per kg of H_2O_2 was utilized for the overall EEO calculation in this study (Rosenfeldt *et al.*, 2006).

To determine the energy effectiveness of the oxidation of the four antimicrobial compounds selected, it is important to consider: (1) practical upper energy limits for UV-based AOPs; e.g. Bolton (2001b) expressed that the most efficient UV-based AOPs have EEO values in the range of 0.2 – 1.0 kWh/order/m^3 and Rosenfeldt *et al.* (2005) indicated an energy feasibility limit of 2.6 kWh/order/m^3; and (2) compare the EEO obtained for a UV-based AOP with the energy requirements for an ozone degradation processes.

Following the EEO criteria for UV-based AOPs, SMX was degraded efficiently by both photolysis and UV/H_2O_2 processes in either neutral or anionic form. The EEO calculated for SMX at all studied conditions was equal to or less than 1 kWh/order/m^3, and the addition of hydrogen peroxide resulted either in no improvement (UPW) or in only a small improvement (LW) of the overall energy requirement for the removal of SMX.

The EEO results for all the experimental conditions studied are shown in Figure 6. Because EEO calculations are related to the pseudo-first order rate model constants (k), the relation between EEO results for neutral and ionic forms is analogous to what is presented in section 4.3.1; eg., the neutral form of SMX requires higher amounts of energy than the ionic form to produce the same amount of transformation, while for SMZ and SDZ, the EEO trend was the opposite than for SMX. For SMZ, SDZ and TMP, energy requirements for 90% parent compound removal by photolysis exceeded the feasibility limit of 2.6 kWh/order/m^3. These compounds required the addition of hydrogen peroxide to create a less energy intensive process. In the case of WWTPE (1.4 times more DOC and 3 times more alkalinity than in LW), the amount of H_2O_2 added must be at least 0.2 mM (6.8 mg L^{-1}) before the UV/H_2O_2 process becomes viable, from an energy consumption perspective.

There are EEO values for the oxidation of many other organic compounds in the literature that can be compared with the results obtained in this study. For example: benzene and its derivates: 2 – 10 kWh/order/m^3, chloroform: 40 – 250 kWh/order/m^3 (Bolton, 2001b), MTBE: 0.2 – 7.5 kWh/order/m^3 (Cater *et al.*, 2000) and MIB and Geosmin: 1.3 - 2.6 kWh/order/m^3 (Rosenfeldt *et al.,* 2005). But, it is important to note that EEO values for UV-based AOPs depend on a number of factors. These factors include: (1) reactor configuration, (2) initial contaminant concentration, (3) initial H_2O_2 concentration, and (4) presence of scavengers (e.g. oxidation products, natural organic matter and alkalinity). And, even though only a firm comparison within EEO values can be made for the oxidation of contaminants occurring at equivalent conditions, the EEO values obtained for SMX, SMZ, SDZ and TMP are in the same order of magnitude as the energy required to remove contaminants such as MTBE, MIB and Geosmin.

Finally, is important to compare the energy consumption of UV-based AOPs to that of molecular ozone. For 90% SMX and TMP removal, Dodd *et al.* (2006) found that an ozone dose of 1.5 mg L^{-1} is needed at pH 7.7 in a secondary wastewater effluent (DOC= 5.3 mg L^{-1} and alkalinity= 3.5 mM as HCO_3^-). An EEO value for ozone can be calculated by multiplying the ozone dose by the energy cost of ozone generation. In the literature, the values for energy cost of ozone generation range from 15 to 26 kWh kg^{-1} of ozone (Gottschalk, *et al.*, 2000 and USEPA, 1999), resulting in values of EEO

equal to 0.023 - 0.039 kWh/order/m^3. Because ozone rapidly and selectively oxidizes the p-sulfanilaniline functional group in sulfonamides and the pyrimidine moiety in TMP (Dodd *et al.,* 2006), ozone processes require less energy to produce the same extent of sulfonamide and TMP removal than UV/H_2O_2 processes.

4.3.3. Antimicrobial activity of photooxidation intermediates.

Figure 7 summarizes growth inhibition data for SMX, SMZ, SDZ and TMP in ultrapure water. In Figure 7 the optical density of the sample (Abs) is normalized by the optical density of the control (Abs_{PC}) and plotted against the antimicrobial compound concentration in solution. Sulfonamides are a group of synthetic antimicrobial compounds that compete with a natural metabolic compound called para-aminobenzoic acid, which is required by bacteria in the synthesis of the coenzyme tetrahydrofolic acid, which is needed in the synthesis of purines and some amino acids (Talaro and Talaro, 2002). As seen in Figure 7, maximum growth inhibition for sulfonamide compounds was between 82% and 83 %, a result that agrees with literature values (EUCAST, 2003). In UPW, SMX, SMZ and SDZ inhibited *E. coli* growth as the concentration increased from 1 to 10 mg L^{-1}, 10 to 200 mg L^{-1} and 1 to 30 mg L^{-1}, respectively. TMP inhibited *E. coli* growth in UPW as the solution concentration increased from 0.06 to 0.4 mg L^{-1}, and growth inhibition approached 100% at the latter concentration.

To evaluate whether photolysis and UV/H_2O_2 oxidation intermediates exhibited antimicrobial activity, equivalent concentrations that represent 50% of the maximum possible growth inhibition (EC50) in treated samples were compared to EC50 values of non treated blanks.

The hill slope equation was used to determine EC50 values:

$$\frac{Abs}{Abs_{PC}} = \left(\frac{Abs}{Abs_{PC}}\right)_{MIN} + \frac{\left(\frac{Abs}{Abs_{PC}}\right)_{MAX} - \left(\frac{Abs}{Abs_{PC}}\right)_{MIN}}{1 + \exp\left(\frac{EC50\text{-}[compound]}{hill\ slope}\right)}$$

where Abs_{PC} is the optical density of the positive control, $\left(\frac{Abs}{Abs_{PC}}\right)_{MAX}$ is equal to 1 and corresponds to the maximum growth and $\left(\frac{Abs}{Abs_{PC}}\right)_{MIN}$ is equal to 0.18 for SMX and SMZ, and 0.17 for SDZ and corresponds to the value representing maximum growth inhibition. For TMP, $\left(\frac{Abs}{Abs_{PC}}\right)_{MIN}$ was zero. The EC50 values for each parent compound in UPW are shown in Figure 8 (SMX), 9 (SMZ), 10 (SDZ) and 11 (TMP). Furthermore, in these figures a 95% prediction band was plotted for the growth inhibition data obtained for the parent compound in UPW. The 95% prediction

band represents the envelope into which 95% of future experimental points would fall (GraphPad Software, Inc).

All photolysis and UV/H_2O_2 experimental conditions were evaluated for SMX and TMP due to its lower EC50 values of 3.50 and 0.17 mg L^{-1}, respectively. Experiments for SMX and TMP at different pHs were conducted to asses the potential difference of products formed from neutral and ionic forms. In the case of SMZ and SDZ, which exhibited EC50 values of 45.90 and 9.43 mg L^{-1}, respectively, only the UV/H_2O_2 experiments with LW and WWTPE were performed. For growth inhibition assays, all UV/H_2O_2 experiments were conducted with a 1:1 on a mass ratio of antimicrobial compound and H_2O_2. The background matrix of LW and WWTPE may also affect on the products speciation compared to experiments with UPW, due to additional reactive species such as carbonate radicals and dissolved natural organic matter that can act as a photosensitizer. Carbonate radicals compete with the hydroxyl radical for organic compounds when the rate constants between carbonate radical and the organic compounds is in the range of 5×10^6 to 2×10^9 M^{-1} s^{-1} (Canonica *et al*., 2005).

Table 2 shows the EC50 values obtained from growth inhibition assays conducted with SMX, SMZ, SDZ and TMP and their photolysis and UV/H_2O_2 oxidation intermediates. As seen in Table 2 only two EC50 values of all tested conditions (SMZ UV/H_2O_2 intermediates in WWTPE and TMP photolysate in UPW at pH 3.6) were

below the lower bound of the 95% prediction band. However the mean of EC50 values were only 4.3 and 1.3 % smaller than the lower bound of the 95 % prediction band; therefore no strong case can be made in terms of claiming that the intermediates from these two tests exerted measurable antimicrobial activity. The remaining EC50 values shown in Table 2 were inside the 95% prediction band or exceeded the upper bound; therefore, one can confidently claim that the photolysis and UV/H_2O_2 oxidation intermediates formed in these tests did not exert any antimicrobial activity and that growth inhibition was a result of the remaining parent compound concentration in the reaction mixture. Furthermore, neither solution pH (for SMX and TMP) nor the background matrices (LW and WWTPE) led to the production of products that exhibited measurable antimicrobial activity. In addition to the EC50 comparisons, Figures 8 – 11 show that the bulk of the experimental data for the oxidation intermediates falls within the 95 % prediction band that was established for the untreated parent compound in UPW.

Finally, the authors compared the EC50 for SMX obtained in this study with the data observed by Wammer *et al.* (2006). Although the *E. coli* strains were different in the two studies, the EC50 values for SMX were similar (12.3 μM or 3.12 mg L^{-1} by Wammer *et al.,* 2006 versus 3.50 mg L^{-1} in this study). Thus, the methodology adapted in this study yielded results that were comparable to previous studies.

4.4. Conclusion

- Removal of SMX in the UV/H_2O_2 process was primarily a result of direct photolysis. In contrast, SMZ, SDZ and TMP, phototransformation rates were slower, and •OH oxidation played a dominant role in their removal. Solution pH affected the photoxidation of the sulfonamides in a non-uniform manner, suggesting that the N-bond substituent of the sulfonamide bond is responsible of the different photochemical behavior within the sulfonamides. For TMP no significant photolysis was achieved.

- Electrical energy per order (EEO) calculations showed that SMX removal is effectively achieved at feasibly energy levels (< 1 kWh/order/m^3). The removal of SMZ, SDZ and TMP in LW and WWTPE required the addition of H_2O_2 (formation of $^{\bullet}$OH) and more energy. The higher EEO for the oxidation in WWTPE compared to LW showed the importance of the background water matrix on the energy consumption of UV-based AOPs.

- For the specific conditions and organism utilized in this research, the growth inhibition assays showed that intermediates formed during photolysis and UV/H_2O_2 oxidation of sulfonamides and TMP did not exhibit measurable

antimicrobial activity. Growth inhibition was principally a result of the parent compound that remained following photolysis or UV/H_2O_2 oxidation.

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Table 1: Effects of pH and background water matrix on pseudo-first order photolysis and UV/H_2O_2 oxidation rate constants (k) for sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfadiazine (SDZ) and trimethoprim (TMP).

Sulfamethaxazole (SMX)		
		k (min^{-1})
UPW pH 3.6	Photolysis only	2.311
	0.038 mM H_2O_2	2.509
UPW pH 7.6	Photolysis only	0.472
	0.038 mM H_2O_2	0.712
LW pH 7.6	Photolysis only	0.313
	0.038 mM H_2O_2	0.338
	0.079 mM H_2O_2	0.352
	0.213 mM H_2O_2	0.468
WWTPE pH 7.85	0.202 mM H_2O_2	0.392

Sulfamethazine (SMZ)		
		k (min^{-1})
UPW pH 3.6	Photolysis only	0.059
	0.037 mM H_2O_2	0.221
UPW pH 9.7	Photolysis only	0.173
	0.043 mM H_2O_2	0.310
LW pH 7.6	Photolysis only	0.075
	0.038 mM H_2O_2	0.100
	0.060 mM H_2O_2	0.119
	0.207 mM H_2O_2	0.227
WWTPE pH 7.85	0.201 mM H_2O_2	0.189

Sulfadiazine (SDZ)		
	k (min^{-1})	
UPW pH 3.6	Photolysis only	0.073
	0.039 mM H_2O_2	0.213
UPW pH 9.7	Photolysis only	0.106
	0.034 mM H_2O_2	0.199
LW pH 7.6	Photolysis only	0.069
	0.035 mM H_2O_2	0.094
	0.078 mM H_2O_2	0.126
	0.178 mM H_2O_2	0.206
WWTPE pH 7.85	0.205 mM H_2O_2	0.143

Trimethoprim (TMP)		
	k (min^{-1})	
UPW pH 3.6	Photolysis only	0.008
	0.039 mM H_2O_2	0.272
UPW pH 9.7	Photolysis only	0.011
	0.034 mM H_2O_2	0.164
LW pH 7.6	Photolysis only	0.008
	0.034 mM H_2O_2	0.042
	0.075 mM H_2O_2	0.088
	0.217 mM H_2O_2	0.272
WWTPE pH 7.85	0.202 mM H_2O_2	0.127

Table 2: EC50 values obtained from growth inhibition assays conducted with sulfamethoxazole (SMX), sulfamethazine (SMZ), sulfadiazine (SDZ), trimethoprim (TMP) and their photolysis and UV/H_2O_2 oxidation intermediates.

	Treatment	EC50 (mg L^{-1})
SMX in UPW	none	3.499 (3.113, 3.885)* (2.609, 4.472)**
	Photolysis at pH 3.6	4.210 (3.751, 4.668)*
	Photolysis at pH 7.6	4.160 (3.832, 4.489)*
	UV/H_2O_2 at pH 3.6	3.540 (3.302, 3.778)*
	UV/H_2O_2 at pH 7.6	4.249 (3.854, 4.644)*
SMX in LW	UV/H_2O_2	5.440 (4.928, 5.952)*
SMX in WWTPE	UV/H_2O_2	5.366 (4.952, 5.779)*
SMZ in UPW	none	45.90 (43.71, 48.09)* (38.93, 53.05)**
SMZ in LW	UV/H_2O_2	45.60 (44.03, 47.16)*
SMZ in WWTPE	UV/H_2O_2	37.25 (35.88, 38.61)*
SDZ in UPW	none	9.583 (9.171, 9.995)* (8.114, 10.88)**
SDZ in LW	UV/H_2O_2	11.52 (11.17, 11.86)*
SDZ in WWTPE	UV/H_2O_2	9.985 (9.520, 10.45)*
TMP in UPW	none	0.168 (0.164, 0.173)* (0.154, 0.183)**
	Photolysis at pH 3.6	0.152 (0.148, 0.156)*
	Photolysis at pH 9.7	0.176 (0.172, 0.180)*
	UV/H_2O_2 at pH 3.6	0.178 (0.161, 0.194)*
	UV/H_2O_2 at pH 9.7	0.164 (0.158, 0.170)*
TMP in LW	UV/H_2O_2	0.193 (0.189, 0.198)*
TMP in WWTPE	UV/H_2O_2	0.197 (0.190, 0.204)*

*values in parenthesis are the lower and upper bound of the 95% confident interval.

* *values in parenthesis are the lower and upper bound of the 95% prediction band.

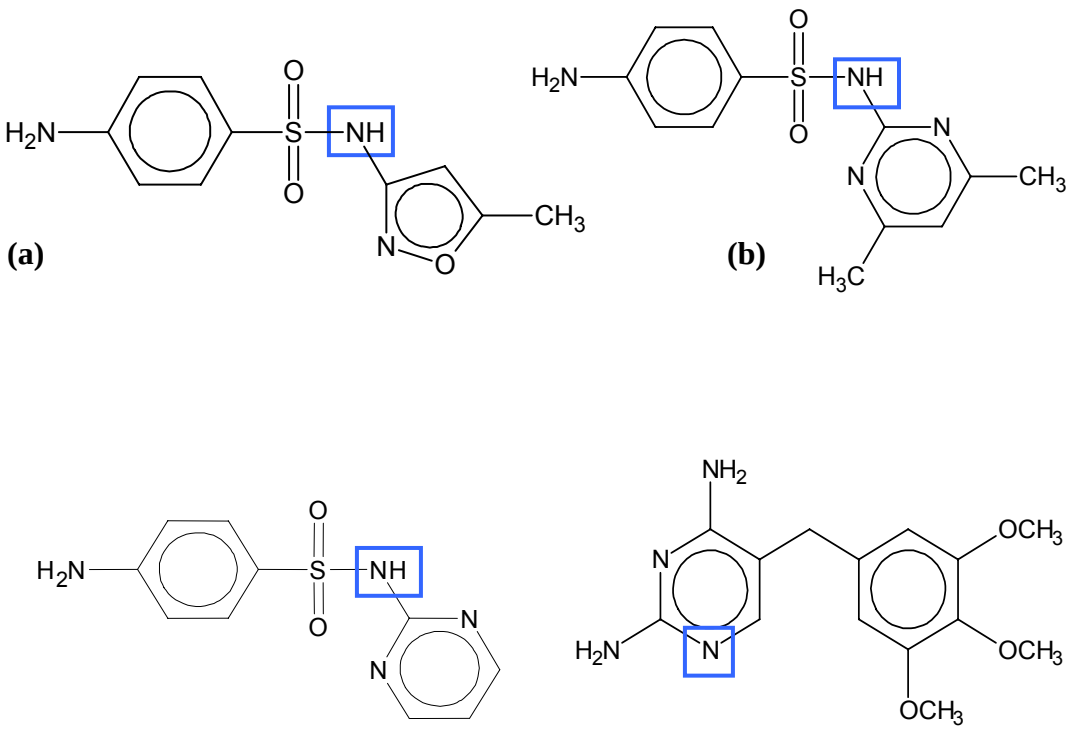

Figure 1. Molecular structures of neutral forms of (a) sulfamethoxazole (SMX) - molecular weight = 253.3 Da, $pK_{a,1}$ = 1.74[1] and $pK_{a,2}$ = 5.65[2]; (b) sulfamethazine (SMZ) - molecular weight = 278.3 Da, $pK_{a,1}$ = 2.26[1] and $pK_{a,2}$ = 7.65[2]; (c) sulfadiazine (SDZ) - molecular weight = 280.2 Da, $pK_{a,1}$ = 2.02[1] and $pK_{a,2}$ = 6.43[2] and (d) trimethoprim (TMP) - molecular weight = 290.3 Da, $pK_{a,2}$ = 6.76[3]. For sulfonamides, SO_2NH changes to SO_2N^- as pH increases. For TMP, aromatic N changes to NH^+ as pH decreases. Ionizable functional groups are highlighted ([1]Lin *et al.*, 1997a, [2]Lin *et al.*, 1997b and [3]Qiang and Adams, 2004)

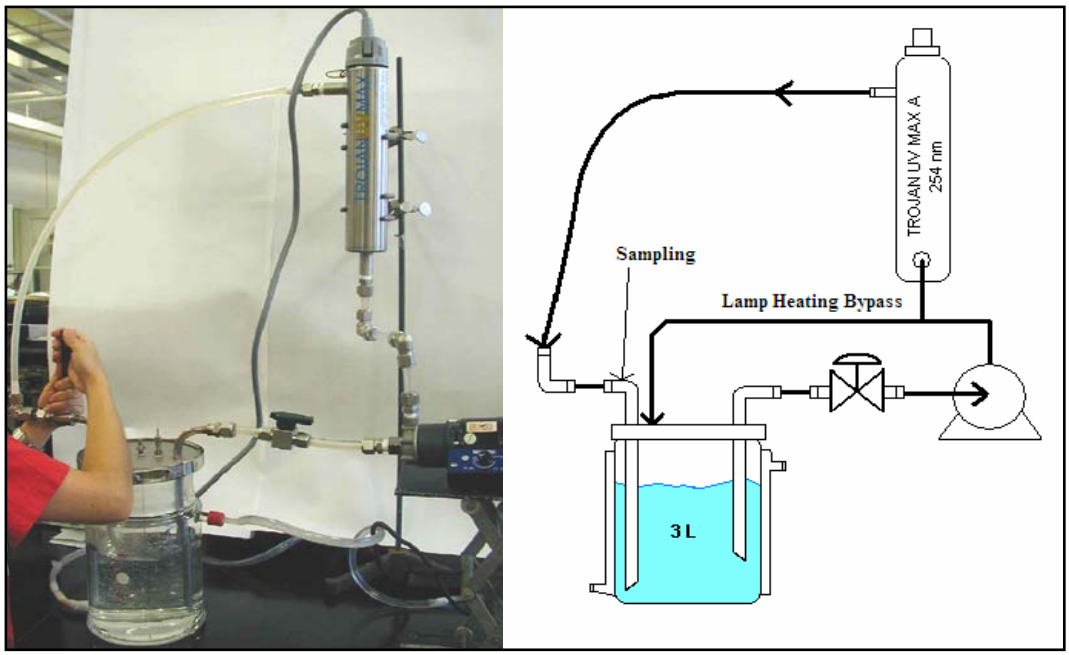

Figure 2. Photoreactor experimental setup

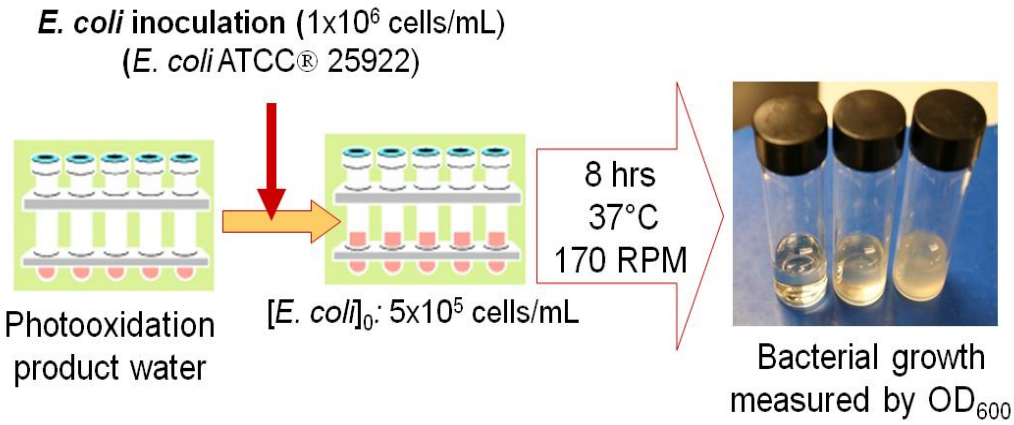

Figure 3. Protocol to determine the antimicrobial activity of photooxidation intermediates

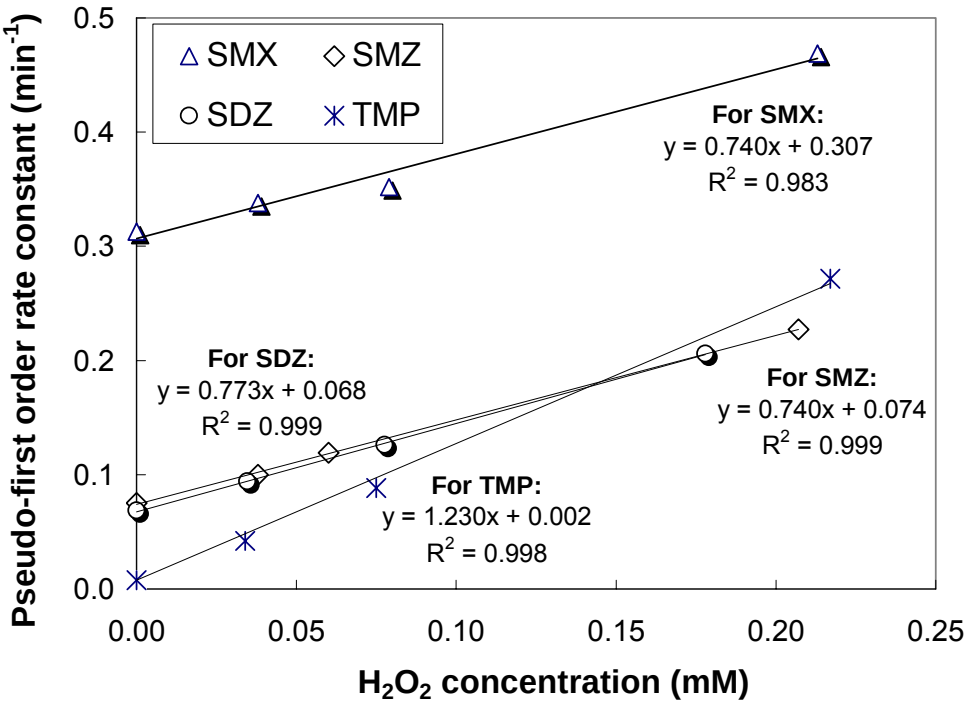

Figure 4. Effect of initial hydrogen peroxide concentration on the pseudo-first order rate constants for the UV/H_2O_2 oxidation of SMX, SMZ, SDZ and TMP in lake water.

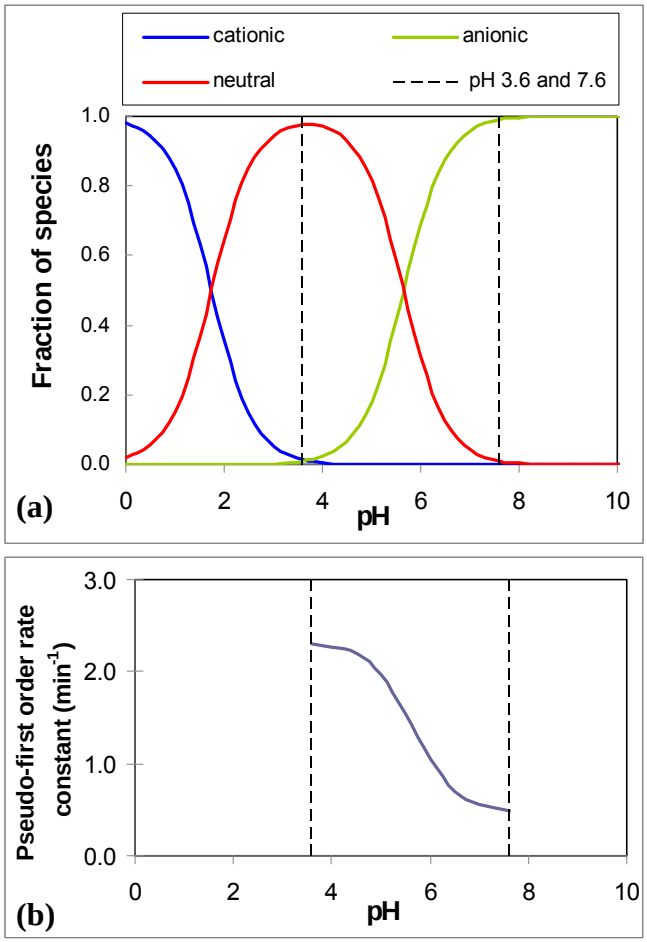

Figure 5. SMX (a) speciation and (b) pH–dependent pseudo-first order photolysis rate constant (k) as function of pH. Figure (b) was calculated with the following expression: $k' = \alpha_{neutral} * k_{neutral} + \alpha_{anionic} * k_{anionic}$, where $\alpha_{neutral}$ and $\alpha_{anionic}$ are the fractions of the neutral and the anionic SMX species in solution, respectively.

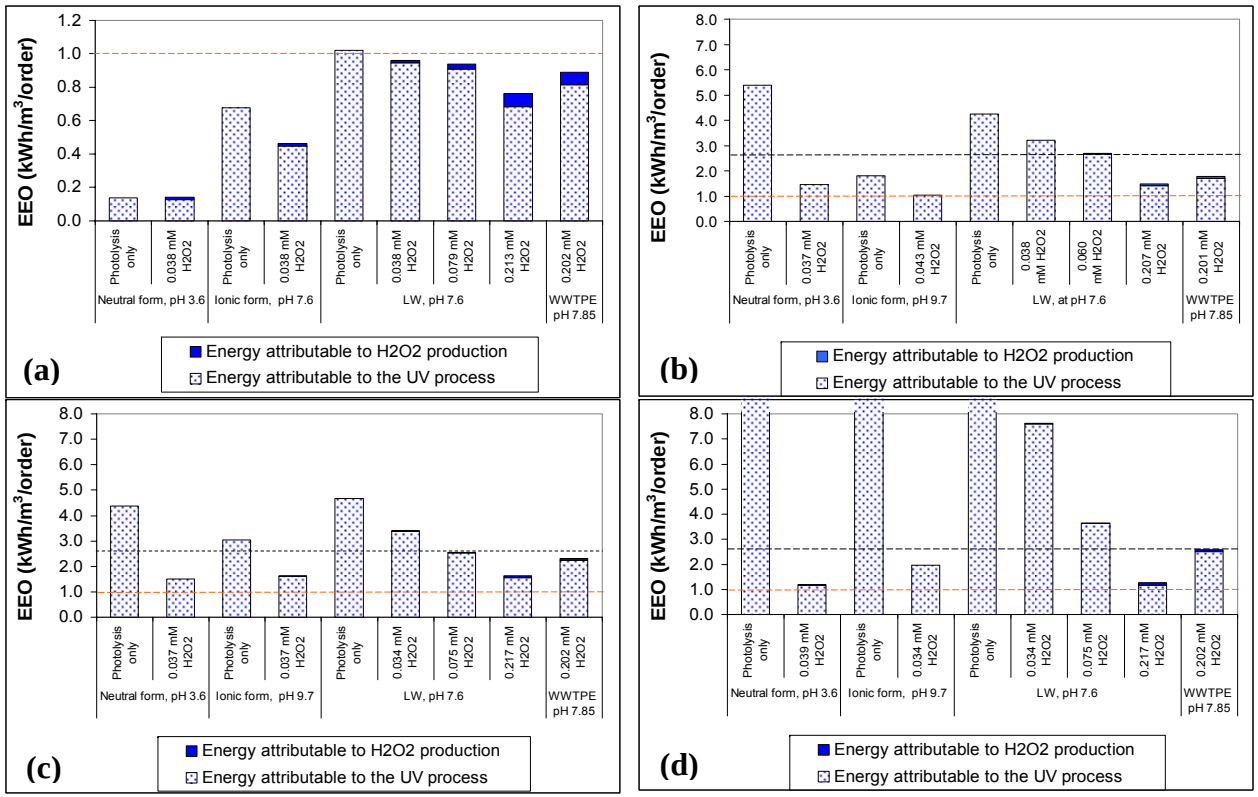

Figure 6: Electrical energy per order for the removal of (a) SMX, (b) SMZ, (c) SDZ and (d) TMP in ultra pure water (neutral and anionic forms of each antimicrobial compound), lake water (LW) and wastewater treatment plant effluent (WWTPE). Lower dashed line (---) indicates an EEO equal to 1 kWh/m^3/order and upper dashed line (---) indicates an EEO equal to 2.6 kWh/m^3/order. Trimethoprim photolysis EEO values exceeded 30 kWh/m^3/order.

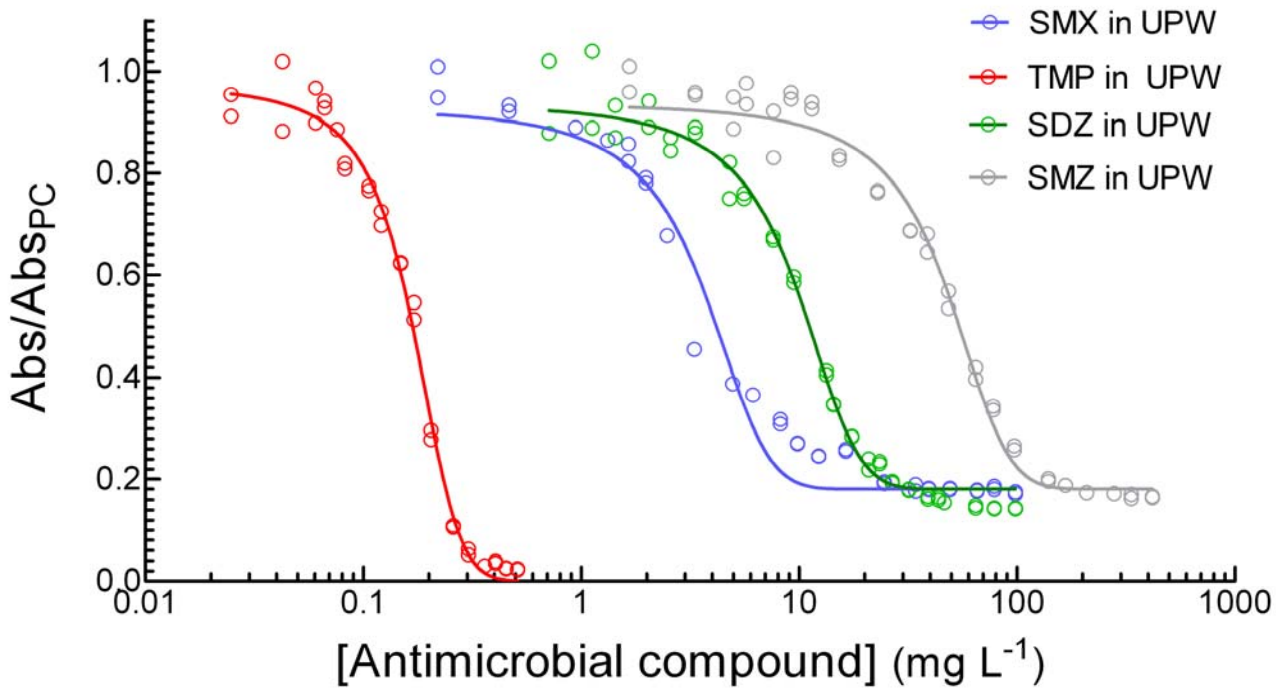

Figure 7: Growth inhibition assay results in ultrapure water for SMX, SMZ, SDZ and TMP.

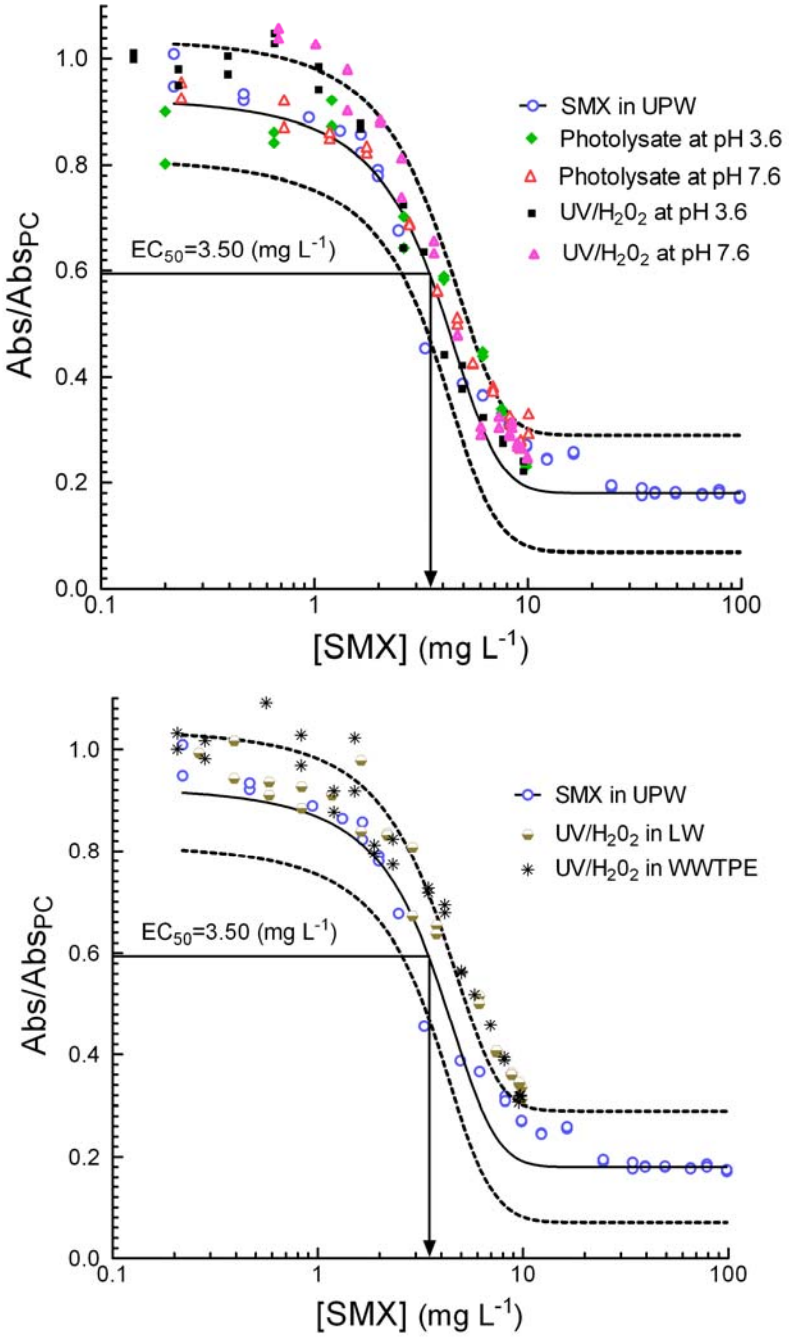

Figure 8: Effect of solution pH and background water matrix on antimicrobial activity of SMX photolysis and UV/H_2O_2 oxidation intermediates. $C_{0,SMX}$ = 20 mg L^{-1}. $C_{0,H2O2}$ = 20 mg L^{-1} in UV/H_2O_2 experiments.

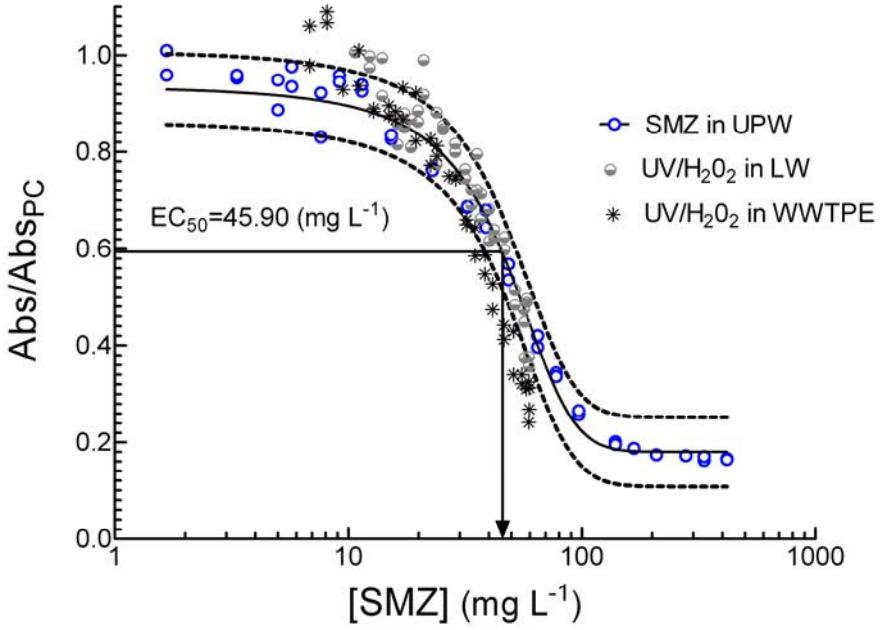

Figure 9: Effect of background matrix on antimicrobial activity of SMZ UV/H_2O_2 oxidation intermediates. $C_{0,SMZ}$ = 120 mg L^{-1}. $C_{0,H2O2}$ = 120 mg L^{-1} in UV/H_2O_2 experiments.

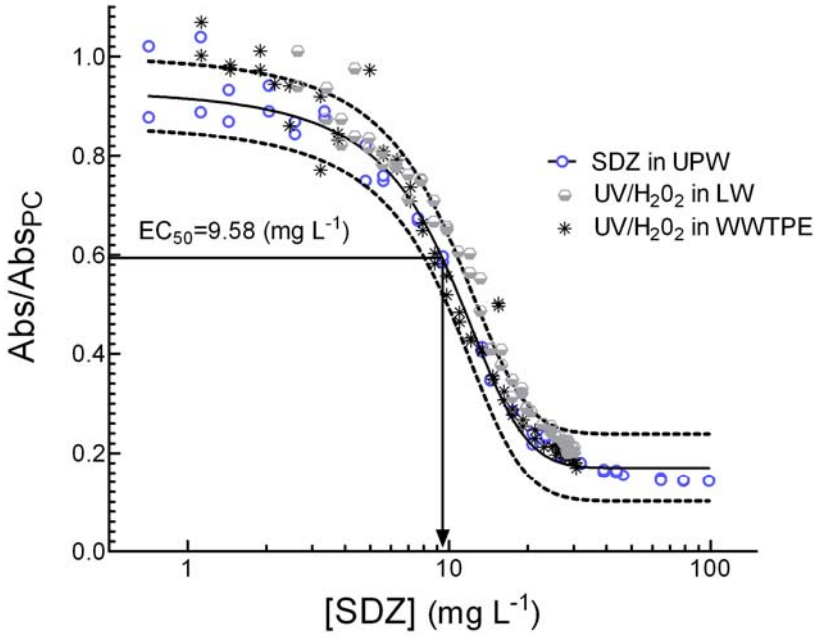

Figure 10: Effect of background matrix on antimicrobial activity of SDZ UV/H_2O_2 oxidation intermediates. $C_{0,SDZ}$ = 60 mg L^{-1}. $C_{0,H2O2}$ = 60 mg L^{-1} in UV/H_2O_2 experiments.

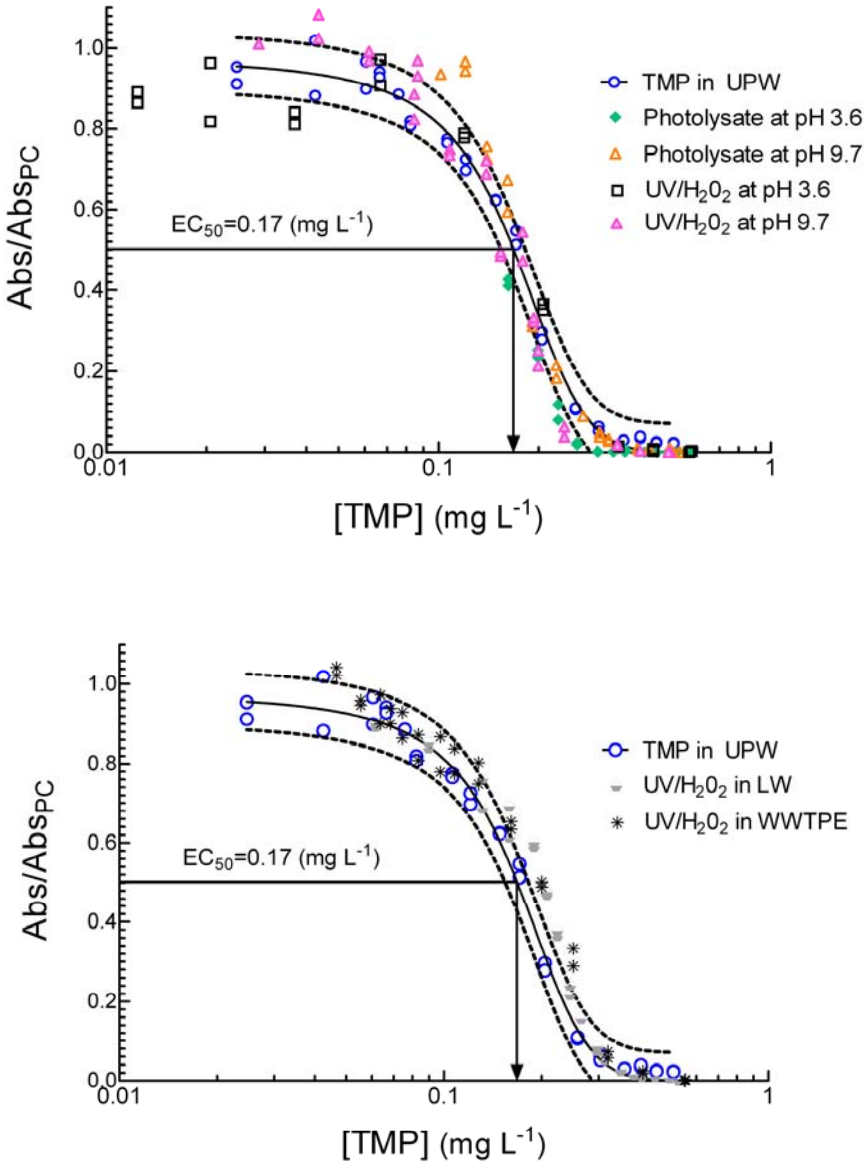

Figure 11: Effect of solution pH and background matrix on antimicrobial activity of TMP photolysis and UV/H_2O_2 oxidation intermediates. $C_{0,TMP}$ = 1 mg L^{-1}. $C_{0,H2O2}$ = 1 mg L^{-1} in UV/H_2O_2 experiments.

Chapter 5.

MINERALIZATION OF BIOCHEMICALLY ACTIVE COMPOUNDS AT TRACE LEVELS BY INTEGRATED UV/H_2O_2 AND BIOLOGICAL OXIDATION PROCESSES.

Abstract

Biochemically active compounds (BACs) are ubiquitous in wastewater treatment plant effluents (WWTPE) at concentrations that range from ng L^{-1} to μg L^{-1} levels. A UV/H_2O_2 oxidation process was used to transform three ^{14}C-labeled BACs [sulfadiazine (SDZ), bisphenol A (BPA) and diclofenac (DCL)] in WWTPE, and the biodegradability of the oxidation intermediates was evaluated in microcosms inoculated with lake water bacteria and lake sediments. The three BACs were studied at equivalent carbon concentrations of 36 μg-C L^{-1}. The UV/H_2O_2 oxidation of the parent compound resulted in the removal of 94%, 88% and +99% for SDZ, BPA and DCL, respectively. Mineralization due to the chemical oxidation step was 27% for DCL and negligible for SDZ and BPA (<2%). Biological mineralization of ^{14}C-labeled oxidation intermediates by surface water bacteria was extremely slow (e.g a maximum of 1.1% in 30 days for SDZ oxidation intermediates). The addition of lake sediments to SDZ oxidation intermediates enhanced the mineralization rate, and 5.2% mineralization was obtained after 30 days. For BPA and DCL, the biological

mineralization rate of their ^{14}C-labeled oxidation intermediates by lake water bacteria did not exceed 0.8% after 30 days. Bacteria in lake water and sediments were capable of mineralizing BAC oxidation intermediates more rapidly than the non-oxidized parent compound. Overall, the results of this research suggest that BAC oxidation intermediates are relatively persistent, and residence times that commonly exist in rivers between wastewater treatment plant discharges and either drinking water treatment plant intakes or ecologically sensitive areas are insufficient to yield substantial levels of biological mineralization.

Keywords: Biochemical active compounds, biodegradation, UV/H_2O_2, natural attenuation.

5.1. Introduction.

Biochemically active compounds (BACs) include endocrine disrupting chemicals (EDCs), antimicrobial compounds, and other pharmaceutically active compounds (PhACs). The presence of BACs in water sources at trace levels has been of concern because (a) bacteria in the presence of antibiotics at sub-therapeutic concentration may develop antimicrobial resistance (Kümmerer, 2001), (b) EDCs may cause intersexuality in fish (Vajda *et al.*, 2008) and (c) little is known about the possible synergistic effects on aquatic life when BAC mixture are present in surface waters

(Snyder *et al*., 2005). BACs are only partially removed during conventional wastewater treatment (Nasu *et al.,* 2001, Ternes, 1998 and Paxeus, 2004), and WWTP discharges are therefore an important source, through which BACs are introduced into the environment. Recent studies have shown that BACs are now ubiquitous in United States surface waters and are detected at concentrations that range from ng L^{-1} to μg L^{-1} levels (Kolpin *et al.,* 2002).

BAC classes and concentrations vary widely in conventional WWTP influents and effluents. For example, antibiotics have been found in the effluents of wastewater treatment plant effluents at concentrations up to 2000 ng L^{-1} (e.g. Gobel *et al.,* 2005, Hartig *et al.,* 1999, Hirsch *et al.,* 1999, Miao *et al.,* 2004). Antimicrobial compounds include β-lactams, sulfonamides, macrolides and fluoroquinones and during conventional activated sludge treatment their removal varied from 30 - 90 % (Carballa *et al.,* 2005, Gobel *et al.*, 2005, Batt et al., 2007). In the case of EDCs, presence in WWTP effluents has been extensively reported; e.g., 17α-ethinylestradiol (EE2) was found at concentrations up to 3.4 ng L^{-1} (Baronti *et al.,* 2000, Williams et al., 2003) and BPA concentrations in WWTP effluents range from <20 – 7625 ng L^{-1} (Fuerhacker, 2003, Aguayo *et al.,* 2004, Hohne and Puttmann, 2008). The removal in conventional activated sludge WWTPs ranges from about 62 – 98% for EE2 (Johnson and Williams, 2004) and from about 73 – 93% for BPA (Fuerhacker, 2003, Hohne and Puttmann, 2008)

Advanced oxidation processes, such as the UV/H_2O_2 process, rely on the hydroxyl radical for the rapid oxidative transformation of many organic contaminants. However, complete mineralization of BACs is not achieved at UV doses and H_2O_2 concentrations that are cost effective. Hence, many unknown oxidation intermediates are formed when BACs are oxidized by hydroxyl radicals. The objective of this study, therefore, was to quantify the effectiveness of combining UV/H_2O_2 and biological oxidation processes for the mineralization of three ^{14}C-labeled BACs, the antimicrobial compound sulfadiazine (SDZ), the EDC bisphenol-A (BPA) and the analgesic diclofenac (DCL), all of which commonly occur in conventionally treated wastewater effluents. By using ^{14}C-labeled compounds, the mineralization potential of ^{14}C- labeled BAC oxidation products was quantified with lake water bacteria and bacteria in lake sediments.

5.2. Material and methods.

5.2.1. Reagents

All ^{12}C compounds were purchased from Sigma Chemical Corporation (St. Louis, MO, USA). ^{12}C-SDZ (>99%), ^{12}C-BPA (>99%) and ^{12}C-DCL (>99%) were stored at room temperature and in the dark. All ^{14}C-labeled BACs were purchased in aqueous solution from American Radiolabeled Chemicals, Inc. (St. Louis, MO,

USA) and stored at 4ºC in the dark. The structures and stock solution characteristics of the selected BACs are shown in Table 1. The radiochemical purity and specific activity of the three ^{14}C-labeled BACs was verified by high-performance liquid chromatography (HPLC) and liquid scintillation counting. ^{14}C-labeled acetate was purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO, USA) and ^{14}C-sodium bicarbonate from Sigma Chemical Corporation (St. Louis, MO, USA). Acetonitrile used in HPLC analyses was HPLC grade (Fisher Scientific, Pittsburgh, PA, USA). Hionic Fluor Scintillation cocktail was purchased from Perkin Elmer (Groningen, Netherlands).

5.2.2. Experimental design

Tests were conducted to determine the biological mineralization potential of ^{14}C-labeled BACs photooxidation intermediates. ^{14}C-labeled BACs were individually spiked at trace levels into wastewater treatment plant effluent (WWTPE), and the biological process was initiated following UV/H_2O_2 oxidation treatment. BAC (SDZ, BPA and DCL) oxidation was performed at a UV dose and H_2O_2 concentration (Table 1) that would yield a high transformation of the parent compound (>85%). Batch microcosms were constructed to evaluate the biological mineralization of ^{14}C-labeled BAC oxidation intermediates with bacteria isolated from lake water and with bacteria associated with lake sediments.

Water Matrix. Experiments were conducted in ultrapure water and in wastewater treatment plant effluent (WWTPE). The pH of the ultrapure water and WWTPE was adjusted to 7 with 1 mM phosphate buffer. WWTPE was obtained from the North Cary Water Reclamation facility (Cary, NC, USA), stored at 4 °C and filtered through a 0.22-μm membrane filter prior to use. Dissolved organic carbon (DOC) concentration of the WWTPE was quantified with a Total Organic Carbon Analyzer (Model TOC-5000A, Shimadzu, Columbia, MD) and was 7.3 mg L^{-1}.

Photochemical oxidation. UV/H_2O_2 oxidation experiments were carried out in a quasi-collimated beam (QCB) apparatus. The QCB apparatus was equipped with four low pressure UV lamps (λ=254 nm), and the delivered UV fluence to the sample was calculated as described by Bolton and Linden (2003). UPW and WWTPE were spiked with a mixture of $^{12}C/^{14}C$-labeled BACs as described in Table 1. At predetermined oxidation levels (Table 1-Applied UV dose), the reaction was stopped by turning off the UV lamp and quenching the remaining hydrogen peroxide with sodium thiosulfate (Liu *et al.*, 2003). Additional UV/H_2O_2 tests were conducted with the WWTPE without the spiked $^{12}C/^{14}C$ compound blend to measure the assimilable organic carbon (AOC) present in the oxidized WWTPE. AOC was measured to determine the overall amount of readily biodegradable carbon that could be used by the bacteria as primary substrate.

Biological oxidation. Upon photochemical oxidation, 50 mL of quenched sample was transferred to batch bioreactors. The batch bioreactors for these tests were described previously by Chen *et al.* (2004) (Figure 1). To obtain reliable results, reactors were set up at least in duplicate. In addition, 50 μL of mineral buffer solution was added to each bioreactor to assure that nutrients and minerals (e.g.: nitrogen, phosphorous, copper, iron, manganese, etc.) were not limiting substrates in the reactor. For the mineral buffer composition see section 5.2.3.1. Each bioreactor was inoculated with a consortium of bacteria that were isolated from lake water as described in 5.2.3.1. Inoculation was done such that the initial concentration of bacteria in the bioreactor was equal to ~1.6×10^4 cell mL^{-1}. Two inocula were prepared in the course of this study (consortium A and B). For ^{14}C-SDZ, additional tests were completed, in which sediments from a local lake served as the bacteria source. Sediments were added to the batch bioreactors before the aqueous sample containing ^{14}C-SDZ oxidation intermediates was transferred to yield a sediment to water ratio (weight/weight) of approximately 0.5. The recorded weight of sediment inside the bioreactors was 25.54 ± 0.14 g.

To assess the improvement in biodegradability due to UV/H_2O_2 oxidation, bioreactors were also set up with the untreated ^{14}C-labeled parent compound. In these tests, the WWTPE was oxidized before spiking the BAC parent compound. Oxidation

conditions were the same as stated in Table 1 to assure that initial AOC concentrations were similar to those in biodegradation tests involving BAC oxidation intermediates.

Bioreactors were equipped with a NaOH trap that captured $^{14}CO_2$ produced from the mineralization of ^{14}C-labeled photooxidation intermediates. Aerobic conditions were maintained in all reactors. In initial experiments, a vial containing 1 mL of 30% (v/v) H_2O_2 and 0.5 mL of 0.5% (wt/wt) $FeCl_2$ served as a source of oxygen to maintain aerobic conditions. During the course of this study, it was realized that the reactor headspace and the aqueous oxygen concentration was sufficient to maintain an aerobic environment inside the bioreactors and therefore the H_2O_2 vial was no longer filled in later experiments. The bioreactors were incubated at 34 ± 1ºC in an incubator shaker operated at 120 rpm (New Brunswick Scientific C24 Classic series, Edison, NJ). The content of the NaOH trap was analyzed periodically to determine the rate of $^{14}CO_2$ production. At each sampling time, the content of the NaOH trap was removed for analysis by scintillation counting and replaced with fresh 2N NaOH solution. All bioreactors were compared to an abiotic control reactor (negative control), to determine $^{14}CO_2$ that was only formed by chemical oxidation and not by microbial activity. Abiotic controls were not inoculated and were spiked with sodium azide (10-15 mg per reactor), which inhibits aerobic biological activity.

Additional abiotic tests were completed by spiking ^{14}C-acetate and ^{14}C-sodium bicarbonate into ultrapure water buffer at pH 7. The purpose of these tests was to determine the effectiveness of the NaOH trap for trapping $^{14}CO_2$ relative to volatile ^{14}C-acids that can form during the UV/H_2O_2 oxidation process. These reactors showed that the NaOH trap recovered 96% of the spiked bicarbonate after 100 hours, while only 1% of the spiked ^{14}C acetate was recovered after 100 hours (For more details see Appendix C).

5.2.3. Lake water bacteria sources.

5.2.3.1. Microbial consortium. The microbial consortium was obtained from Jordan Lake, North Carolina, USA. To obtain the inoculum, 40 mL of lake water was filtered through PVDF filter membranes (0.22-µm pore size, Millex-GV). Prior to use, filters were made AOC-free by rinsing the filters with 2L of ultrapure water at a rate of ~ 4 ml min^{-1} (Berger *et al.,* 2005). The filtrate was inoculated with ~100 µL of unfiltered water and incubated at 34ºC for 14 days. The cells were harvested by centrifugation (3000 rpm, 30 minutes), and subsequently re-suspended in HPLC grade water amended with mineral buffer (for 1L: 1.0 g $(NH_4)_2SO_4$, 3.0 g KH_2PO_4, 7.0 g K_2HPO_4, 0.2 g KCl, 0.1 g NaCl, 50 mg $MgSO_4$, 4.1 mg $CoCl_2{\cdot}6H_2O$, 5.4 mg $CuCl_2{\cdot}6H_2O$, 5.0 mg $MnSO_4.7H_2O$, 2.1 mg $ZnCl_2$, 1.3 mg $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1 mg $FeSO_4{\cdot}7H_20$; Berger *et al.*, 2005). The re-suspended cells were incubated for 7

additional days to assure that the AOC concentration of the inoculum was negligible. To obtain a cell count of the prepared inoculum, a sub-sample was analyzed by flow cytometry. The inoculum was stored for at least six months at 4ºC and; remained viable and maintained similar growth characteristics throughout this period as confirmed by tests that were periodically conducted with ^{14}C-acetate. This procedure was adapted from Hammes and Egli (2005).

5.2.3.2. Sediments. Sediments were collected from Lake Wheeler, North Carolina, USA. One day prior to setting up experiments, 1-L of sediments was collected from the top layer of a shallow part of the lake to assure that sediments were aerobic. Sediments were stored overnight at 4ºC. The collected sediments had a moisture content of 30.4 ±0.4% at the start of the experiment. Additional tests were completed with abiotic sediments to determine the partitioning of ^{14}C-SDZ or/and ^{14}C-SDZ oxidation byproducts between the water and sediments. Abiotic sediment tests were prepared in baked, amber 20 mL vials at the same sediment-to-water mass ratio as the bioreactors. Sediments were sterilized by the following succeeding steps: (1) dried over 2 days at 105ºC, (2) autoclaved for 20 minutes at 121ºC, and (3) mixed with a sodium azide solution (240 mg L^{-1}) such that sediments regained their initial moisture content of 30.4 %. Vials were shaken at 34 ± 1ºC in an incubator shaker operated at 120 rpm. After 40 days, the supernatant of the samples was removed and filtered through a 0.22 μm membrane (Millex-GV, PVDF). Sample aliquots of 1 ml were

analyzed by scintillation counting and compared with a blank sample (without sediments) that corresponded to the initial concentration in solution.

5.2.3.3. Positive controls. To determine the viability of both, lake water and sediment bacteria, additional bioreactor experiments were completed with ^{14}C-acetate. ^{14}C-acetate was added to ultrapure water buffered at pH 7 and to WWTPE that was preoxidized at the same experimental conditions as those used for BAC biodegradation tests.

5.2.4. Analytical methods.

5.2.4.1. BAC concentration*:* Analyses of ^{12}C-BAC concentrations were conducted with a HPLC system (Breeze, Waters, Milford, MA) equipped with a C18-AQ HPLC column (5 μm, 4.6 x 250 mm, Alltima HP, Grace) and a dual-wavelength UV detector. The mobile phase flow rate was 1.0 mL/min. For SDZ, the mobile phase was 20% v/v acetonitrile and 80% v/v 25 mM ammonium acetate buffer (pH 5). For BPA and DCL, the mobile phase was 42% v/v acetonitrile and 58% v/v 25 mM ammonium acetate buffer (pH 5). The detector wavelength was set at 266 nm for SDZ, 225 nm for BPA, and 220 nm for DCL.

5.2.4.2. Liquid scintillation counting (LCS): To measure $^{14}CO_2$ production the entire content of the NaOH trap was removed at each sampling point and divided into 1 mL aliquots that were each mixed with 12 mL of Hionic Fluor Scintillation cocktail (Perkin Elmer, Groningen, Netherlands) in 20 mL scintillation vials. The Hionic Fluor Scintillation cocktail is specifically made for samples in strong alkaline media and reduces the chemiluminescence of basic samples that occurs with traditional scintillation cocktails. LCS samples were counted by a TRI-CARB 2800TR scintillation counter (Packard Instrument Company, Downers Grove, IL, USA).

5.2.4.3. Flow cytometry: Bacteria in the lake water consortium were enumerated with a Beckman-Coulter EPICS Altra flow cytometer (Fullerton, CA). Samples were prepared as described in Hammes and Egli (2005). Samples were stained with 10 μL mL^{-1} of SYBR green I (Invitrogen, Molecular Probes. 10,000X concentrate in DMSO) previously diluted in DMSO to a concentration of 1:100. The samples were left in the dark for at least 20 minutes prior to analysis. SYBR green stains total nucleic acids and emits a bright fluorescent signal at 530nm +/- 30nm (Green Fluorescence) upon excitation with a laser at 488nm. Another signal is also detected above 590nm (Red Fluorescence). Data was analyzed by a compatible shareware software called WinMDI Version 2.8 (Windows Multiple Document Interface for Flow Cytometry) developed by Joe Trotter, Scripps Institute.

5.2.4.4. Assimilable Organic Carbon (AOC). AOC was measured according to the method of Hammes and Egli (2005). Additional details of the AOC method are presented in Appendix D. For this method, all glassware and materials was made AOC-free as described by Standard Methods 9217 (AAWA, 1998) and Charnock and Kjonno (2000) (the same method was used for the oxidation experiments and bioreactors glassware). The microbial consortium utilized in the AOC protocol was the same as that for the bioreactors. The growth of the microbial consortium was converted to AOC concentration by a yield factor, which represents the number of cells produced per μg organic carbon used. To obtain the yield factor for the inoculum, a calibration was developed with acetate as the carbon source. The same mineral buffer described in 5.2.3.1. was added to all samples to assure that minerals were not limiting growth. The mineral buffer was added at a ratio of 1 μL of mineral buffer per mL of sample. Two inocula were prepared during the course of this study. Using acetate concentrations ranging from 0 – 300 μg-C L^{-1}, a yield factor of 6.35×10^{6} cells (μg C-acetate)$^{-1}$ was obtained for consortium A and 4.36×10^{6} cells (μg C-acetate)$^{-1}$ for consortium B.

5.3. Results and discussion.

5.3.1. ^{14}C-acetate biodegradation.

To asses the validity of the microbial consortia used in this study, bioreactors experiments with ^{14}C-acetate were performed. These experiments served as positive controls and determined the aptitude of microorganisms to mineralize an easily biodegradable carbon source at trace levels. Figure 2 shows the recovery of ^{14}C in the NaOH trap as a function of time after inoculation. In Figure 2, results obtained for inoculated reactors are compared to those obtained for matching abiotic reactors (negative controls with 10-15 mg of azide per reactor). Figure 2(a) depicts the rate of ^{14}C-acetate mineralization in ultrapure water buffered at pH 7. The AOC of the ultrapure water prior to spiking ^{14}C-acetate was negligible (0.018± 0.092 μg L^{-1}); therefore, the sole carbon source that was available to the bacteria was the ^{14}C-acetate spiked at a concentration of 1 μg L^{-1} = 0.33 μg-C L^{-1}. As shown in Figure 2(a) the consortium was clearly able to mineralize acetate at trace levels. However, the rate of $^{14}CO_2$ evolution was slow, and a comparison between the inoculated sample and the negative control suggests that only 3.2% of ^{14}C-acetate was mineralized after 53 days. It was expected that no exponential growth and/or substrate uptake will take place in these reactors knowing that (1) van der Kooij (1992) suggested that to prevent microbial growth and create a stable water, AOC should not exceed a concentration of

10 μg acetate-C L^{-1} and (2) Ritmann (2001) states that Smin for easily biodegradable organic mater is of 37 μg BOD_L L^{-1}.

Figure 2(b) depicts the mineralization rate of ^{14}C-acetate in UV/H_2O_2 oxidized WWTPE that was buffered at pH 7 and spiked at an initial ^{14}C-acetate concentration of 1 μg L^{-1}. Prior spiking ^{14}C-acetate, the WWTPE was treated with a UV dose of 2000 mJ cm^{-2} and H_2O_2 initial concentration equal to 6 mg L^{-1}. After the UV/H_2O_2 treatment the AOC of the oxidized WWTPE was 136.6 ± 4.3 μg L^{-1}. Before oxidizing the WWTPE, the non-treated WWTPE had an AOC of 85.8 ± 0.5 μg L^{-1}. The AOC increase of 60% can be explained by the UV/H_2O_2 oxidation of organic matter present in WWTPE to more biodegradable compounds (e.g. low molecular weight organic compounds with higher oxygen content).

For the bioreactor, for which results are shown in Figure 2(b), 48.5% of ^{14}C-acetate was mineralized after 25 days, and a lag phase was observed for the first 3-5 days. Although the ^{14}C-acetate concentration in the UPW reactor (Figure 2a) and the pre-oxidized WWTPE reactor (Figure 2b) was the same, the rate of ^{14}C-acetate mineralization was higher in the WWTPE reactor. This result can be explained by the higher AOC of the pre-oxidized WWTPE (136.6 ± 4.3 μg L^{-1}) that provided an additional carbon source for the bacteria.

Results shown in Figure 2(c) were obtained with bioreactors that contained pre-oxidized WWTPE and 36 μg L^{-1} of a $^{12}C/^{14}C$-acetate mixture (6 μg L^{-1} of ^{14}C-acetate). The purpose of these experiments was to (1) assess the mineralization rate of a readily biodegradable organic molecule at the same initial carbon concentration at which tests were performed with ^{14}C-BACs and (2) test the variability between consortium A and B. WWTPE for bioreactors inoculated with consortium A was oxidized with a UV dose of 2000 mJ cm^{-2} and an initial H_2O_2 concentration of 6 mg L^{-1}. WWTPE for bioreactors inoculated with consortium B was oxidized with a UV dose of 575 mJ cm^{-2} and an initial H_2O_2 concentration of 6 mg L^{-1}. For the treatment with the lower UV dose, the AOC of the oxidized WWTPE was 87.7 ± 0.9 μg L^{-1}; compared to the non-treated WWTPE (85.8 ± 0.5 μg L^{-1}) no significant change on the AOC content was observed. Figure 2(c) results showed that for the bioreactors inoculated with consortium A and B the ^{14}C-acetate mineralization after 20 days ranged from 45% to 64%, and a lag phase of 3-5 days was apparent for both consortia. The lag-phase may represent the time that the consortium needs to transition from a maintenance mode to growth before a more rapid ^{14}C-acetate mineralization can proceed.

Figure 2(d) shows the results obtained with ^{14}C-acetate bioreactors that were inoculated with lake sediments. The ^{14}C-acetate concentration spiked into these reactors was 1 μg L^{-1} and the WWTPE was previously oxidized with a UV dose of

2000 mJ cm^{-2} and H_2O_2 initial concentration equal to 6 mg L^{-1}. For these reactors, there was no measurable lag phase for the ^{14}C-mineraliztion process and ^{14}C-acetate mineralization reached 70.3% after 15 days (Figure 2d).

Mass balances for the ^{14}C-acetate reactors were determined once the $^{14}CO_2$ recovery began to reach a plateau. The mass balance for the abiotic samples was complete (100±3%) and verified that losses to reactor components and/or leaks were negligible. For the biotic reactors, the mass balance ranged from 80 – 87 %. When ^{14}C is incorporated into biomass, reflection from intracellular walls can interfere with the measurement of radioactivity by LSC. Similar ^{14}C recovery percentages were found by Ingerselv and Nyholm (2000), who used aniline as a readily biodegradable substrate. No mass balances were performed for the bioreactors inoculated with sediments.

5.3.2. Biodegradation of BACs and oxidized BAC intermediates by a natural microbial consortium.

Sulfadiazine. Figure 3 shows results from ^{12}C /^{14}C-Sulfadiazine biodegradation tests that were inoculated with consortium A. Figure 3(a) represent results from a biodegradation test conducted in buffered ultrapure water after UV/H_2O_2 oxidation treatment with a UV dose of 340 mJ cm^{-2} and an initial H_2O_2 concentration of 6 mg L^{-}

[1]. For this experiment, 91% of the parent compound was transformed and the resulting oxidation intermediates were the sole carbon source in solution due to the negligible AOC of the UPW. The biological mineralization of ^{14}C-labeled SDZ intermediates was negligible with a recovery of 0.3% above the abiotic control. Figure 3(b) depicts results from a biodegradation test conducted in buffered pre-oxidized WWTPE into which the untreated SDZ parent compound was spiked. No $^{14}CO_2$ above the abiotic controls was recovered in these bioreactors over a period of 65 days. Studies evaluating the biodegradability of sulfonamides in activated sludge systems showed that degradation of structurally similar sulfonamides occurred at similar rates on previously acclimated sludge (Ingerslev and Halling-Sorensen, 2000, Perez *et al.*, 2005), suggesting that the enzyme required for sulfonamide degradation attacks the common 4-aminobenzene moiety (phenyl ring) of sulfonamide compounds (Perez *et al.*, 2005). However, no mineralization of the ^{14}C-labeled phenyl ring of the parent compound was observed in the samples that were inoculated with non-acclimated surface bacteria (Figure 2b).

Figure 3(c) depicts results from a biodegradation test conducted with SDZ-spiked (36 μg-C L^{-1}) buffered WWTPE after UV/H_2O_2 oxidation treatment with a UV dose of 2000 mJ cm^{-2} and an initial H_2O_2 dose of 6 mg L^{-1}. In this experiment, 94 % of the parent compound was transformed during UV/H_2O_2 treatment, but the biodegradation test showed that only 1.8% of ^{14}C-labeled oxidation intermediates was mineralized

after 65 days. Also, a 10-day lag phase was observed before the bacteria began to mineralize the oxidation intermediates. A longer lag period compared to that observed for ^{14}C-acetate biodegradation tests was expected because the cells in the consortium were not adapted to the ^{14}C-labeled oxidation intermediates. The SDZ phenyl ring, which was uniformly ^{14}C labeled, is a preferable site to for an $^{\bullet}$OH radical attack via electrophilic addition to the aromatic ring; in addition, ring cleavage can be expected upon addition of two or more OH groups to the aromatic ring (Patience, 1986). Therefore, ^{14}C-labeled oxidation products should be more biodegradable than the SDZ parent compound. A comparison of results presented in Fig 3(b) and (c) shows that the oxidation intermediate were indeed more biodegradable, but the difference was small.

Finally, the mineralization due to chemical oxidation only was determined by comparing the cumulative ^{14}C-recovery of the abiotic controls. For the untreated SDZ parent compound, 0.3 % of the ^{14}C-radiolabeled carbon was recovered; it is likely that this result was attributable to ^{14}C impurities in the ^{14}C-SDZ, and this value was used to correct the mineralization percentages obtained during UV/H_2O_2 treatment. For the experiment conducted in ultrapure water at a UV dose of 340 mJ cm^{-2}, the ^{14}C recovery of the abiotic control was equal to that obtained with untreated sulfadiazine; consequently no mineralization of the ^{14}C-phenyl ring was achieved during the chemical oxidation step. For the experiment conducted in WWTPE at a UV dose of 2000 mJ cm^{-2}, the ^{14}C recovery of the abiotic control was of 2.2 %, suggesting

therefore, that 1.9% of the ^{14}C-phenyl ring in SDZ was minerlized in the chemical oxidation step.

Bisphenol A. Figure 4 summarizes results of the bisphenol A biodegradation tests. Figure 4(a) depicts results of the biodegradation test conducted with buffered oxidized WWTPE into which the bisphenol A parent compound was spiked. Comparing ^{14}C recoveries between the inoculated and the abiotic bioreactors, only a 0.2% difference in ^{14}C recovery was achieved after 36 days. This result shows that bisphenol A is poorly degradable in the oxidized WWTPE matrix

Figure 4(b) shows results from the biodegradation test conducted with BPA oxidation intermediates in buffered WWTPE after UV/H_2O_2 oxidation treatment with a UV dose of 2000 mJ cm^{-2} and an initial H_2O_2 dose of 6 mg L^{-1}. BPA parent compound transformation by the UV/H_2O_2 treatment was 88% and 2.0% of the ^{14}C was mineralized during the photochemical oxidation step. Ring-labeled ^{14}C-BPA was used in this study; therefore, a small percentage of the phenolic rings was mineralized via hydroxyl radical oxidation. In terms of biodegradability, ^{14}C recovery in the inoculated reactors after 36 days was only 0.8% above that measured in the abiotic reactor. Therefore, the UV/H_2O_2 oxidation step offered little advantage in terms of improving biodegradability. Fukahori *et al.* (2003) studied BPA oxidation by TiO_2 photocatalysis and suggested an oxidation pathway in which the ring structure was

largely preserved during $^{\bullet}OH$ radical attack, and this may be the reason why UV/H_2O_2 treatment had little effect on biodegradability.

Diclofenac. Figure 5 shows results of diclofenac biodegradation tests. When diclofenac was spiked into buffered oxidized WWTPE, Figure 5(a) shows that similar ^{14}C recoveries were obtained with the inoculated and the abiotic bioreactor over the study period of 36 days. The ^{14}C recovery of 1.5% for untreated diclofenac in the abiotic reactor was likely attributable to volatile ^{14}C impurities. A lack of biological diclofenac mineralization was expected, in agreement with results from prior studies that showed very limited diclofenac removal in wastewater treatment plants (Heberer *et al,.* 2002 and Quintana *et al.,* 2005). Groning *et al.* (2007) identified p-benzoquinone imine and 5-hydroxyquinone as microbial transformation intermediates of diclofenac, but no evidence of complete mineralization was reported. In addition, Groning *et al.* (2007) pointed out that the biotransformation of diclofenac in different river sediments may be restricted only to a specific group of microorganisms and is not controlled by the total microbial activity. The results obtained here suggest that this group of microorganisms was not present in the tested microbial consortium.

Figure 5(b) depicts results from the biodegradation test conducted with diclofenac oxidation intermediates in buffered WWTPE after UV/H_2O_2 oxidation treatment with a UV dose of 575 mJ cm^{-2} and an initial H_2O_2 dose of 6 mg L^{-1}. For diclofenac, the

removal of the parent compound by the UV/H_2O_2 treatment exceeded 99%, and 27.1% of the ^{14}C was mineralized by the photochemical oxidation step. Diclofenac was labeled at the carboxyl carbon; therefore, the relatively high degree of ^{14}C mineralization suggests that the hydroxyl radical attacked the carboxylic acid moiety of diclofenac. Perez-Estrada *et al.* (2005) studied diclofenac photo-Fenton reactions and suggested a partial degradation pathway by which the hydroxyl radical reacts with diclofenac. Decarboxylation was one of the steps involved in the proposed pathway, and the results obtained here support the decarboxylation pathway. The ^{14}C recovery in the inoculated and abiotic bioreactors (Figure 5b) was similar over the 36-day study period and only 1.3% of ^{14}C was recovered above the abiotic control on day 36. This result suggests that mineralization of ^{14}C-labeled diclofenac oxidation intermediates by the microbial consortium was negligible. The relatively high ^{14}C recovery in the abiotic blank indicates the level of mineralization that was achieved at the carboxyl moiety during the UV/H_2O_2 oxidation step.

5.3.3. Biodegradability of ^{14}C-SDZ oxidation intermediates by bacteria present in lake sediments.

Figure 6 shows results for the biological mineralization of ^{14}C-labeled sulfadiazine and sulfadiazine oxidation intermediates after inoculation with lake sediments. To tests the biological mineralization of ^{14}C-labeled oxidation intermediates, SDZ was spiked into buffered WWTPE prior to UV/H_2O_2 treatment (Table 1). A comparison of ^{14}C recoveries for the inoculated and abiotic reactors shows that both ^{14}C-SDZ and ^{14}C-labeled SDZ oxidation intermediates were mineralized by sediment-associated microbial activity (Figure 6). Mineralization of SDZ parent compound reached 1.6% after 52 days (Figure 6a), suggesting that biodegradation of the ^{14}C-labeled 4-aminobenzene moiety of SDZ is possible. For the ^{14}C-labeled UV/H_2O_2 oxidation intermediates, 6.7 % mineralization was measured after 52 days (Figure 6b). As seen in Figure 6, the lag period was eliminated and degradation rates were faster than those obtained with the lake water-derived microbial consortium. The sediments collected were not acclimated to sulfonamides in the laboratory prior to use in the bioreactors. It is possible that trace levels of sulfonamides were present in the lake water from which sediments were collected, suggesting a possible natural adaptation of the sediment bacteria to SDZ. However, the faster mineralization rate observed with sediments (Figure 6) compared to that observed with lake water bacteria (Figure 3) was more likely a result of the increased biomass and/or background AOC concentration present

in the sediments. These reasons can also explain the absence of a lag phase in Figure 6.

Sorption of SDZ and SDZ oxidation intermediates is a factor that affects the availability of this carbon source for the bacteria. At the tested solid/liquid ratio (500 g L^{-1}) uptake of ^{14}C in SDZ and ^{14}C SDZ oxidation intermediates were 73.5 ± 0.5 and 74.7 ± 2.1%, respectively. Partition coefficients for ^{14}C-SDZ and ^{14}C-labeled SDZ oxidation intermediates were 6.25 ± 0.14 and 6.82 ± 0.79 L Kg^{-1}, respectively. Sulfonamides are polar compounds and these results are in agreement with low sulfonamide sorption to soils; e.g. Thiele-Bruhn *et al*. (2004) found partition coefficients values for sulfapyridine were 7.0 and 20.9 L Kg^{-1} sorption to two sandy soils. The similarities of the partition coefficients obtained in this study show that the characteristics of ^{14}C-labeled oxidation intermediates that affect sorption (e.g., solubility, polarizabilty) are similar to those of the parent compound. For the bioreactors inoculated with lake sediments, the extent of the biological mineralization of spiked ^{14}C was less than the aqueous ^{14}C concentration that remained in solution once sorption equilibrium was obtained; therefore, it is unlikely that sediments interfered with the bioavailability of ^{14}C-SDZ and ^{14}C- labeled SDZ oxidation intermediates.

5.4. Environmental significance.

When BACs are oxidized by the UV/H_2O_2 process at conditions typically used in water treatment plants, mineralization does not occur, and the identity and toxicity of the oxidation product mixture is unknown. Therefore, the purpose of this study was to investigate whether the UV/H_2O_2 oxidation process produces intermediates that can be mineralized faster by natural microbial consortia than their respective parent compound.

For the three BACs studied here, the mineralization rates measured for the parent compound and the ^{14}C-labeled oxidation intermediates were extremely slow; <1.8% after 65 days of biodegradation when bioreactors were inoculated with bacteria isolated from lake water and <6.7% after 52 days when bioreactors were inoculated with lake sediments. For BPA and DCL, pretreatment by UV/H_2O_2 oxidation yielded negligible improvement in biodegradability of ^{14}C-labeled moieties with lake water bacteria (<1.3 % after 36 days).

The three tested BACs were studied at a concentration 36 μg-C L^{-1}. In the presence of other organic substrates found in oxidized WWTPE; the BAC parent compounds or their oxidation products were not a useful energy source for the bacteria, and cometabolism can be contemplated as the mechanism responsible for their

biodegradation. The fundamental nature of cometabolism is that a primary electron donor needs to be present to provide the energy for growth and cometabolic enzyme production. When the primary substrate becomes limiting, the organisms no longer participate in the cometabolic process (Rittmann, 2001). In the biodegradation tests performed here, background organic matter in oxidized WWTPE and in lake sediments served as the primary substrate for the microorganisms. This may lead to a limited supply of the primary substrate in the bioreactors after a long period of time. Additional experiments with intermittent spiking of a readily biodegradable carbon source (e.g., acetate) would need to be conducted to determine the role of the primary carbon source in the mineralization of the ^{14}C-labeled oxidation intermediates and to establish whether cometabolism is the mechanism for the biodegradation of BACs and their oxidation intermediates.

Results from these experiments yield a first approximation of the expected environmental fate of BACs and BAC photooxidation intermediates. This information is useful to estimate the degree of mineralization that can be accomplished by stream microorganisms between a WWTP discharge location and a downstream water treatment plant intake or an ecologically sensitive area. For example, in North Carolina, the Neuse river basin includes rapidly expanding urban areas (Durham, Cary, Raleigh), and conventionally treated wastewater from these municipalities discharged into the Neuse or its tributaries. The Neuse flows into the ecologically

sensitive Pamlico Sound, which is the second largest estuary in the United States and serves as a breading and rearing ground for many species of fish. The presence of EDCs may cause intersexuality in fish (Vajda, et al. 2008); hence, it is of interest to estimate the expected EDC mineralization that would occur over relevant time scales (e.g, in the Neuse River between a WWTP discharge and Pamlico Sound). The Neuse River flows in a southeasterly direction from its origins north of Durham, NC, for about 150 miles before it connects with the estuary. To determine an estimate of the time that it takes for a parcel of water to travel downstream from the city of Durham to the estuary, the stream velocity of the Neuse River near Fort Barnwell was used. The monthly mean stream velocities for this site (USGS-02091814 Neuse River near Fort Barnwell, NC; http://waterdata.usgs.gov/nwis) for the period of June 2004 to February 2007 ranged from 0.697 to 1.36 ft s^{-1}. With these data, the travel time from Durham to the estuary was calculated to be 7 – 13 days. Assuming an average travel time of 10 days, SDZ mineralization in aerobic sediments would be only of 0.5% for the parent compound and 3.5 % for SDZ oxidation intermediates. In contrast, mineralization of BPA and DCL or their oxidation intermediates by planktonic bacteria would be negligible. Experiments conducted in this study were conducted at a temperature of 34ºC and did not consider all of the factors controlling contaminant fate and transport in a flowing river. However, the use of natural microbial consortia (instead of pure cultures) in this study provides a first basis for the biodegradability of BACs and their oxidation intermediates in surface waters.

UV/H_2O_2 oxidation of antimicrobial compounds and EDCs reduces the level of antibiotic activity and endocrine disrupting activity in water (e.g. Chapter 4 of this manuscript, Wammer at al., 2006 and Rosenfeldt et al., 2007). However, the mineralization of the BAC oxidation intermediates was extremely slow, suggesting that oxidation products may be persistent in the environment. Toxicological studies should therefore be conducted to determine the possible effects of BAC oxidation intermediates on aquatic life.

5.5. Acknowledgments

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5.6. References

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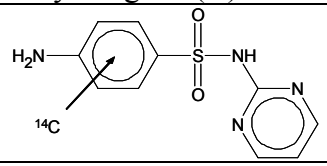
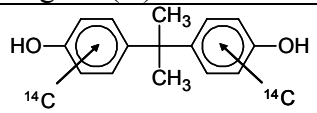
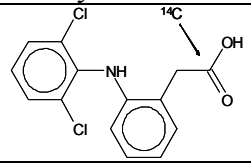
Table 1: Characteristics of ^{14}C- labeled BACs and conditions used to conduct UV/H_2O_2 oxidation experiments.

Characteristics of ^{14}C stock solution				
Compound	^{14}C-Sulfadiazine		^{14}C-Bisphenol A	^{14}C-Diclofenac
Molecular weight	250.25		228.29	318.13
	$pK_{a,1}$ = 2.02 ; $pK_{a,2}$ = 6.43		$pK_{a,1}$ = 9.78 ; $pK_{a,2}$ =10.52	pK_a = 4.15
Radiolabel site	Phenyl-ring-^{14}C(U)		Ring-^{14}C(U)	Carboxyl-^{14}C
Structure				
Solvent	Sterile water		Sterile water	Sterile water
Specific activity	67 mCi/mmol		200 mCi/mmol	50 mCi/mmol
UV/H_2O_2 experimental design				
Water matrix	UPW	WWTPE	WWTPE	WWTPE
Initial parent compound concentration (μg L^{-1})*	75	75	46	68
^{12}C /^{14}C initial mass ratio	0.8 / 0.2	0.8 / 0.2	0.8 / 0.2	0.8 / 0.2
Applied UV dose (mJ cm^{-2})	340	2000	2000	575
H_2O_2 initial concentration (mg L^{-1})	6	6	6	6
Expected parent compound degradation (%)**	90	90	85	99
Measured parent compound degradation (%)	91	94	88	>99 (L.D.L***)

* All experiments were conducted with an initial carbon concentration of 36 μg-C L^{-1}.
** Expected compound degradation was calculated with fluence–based pseudo first order rate constants.
***Final concentration after UV/H_2O_2 treatment was less than HPLC detection limit.

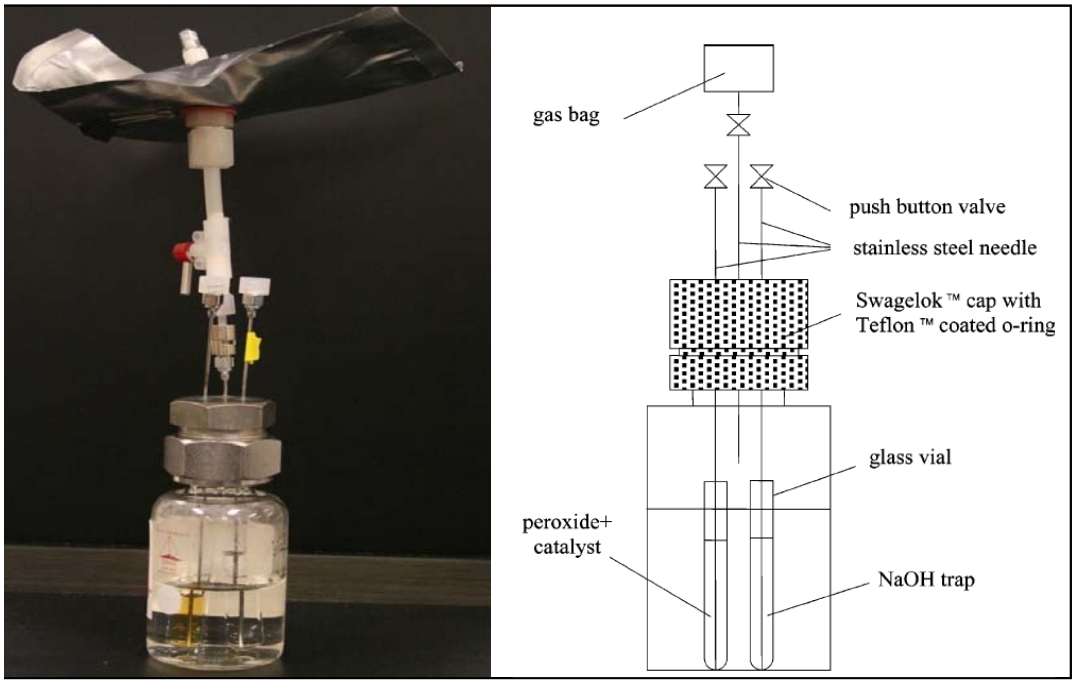


Figure 1. Reactor design for aerobic biodegradation test. (Chen et al., 2004)

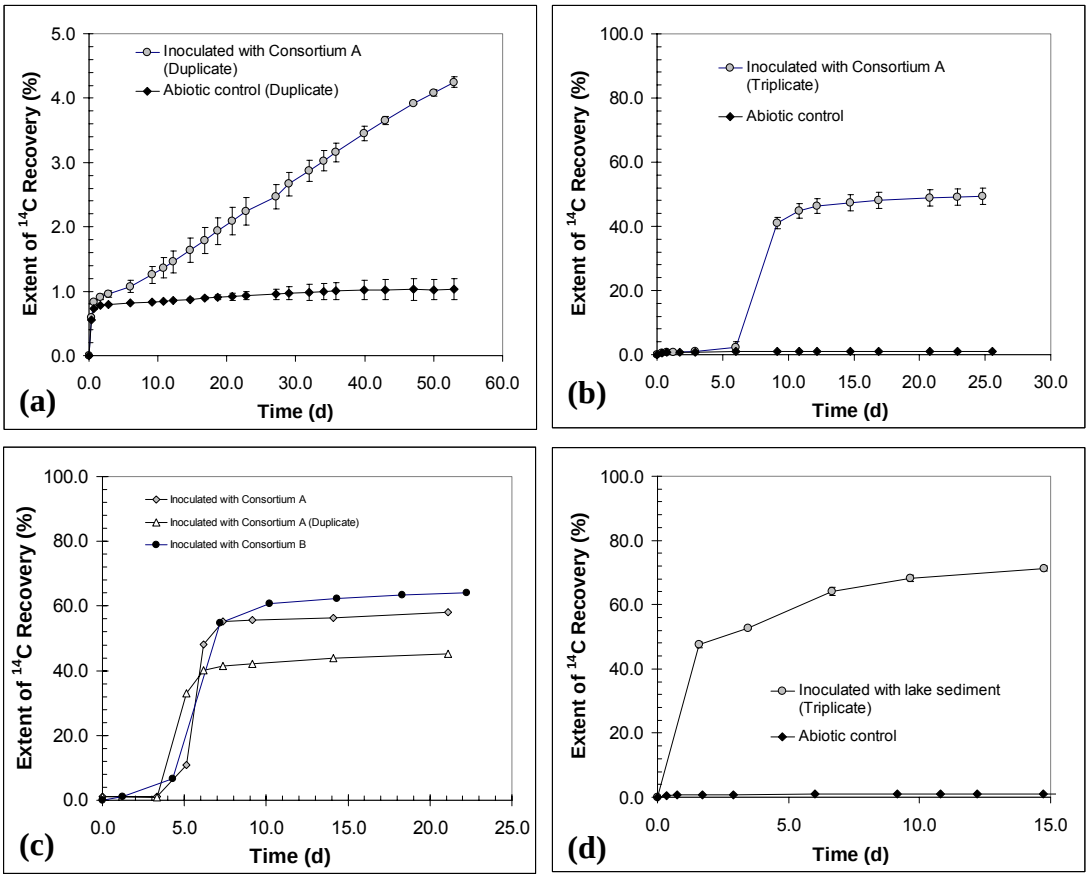


Figure 2. Mineralization of ^{14}C-labeled acetate by lake water bacteria in (a) buffered ultrapure water (C_o= 1 μg L^{-1}), (b) buffered, UV/H_2O_2 treated WWTPE (C_o= 1 μg L^{-1}), and (c) buffered, UV/H_2O_2 treated WWTPE (C_o= 36 μg-C L^{-1}, of which 6 μg L^{-1} was ^{14}C-labeled). Figure (d) depicts mineralization of ^{14}C-acetate by bacteria associated with lake sediments in buffered, UV/H_2O_2 treated WWTPE (C_o= 1 μg L^{-1}).

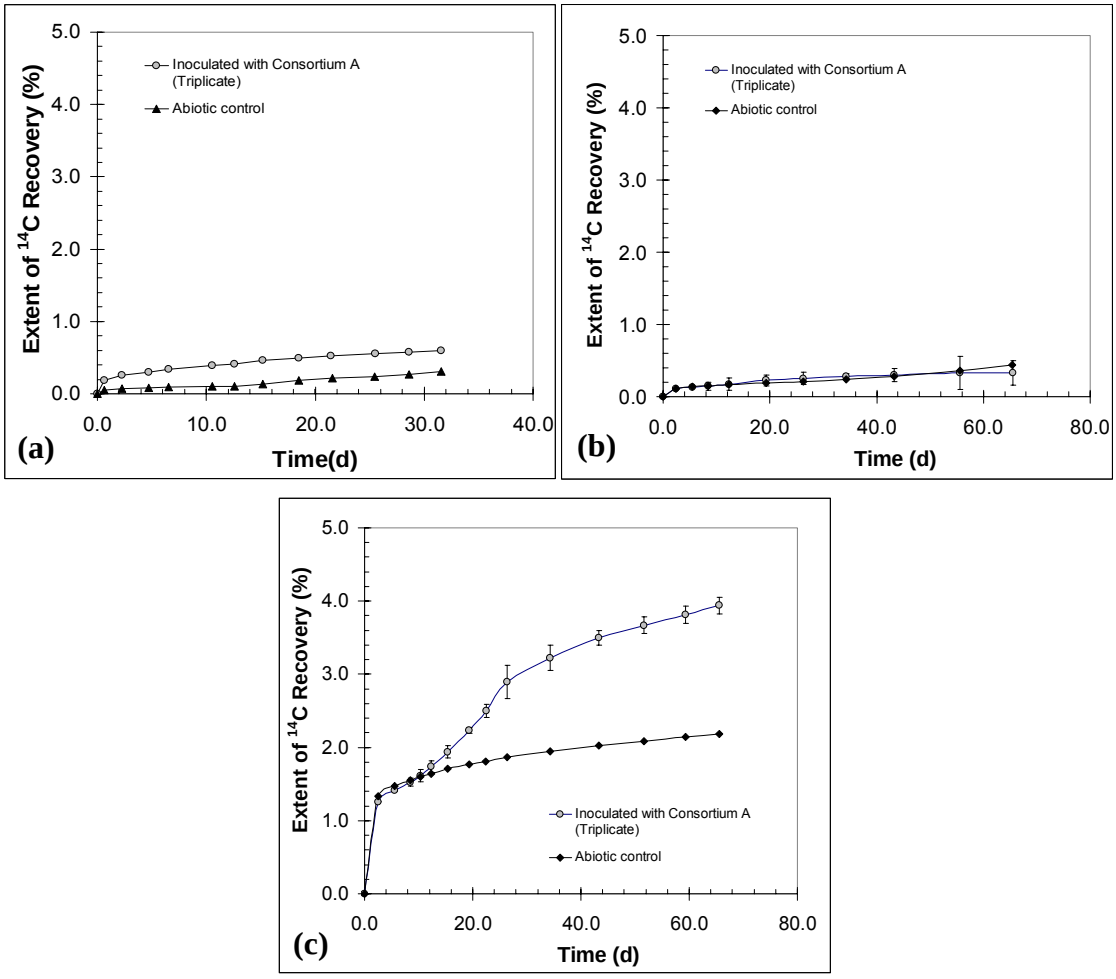


Figure 3. Mineralization of ^{14}C label associated with (a) SDZ oxidation intermediates in buffered, ultrapure water, (b) SDZ in buffered UV/H_2O_2 treated WWTPE, and (c) SDZ oxidation intermediates in buffered UV/H_2O_2 treated WWTPE by lake water bacteria. For UV/H_2O_2 oxidation conditions refer to Table 1.

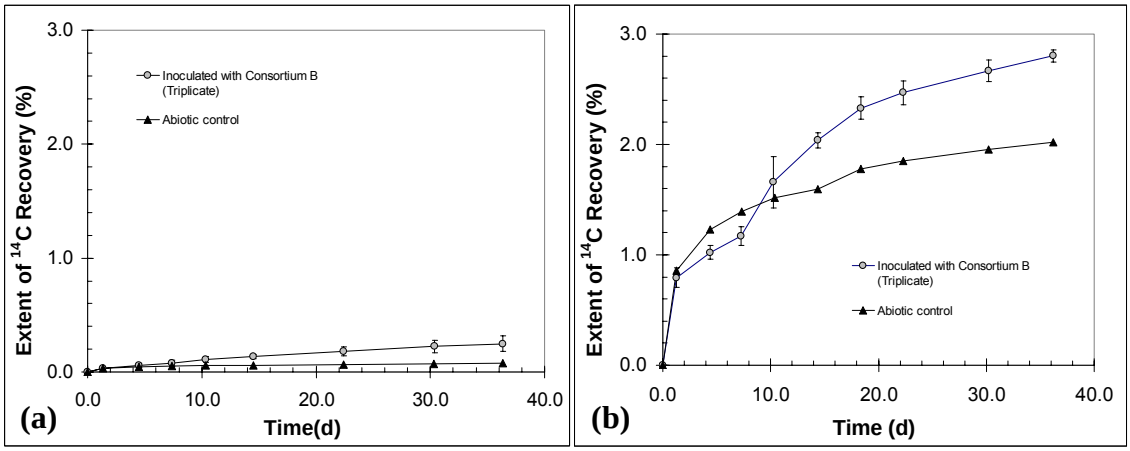


Figure 4. Mineralization of ^{14}C label associated with (a) BPA and (b) BPA oxidation intermediates by lake water bacteria. Experiments were conducted in buffered, UV/H_2O_2 treated WWTPE. For UV/H_2O_2 oxidation conditions refer to Table 1.

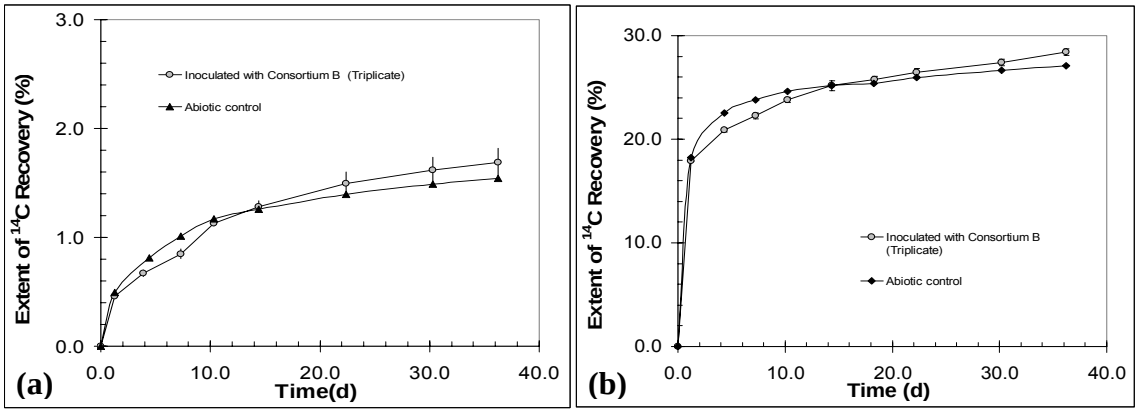


Figure 5. Mineralization of ^{14}C label associated with (a) DCL and (b) DCL oxidation intermediates by lake water bacteria. Experiments were conducted in buffered, UV/H_2O_2 treated WWTPE. For UV/H_2O_2 oxidation conditions refer to Table 1.

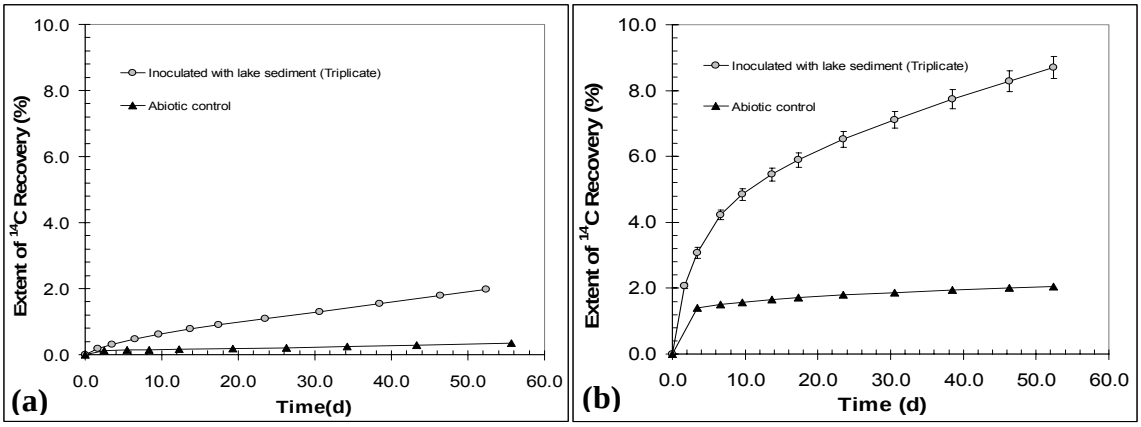


Figure 6. Mineralization of ^{14}C label associated with (a) SDZ and (b) SDZ oxidation intermediates by bacteria in lake sediments. Experiments were conducted in buffered, UV/H_2O_2 treated WWTPE. For UV/H_2O_2 oxidation conditions refer to Table 1.

Chapter 6.

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

The performance of low pressure UV photolysis and UV/H_2O_2 oxidation processes for the removal of six biochemically active compounds (BACs) was evaluated in this study. Following a detailed characterization of BAC parent compound removal rates in photolysis and hydroxyl radical oxidation processes (Chapter 3), this study focused on the characterization of photolysis and oxidation intermediates by assessing their antimicrobial activity (Chapter 4) and their biodegradability by microbial consortia and lake sediments (Chapter 5).

Chapter 3 provides a detailed analysis of SMX, SMZ, SDZ, TMP, BPA and DCL removal by low pressure UV photolysis and UV/H_2O_2 oxidation. Experiments were conducted at a wavelength of 254 nm in a quasi-collimated beam (QCB) apparatus. Experiments were conducted in UPW, LW, and WWTPE. Decadic molar absorption coefficients were measured and quantum yields were calculated to describe BAC transformation rates by direct photolysis, and the results showed that only SMX and DCL were rapidly photolyzed. Effective removal of SMZ, SDZ, TMP, and BPA required the addition of hydrogen peroxide to enhance removal rates. Second order

rate constants describing the oxidation of the six BACs by the hydroxyl radical ranged from 5.30×10^9 to 9.26×10^9 M^{-1} s^{-1}. In addition fluence-based pseudo first order constants were determined and used to calculate the UV fluence required to achieve a desired treatment goal at a given H_2O_2 dose in UPW, LW and WWTPE. The ranking of the investigated BACs in terms of UV/H_2O_2 transformation efficiency was DCL>SMX>SMZ>SDZ>TMP>BPA. For an H_2O_2 dose of 10 mg L^{-1} the required UV dose to achieve 90% of SMX and DCL transformation was ≤1000 mJ cm^{-2}, while UV doses ≥1000 mJ cm^{-2} were required to achieve 90% removal for the remaining BACs

.

Additional experiments were conducted with SMX, SMZ, SDZ and TMP to determine pH effects on photolysis and UV/H_2O_2 oxidation rates. For the sulfonamides, pH-related differences in transformation rates were mainly due to differences in the photolysis rate between the neutral and anionic species. For TMP, on the other hand the reaction rate between TMP and the hydroxyl radical was pH dependent, where the protonated form reacted more readily than the neutral form. Finally, background water quality parameters (DOC, alkalinity, major anions) for LW and WWTPE were measured and used to characterize their effects on steady state hydroxyl radical concentrations and BAC oxidation rates. The major scavenger in both LW and WWTPE was the background organic matter (or dissolved organic carbon).

In Chapter 4, the effects of photolysis and UV/H_2O_2 oxidation on the removal of antimicrobial activity are described for four antimicrobial compounds (SMX, SMZ, SDZ and TMP). Results showed that photolysis and oxidation intermediates did not exhibit measurable antimicrobial activity. Neither solution pH not background water matrix constituents affected this conclusion. Therefore, both low pressure UV photolysis and UV/H_2O_2 processes destroy the biochemically active moieties of the studied antimicrobial compounds. Experiments were conducted in a commercial annular photoreactor, and the electrical energy per order (EEO) concept was used to assess the energy efficiency of UV photolysis and UV/H_2O_2 oxidation processes for SMX, SMZ, SDZ and TMP removal. The EEO values showed that SMX was efficiently removed by photolysis alone and that H_2O_2 addition provided little additional benefit. For poorly photolyzable antibiotics (SMZ, SDZ and TMP), H_2O_2 addition was required to make the oxidation process feasible from an energy perspective.

Chapter 5 introduces the results of bioreactor experiments that were conducted to assess the biodegradability of BACs and their oxidation intermediates in WWTPE. ^{14}C-labeled BACs (SDZ, BPA, DCL) were used in bioreactors that were inoculated with (1) microbial consortia derived from lake water and (2) lake sediment. The results showed negligible mineralization of ^{14}C-labeled parent compound moieties by lake water bacteria. Upon UV/H_2O_2 oxidation, mineralization rates of ^{14}C-labeled

BAC oxidation intermediates were faster than those of the respective parent but still very low; i.e., <1.1 % of SDZ, <0.8 % of BPA and <0.8 % of DCL was mineralized after 30 days of incubation. The use of lake sediments enhanced the biodegradation rate of SDZ and its UV/H_2O_2 oxidation intermediates, but mineralization rates were again low (after 30 days of incubation 1.1 % of SDZ and 5.2% of SDZ UV/H_2O_2 oxidation intermediates was mineralized). While these results provide evidence that natural microbial consortia are capable of mineralizing some BACs and their oxidation intermediates, the slow rate at which the biological mineralization occurred suggests that BACs and their UV/H_2O_2 oxidation intermediates are relative persistent in the environment.

6.2. Recommendations for Future Work

1) Study the biodegradability of BAC oxidation products of other relevant oxidants such as ozone and chlorine. Ozone and chlorine are more selective oxidants than the hydroxyl radical and may therefore form oxidation intermediates that differ from those formed in UV/H_2O_2 processes. Furthermore, chlorine may lead to the production of halogenated oxidation intermediates.

2) Use liquid chromatography coupled with mass spectrometry to identify the main intermediates formed by chemical and biological oxidation processes such that mechanistic pathways of BAC oxidation and biodegradation can be developed.

3) The result of this research showed that BACs and their UV/H_2O_2 oxidation intermediates are slowly mineralized at best and thus are relatively persistent in the environment. While oxidation intermediates of antimicrobial compounds did not exhibit measurable antimicrobial activity and oxidation intermediates of endocrine disrupting chemicals exhibit negligible or significantly reduced endocrine disrupting properties (Chapter 4 of this manuscript, Wammer *at al.,* 2006 and Rosenfeldt *et al.,* 2007), other toxicological endpoints should be evaluated before it is possible to say that the presence of oxidation intermediates in the aquatic environment is safe. Such studies should evaluate synergistic effects when aquatic organisms are exposed to mixtures of oxidation intermediates that are formed during the oxidative treatment of whole wastewater treatment plant effluents.

4) Using molecular methods, identify bacteria present in lake water and sediments that participate in the mineralization of BAC oxidation intermediates. A better understanding of the microbial ecology of the organisms involved in the biodegradation of BAC oxidation intermediates may ultimately lead to the design of

effective biological treatment processes for BAC removal in wastewater treatment plants.

APPENDICES

Appendix A: Supplementary Information for Chapter 3.

Photolysis and UV/H_2O_2 time and fluence based pseudo-first order constants obtained in ultrapure water (UPW) at pH 3.6, 7.85, lake water (LW) and wastewater treatment plant effluent (WWTPE) for the six BACs studied.

A) Sulfamethoxazole (SMX)

UPW at pH 3.6				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	4.331E-01	0.9990	1.004E-02	0.9993
0.00	4.223E-01	0.9999	9.483E-03	0.9997
1.98	4.740E-01	0.9991	1.028E-02	0.9995
2.03	4.688E-01	0.9991	1.050E-02	0.9997
5.82	5.288E-01	0.9912	1.217E-02	0.9997
9.90	5.496E-01	0.9987	1.296E-02	0.9995
UPW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	9.458E-02	0.9999	2.352E-03	0.9994
0.00	1.027E-01	0.9982	2.450E-03	0.9982
2.39	1.370E-01	0.9920	3.302E-03	0.9986
6.02	1.870E-01	0.9992	4.284E-03	0.9998
10.10	2.525E-01	0.9985	6.148E-03	0.9999
WWTPE at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	5.779E-02	0.9936	2.148E-03	0.9992
0.00	5.908E-02	0.9991	2.189E-03	0.9996
1.95	5.971E-02	0.9947	2.194E-03	0.9966
5.23	6.696E-02	0.9963	2.435E-03	0.9998
10.34	7.185E-02	0.9973	2.727E-03	0.9999
LW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	5.630E-02	0.9967	2.014E-03	0.9999
2.03	6.446E-02	0.9974	2.259E-03	0.9999
5.34	7.624E-02	0.9963	2.891E-03	0.9998
10.06	9.188E-02	0.9979	3.520E-03	0.9953
10.94	9.746E-02	0.9961	3.691E-03	0.9967

B) Sulfamethazaine (SMZ)

UPW at pH 3.6				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	8.202E-03	0.9986	2.086E-04	0.9994
0.00	8.562E-03	0.9992	2.247E-04	0.9987
0.00	8.790E-03	0.9984	2.351E-04	0.9973
2.12	3.693E-02	0.9983	9.945E-04	0.9993
6.19	8.724E-02	0.9992	2.335E-03	0.9998
6.58	1.000E-01	0.9980	2.520E-03	0.9952
10.19	1.482E-01	0.9914	3.869E-03	0.9920
UPW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	2.931E-02	0.9977	7.672E-04	0.9976
0.00	2.958E-02	0.9949	7.882E-04	0.9973
0.00	3.027E-02	0.9943	8.065E-04	0.9968
2.10	5.656E-02	0.9996	1.509E-03	0.9998
2.10	5.064E-02	0.9998	1.448E-03	0.9996
6.03	1.114E-01	0.9964	3.075E-03	0.9979
6.20	1.123E-01	0.9982	3.080E-03	0.9974
10.18	1.555E-01	0.9989	4.362E-03	0.9995
WWTPE at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.727E-02	0.9970	7.556E-04	0.9991
2.05	2.408E-02	0.9967	1.004E-03	0.9998
6.20	3.242E-02	0.9979	1.438E-03	0.9999
6.54	3.414E-02	0.9965	1.435E-03	0.9999
10.09	4.138E-02	0.9972	1.844E-03	0.9998
LW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.927E-02	0.9995	7.537E-04	0.9990
2.06	2.558E-02	0.9996	1.046E-03	0.9981
6.25	4.239E-02	0.9995	1.729E-03	0.9995
10.07	5.950E-02	0.9961	2.406E-03	0.9992

C) Sulfadiazine (SDZ)

UPW at pH 3.6				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.211E-02	0.9962	3.117E-04	0.9950
0.00	9.839E-03	0.9998	2.520E-04	0.9999
0.00	1.169E-02	0.9982	2.889E-04	0.9990
2.06	3.820E-02	0.9991	1.000E-03	0.9997
2.12	3.747E-02	0.9978	9.561E-04	0.9966
6.31	1.108E-01	0.9967	2.869E-03	0.9977
6.20	9.987E-02	0.9960	2.567E-03	0.9965
9.96	1.591E-01	0.9966	4.125E-03	0.9974
UPW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	2.243E-02	0.9989	6.242E-04	0.9980
0.00	1.964E-02	0.9990	5.172E-04	0.9969
0.00	2.021E-02	0.9940	5.750E-04	0.9917
2.07	5.372E-02	0.9960	1.464E-03	0.9962
9.98	1.675E-01	0.9998	4.672E-03	0.9987
WWTPE at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.180E-02	0.9972	4.258E-04	0.9999
2.06	1.650E-02	0.9989	6.126E-04	0.9992
6.05	2.750E-02	0.9995	9.939E-04	0.9975
6.13	2.631E-02	0.9973	9.705E-04	0.9998
9.87	3.511E-02	0.9958	1.298E-03	0.9999
LW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.230E-02	0.9985	4.778E-04	0.9997
1.96	2.071E-02	0.9994	7.439E-04	0.9999
5.97	3.480E-02	0.9989	1.331E-03	0.9982
9.86	4.902E-02	0.9971	1.888E-03	0.9996

D) Trimethoprim (TMP)

UPW at pH3.6				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	7.980E-04	0.9313	1.917E-05	0.9290
0.00	4.380E-05	0.9540	9.300E-06	0.9542
2.10	5.791E-02	0.9980	1.256E-03	0.9990
5.84	1.517E-01	0.9976	3.332E-03	0.9980
9.67	2.248E-01	0.9989	4.959E-03	0.9910
UPW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	8.269E-04	0.9960	1.804E-05	0.9960
0.00	7.156E-04	0.9960	1.588E-05	0.9960
2.14	4.175E-02	0.9999	9.202E-04	0.9999
6.11	1.074E-01	0.9978	2.508E-03	0.9998
10.21	1.592E-01	0.9994	3.342E-03	0.9991
10.08	1.542E-01	0.9984	3.498E-03	0.9982
WWTPE at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.050E-03	0.9976	3.703E-05	0.9958
2.04	7.785E-03	0.9986	2.756E-04	0.9958
5.91	1.903E-02	0.9999	6.715E-04	0.9988
9.73	3.146E-02	0.9996	1.112E-03	0.9997
LW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	7.380E-04	0.9552	2.511E-05	0.9647
2.10	1.226E-02	0.9997	3.988E-04	0.9988
6.22	3.283E-02	0.9987	1.091E-03	0.9999
9.90	4.862E-02	0.9991	1.643E-03	0.9998

E) Bisphenol A (BPA)

UPW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	7.260E-04	0.9764	1.575E-05	0.9763
0.00	8.100E-04	0.8807	1.796E-05	0.8801
2.01	3.458E-02	0.9999	7.581E-04	0.9999
6.04	1.014E-01	0.9993	2.209E-03	0.9993
9.99	1.580E-01	0.9933	3.591E-03	0.9994
WWTPE at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.512E-03	0.9486	5.250E-05	0.9590
2.04	7.566E-03	0.9986	2.602E-04	0.9956
6.04	2.175E-02	0.9983	7.448E-04	0.9958
10.22	3.139E-02	0.9997	1.099E-03	0.9981
LW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.398E-03	0.9954	4.651E-05	0.9936
1.96	1.260E-02	0.9997	4.141E-04	0.9984
6.17	3.677E-02	0.9992	1.179E-03	0.9999
10.05	5.848E-02	0.9971	1.855E-03	0.9993

F) Diclofenac (DCL)

UPW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	2.418E-01	0.9994	5.424E-03	0.9994
0.00	2.400E-01	0.9972	5.407E-03	0.9981
2.16	2.996E-01	0.9998	6.760E-03	0.9996
2.28	3.023E-01	0.9967	6.827E-03	0.9994
6.56	3.701E-01	0.9997	8.374E-03	0.9997
9.13	4.300E-01	0.9997	9.650E-03	0.9996
10.05	4.374E-01	0.9998	9.870E-03	0.9994
WWTPE at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.625E-01	0.9982	5.818E-03	0.9995
2.00	1.694E-01	0.9990	6.000E-03	0.9992
6.07	1.845E-01	0.9999	6.567E-03	0.9994
9.62	1.964E-01	0.9999	7.039E-03	0.9996
LW at pH 7.85				
$[H_2O_2]$	k'-time based		k'-dose based	
mg L^{-1}	min^{-1}	R^2	cm^2 mJ^{-1}	R^2
0.00	1.676E-01	0.9997	5.779E-03	0.9991
2.09	1.789E-01	0.9997	6.197E-03	0.9993
6.17	2.169E-01	0.9904	7.075E-03	0.9970
10.19	2.293E-01	0.9986	7.988E-03	0.9996

Appendix B. Supplementary Information for Chapter 3.

Regression equation describing the fluence-based pseudo-first order constants as a function of initial H_2O_2 concentration in ultrapure water (UPW) at pH 7.85, lake water (LW) and wastewater treatment plant effluent (WWTPE).

		Linear regression	R^2
SMX	UPW at pH 7.85	y = 0.000360 x + 0.00238	0.9903
	LW at pH 7.85	y = 0.000154 x + 0.00200	0.9960
	WWTPE at pH 7.85	y = 0.000055 x + 0.00215	0.9768
SMZ	UPW at pH 7.85	y = 0.000360x + 0.000781	0.9972
	LW at pH 7.85	y = 0.000165x + 0.000727	0.9986
	WWTPE at pH 7.85	y = 0.000106x + 0.000767	0.9980
SDZ	UPW at pH 7.85	y = 0.000411x + 0.000581	0.9994
	LW at pH 7.85	y = 0.000144x + 0.000471	0.9999
	WWTPE at pH 7.85	y = 0.0000890x + 0.000431	0.9983
TMP	UPW at pH 7.85	y = 0.000336x + 0.000117	0.9857
	LW at pH 7.85	y = 0.000164x + 0.0000445	0.9988
	WWTPE at pH 7.85	y = 0.000109x + 0.0000404	0.9993
BPA	UPW at pH 7.85	y = 0.000358x + 0.0000252	0.9999
	LW at pH 7.85	y = 0.000180x + 0.0000560	0.9998
	WWTPE at pH 7.85	y = 0.000104x + 0.0000624	0.9941
DCL	UPW at pH 7.85	y = 0.000437x + 0.00559	0.9906
	LW at pH 7.85	y = 0.000217x + 0.00576	0.9995
	WWTPE at pH 7.85	y = 0.000130x + 0.00578	0.9967
y: fluence based pseudo-first order constants (cm^2 mJ^{-1}); x: H_2O_2 concentration (mg L^{-1})			

Appendix C: Supplementary Information for Chapter 5.

Tests to determine NaOH trap effectiveness for $^{14}CO_2$ recovery.

Abiotic experiments were performed to determine if the NaOH trap in the bioreactor used for assessing biodegradation was effective for $^{14}CO_2$ recovery. These tests employed ^{14}C-acetate to determine if low molecular weight ^{14}C-labeled organic acids (formed through chemical or biological oxidation) may be found inside the NaOH trap and interfere with the assessment of mineralization rates that are determined on the basis of $^{14}CO_2$ trapping. These experiments were performed in bioreactors with 50 ml of buffered ultrapure water at pH 7.0. Two reactors were spiked with ^{14}C-sodium carbonate and two reactors were spiked with ^{14}C-acetate. Both compounds were spiked at the same concentration (30,000 DPM per reactor). After spiking, reactors were closed rapidly to minimize ^{14}C loss. The 2N NaOH trap was sampled periodically. At each sampling time, the content of the NaOH trap was removed for analysis by scintillation counting and replaced with fresh 2N NaOH solution. The content from the basic trap was divided in two equal aliquots and each was mixed with 12 mL of Hionic Fluor Scintillation cocktail (Perkin Elmer, Groningen, Netherlands). Samples were counted by a TRI-CARB 2800TR scintillation counter (Packard Instrument Company, Downers Grove, IL, USA).

Figure 1 shows the ^{14}C recovery in the NaOH trap as a function of time.

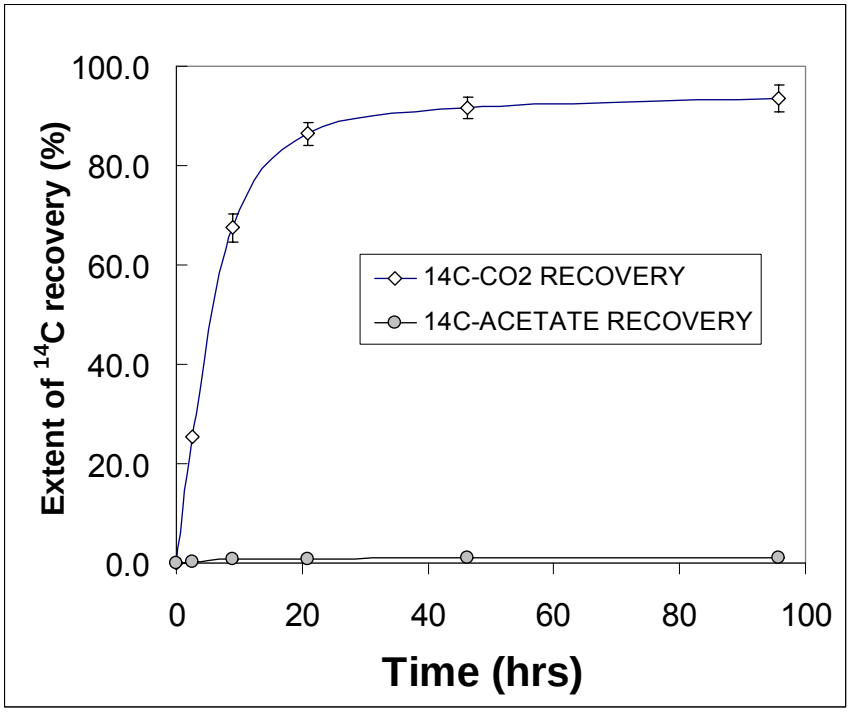


Figure 1. ^{14}C recovery in the NaOH trap for $^{14}CO_2$ and ^{14}C-acetate experiments as a function of time.

Appendix D: Supplementary Information for Chapter 5.

Assimilable Organic Carbon (AOC) method

Introduction.

The conventional AOC method was first developed by the van der Kooij research group (1982). This method was later adapted and simplified by Kaplan *et al.* (1993) and serves as the basis for *Standard Method* 9217 for AOC measurements. The AOC bioassay is based on the growth of *Pseudomonas fluorescens* P17 and *Spirilum* NOX until a stationary phase is reached. *Pseudomonas fluorescens* P17 is able to take up a broad range of low molecular weight compounds at low concentrations. *Spirilum* NOX grows only on carboxylic acids, such as formate, oxalate, glycollate and glyoxalates, compounds that are not utilized by the *Pseudomonas fluorescens* P17. AOC samples are incubated with the pure culture mixture for 9 days at 15°C. Microbial growth is measured on days 7, 8 and 9 by preparing three sample dilutions and plating each dilution in triplicate on nutrient agar. Plates are incubated for 3 – 5 days at 25°C. Overall, the AOC method will require approximately 10 - 14 days and a minimum of 27 enumeration plates to obtain a result. LeChevallier *et al.* (1993) suggested reducing the incubation time by increasing the temperature and the density of the inocula. In addition, this author used the adenosine triphosphate (ATP) luciferin-luciferase technique to quantify organism growth. As a result the AOC concentration could be obtained in 2 – 4 days. Even though the ATP enumeration

methodology is faster and simpler than the plate count procedure, plating is still more commonly used as an enumeration technique (Hammes and Egli, 2005).

A novel method for AOC determination was published by Hammes and Egli (2005) and utilized in this study. This new method utilizes flow cytometry in combination with a fluorescent dye for organisms enumeration. The AOC flow-cytometric method permits a more rapid AOC analysis, producing results in 2-3 days. An additional advantage of the flow cytometric method is the use of a natural consortium for inoculation; therefore, the AOC measurement is based on microbial communities that more appropriately describe environmental growth/re-growth.

Methodology

AOC was measured according to the protocol described by Hammes and Egli (2005). The following section is a summary of the AOC methodology that was used.

AOC free material. All glassware and screw caps were cleaned according to the procedure described in *Standard Methods* 9217 and Charnock and Kjonno (2000). Borosilicate glass was washed with detergent, rinse thrice in ultrapure deionized water, submerged overnight in 0.2 N HCl and again rinse thrice with ultrapure deionized water. Removal of trace carbon from glassware was achieved by baking all glassware at 550ºC for 6 hours. Baked glassware covered with aluminum foil was

stored for a maximum of one week. Screw caps had teflon-lined silicone septa and were washed with detergent, rinse thrice in ultrapure deionized water and submerged in 10% sodium persulphate solution at 60°C for at least 1 h, rinse thrice in ultrapure deionized water and twice with 0.22 μm filtered ultrapure deionized water. AOC free pipette tips were prepared by rinsed 5 times with 0.22 μm filtered ultrapure deionized water.

AOC free gloves. Use either latex or nitrile gloves and rinsed in ultrapure deionized water for all steps to minimize carbon contamination. Change gloves between samples to minimize cross contamination.

Filter conditioning: All filters used (0.22 μm filter, Millex-GV - PVDF, Millipore) in this study were previously rinsed by passing 2 L of ultrapure deionized water overnight. (Berger *et al.,* 2005).

Mineral buffer. Prepared with sterilized ultrapure deionized water filtered through a condition PVDF membrane filters (0.22 μm filter, Millex-GV, Millipore) For 1L; 1.0 g $(NH_4)_2SO_4$, 3.0 g KH_2PO_4, 7.0 g K_2HPO_4, 0.2 g KCl, 0.1 g NaCl, 50 mg $MgSO_4$, 4.1 mg $CoCl_2{\cdot}6H_2O$, 5.4 mg $CuCl_2{\cdot}6H_2O$, 5.0 mg $MnSO_4.7H_2O$, 2.1 mg $ZnCl_2$, 1.3 mg

$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, 1 mg $FeSO_4\cdot 7H_20$). (Berger *et al,* 2005 and LeChevallier 1991). Kept refrigerated at 4ºC.

Natural Microbial Consortium. The natural microbial consortium was obtained from Jordan Lake, North Carolina, USA. To obtain the inoculum, 40 mL of lake water was filtered through PVDF filter membranes (0.22-µm pore size, Millex-GV, Millipore). Prior to use, filters were conditioned as described above. The filtrate was inoculated with ~100 µL of unfiltered water and incubated at 34ºC for 14 days. The cells were harvested by centrifugation (3000 rpm, 30 minutes), and subsequently re-suspended in HPLC water amended with mineral buffer. The re-suspended cells were incubated for 7 additional days to assure that no residual AOC is present in the inoculum. To obtain a cell count of the inoculum prepared, a sub-sample was taken and analyzed by flow cytometry. The inoculum can be stored for at least eight months at 4ºC.

Calibration of cell counts to µg L^{-1}. The natural consortium growth is converted to AOC concentration by a yield factor, which represents the number of cells produced per µg organic carbon used. To obtain the yield factor for the inoculum a calibration was done with acetate as the organic source. The same mineral buffer used in 5.2.3.1. was added to all samples to assure that minerals were not limiting. The mineral buffer was added at ratio of 1 µL of mineral buffer per mL of sample. Two different inoculum were prepared in the course of this study, and the calibration method (0 –

300 μg L^{-1} C-acetate) yielded a growth factor of (1) 6.35×10^6 cells / μg C-acetate for consortium A and (2) 4.36×10^6 cells / μg C-acetate for consortium B, showing the reproducibility of the method. Figure 1 shows the

Preparation of AOC sample. AOC samples were collected in baked, AOC-free glass bottles. Samples containing hydrogen peroxide were quenched with sodium thiosulfate (Liu *et al.*, 2003). Liu *et al.*, 2003 found that no peroxide was detected after 5-10 minutes when a 10 mg/L H_2O_2 concentration quenched with a solution containing 97.5 mg/L sodium thiosulfite. Samples were placed in the dark until no detectable peroxide concentration remains. Then, samples were filtered with pre-condition 0.22 μm filters into AOC free 40ml EPA vials and capped. The bacteria consortium (corresponding to approximately $1x10^5$ organisms/ml) was then spiked into each 40 ml sample. The samples were mixed and incubated at 34°C, and enumerated using the above described flow cytometer method on the fourth day of inoculation. AOC measurements were performed in duplicate or triplicate, and uninoculated samples were included for each AOC measurement to account for any growth of background organisms. These uninoculated samples never yielded organism growth.

Flow Cytometry analysis. Natural consortium bacteria enumeration was performed using a Beckman-Coulter EPICS Altra flow cytometer (Fullerton, CA). Samples were prepared as described in Hammes and Egli (2005). Samples were stained with 10 μL

mL^{-1} of SYBR green I (Invitrogen, Molecular Probes. 10,000X concentrate in DMSO) previously diluted in DMSO to a concentration of 1:100. SYBR green stains total nucleic acids and emits a bright fluorescent signal at 530nm +/- 30nm (Green Fluorescence) upon excitation with a laser at 488nm. The samples are left in the dark for at least 20 minutes before analysis. Another signal is also detected above 590nm (Red Fluorescence). Utilizing these two fluorescent signals, bacteria was enumerated by gating the flow cytometer counts. Data was analyzed by a compatible shareware software called WinMDI Version 2.8 (Windows Multiple Document Interface for Flow Cytometry) developed by Joe Trotter, Scripps Institute. Figure 2 displays the gates utilized in this work, applied to a sample of pure *e. coli* and a natural consortia (A and B) harvested from Jordan Lake, in North Carolina.

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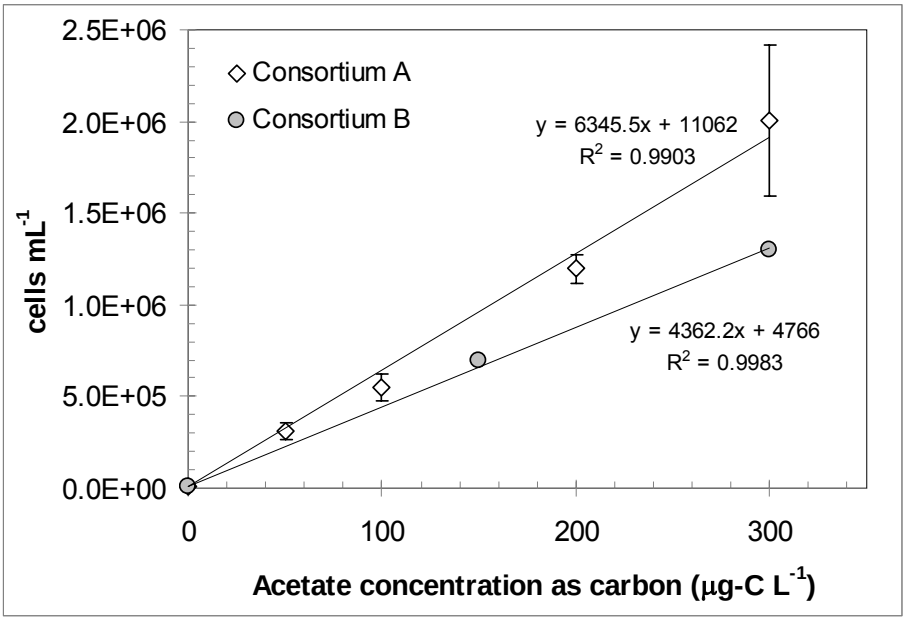

Figure 1. Calibration of cell counts to µg-C L^{-1} for consortium A and B. Acetate was used as the carbon source.

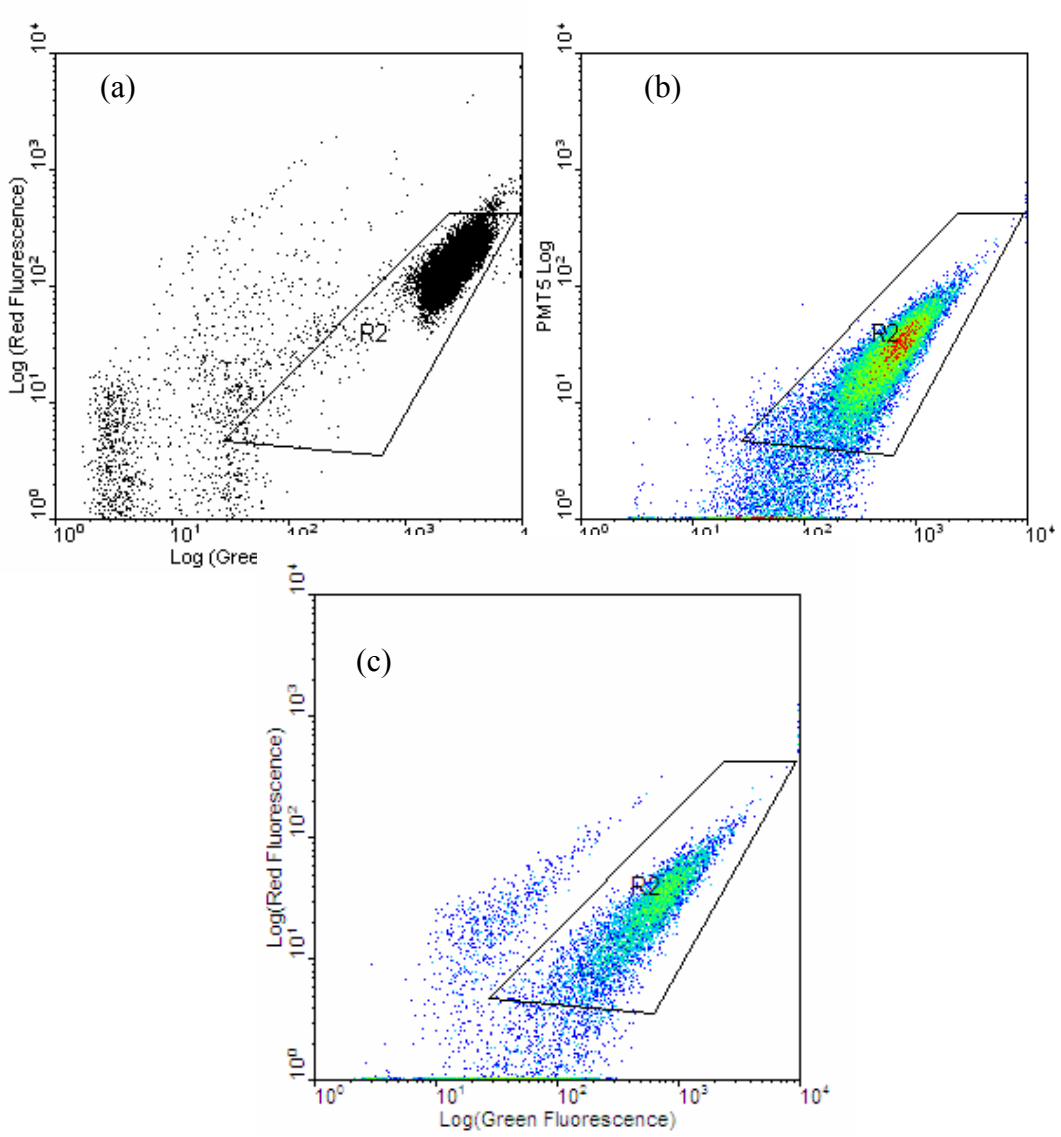

Figure 2: Flow cytometer output, with gating, for (a) pure culture *E. coli* ATCC® 25922, (b) Jordan Lake natural consortium A and (c) Jordan Lake natural consortium B.