PHOTOLYSIS AND DIGESTION AS POLISHING STEPS FOR THE REMOVAL OF ANTIBIOTICS FROM MUNICIPAL WASTEWATER TREATMENT PLANT EFFLUENT AND BIOSOLIDS

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Abstract

The degradation of five pharmaceuticals and personal care products, tetracycline, triclosan, tylosin, sulfamethoxazole, and trimethoprim was examined. The photolysis of these compounds was studied in pure water, wastewater treatment plant effluent, and natural waters. The biodegradation of the substances, excluding tetracycline, was examined during both aerobic and anaerobic sludge digestion. Tetracycline, triclosan, and tylosin were shown to degrade rapidly under both natural and simulated sunlight. Sulfamethoxazole and trimethoprim did not photodegrade as rapidly as the other compounds. These two compounds, however, did degrade more quickly in wastewater treatment plant effluent than in pure water or natural water. This observation indicated indirect photolysis was an important loss process for these compounds in the effluent. The cause of indirect photolysis was shown to be both triplet-excited dissolved organic matter and hydroxyl radicals reacting with the sulfamethoxazole and trimethoprim. During aerobic digestion, trimethoprim degraded in the room temperature, 45 °C, and 55 °C digesters, and in the 35 °C digester during anaerobic digestion. Degradation did not occur in the 35 °C aerobic digester. Tylosin degraded during aerobic digestion in the 35 °C and 55 °C digesters. Degradation did not occur in the room temperature aerobic digester or in the 35 °C anaerobic digester. Sulfamethoxazole was not detected in any of the digester samples, despite being spiked in initially. Triclosan concentrations in the digester samples were always at least an order of magnitude higher than expected based on amount spiked in, indicating that a large amount of the compound was present in the digester sludge prior to spiking.

Table of Contents **Page**

List of Symbols and Abbreviations

Φ	:	Quantum yield
ε	:	Molar absorptivity
λ_i	:	Initial wavelength
λ_f	:	Final wavelength
$^{\bullet}OH$	:	Hydroxyl radical
^1SMX	:	Singlet-excited sulfamethoxazole
^3SMX	:	Triplet-excited sulfamethoxazole
BLE	:	Blue Lake effluent water
BOD	:	Biochemical oxygen demand
C	:	Concentration
DI	:	Deionized water
DOC	:	Dissolved organic carbon
DOM	:	Dissolved organic matter
$h\nu$	:	Light
HPLC	:	High performance liquid chromatography
I	:	Light intensity
I_o	:	Incident light intensity
IPA	:	2-propanol
k_{direct}	:	Direct photolysis rate constant
l	:	Path length
LJW	:	Lake Josephine water
MQW	:	Milli-Q water
PPCPs	:	Pharmaceuticals and personal care products
qPCR	:	Quantitative polymerase chain reaction
SMX	:	Sulfamethoxazole
TCS	:	Triclosan
TMP	:	Trimethoprim
TTC	:	Tetracycline
TYS	:	Tylosin
UV	:	Ultraviolet
UVT	:	Ultraviolet light transmitting
WWTP	:	Wastewater treatment plant

List of Tables **Page**

List of Figures **Page**

Chapter 1: Introduction

Pharmaceuticals and personal care products (PPCPs) are a class of environmental pollutants that have been studied extensively for the past decade because of their potential to negatively impact environmental systems. The group consists of hundreds of compounds which are found in daily-use products, including perfumes, soaps, cleaning agents, medicines, and cosmetic products. Two important questions need to be answered before it can be determined whether or not a particular PPCP will be a problematic pollutant. The first is, "What is the concentration of the compound in the environment?" and the second is, "Does a compound exhibit biological effects at the concentrations found for environmental systems?" Recent studies have been examining both of these questions.

PPCPs are produced on the scale of thousands of tons per year (1). Many of these compounds are flushed directly down the drain as a result of their use. Other compounds, particularly pharmaceuticals, are ingested, incompletely metabolized in the body, and then excreted into the sanitary sewer system. During the wastewater treatment process some PPCPs are substantially removed while others continue to persist (2-5). Depending on the amount of a particular compound used and the percentage of it that is removed during waste treatment, some quantity of the compound may enter the environment.

Hundreds of PPCPs have been detected in surface waters around the world at concentrations in the ng/L to μg/L range (6-8). There are concerns that even at these concentrations, the chemicals may exhibit adverse effects on aquatic organisms. Studies have found some PPCPs to cause feminization in fish, amphibians, and reptiles

(9-12). Other studies have linked the presence of antibiotic and antibacterial compounds to hindering the growth of both microbial organisms, including bacteria, algae, and protozoa, and macroscopic organisms, including crustaceans and amphibians (13-15). The potential also exists for impacts on human health resulting from populations of antibiotic-resistant bacteria being generated by PPCPs in the environment (16).

Although PPCPs have been found in the environment, multiple processes lead to their removal, including sorption to sediments, hydrolysis, biological degradation, and volatilization. Photolysis is an additional process which has been found to degrade many PPCPs in the environment (17-24). Photolysis could also be used as an engineered strategy for removing PPCPs in the wastewater stream.

The goal of this study is to investigate methods of removing PPCPs following the wastewater treatment process, but before discharging the effluent to surface waters or spreading the biosolids on agricultural land. More specifically, direct and indirect photolysis are examined for their roles in the destruction of antibiotic compounds dissolved in the effluent from a wastewater treatment plant. Information gathered is used to assess the feasibility of placing a stabilization pond after secondary treatment to provide time so that photolysis could occur. Aerobic and anaerobic digestion, processes used to reduce the amount of organic matter in biosolids, are also examined to look at the potential for degradation of PPCPs sorbed to biosolids during the digestion process.

The findings of this study suggest that photolysis could be used to appreciably degrade several compounds commonly detected in wastewater. Evidence was also uncovered during this study that indicates that the rate of photodegradation for some

compounds may increase in effluent waters as compared to natural waters. Additional findings show that biological degradation occurred for some antibiotic compounds during sludge digestion.

This thesis includes:

Chapter 1: Introduction

Chapter 2: Literature Review. Background information on the occurrence of pharmaceuticals and personal care products in the environment, their removal during the wastewater treatment process, and their photochemical degradation under sunlight.

Chapter 3: Sunlight Exposure as a Treatment Step for Pharmaceutical and Personal Care Products (PPCPs) in Treated Municipal Wastewater

Chapter 4: Enhanced Photolysis of Sulfamethoxazole and Trimethoprim in Wastewater Treatment Plant Effluent Waters Compared to Natural Surface Waters

Chapter 5: Degradation of Three Antibiotic Compounds and Triclosan During Anaerobic and Aerobic Sludge Digestion

Chapter 6: Conclusions and Recommendations

Chapter 2: Literature Review

2.1 Background

Pharmaceuticals and personal care products (PPCPs) have been receiving an increasing amount of attention over the last decade since the term was coined in 1999 by the US EPA (25). This group of pollutants consists of hundreds of chemicals commonly found in human and veterinary drugs, cosmetic products such as soaps, lotions, make-ups, and shampoos, and cleaning products of nearly every kind. Although these chemicals often make up only a small fraction of the actual product, the widespread use of the products result in the chemicals being manufactured on an industrial scale in the range of thousands of tons a year (1). Often the lifecycle of the products ends with the chemicals reaching the sanitary sewer system. Once down the drain, a portion of the compounds is removed or destroyed in wastewater treatment plants (WWTPs). The remaining portion passes through the treatment plants unchanged, and enters into environmental systems.

Pollutants leave wastewater treatments in two principal ways, either dissolved in the liquid effluent or sorbed to biosolids. The liquid effluent from the plant is normally discharged to a receiving body, typically a river, lake, or ocean. In areas where freshwater is scarce, the effluent is increasingly being injected back into the ground to replenish groundwater reserves. The process of disposing of solids from WWTPs often involves dewatering and concentrating solids to some degree, and then spreading the concentrated material over the land. These disposal practices result in the PPCPs being sent out to the environment.

From the earliest reports of PPCPs in the environment in the late 1970's until the EPA report in 1999, studies working to detect PPCPs were rare (26, 27). During this time, much of the work focused on environmental contamination from agricultural antibiotic use and disposal of waste from drug production (28). Recent studies from the last decade have found trace levels of PPCPs in a wide range of environmental systems. Surface waters from around the world have been found to contain many of these of compounds at ng/L to μg/L levels (6-8). PPCPs have also been detected in groundwater contaminated from both WWTP effluent and landfill leachate (29, 30). Soils irrigated using wastewater or fertilized using WWTP biosolids and agricultural manure have also been found to be contaminated with a wide range of PPCPs (31, 32).

Concern about PPCPs in the environment at trace levels comes from the fact that the majority of these compounds are used because of the biological effects that they illicit. Studies have linked the trace-level presence of estrogenic compounds in receiving streams of WWTPs to the feminization of aquatic organisms (9-12). At environmentally relevant concentrations, antibiotic and antibacterial compounds have been found to found to be harmful to both microscopic and macroscopic aquatic organisms (13-15). The potential also exists for the selection of antibiotic resistant bacteria in natural systems being caused by antibiotic compounds being discharged from WWTPs (16). Studies have also shown the potential for PPCPs to bioaccumulate in organisms that are continually exposed to low levels of compounds (32, 33)

2.2 Removal in WWTP

As stated above, many PPCPs are partially removed from wastewater during the various processes that take place in WWTPs. In a conventional plant, treatment

typically begins with a bar rack to remove large objects in the waste stream. This is followed by primary clarification, where solids are removed as they settle out of the liquid. Secondary treatment typically involves biological treatment, such as the activated sludge process or trickling filters, followed by secondary clarification. The final step in wastewater treatment is often a disinfection step prior to effluent discharge. During each of these treatment steps, there is potential for chemicals to be removed from the waste stream through physical, biological, or chemical pathways. In conventional plants, typical removal rates of pharmaceutically active compounds vary anywhere from under 30% for persistent compounds like carbmazapine to greater than 98% for compounds like ibuprofen (34, 35). For estrogenic compounds, removal rates were found to be near 90%, mainly as a result of biodegradation during the nitrification and denitrification steps (36).

To achieve a cleaner effluent water, some WWTPs use advanced treatment processes, such as ozonation, ultraviolet light and hydrogen peroxide, or membranes to treat their final effluent. Along with disinfecting the effluent, these processes remove a vast majority of the PPCPs found in the effluent. Ozonation has been found destroy 90 to 99% of the antibiotics present in waste stream but only reduced the concentration of X-ray constant media by 40% (37). Reverse osmosis, which physically separates compounds from the water, was found to have removals rates that were at least ten times greater than what was found for a conventional activated sludge plant for several antibiotic compounds (38). Implementing these advanced technologies would help to eliminate PPCPs entering the environment by way of WWTPs. The trade off of adding

these technologies to the end of the treatment process is the additional cost and energy that would be associated with their use.

In addition to PPCPs being dissolved in the liquid effluent, these chemicals may be contained by biosolids generated in WWTPs. Digestion is a process which uses biological degradation to decrease the amount of solids that must be disposed after wastewater treatment. In addition to degrading solids, digestion has potential to biologically degrade PPCPs that are sorbed to solids. A study operating lab-scale, thermophilic and mesophilic anaerobic digesters found that some estrogenic and antibiotic compounds were removed with greater than 80% efficiency. Other compounds such as the anti-epileptic, carbamazapine, and the X-ray contrast media chemical, iopromide were removed with less than 25% efficiency (39).

2.3 Photochemistry

2.3.1 Direct Photolysis

A process with the potential to remove PPCPs from WWTP effluent without excess costs associated with it is photolysis. By retaining the effluent in a stabilization pond prior to final discharge, sunlight could be used to degrade chemicals, and to potentially disinfect the effluent. Photolysis can proceed by two mechanisms, direct and indirect photolysis. Direct photolysis occurs when a molecule breaks apart after absorbing a photon of light. To quantify to what extent this process will occur for any given substance, three things need to be known: the quantum yield of the compound, the absorbance spectrum of the compound, and the emission spectrum of the light source. The quantum yield, which is given the symbol, Φ, is essentially a fraction described by of the number of photon absorption events that lead to destruction of the

compound divided by the number of photon absorption events overall. The quantum yield is wavelength dependent and must be found experimentally for every compound. The absorbance spectrum is typically found by placing a dilute solution of the compound in a spectrophotometer. This instrument measures the fraction of light that is absorbed by the solution at specific wavelengths. The absorbance is a function of the solution concentration, *C*, the distance that the light beam travels through solution, *l*, and the molar absorptivity of the compound, ε. The absorbance is also defined as the negative logarithm of incident light intensity on the solution, I_o, divided by the light intensity leaving the solution, *I*. Absorbance is described by the Beer-Lambert law as shown below.

$$A = \varepsilon l C = -\log_{10}\left(\frac{I_o}{I}\right)$$

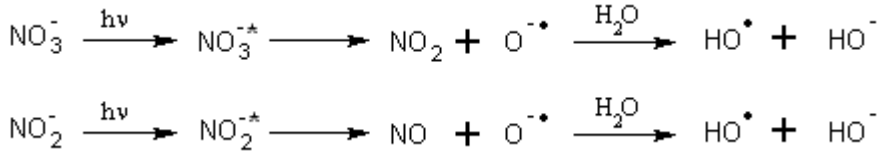
The intensity of the light source at various wavelengths must be a known or measured quantity. The expression for calculating the first-order rate constant for the direct photolysis of a compound is given below.

$$\Phi(\lambda) * \int_{\lambda_i}^{\lambda_f} \varepsilon * I \, d\lambda = k_{direct}$$

2.3.2 Indirect Photolysis

Indirect photolysis occurs when light interacts with some material dissolved in a water to generate a reactive species, which then can degrade a substrate. The light-absorbing material can be excited and react directly with the substrate of interest or it can react with dissolved oxygen to produce reactive oxygen species, which also have the potential to react with a substrate.

Dissolved organic matter absorbs the majority of the light that passes through natural waters. When light interacts with this material it can break bonds within the molecular structure or it can produce excited species. These reactive species include oxyl and peroxyl radicals, singlet oxygen, and the superoxide radical anion, and can also give rise to hydrogen peroxide and hydroxyl radicals. Besides the DOM, the principle species in most natural waters which produce reactive oxygen species are dissolved nitrate and nitrite. Light interacts with nitrate and nitrite to form hydroxyl radicals following the pathway shown below (40).



Hydroxyl radicals are perhaps the most unstable, and therefore, the most reactive species that is important for indirect photolysis. Hydroxyl radicals react by adding to a double bond or by abstracting a hydrogen atom from a molecule. For many organic compounds, this process occurs with second-order reaction rates greater than 10^9 M^{-1} s^{-1}, which is near the diffusion controlled limit. Slower reaction rates occur for compounds without double bonds, aromatic bonds, or easily accessible hydrogen atoms (41).

Singlet oxygen is produced by dissolved oxygen quenching triplet-excited DOM. It is an electrophilic species that only provides significant degradation for reactive compounds which possess electron-rich double bonds or functional groups that are easily oxidized such as sulfides, anilines, and phenols. Electron withdrawing substituents decrease the susceptibility of a compound to react with singlet oxygen,

while electron donating substituents increase this susceptibility. Anionic compounds are much more reactive with singlet oxygen, which often translates to greater compound reactivity at higher pH values (41).

As stated above, compounds also have the potential to degrade from interacting directly with excited DOM. When light is absorbed by DOM it often excites a molecule to a singlet-excited state. This short-lived singlet state can intersystem cross over to a longer lived triplet-excited state. The triplet-state can react with dissolved oxygen, to produce singlet oxygen, or with organic molecules present in the water to transform the molecule. This second type of reaction can proceed by three separate mechanisms, energy transfer, electron transfer, or proton transfer mechanisms (42). Energy transfer reactions will often occur for compounds with triplet state energies below 250 kJ/mol (43). The electron and hydrogen transfer reactions oxidize the substrate and have been found to occur for electron-rich phenolic compounds (42).

2.4 Compounds in this Study

This study focuses on five compounds with antibiotic or antibacterial properties commonly detected in water bodies affected by municipal wastewater (tetracycline, triclosan, sulfamethoxazole, trimethoprim) and by agricultural practices (tetracycline, tylosin).

Tetracycline is an antibiotic compound used for human and veterinary treatment that has commonly been detected in WWTP effluent (44, 45). It has been found to undergo rapid photolysis under natural conditions, with a predicted half-life of anywhere from 44 to 220 minutes in natural water samples under summer sunlight in Minneapolis, MN, depending on the pH and hardness of the water (23). The most

common photoproduct of this compound was found to be 5a,6-anhydrotetracycline, which is 85% less potent as an antibiotic compared to the parent compound (46, 47).

Triclosan is a chemical disinfectant used in many antibacterial cleaning products and toothpastes, and as an additive to plastics. The compound has been commonly found in natural waters up to μg/L levels (6). The photolysis of triclosan has been found to occur rapidly in natural systems with a half-life of anywhere from one to five hours under summer sunlight near 45° N latitude (21, 48). The major photoproducts formed in natural waters are higher molecular weight molecules resulting from coupling reactions of the compound with itself and with dissolved organic matter. Another important photoproduct of this molecule is the toxic 2,8-dichlorodibenzo-*p*-dioxin. This product was found to form with a yield of one to five percent under environmentally relevant conditions (49). None of the photoproducts of triclosan was found to exhibit significant antimicrobial activity when compared to the parent compound (50).

Tylosin is an antibiotic that is typically used in agricultural practices as a growth promoter for swine and cattle. Concern for the use of tylosin in this manner comes from evidence that it promotes bacterial resistance to erythromycin, a human-use antibiotic (51). Tylosin has also been widely detected in surface waters as a trace organic pollutant (6). The photochemistry of the compound proceeds via two processes. The first occurs quickly and involves a reversible isomerization to a product which was found to have 26% of the antibiotic activity of the parent compound (24, 52). The second process involves the irreversible degradation of the two isomers, and occurs on a longer timescale. This degradation step provides a half-life of four hours for tylosin in summer sunlight in Minneapolis, MN (24).

Sulfamethoxazole is a human-use antibiotic, which is typically used in tandem with trimethoprim. Studies have found trace levels of the compound in WWTP effluent and in surface waters around the United States (6, 44, 45). In natural waters, only direct photolysis was found to be important for this compound, although it was found to react with hydroxyl radicals generated in aqueous solutions (18, 20). The calculated half-life for sulfamethoxazole in pure water, under summer sunlight in Minneapolis, MN was approximately 16 hours for a solution with environmentally relevant pH values (18). Major photoproducts of the compound's degradation include 3-amino-5-methylisoxazole, sulfamic acid, and sulfanamide (18, 20). These photoproducts were not found to possess significant antibiotic activity when compared to the parent compound (50).

Trimethoprim, along with the previously mentioned compounds, has been commonly found in both WWTP effluent and surface waters of the United States (6, 45). Studies have shown this compound to react slowly by photolysis, whether direct or indirect (19, 53). The half-life of this compound was found to be approximately 10 days under natural summer sunlight in Toronto, Canada (19). It was found to exhibit limited suspectibility towards indirect photolysis from singlet oxygen and triplet-excited organic matter (53). The products of the photodegradation of trimethoprim include 3,4,5-trimethylbenzaldehyde, trimethoxylbenzoylpyrimidine, and two tetraarylethanes (53). The bacterial potency of these compounds could not be found in the literature.

The behavior of compounds during sludge digestion is another topic of this research. For triclosan, biological degradation has been suggested to occur for aerobic processes, including aerobic digestion, the activated sludge process, and trickling filters

(54, 55). Degradation has not been found to occur during anaerobic digestion (54). Little data was found on the biological degradation of tetracycline. One study that examined this compound during the activated sludge process concluded that it was removed as a result of sorption to solids and that biodegradation played an insignificant role in its removal (56). Information on the biodegradation of tylosin was also difficult to obtain. A study looking at an anaerobic reactor designed to treat pharmaceutical waste showed that 95% of tylosin could be removed from the waste stream (57). Data on the biodegradation of trimethoprim and sulfamethoxazole was more prevalent. Sulfamethoxazole was found to exhibit 95% removal in both mesophilic and thermophilic anaerobic digesters located at a municipal WWTP (39) . Both sulfamethoxazole and trimethoprim were found to be removed with greater than 99% efficiency from an anaerobic digester set up to process swine farm waste (58). In an example of an aerobic process, sulfamethoxazole was found to degrade by 60% and trimethoprim by 20% during the activated sludge process (3).

Dealing with wastewater containing PPCPs is a difficult problem. To find the best method for removing these compounds, multiple strategies need to be considered and evaluated based on their construction and operating costs and their effectiveness at removing these compounds from the waste stream. This study examines the use of photolysis to treat PPCPs in the liquid effluent from WWTPs and sludge digestion to treat PPCPs in the solids generated from wastewater treatment.

Chapter 3:

Sunlight Exposure as a Treatment Step for the Removal of Pharmaceutical and Personal Care Products (PPCPs) From Treated Municipal Wastewater

3.1 Introduction

The primary purpose of wastewater treatment plants (WWTPs) is to manage the sewage generated by the public. Conventional treatment aims to remove the biochemical oxygen demand (BOD) of the waste. Steps are now increasingly being taken to implement treatment strategies which remove nutrients, like nitrogen and phosphorus, from the waste stream and disinfect the final effluent leaving a WWTP. In addition to nutrients and BOD, pharmaceutical and personal care products (PPCPs) are present in the waste streams. Because WWTPs are not specifically designed to treat these trace-level compounds, PPCPs can enter into the environment through the waste stream and may cause problems for the ecosystems to which they are discharged. Research into how to treat this class of pollutants is ongoing, and at present, the implementation of strategies to remove them is limited.

Included in the group of PPCPs are estrogenic, antibiotic, and disinfecting compounds, all of which have been detected in WWTP effluents (7, 48, 59). The problem with allowing these compounds to pass through WWTPs and into the environment is that the initial purpose of these compounds is to induce a biological response. When these compounds enter the environment through WWTP effluent, they may continue to exert a biological response in unintended or harmful ways. Many studies have linked the trace-level presence of estrogenic compounds in receiving streams of WWTPs to the feminization of aquatic organisms (9-12). Antibiotic and

disinfecting compounds have been found to have adverse biological impacts on both microscopic and macroscopic aquatic organisms (13-15). There is also concern about the selection of antibiotic resistant bacteria in natural systems caused by these compounds being discharged from WWTPs (16).

Several strategies that have been shown to remove PPCPs from WWTP effluent. Advanced oxidation processes, including the use of ozone, hydrogen peroxide, and ultraviolet (UV) light, either alone or combination, have been shown to be effective at degrade these compounds (60, 61). Treatment with specific types membranes also effectively removes PPCPs by physically separating them from the effluent water (62). The problem with wide- scale implementation of these technologies is the cost and energy intensity associated with their use. For effective management of PPCPs, a low cost, low maintenance solution would be ideal. Using sunlight to degrade these compounds is one possible solution that fits this criteria.

The purpose of this study is to examine the effect that sunlight has on five organic compounds commonly found in wastewater treatment plant effluent at trace levels, tetracycline (TTC), triclosan (TCS), Tylosin (TYS), trimethoprim (TMP), and sulfamethoxazole (SMX). Lab experiments were conducted to assess the susceptibility of each of the compounds to solar degradation and to examine the effect that various materials would have on the photolysis of the compounds if placed between them and a light source. Additionally, a bench-scale, flow-through reactor was constructed. The degradation of the compounds was monitored as they passed through the reactor to assess the plausibility of using sunlight as final treatment step to remove PPCPs from a municipal wastewater stream. A possible additional benefit of exposing WWTP effluent

to sunlight would be disinfection. As a result, experiments were conducted with the solar reactor to test for the inactivation of bacteria and for the destruction of genes encoding for tetracycline resistance. A cover was also placed over the reactor to examine if trapping heat would accelerate disinfection.

3.2 Experimental

3.2.1 Materials

SMX (98%), TTC (98%), TYS (95%), TCS (97%), and TMP (98%) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). All solvents were high-performance liquid chromatography (HPLC) grade. Chemicals were used as received. Acrylic plating (1/8" thick), borosilicate glass plating (1/4" thick), and quartz glass plating (1/8" thick) were purchased from McMaster-Carr (Princeton, NJ, USA). Ultra-violet transmitting (UVT) acrylic plating (1/8" thick) was purchased from Gold Leaf Plastics (St. Cloud, MN).

3.2.2 Instrumental Analysis

Concentrations were quantified using an Agilent Technologies (Santa Clara, CA) 1200 Series HPLC equipped with a photo-diode array detector. All compounds were analyzed on a Supelco (Park Bellefonte, PA, USA) Ascentis RP Amide 150 mm × 4.6 mm, 5 μm column. For SMX and TMP a methanol:pH 3 phosphate-buffer gradient method was used, starting at 20:80 and changing to 50:50 over one minute and then holding for four minutes, with a 1 ml/min flow rate and a detection wavelength of 274 nm. TTC was analyzed using an acetonitrile:pH 3 phosphate buffer gradient method going from 10:90 initially to 35:65 over five minutes. The detection wavelength was 270 nm and the flow rate was 1 ml/min. TYS was analyzed using an isocratic method

with a 30:70 acetonitrile:pH 3 phosphate buffer with a flow rate of 1 ml/min and a detection wavelength of 286 nm. TCS analysis was performed using a 90:10 acetonitrile:pH 3 phosphate buffer mobile phase, at a flow rate of 1 ml/min, and a detection wavelength of 230 nm.

3.2.3 Laboratory Photolysis

Indoor photolysis was conducted using a Suntest CPS+ solar simulator with a UV-Suprax optical filter (Atlas Materials Testing Solutions, Chicago, IL, USA) with the light intensity set at either 250 W/m^2 for experiments with TCS alone or 765 W/m^2 for experiments with other compounds. Samples were held in 150 mm × 25 mm (i.d.) borosilicate glass test tubes with the walls wrapped in a layer brown paper and a layer of aluminum foil to prevent light from shining through the tube walls or reflecting inside of the tubes as shown in Figure 3-1. The tubes were positioned vertically so that the tube openings were held directly below the xenon-arc lamp used in the simulator as given in Figure 3-2.

For experiments examining direct photolysis, each compound was tested separately at an initial concentration of approximately 1 μM in pH 8 phosphate buffered ultrapure Milli-Q water (MQW). Test tubes were filled to a liquid depth of 5 cm. Samples of approximately 1 ml volume were taken at regular time intervals, which depended on the half-life of the compound being examined. Chemical concentrations were quantified using HPLC as described above. To examine the effect of light screening and the impact of indirect photolysis on compound degradation, similar experiments were conducted with effluent from the Blue Lake wastewater treatment plant (BLE) in Shakopee, MN that were adjusted to pH 8 using dilute phosphoric acid

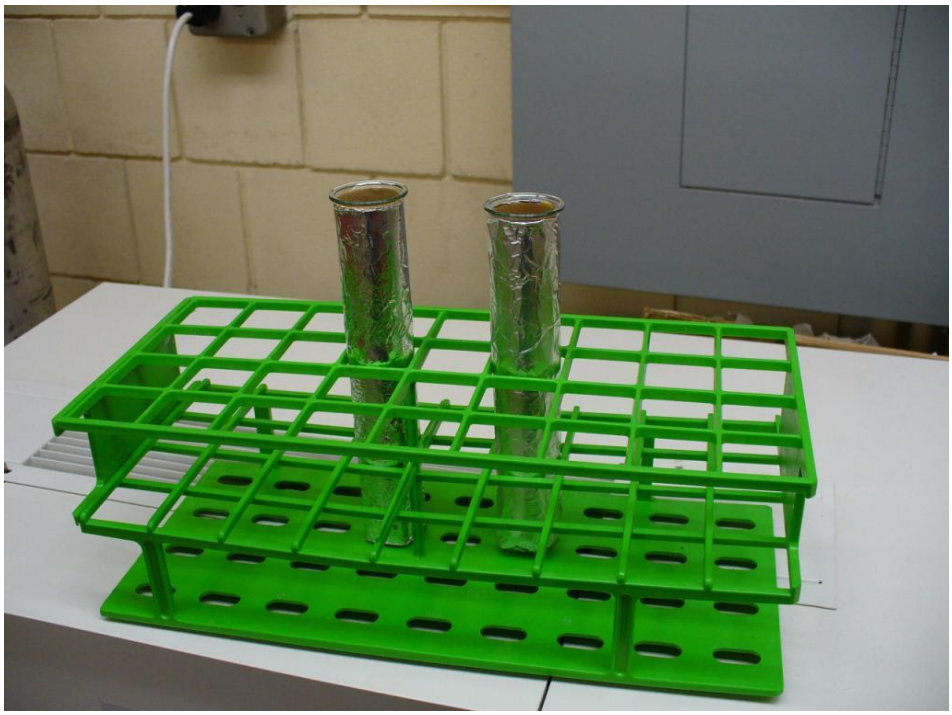


Figure 3-1. Test tubes used for indoor photolysis experiments with foil-paper cover.

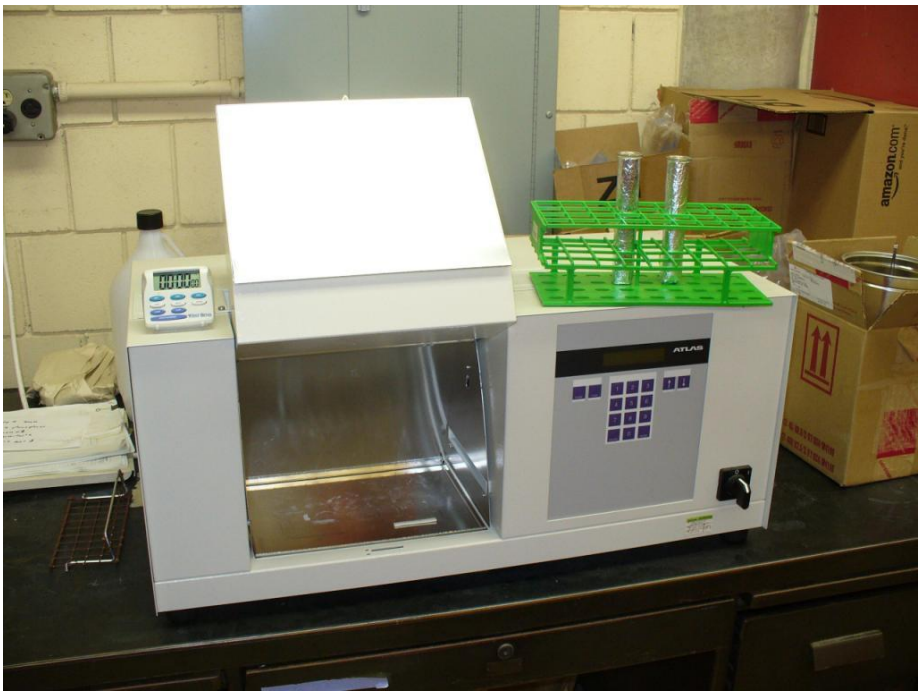


Figure 3-2. Test tubes and the solar simulator.

or sodium hydroxide. The alteration of photolysis rates from placing various cover materials over the top of the test tubes was also studied. All compounds were tested for nonphotolytic degradation by monitoring the concentration of control samples placed in the dark during the photolysis experiments.

3.2.4 Outdoor Photolysis

Outdoor photolysis experiments were conducted in 2008 or 2009 during the months of June, July, August, and September in Minneapolis, MN, USA (~45° N latitude) on clear days. Solutions used for photolysis experiments were prepared in 20 L plastic carboys with either pH 8 phosphate buffered MQW or in BLE at ambient pH (pH 7.6). The initial concentration of the compounds was approximately 5 μM. Solutions containing TYS, TCS and TTC were prepared using methanol as a co-solvent

Figure 3-3. Solar reactor, peristaltic pump, and carboy.

at a concentration of 0.1 % methanol or less. Solutions containing both SMX and TMP were prepared by adding a concentrated Milli-Q water solution to the 20 L carboys at a rate of 25 ml of the concentrated solution per liter of the bulk solution.

The solar reactor, which measured 20.5 inches wide, 22 inches long, and 8 inches tall, is shown in Figure 3-3. The walls were constructed using 0.5 inch thick acrylic plastic and the base was constructed with 0.25 inch thick mirror-extruded acrylic plastic. The outer walls of the reactor were covered with brown paper so that wavelengths of light were not selectively allowed to penetrate into the reactor through the acrylic walls. Five, 19 inch long baffles constructed using the 0.5 inch thick acrylic plastic and were placed in the reactor to create 3 inch wide channels for the liquid to flow through. A schematic of the reactor is given in Figure 3-4. With a 2 inch liquid depth, the reactor contained a volume of 13.2 liters. A pulse-input tracer study, using potassium permanganate as the tracer, showed the reactor to behave principally as a plug flow reactor, as can be seen in Figure 3-5. A step input tracer study, again using

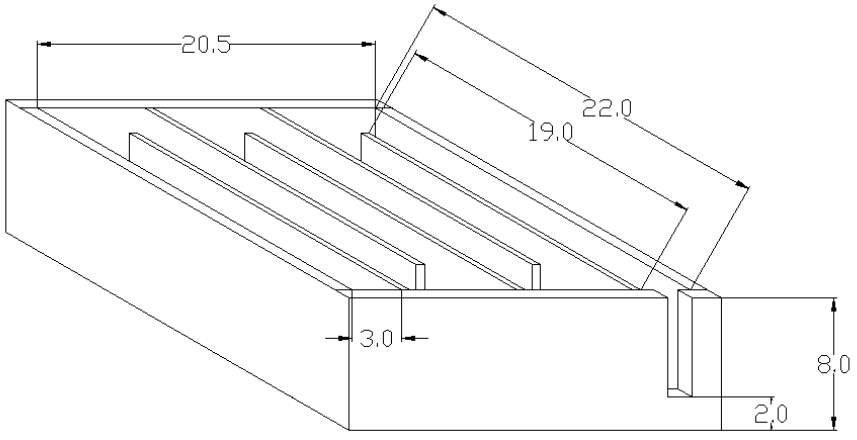


Figure 3-4. Schematic showing dimensions of the solar reactor in inches.

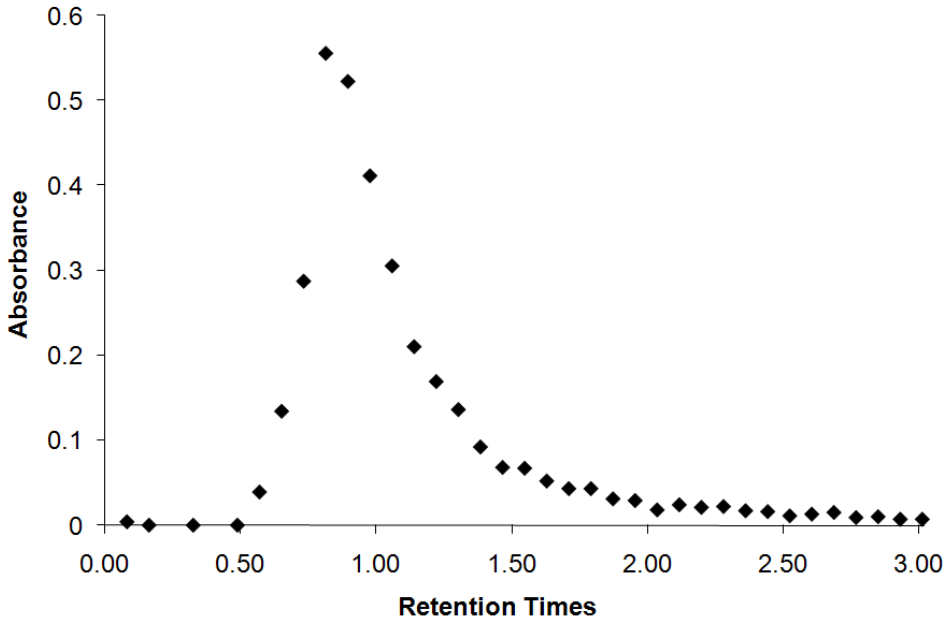


Figure 3-5. Pulse input analysis of solar reactor. Potassium permanganate was used as the tracer and the absorbance was monitored at 525 nm.

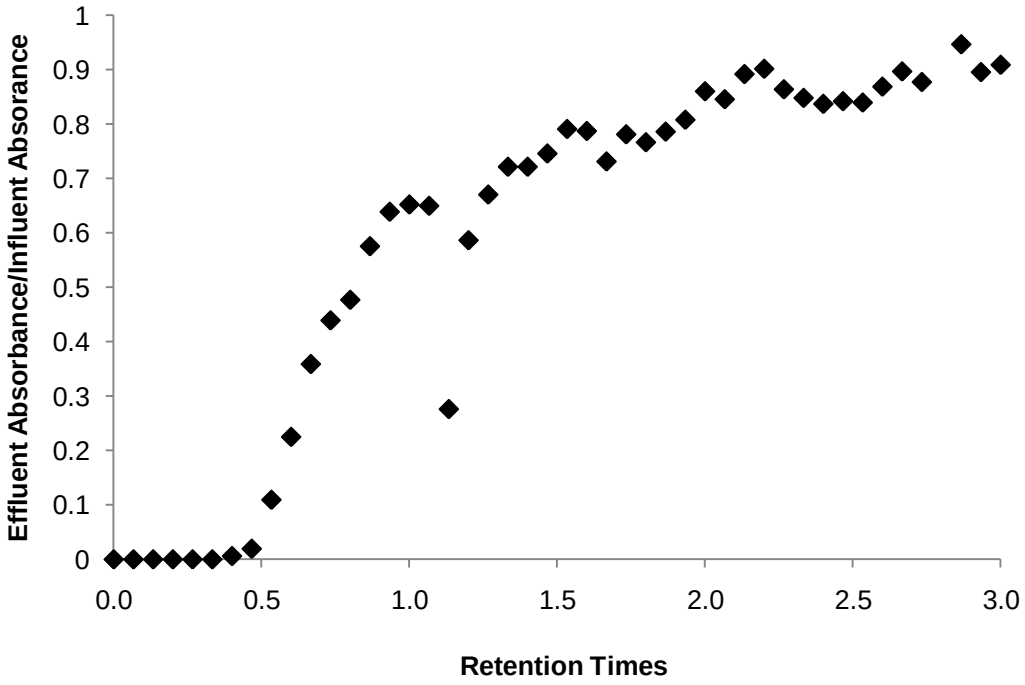


Figure 3-6. Step input analysis of solar reactor. Potassium permanganate was used as the tracer and absorbance was monitored at 525 nm.

potassium permanganate as the tracer, showed that the concentration of the compound in the effluent was 90% of that in the influent after approximately two hydraulic retention times, as shown in Figure 3-6. For the purpose of the experiments in this study, it was assumed that the reactor reached steady-state after the two reactor volumes had passed through.

Solutions were pumped through the bench scale reactor at a rate of 75 ml/min using a peristaltic pump with a Cole-Parmer (Vernon Hills, IL) Masterflex console drive and Masterflex Easy-Load II pump head and Masterflex Tygon LFL #16 tubing. The pumping rate gave a hydraulic retention time of approximately 3 hours in the solar reactor. Samples were taken every 45 minutes from the influent, middle, and effluent of the reactor. Analysis of samples was performed with HPLC as described above.

3.2.5 Solar Disinfection

On July 16, 2009 a solar reactor run testing for bacterial disinfection and removal of genes encoding for tetracycline resistance was conducted with BLE. To monitor fecal coliform concentrations, samples of the influent and effluent to the solar reactor were taken on an hourly basis during the nine hour experiment. Methods to quantify the fecal coliform concentration followed EPA standard method 9222D.

To quantify the presence of gene encoding for tetracycline resistance, samples of 100 ml were taken hourly, in duplicate from the effluent of the solar reactor. One time, 100 ml samples were also taken in duplicate from each of the three carboys containing the BLE source water. Each sample was passed through a separate 0.22 μm filter. The filter was then placed in a 1.5 ml microcentrifuge tube containing 0.5 ml of lysis buffer. DNA extractions were done using a Promega FastDNA extraction kit. The process of

quantitative polymerase chain reaction (qPCR) was performed on the samples using a thermocycler. A mastermix including SyberGreen (Applied Biosystems) was used for each PCR. qPCR was performed for the tetA, tetX, and tetO genes, as well as the 16S ribosomal RNA. A set of ten standards were used for each gene. The 16S gene copy was used to quantify the biomass contained in each samples and to normalize results.

3.2.6 Trace Analysis of TCS in BLE

One liter samples were taken from the influent and effluent of an aeration pond, at the Blue Lake WWTP in Shakopee, MN. The pond is used as a polishing treatment step for BOD removal following primary and secondary treatment. It is characterized by an eight foot depth and an 18 hour retention time during average flow conditions. The samples were taken at sunset on May 28, 2009 and June 22, 2009. After transportation to the laboratory, the samples were acidified and filtered to prevent biological activity and were placed in a refrigerator until analysis was conducted. Biological control samples were taken from the influent to the pond. These samples were filtered and acidified after sitting for 18 hours at room temperature.

Samples were extracted and concentrated using solid-phase extraction. Concentrations were quantified using liquid chromatography coupled to quadrapole mass spectrometry. For full details on the analysis procedure, see the doctoral thesis of Jeff Buth (63).

3.3 Results and Discussion

3.3.1 Indoor Photolysis

Preliminary experiments investigating the rates of photolysis of the compounds in this study were conducted in the laboratory using the solar simulator. As shown in

Figure 3-7, TCS was found to degrade the fastest from direct photolysis of all the compounds tested. TTC was found to degrade with the second fastest rate. TYS was also shown to degrade at a substantial rate when in MQW. For SMX, direct photolysis occurred at a much slower rate than for the previous three compounds, and for TMP, direct photolysis did not occur on a reasonable time scale. None of the compounds showed degradation in the dark control samples.

Photolysis experiments using the solar simulator were also conducted with the compounds of interest dissolved in BLE. These experiments examined the impact of light screening caused by the organic matter in the effluent, and also assessed the role of indirect photolysis on the compounds. Looking at Tables 3-1 and 3-2, it is apparent that for TCS, degradation was substantially slower in BLE. Conversely, for TTC and TYS the photolysis rates were nearly the same regardless of whether the compounds were in MQW or BLE. One possible explanation for this difference has to do with the absorbance spectra of these compounds. For TCS, as seen in Figures A-1 and A-2, there is very little overlap between its absorbance spectrum and the solar spectrum. At lower wavelengths, nearing 290 nm, the absorbance of BLE organic matter is strongest, as shown in Figure A-9. Thus, light screening may inhibit the degradation of TCS to a greater extent than for the other compounds. The situation is different for TTC and TYS. Both of these compounds absorb farther into the solar spectrum, as can be seen in Figures A-3 and A-4. Because SMX and TMP did not degrade substantially via direct photolysis, testing the impact of screening by BLE organic matter was not possible with the experimental set up. Briefly, indirect photolysis in BLE did not have a large

Table 3-1. First-order degradation rate constants for samples irradiated in the solar simulator, with units of hr^{-1}. Samples are dissolved in Milli-Q water.

Compound	Open	Quartz	Pyrex	Acrylic	UVT Acrylic
TTC	1.63 ± 0.02	1.48 ± 0.02	1.45 ± 0.02	0.73 ± 0.02	-
TCS	0.76 ± 0.08	0.69 ± 0.04	-	-	0.49 ± 0.02
TYS	0.30 ± 0.03	0.27 ± 0.02	0.24 ± 0.02	-	-

Table 3-2. First-order degradation rate constants for samples irradiated in the solar simulator, with units of hr^{-1}. Samples are dissolved in Blue Lake Effluent Water.

Compound	Open	Quartz	Pyrex
TTC	1.66 ± 0.07	1.60 ± 0.07	1.62 ± 0.07
TCS	0.48 ± 0.05	0.48 ± 0.02	0.37 ± 0.05
TYS	0.30 ± 0.06	0.22 ± 0.03	0.17 ± 0.05

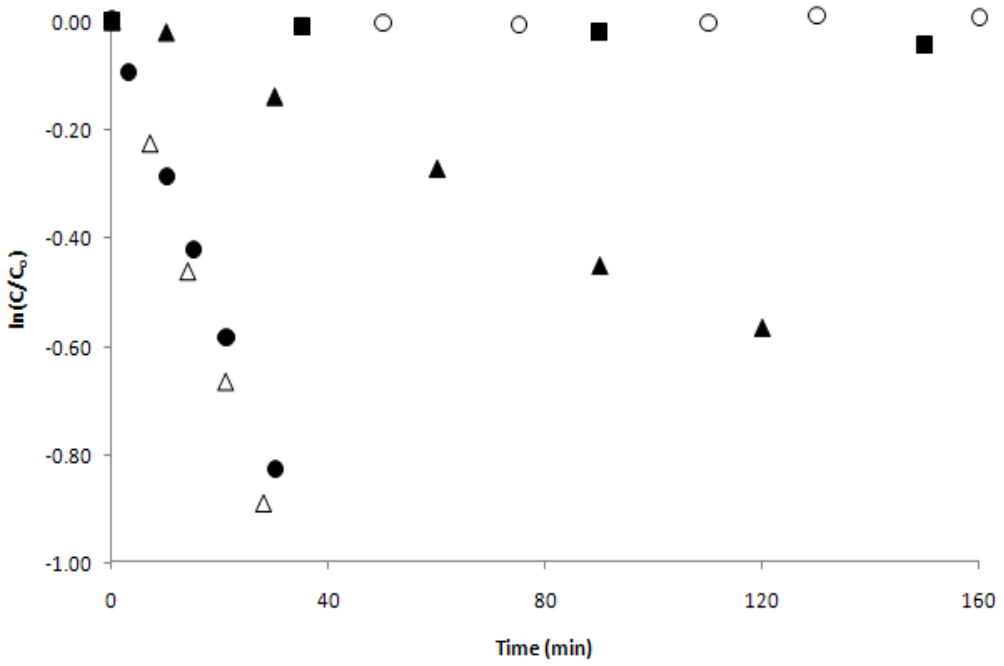


Figure 3-7. Degradation of compounds in Milli-Q water in the solar simulator with no cover material. ○ = trimethoprim, ■ = sulfamethoxazole, ▲= tylosin, ● = tetracycline, △ = triclosan.

effect on the degradation rates of TCS, TYS, TTC. For SMX and TMP, substantial indirect photolysis did occur in BLE. Details on this subject will be the topic of the following chapter.

The impact of cover materials on the photolysis rates of the compounds is summarized in Table 3-1 and 3-2. Again, because direct photolysis did not occur rapidly for SMX and TMP, quantifying the impact of the cover materials on the photolysis of these compounds was not possible with the experimental set up. In Figures 3-8 and 3-9 it is shown that the borosilicate glass and quartz glass covers did not have a substantial impact on the photolysis of TTC in either MQW or BLE. The acrylic cover, however, substantially slowed the degradation rate in BLE. For TCS, Figures 3-10 and 3-11 show that degradation rates were not largely affected by quartz glass. Borosilicate glass and UVT acrylic plates both substantially slowed the degradation rates, while acrylic plating nearly stopped degradation from occurring. Figures 3-12 and 3-13 show the effect of cover materials on the photolysis rates of TYS. Compared to the case when no cover was present, the quartz cover slowed the degradation slightly and the borosilicate glass plate slowed the degradation further. The acrylic cover almost entirely prevented TYS from degrading in the solar simulator.

The behavior of the compounds when cover materials were present had to with absorbance spectrum of the cover materials relative to the absorbance of the spectrum of the compound being examined. If a particular cover material absorbed light strongly at the same wavelengths of light that a compound absorbed light, then it greatly

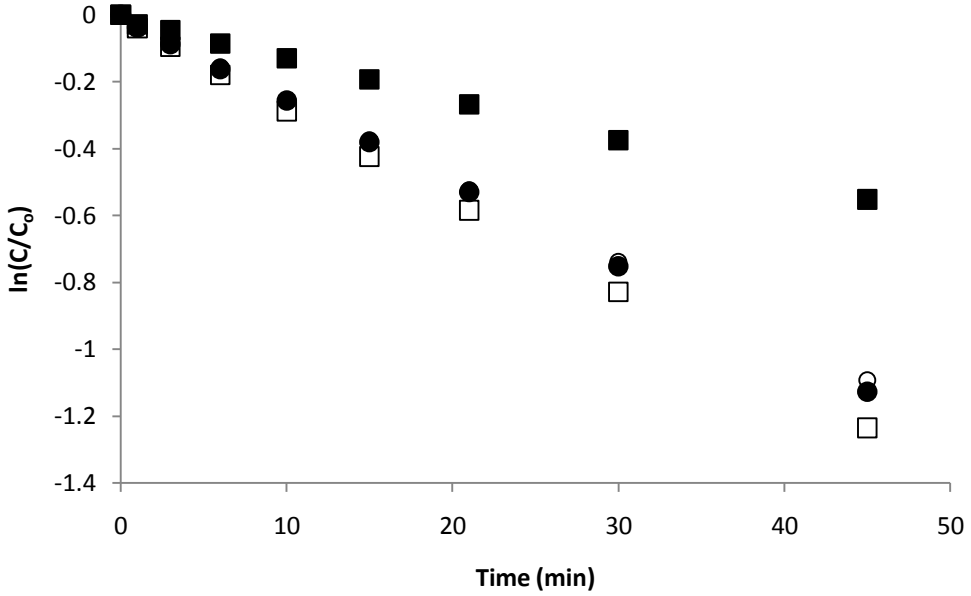


Figure 3-8. Tetracycline photolysis in Milli-Q water with various cover materials. Conditions are as follows: ■ = acrylic cover, △ = UVT acrylic, ● = quartz, □ = no cover.

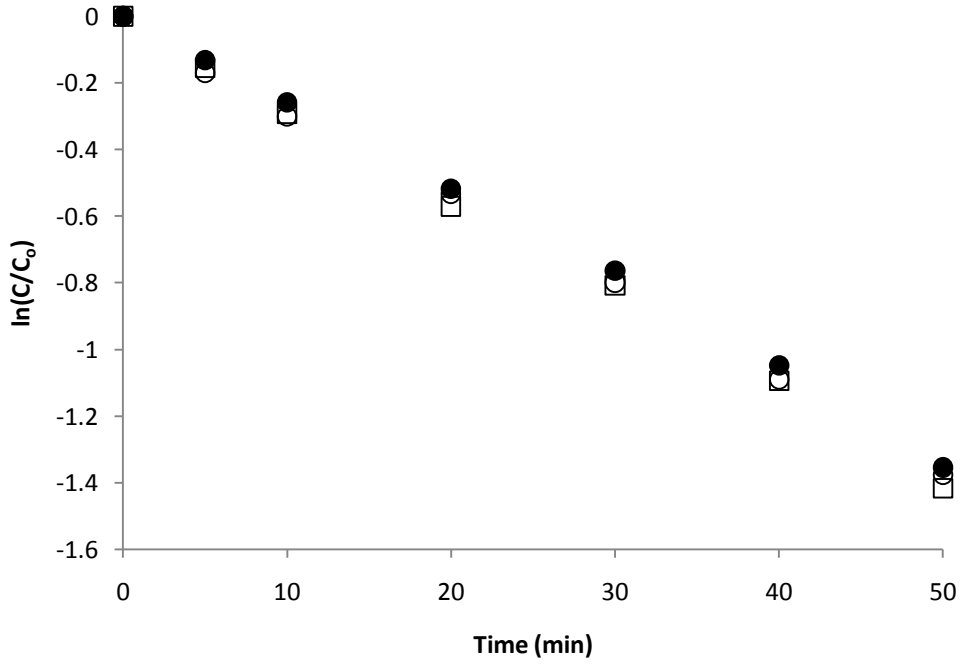


Figure 3-9. Tetracycline photolysis in Blue Lake effluent water with various cover materials. Conditions are as follows: ○ = Pyrex glass cover, ● = quartz, □ = no cover.

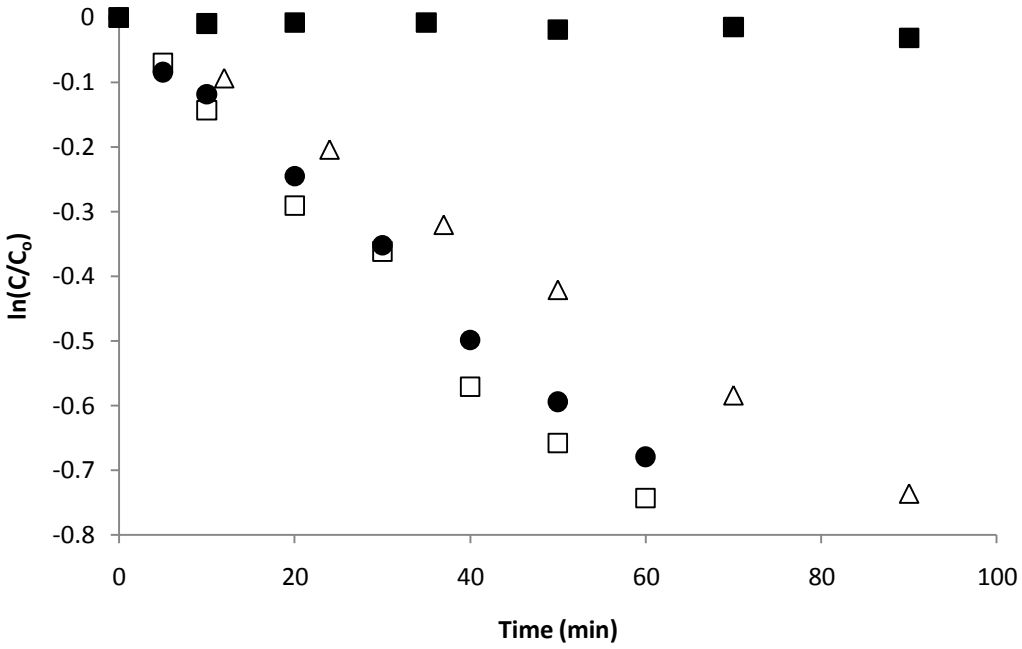


Figure 3-10. Triclosan photolysis in Milli-Q water with various cover materials. Conditions are as follows: ■ = acrylic cover, △ = UVT acrylic, ● = quartz, □ = no cover.

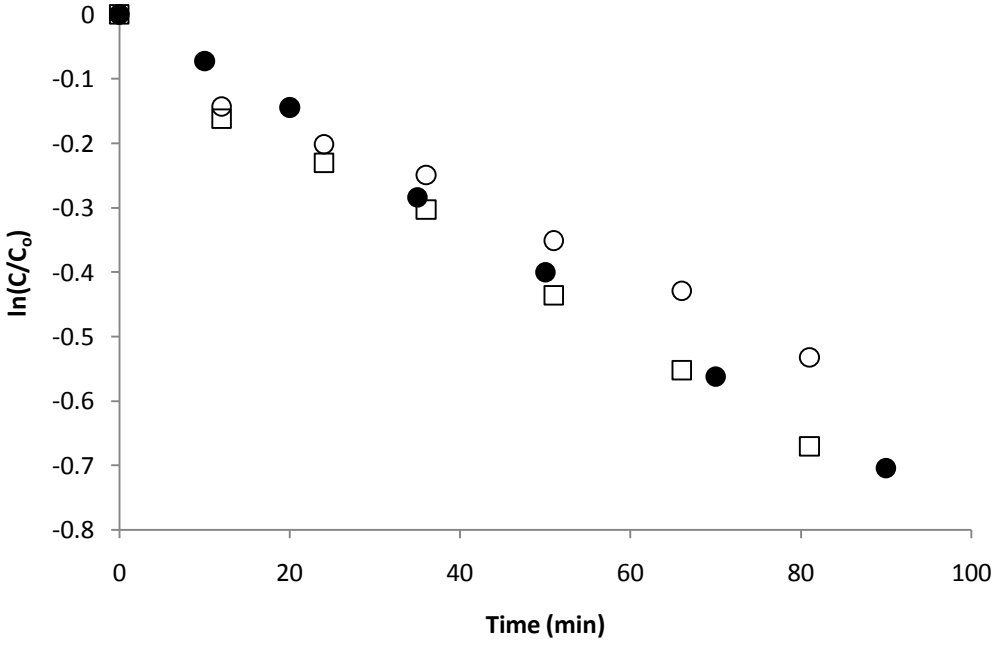


Figure 3-11. Triclosan photolysis in Blue Lake effluent water with various cover materials. Conditions are as follows: ○ = Pyrex glass cover, ● = quartz, □ = no cover.

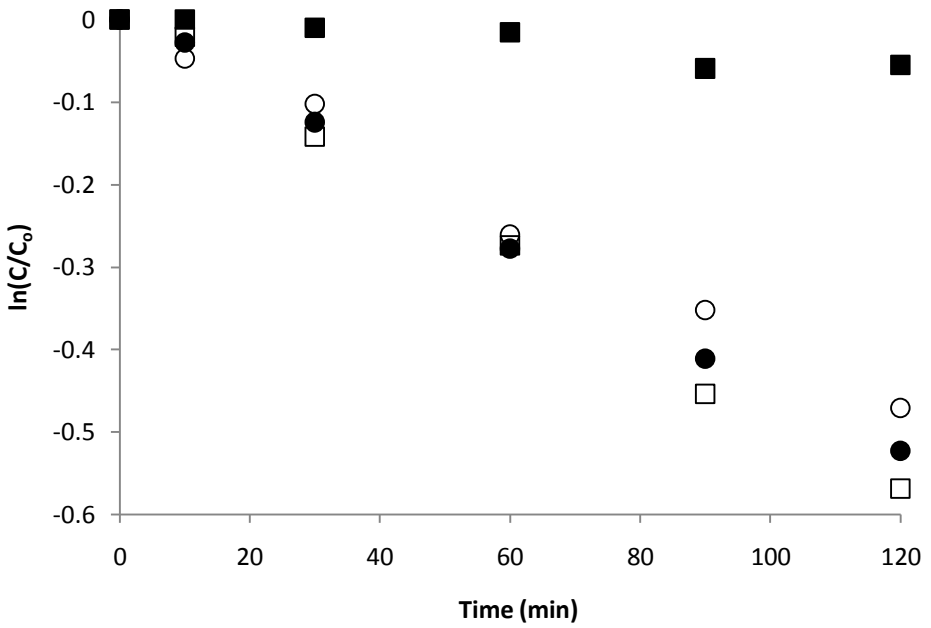


Figure 3-12. Tylosin photolysis in Milli-Q water with various cover materials. Conditions are as follows: ■ = acrylic cover, △ = UVT acrylic, ● = quartz, □ = no cover.

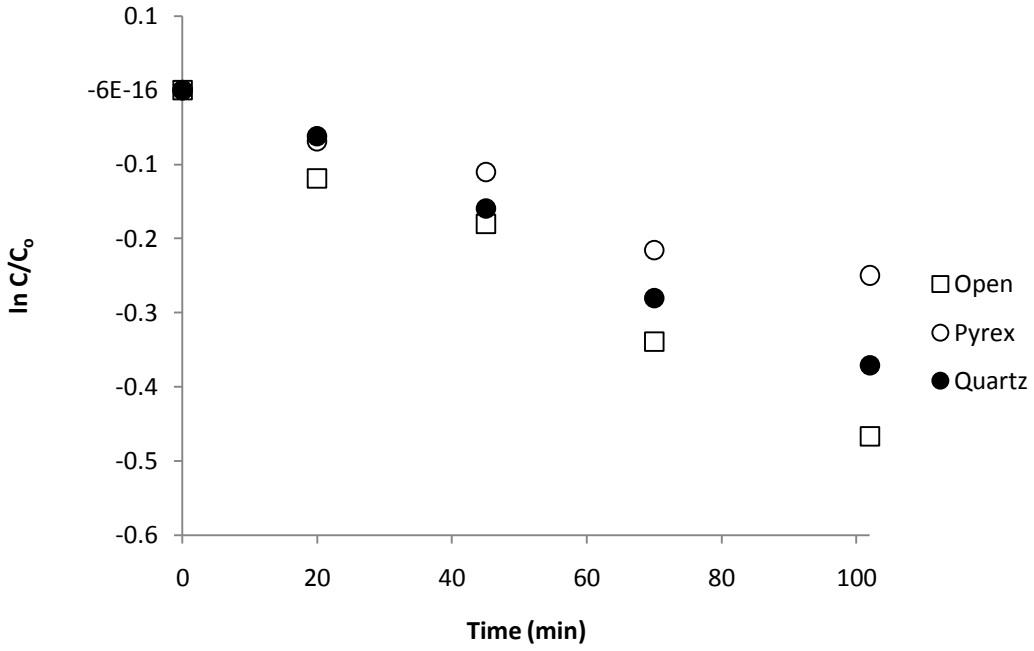


Figure 3-13. Tylosin photolysis in Blue Lake effluent water with various cover materials. Conditions are as follows: ○ = Pyrex glass cover, ● = quartz, □ = no cover.

inhibited the degradation of that compound. Borosilicate glass and quartz glass both absorb light relatively weakly over a wide range of the solar spectrum, as can be seen in Figures A-5 and A-6. As a result, these materials only slightly inhibited direct photolysis of the compounds that were examined. The acrylic material absorbs wavelengths below 375 nm very strongly as is shown in Figure A-7. These wavelengths are primarily responsible for the degradation of TCS and TYS. Thus, when an acrylic cover was placed over solutions of TCS and TYS, almost no degradation occurred. Alternatively, TTC absorbs light strongly up to 425 nm, where the acrylic material does not absorb light as strongly. Therefore, some degradation of TTC was able to take place when an acrylic cover was present. UVT acrylic is different from regular acrylic in that it strongly absorbs light only at lower wavelengths outside of the solar spectrum, as can be seen in Figure A-8. This property allowed for TCS to still degrade when a UVT acrylic cover was present, although at a slower rate because UVT acrylic does absorb some light over the solar spectrum.

3.3.2 Outdoor Photolysis

The results from the indoor photolysis experiments were used to predict the behavior of the compounds in natural sunlight. The data generated from experiments involving cover materials were also used to assess what cover material might be effective in trapping heat in a bench-scale solar reactor. The purpose of the cover material would be to increase the reactor temperature, and therefore, increase photolysis rates or bacterial inactivation rates in WWTP effluent. Based on the data presented above, TCS, TTC, and TYS should degrade quickly under natural sunlight, while SMX and TMP mostly likely will not degrade at a reasonable pace. Considering possible

covers to a solar reactor, the acrylic material would not seem to fit well, because it would greatly inhibit photolysis for all of the compounds in this study. Quartz, Pyrex, and UVT acrylic covers would all allow for photolysis to occur. For practical considerations, however, UVT acrylic would be the best cover material. This material would lose heat at a slower rate than a quartz glass or borosilicate glass cover, which would theoretically allow for higher reactor temperatures. Additionally, this material is much more durable than quartz glass or borosilicate glass, making it much easier to handle.

The solar reactor was operated to examine the effect that effluent water would have on photolysis rates of the compounds versus deionized water (DI), and also to assess whether or not adding a UVT cover to the reactor would increase or decrease photolysis rates. The first trial with the solar reactor was completed on August 26, 2008. For this run TCS, TYS, and TTC were dissolved in DI water. The solution was run through the reactor with no cover. The second reactor trial was completed on September 26, 2008, with TCS, TYS, and TTC being dissolved in BLE and with no cover. The third solar reactor run was done on June 6, 2009 and involved SMX and TMP in BLE without a cover. The fourth run occurred on June 25, 2009 and had TCS, TYS, and TTC dissolved in BLE with the UVT acrylic cover over the reactor. The fifth and final solar reactor run took place on August 10, 2009 and involved TCS, TYS, and TTC dissolved in DI, with the UVT acrylic cover on the reactor.

During each of the reactor runs involving TCS, TYS, and TTC similar behavior was observed. As is evident from Table 3-3, of the three compounds, TTC experienced the highest percentage degradation, followed by TCS and then TYS. Looking at

Table 3-3. Maximum number of degradation half-lives observed during runs with the solar reactor when comparing the effluent concentration to the influent concentration.

Compound	DI No Cover 8/26/2008	BLE No Cover 9/26/2008	BLE Cover 6/25/2009	DI Cover 8/11/2009
TYS	0.33	0.34	0.48	0.49
TCS	1.64	0.91	1.86	1.32
TTC	3.16	2.08	3.18	3.06

Figures 3-14 to 3-16, it can be seen that for all three compounds the effluent concentration gradually dropped as the day progressed. The lowest effluent concentrations occurred near 3:00 PM and the concentration in the effluent increased slightly thereafter. For TCS and TTC, it appears that a larger concentration drop occurred going from the influent to the middle of the reactor than from the middle of the reactor to the effluent. This is deceiving, however, because the compounds decayed exponentially and the vertical scale of the plots is linear. The best way to consider this issue is to realize that for each compound the concentration in the middle of the reactor is a certain fraction of the influent concentration. The effluent concentration is also that same fraction of the concentration in the middle of the reactor, but not in reference to the influent concentration.

In general, there were three phases that occurred while the reactor was operated. The first phase occurred from when the reactor was first started to when one retention time had passed. Initially, during this time period, none of the solution had received any sunlight and the concentrations at the influent, middle and effluent were the same. As time passed, the solution leaving the reactor had received more sunlight and greater degradation occurred. If the reactor behaved as a plug flow reactor, then the effluent

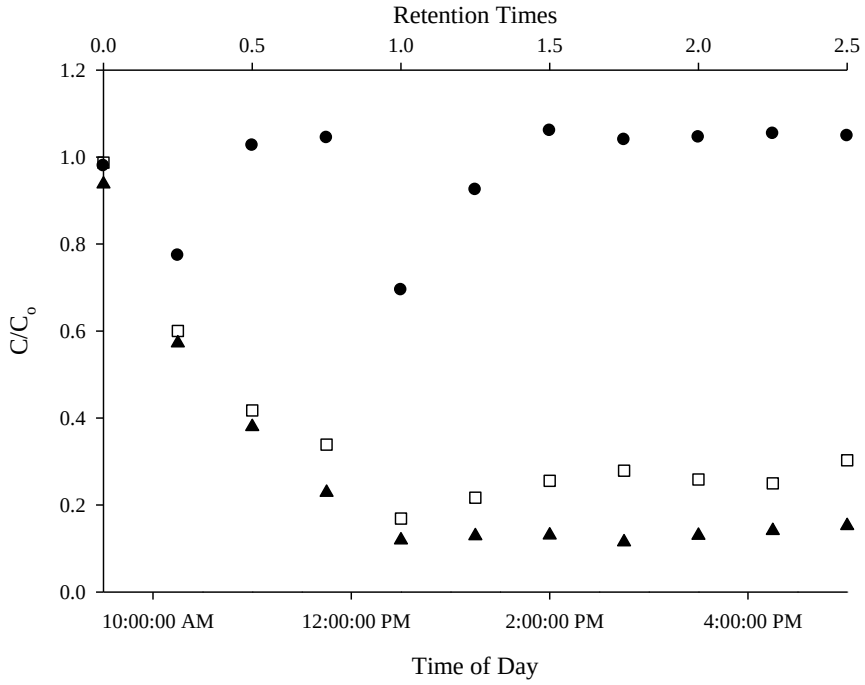


Figure 3-14. Degradation of tetracycline during a solar reactor run on August 26, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

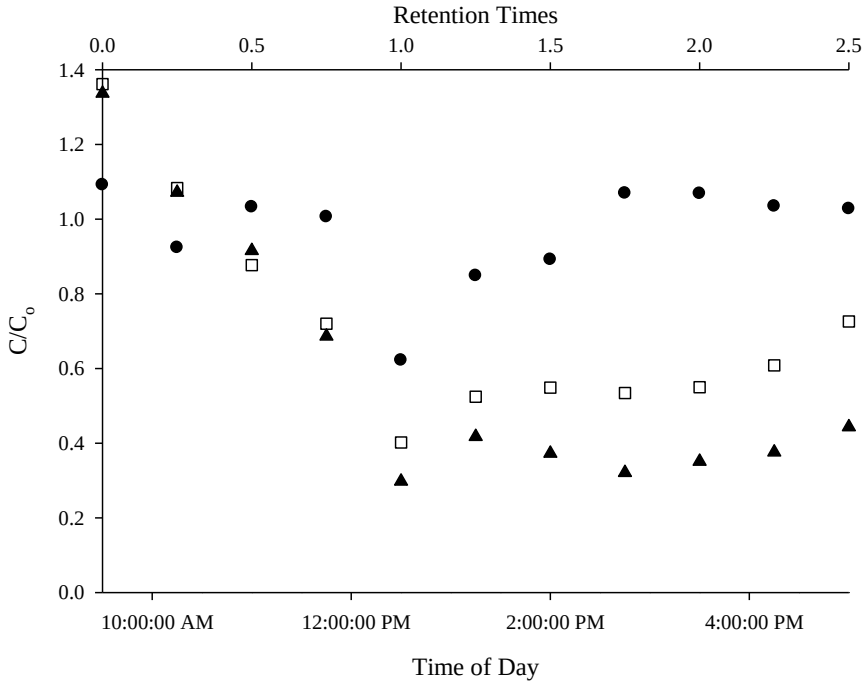


Figure 3-15. Degradation of triclosan during a solar reactor run on August 26, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

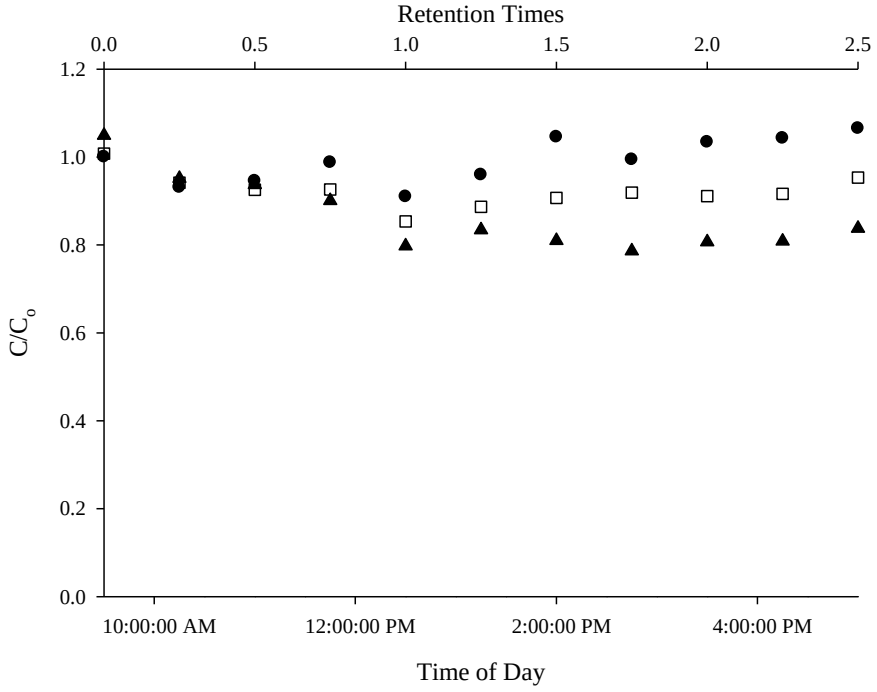


Figure 3-16. Degradation of tylosin during a solar reactor run on August 26, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

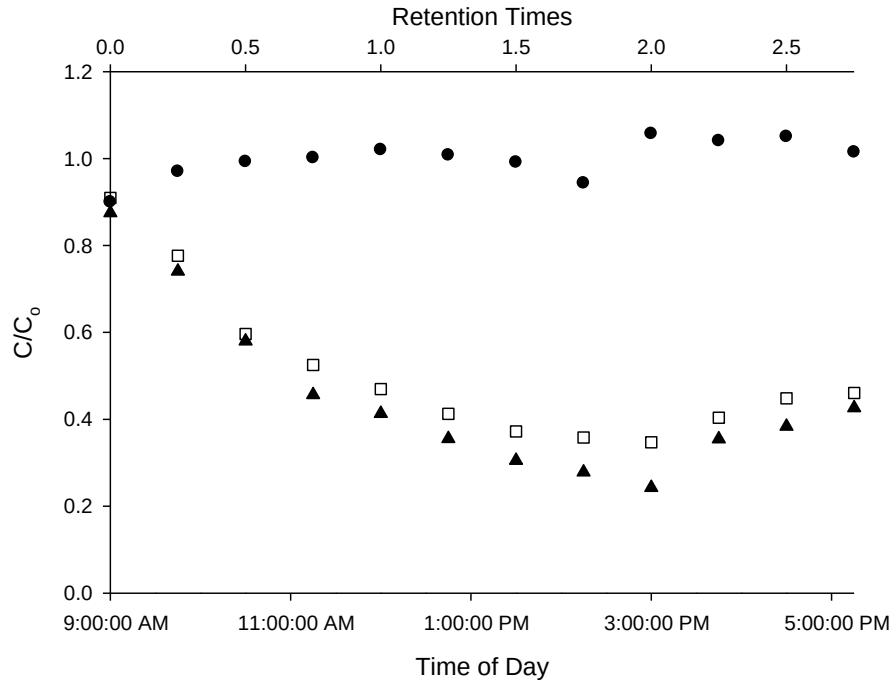


Figure 3-17. Degradation of tetracycline during a solar reactor run on September 26, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

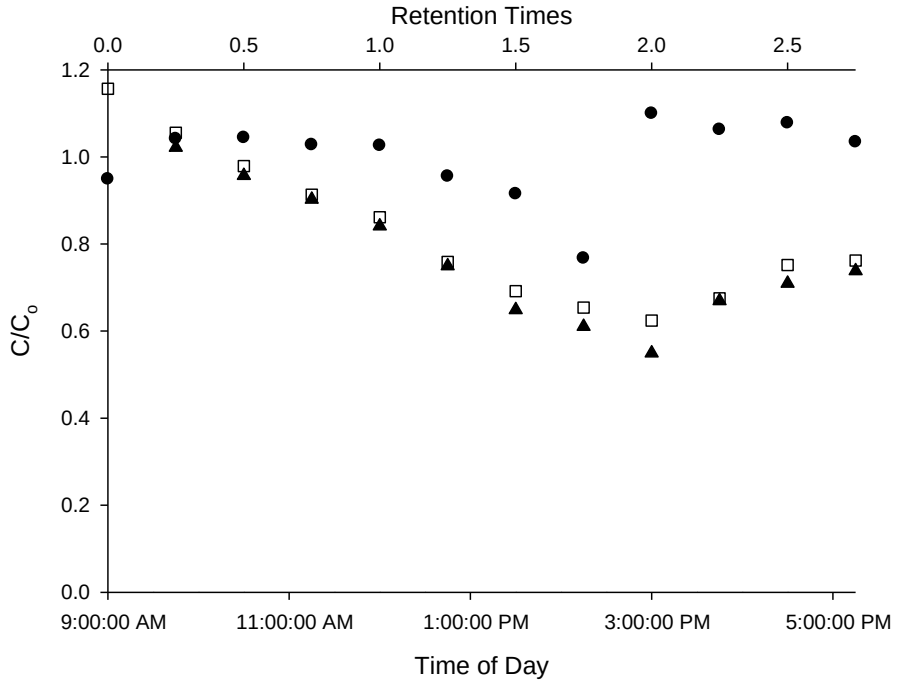


Figure 3-18. Degradation of triclosan during a solar reactor run on September 26, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

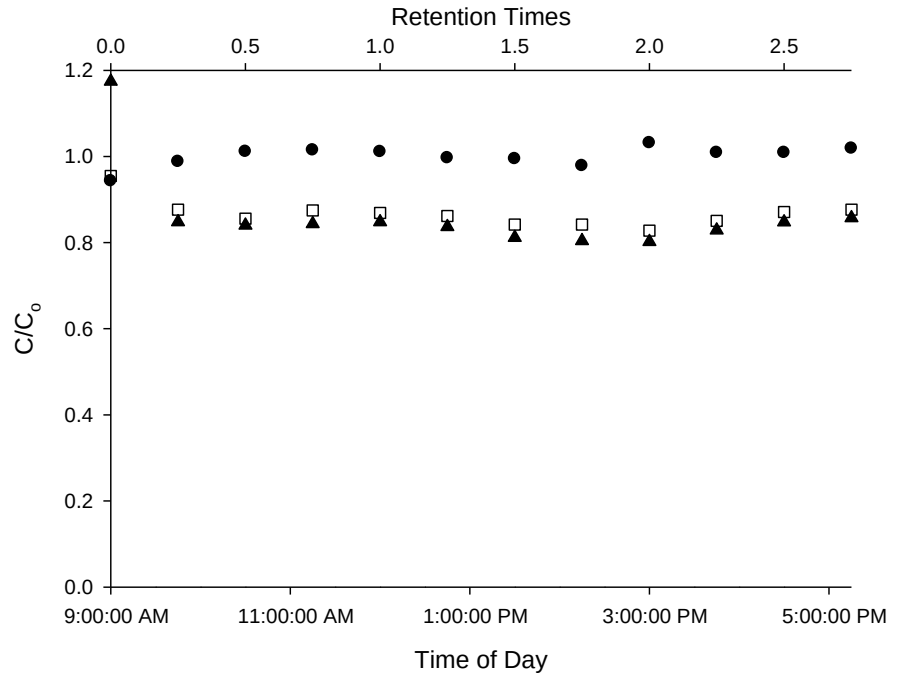


Figure 3-19. Degradation of tylosin during a solar reactor run on September 26, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

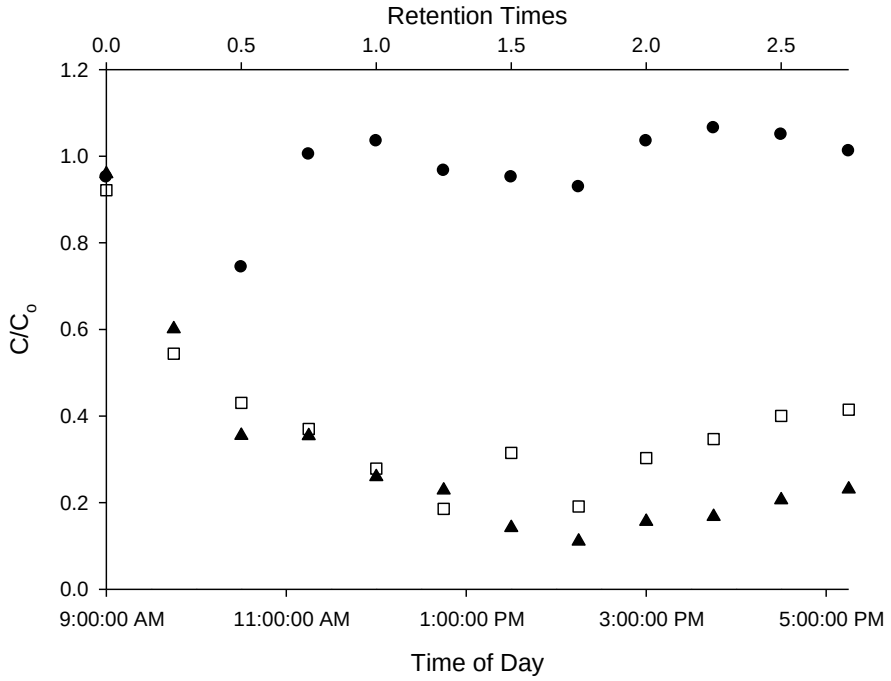


Figure 3-20. Degradation of tetracycline during a solar reactor run on June 25, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

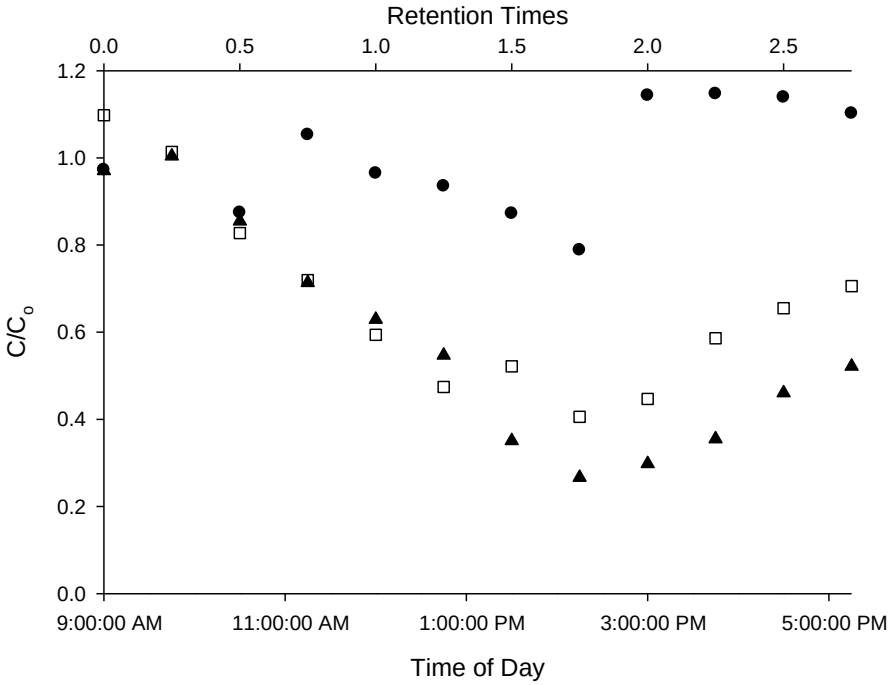


Figure 3-21. Degradation of triclosan during a solar reactor run on June 25, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

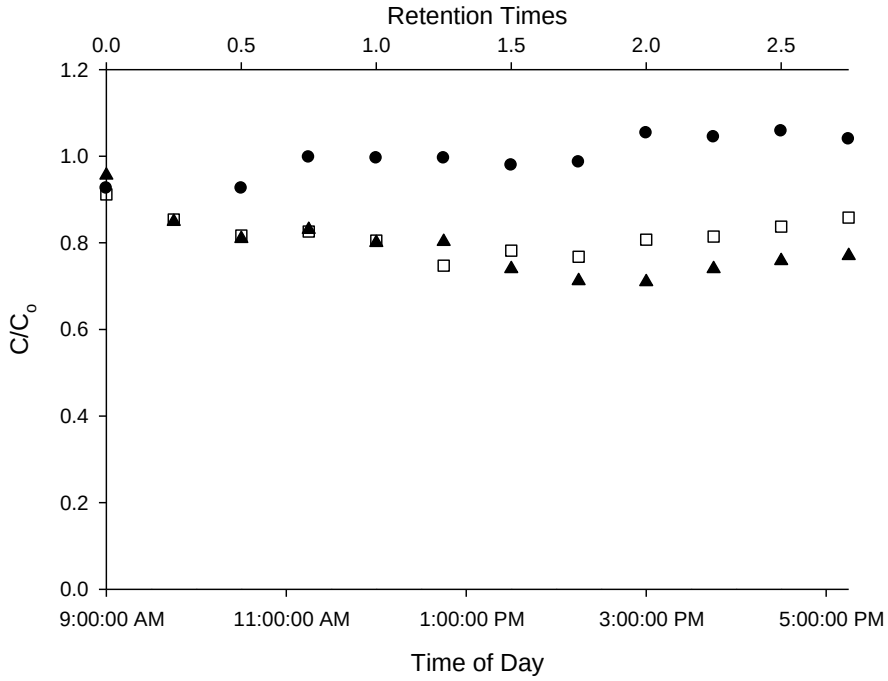


Figure 3-22. Degradation of tylosin during a solar reactor run on June 25, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

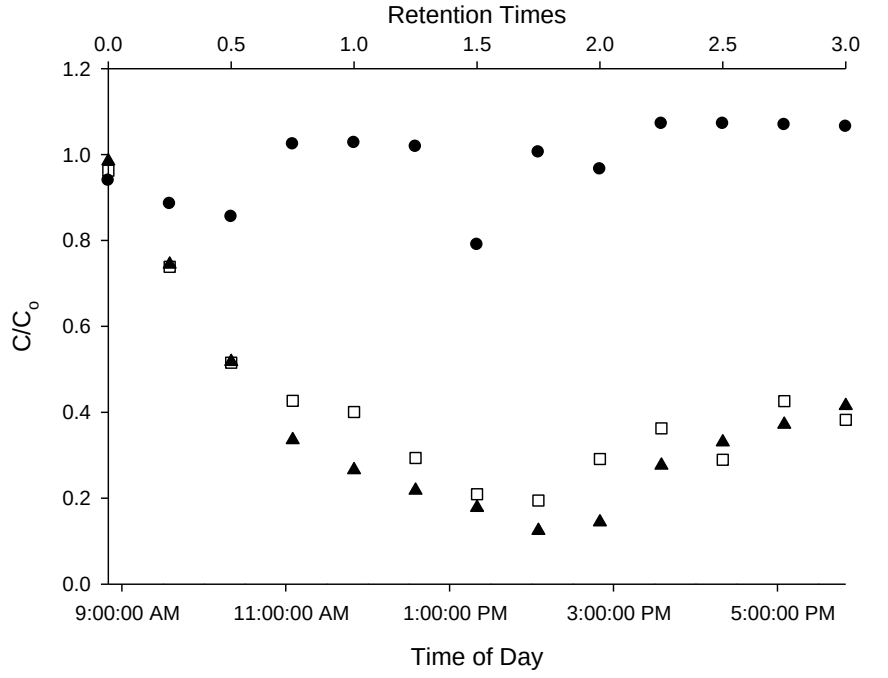


Figure 3-23. Degradation of tetracycline during a solar reactor run on August 10, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

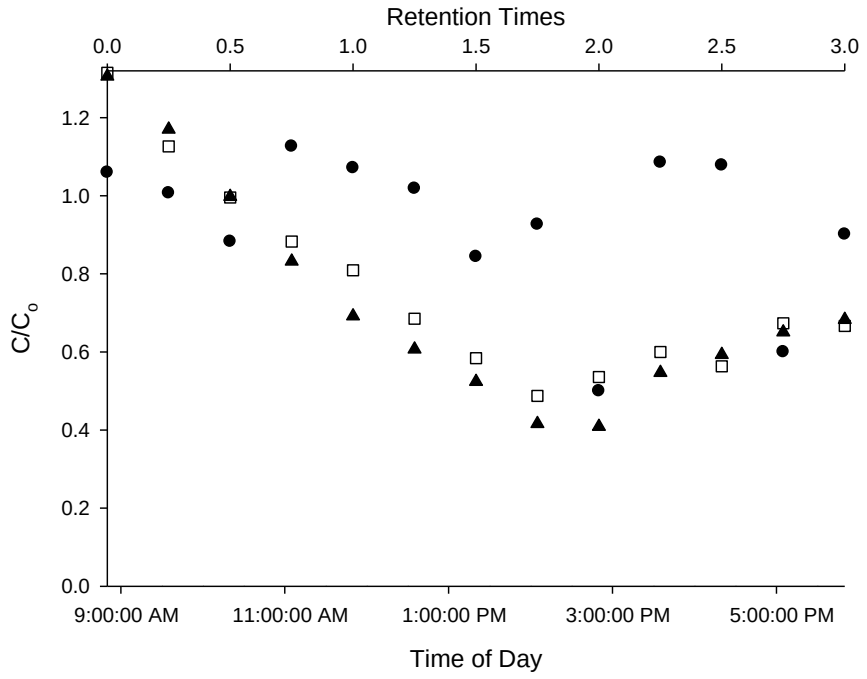


Figure 3-24. Degradation of triclosan during a solar reactor run on August 10, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

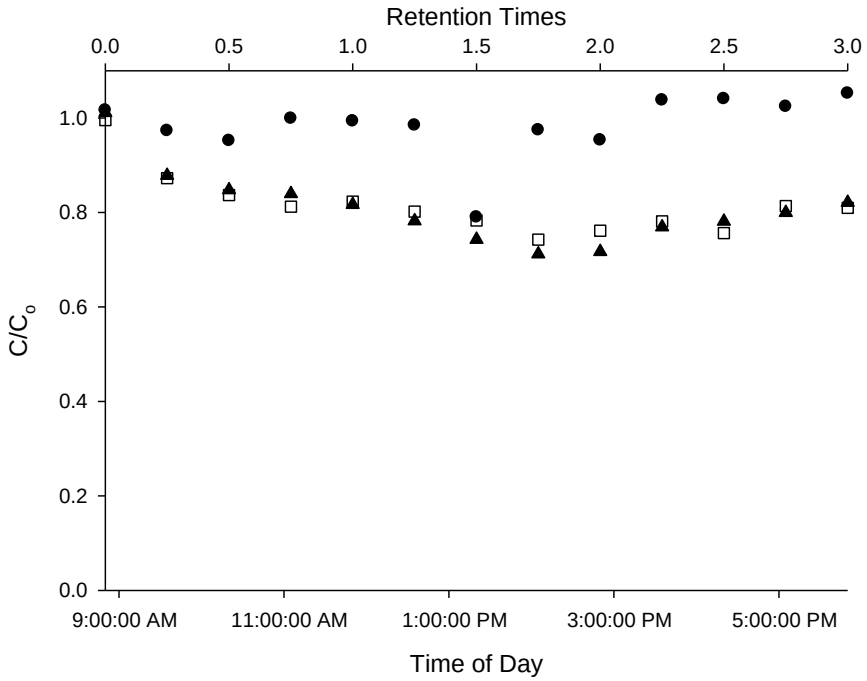


Figure 3-25. Degradation of tylosin during a solar reactor run on August 10, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

from the reactor did not receive the maximum dose of sunlight until the reactor had been operated for a full retention time. Essentially the rapid drop in the effluent concentration up until one retention time could be explained by the effluent receiving a larger dose of sunlight as time passed. In the second phase of the reactor operation, starting after one retention time, the effluent concentration continued to decrease, although at a slower pace. To understand this behavior it is necessary to consider that the reactor did not display perfect plug flow behavior, and so it was still nearing a steady-state after one retention time of operation. Additionally, as the day progressed the solution was "seeing" more and higher intensity sunlight while in the reactor. Early in the day the sun was at a lower angle to the horizon and much of the light was blocked from the solution by the reactor walls. As the sun proceeded to being directly overhead, the amount of light that was blocked decreased. Also, as the day progressed the sunlight became more intense, again causing greater compound degradation. The third phase of the reactor behavior went from when the concentration of compounds in the effluent was at its lowest and until the end of the reactor run. During this phase of operation, the effluent concentration slowly increased. The explanation for this behavior had to do with the sun getting lower in the sky and with the sunlight intensity decreasing later in the day. Effectively, the solution continually "saw" less sunlight during this phase of operation and so less degradation occurred for each compound.

During indoor photolysis experiments TCS and TTC displayed very similar degradation rates with TCS degrading slightly faster, as is shown in Figure 3-7. In all of the solar reactor experiments, however, TTC degraded to a much greater extent than did TCS. There are several possible explanations for this behavior, all of which deal

with the differences in the absorbance spectra of the two compounds. First explanation deals with a bias between the solar simulator lamp spectrum and the solar spectrum. In Figure A-1, it can been seen that these two spectra do not match perfectly. Among other differences, the spectrum of the simulator lamp reaches farther in the ultraviolet region and the wavelengths in the ultraviolet region are more intense than in natural sunlight. TCS does not absorb over a wide range of the solar spectrum, so these lower wavelengths are crucial for its degradation. Because of the simulator bias, TCS degrades at a faster pace than what would be expected for natural sunlight. The other explanations for the behavior of TCS and TTC deal with the fact that TTC degradation can be caused by a wide range of wavelengths emitted by both the simulator and the sun and had to do with the design of the solar reactor itself. The base of the solar reactor was constructed of a mirror-extruded acrylic plate. The mirrored surface was below the acrylic plate. As is evident in the test tubes experiments, acrylic material absorbs the wavelengths of light responsible for TCS degradation. Because the mirror was below the acrylic material these wavelengths did not reach the mirror to be reflected back onto the solution. The acrylic material allowed some wavelengths to pass through and reflect back onto the solution. These wavelengths caused further degradation for TTC but not of TCS.

Indoor photolysis experiments with the solar simulator showed that in BLE degradation of both TMP and SMX increased because of indirect photolysis (Chapter 4). When these drugs were tested in the solar reactor while dissolved in BLE little degradation occurred. For SMX, the maximum degradation going from the influent to the effluent was less than five percent, excluding the non-representative point at 1.25

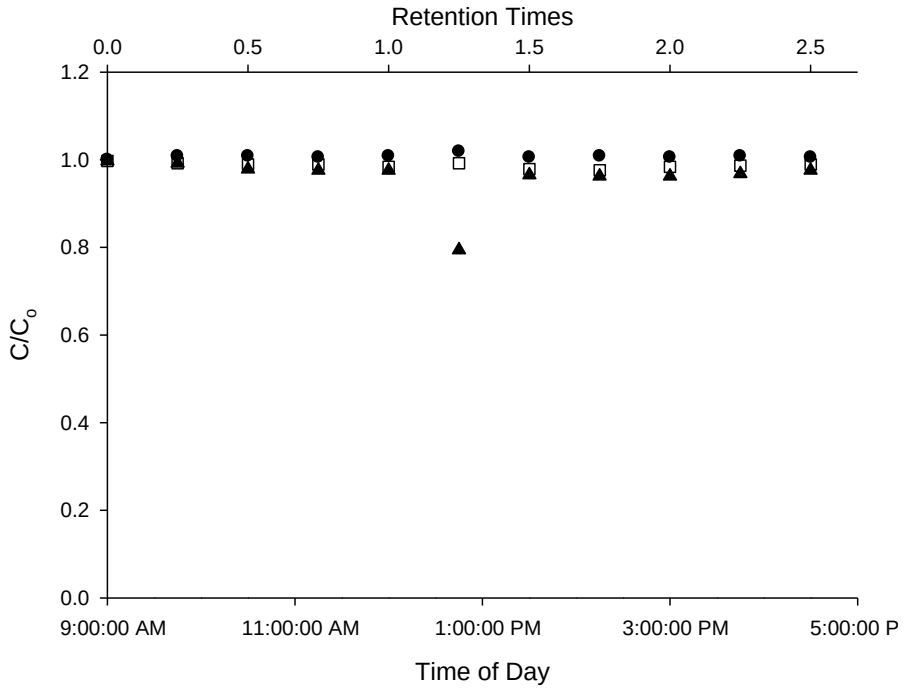


Figure 3-26. Degradation of sulfamethoxazole during a solar reactor run on June 3, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

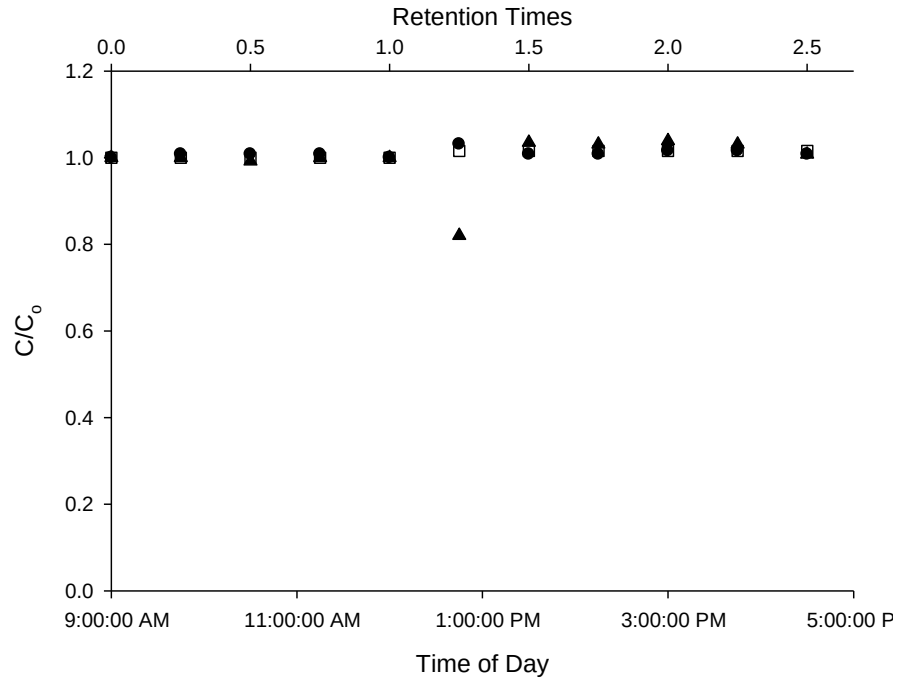


Figure 3-27. Degradation of trimethoprim during a solar reactor run on June 3, 2009. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

retention times in Figure 3-26. The plot of the TMP concentration during this run, given in Figure 3-27, shows that the influent, middle, and effluent concentrations were constant during the entire run, meaning no appreciable degradation occurred.

To compare the degradation of compounds between different days of solar reactor operation the amount of sunlight that was absorbed by each compounds was quantified for each day of the solar reactor operation. To calculate the amount of solar irradiation that was incident on the reactor during runs the sunlight intensity at 1:30 PM for each day of the reactor operation was calculated using the SMARTS computer program (64). This time of day was used because compound degradation in the effluent was found to be greatest near 3:00 PM. The sunlight intensity halfway through a retention time could be considered a good approximation of the average sunlight intensity during that retention time. Because the retention time for the reactor was three hours, using the sunlight intensity at 1:30 PM was a good approximation for the average light intensity received by a sample leaving the reactor at 3:00 PM. By using the absorbance spectra of each compound and comparing it to the intensity of the solar spectrum on each day of reactor operation the amount of solar energy per unit area of the reactor was quantified. To determine the horizontal area of the reactor that was exposed to sunlight, the maximum solar angle on each day of reactor operation and the geometry of the solar reactor were considered. To compare between the days of the reactor operation the relative amount of solar energy absorbed by each compound was normalized to the maximum value, as shown in Table 3-4. These factors were then multiplied by the values in Table 3-3 to obtain Table 3-5.

Table 3-4. Relative amount of sunlight theoretically absorbed by each compound on days of solar reactors runs without consideration of light screening.

Compound	8/26/2008	9/26/2008	6/25/2009	8/11/2009
TYS	0.74	0.47	1.00	0.85
TCS	0.69	0.39	1.00	0.82
TTC	0.82	0.61	1.00	0.90

Table 3-5. Corrected maximum number of degradation half-lives observed during runs with the solar reactor when comparing the effluent concentration to the influent concentration.

Compound	DI, No Cover 8/26/2008	BLE, No Cover 9/26/2008	BLE, Cover 6/25/2009	DI, Cover 8/11/2009
TYS	0.44	0.73	0.48	0.58
TCS	2.39	2.35	1.86	1.61
TTC	3.84	3.39	3.18	3.41

When the degradation of compounds across different days of reactor operation was compared and normalized to the amount of sunlight available, patterns emerged. TCS degradation was greater for trials without the cover over the reactor than for when it was in place. This observation is not unexpected, as the UVT acrylic plate was shown to inhibit TCS degradation during indoor photolysis experiments. The maximum degradation for TYS also occurred during one of the reactor trials with no cover present. The minimum amount of degradation, however, also occurred on one of these days of operation. A pattern for TYS degradation, therefore, is less evident, although it would be expected that the UVT acrylic cover would considerably inhibit TYS degradation. TTC degradation was greatest for the trial without a cover and with a DI solution running through the reactor. TTC degradation was the least for the trial with a cover and a BLE solution. The differences between the trials experiencing the greatest

and least amount of degradation were less than those observed to TCS and TYS. These results seem reasonable as both the cover and BLE would absorb a small amount of light across the entire absorbance spectrum of TTC. Also, the cover and effluent absorb more light at lower wavelengths which are crucial for TCS and TYS degradation, but not for TTC degradation.

3.3.3 Disinfection

The effect that the reactor had on bacterial communities in BLE was also examined. A solar reactor trial was conduct on July 16, 2009 using BLE without any chemical compounds added. Both the influent and effluent of the reactor were monitored for fecal coliform counts and for tetracycline resistant gene concentrations.

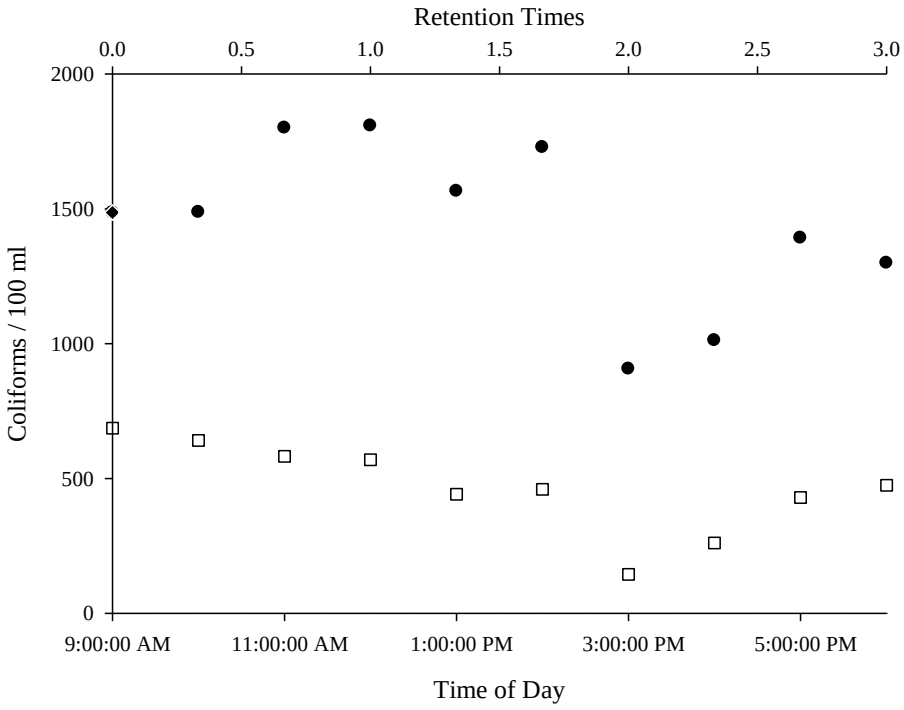


Figure 3-28. Monitoring the coliform concentration present in Blue Lake effluent (BLE) water during a solar reactor run conducted on July 16, 2009. No cover was placed over the reactor and no compounds were in the BLE. Conditions are as follows: ● = influent concentration, □ = effluent.

The results for the fecal coliform data are given in Figure 3-28. It is evident that there was solar disinfection taking place during the reactor run, although not to a great extent. A pattern does emerge in the data, however. As with the reactor runs involving the compound degradation discussed above, the bacterial disinfection increased during the first part of the day, reached a maximum near 3:00 PM, and then slowly decreased as the day progressed. For tetracycline resistant genes, there was no observable pattern to the data as is evident in Figure 3-29. This observation indicates that sunlight does not eliminate the tetracycline resistant genes, even while the bacterial numbers decrease.

3.3.4 TCS Degradation in an Effluent Holding Pond

To examine the effectiveness of using a holding pond to allow sunlight to degrade PPCPs the Blue Lake WWTP was studied. This plant uses an aeration pond to further treat the effluent following primary and secondary treatment. Samples were

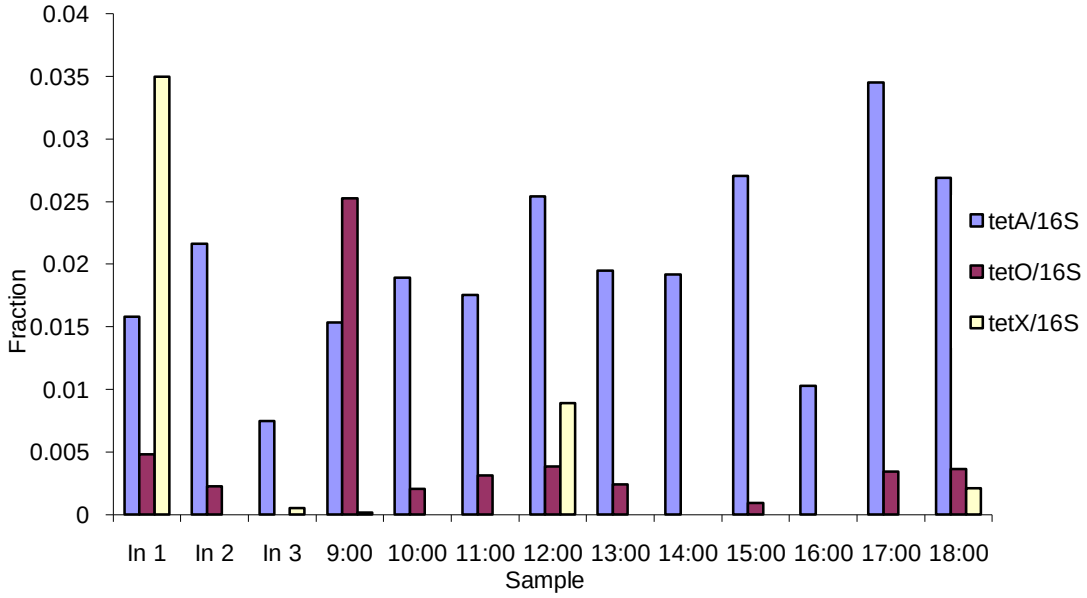


Figure 3-29. Presence of tetracycline resistant genes in the influent and effluent of the solar reactor. The trial was conducted on July 16, 2009. The presence of tetA, tetO, and tetX genes were quantified and normalized using the 16S ribosomal RNA gene as a surrogate for biomass concentration.

taken at sunset from the influent and effluent of the pond, so that the effluent samples would have received a full day worth of sunlight. The TCS concentration in the samples was quantified. The first samples were taken on May 28, 2009. The concentration in the influent to the pond, using duplicate samples, was found to be 75 ng/L, with a standard deviation of 2 ng/L. The effluent from the pond was found to have a TCS concentration of 53 ng/L and a standard deviation of 1 ng/L. Assuming a constant TCS input, these results suggest a 29% drop in the TCS concentration occurred during the sampling day. The biological control samples from this day had a concentration of 70 ng/L with a standard deviation of 1 ng/L, suggesting TCS was not being degraded biologically. The second set of samples were taken on June 22, 2009. The influent TCS concentration was found to be 90 ng/L, with a standard deviation of 2 ng/L. The effluent concentration was found to be 49 ng/L, with a standard deviation of less than 1 ng/L. These values suggest a 57% drop in the TCS concentration during the sampling day. The single biological control sample gave a TCS concentration of 166 ng/L, again suggesting that no biological degradation occurred. Degradation in the pond was less than what was found for the solar reactor. This can be explained by the water depths in each situation and light screening. Light screening by DOM is not important for the two inch water depth that was used during solar reactor runs. The depth of the Blue Lake pond was six feet. At this depth, light screening becomes important, and likely eliminates photolysis one foot below the surface or deeper. As a result, photolysis in the pond would be expected to be slower than what was found during solar reactor runs.

The findings of this chapter show that sunlight is effective at eliminating some compounds that are present in WWTP effluent. These compounds, which include TTC, TCS, and TYS, must react quickly under sunlight for degradation to be substantial on a reasonable timescale. Other compounds, such as TMP and SMX, will not experience substantial degradation under natural sunlight. Other methods will need to be used to remove these compounds from wastewater treatment effluent. Solar disinfection was shown to occur in BLE. The amount of disinfection that occurred, however, was not substantial (less than 90% removal). No degradation of tetracycline resistance genes was shown to occur during operation of the solar reactor.

Chapter 4:

Enhanced Photolysis of Sulfamethoxazole and Trimethoprim in Wastewater Treatment Plant Effluent Waters Compared to Natural Surface Waters

4.1 Introduction

Sulfamethoxazole (SMX) and trimethoprim (TMP) are two human-use antibiotic compounds that are often prescribed together to treat various bacterial infections under the trade names of Bactrim, Septra, and Sulfatrim. Sulfamethoxazole belongs to the sulfonamide classes of antibacterial compounds, while trimethoprim does not belong to any specific class of compounds. These two compounds inhibit bacterial growth by working together to prevent folic acid production, which is essential to DNA synthesis. Humans obtain folic acid from the food they ingest, while bacteria must synthesize this compound. This difference allows the drugs to target bacterial cells while not affecting human cells. SMX and TMP block folic acid production under two different mechanisms and can inhibit bacterial growth each on their own. When they are used together, however, they are effective against a much wider range of bacterial species and have a decreased potential for bacterial resistance to be developed than when either of the drugs are used alone (65).

Drugs that are ingested by humans are not entirely metabolized by the digestive system and pass into the sanitary sewer system. At wastewater treatment plants, some portion of the drugs entering the plants are degraded, but a large portion may pass through, either sorbed to the waste solids or dissolved in the liquid effluent (2-5). As these waste streams enter the environment, they can contaminate a wide range of environmental systems with the compounds (6, 32, 66). Concerns about SMX and TMP

are related to the potential for antibiotic resistance to be developed to this drug combination because of its widespread use. Since these compounds first began being used in combination in 1968, the frequency of bacterial isolates showing resistance to the combination has gradually increased (67). Besides resistance developed through normal use, concerns exist about resistance developing due to bacteria being exposed to the drugs at low concentrations in the environment.

When SMX and TMP reach environmental systems, there are multiple routes for their possible removal, including photodegradation, biodegradation, and partitioning to sediments. Focusing on photodegradation, previous work has found SMX to degrade by direct photolysis. These studies, however, did not find indirect photolysis to be an important process in natural waters (18, 20). Indirect photolysis was found to be important for SMX in one study that showed adding nitrate or humic acids to solutions increased the degradation rates above what was observed for distilled water solutions (17). Photodegradation has been found to be relatively less important for TMP, with studies showing little degradation taking place under environmentally relevant conditions (22, 68).

The goal of this study was to examine various aspects of the direct and indirect photolysis of SMX and TMP. The photodegradation taking place in ultrapure water, natural water, and wastewater treatment plant (WWTP) effluent was compared. By using chemicals with known photochemical behavior, work was done to uncover the importance of direct and indirect photolysis for SMX and TMP in each of these waters with differing compositions.

4.2 Materials and Methods

4.2.1 Materials

SMX (98%), TMP (98%), 4-chlorobenzoic acid (99%), perinaphthenone (97%), 2'-acetonaphthone (99%), furfuryl alcohol (99%), and 2-propanol (IPA) (99.5%) were purchased from Sigma-Aldrich (Milwaukee, WI). Sodium azide (99%) was purchased from Janessen Chimica (New Brunswick, NJ). Isoprene (99%) and 3'-methoxyacetophenone (98%) were purchased from Acros Organics (Morris Plains, NJ). All solvents were high-performance liquid chromatography (HPLC) grade. Chemicals were used as received.

4.2.2 Instrumental Analysis

Concentrations were quantified using an Agilent Technologies (Santa Clara, CA) 1200 Series HPLC equipped with a photo-diode array detector. All compounds were analyzed on a Supelco (Park Bellefonte, PA, USA) Ascentis RP Amide 150 mm × 4.6 mm, 5 μm column. For SMX and TMP a methanol:pH 3 phosphate-buffer gradient method was used, starting at 20:80, changing to 50:50 over one minute, and then held at 50:50 for four minutes. The mobile phase flow rate was 1 ml/min and the detection wavelength was 274 nm. Analysis for furfuryl alcohol was completed with an acetonitrile:pH 3 phosphate buffer gradient method initially at 35:65, going to 20:80 over three minutes, and then held at 20:80 for 2 minutes. The flow rate was 1 ml/min and the detection wavelength was 200 nm. Analysis for 4-chlorobenzoic acid used an isocratic 75:25 methanol:pH 3 phosphate buffer mobile, phase at a flow rate of 1 ml/min, with a detection wavelength of 240 nm.

4.2.3 Photolysis

Indoor photolysis was conducted using a Suntest CPS+ solar simulator with a UV-Suprax optical filter (Atlas Materials Testing Solutions, Chicago, IL, USA) with the light intensity set at 765 W/m^2. Samples were held in quartz test tubes (o.d. = 1.3 cm, i.d. = 1.1 cm, V = 10 ml) set at an angle of 30° from horizontal. Tubes were filled with approximately 7 ml of solutions of varying compositions. Deoxygenated samples had nitrogen or argon gas bubbled through them for 5 minutes and were subsequently capped and sealed. As subsamples were taken from the sealed tubes, the appropriate gas, either nitrogen or argon, was injected into the headspace of the vials to replace the lost volume. Each compound was tested for nonphotolytic degradation by place control samples in the dark during the photolysis experiments.

Experiments investigating indirect photolysis used dilute solutions of either SMX or TMP at an initial concentration of approximately 1 µM in pH 8 phosphate buffered Milli-Q water (MQW), BLE water (Shakopee, MN, DOC=7.49 mg/L, NO_3^- =16.5 mg/L as N, pH=8.0), Lake Josephine water (LJW) (Roseville, MN, DOC=6.03 mg/L, NO_3^-= 0.4 mg/L as N, pH=8.0), or Metro wastewater treatment plant effluent water (MPE) (St. Paul, MN, DOC=8.12 mg/L, NO_3^-=11.8 mg/L as N, pH=8.0). Chemical additives were also used. This included solutions of 1% isopropyl alcohol (a radical scavenger) in both MQW and BLE, and solutions of sodium azide (a singlet oxygen quencher) at a concentration of 1.5 mM. Perinaphthenone, 2'-acetonaphthone, and 3'-methoxyacetophenone (triplet sensitizers) were used in MQW, at a concentration of approximately 1 µM. Isoprene (triplet scavenger), at a concentration of 0.1 %, was added to BLE containing 1 µM SMX. The involvement of a triplet-excited state in the

degradation of TMP was also examined using 40 µM TMP and 2 µM furfuryl alcohol in Milli-Q water solutions. The degradation of both TMP and furfuryl alcohol were monitored and compared in solutions that were oxygenated with pure O_2 gas, deoxygenated with pure N_2 gas, or left at standard conditions.

Experiments quantifying the interaction of SMX and TMP with hydroxyl radicals were conducted outdoors on August 3, 2009 in Minneapolis, MN, USA (~45° N latitude). This experiment involved comparing the degradation rates of TMP, SMX, and 4-chlorobenzoic acid in MQW, BLE, and solutions containing 1 mM potassium nitrate and 1 mg/L octanol (69). 4-chlorobenzoic acid was used to as a hydroxyl radical probe to quantify hydroxyl radical steady-state concentrations. Nitrate was added to generate hydroxyl radicals and octanol was added to scavenge for the radicals to create a stable steady-state hydroxyl radical concentration. The information from the experiments was used to determine the fraction of indirect photolysis of SMX and TMP that was attributable to interaction with hydroxyl radicals.

4.3 Results and Discussion

4.3.1 Indirect Photolysis of Sulfamethoxazole

SMX was found to degrade slowly in MQW. When present in BLE, the rate of photodegradation of SMX increased substantially, indicating that indirect photolysis was an important loss process for this compound in BLE, as shown in Figure 4-1. Interestingly, Boreen et. al. 2004 (18) found that indirect photolysis of SMX did not occur in natural waters. The finding that indirect photolysis occurred in BLE suggests the possibility that effluent waters from wastewater treatment plants possess a unique

property compared to natural waters and that this property may promote the indirect photolysis of some compounds.

To investigate this possibility the mechanism of indirect photolysis for SMX in BLE needed further study. The first experiments repeated the experiments conducted by Boreen et al. 2004. The results were similar to those observed by Boreen et. al., with the degradation rates in LJW and Minnesota River water being statistically the same as with MQW. The first-order degradation rate constants were 0.40 ± 0.02 hr^{-1} in MQW, 0.42 ± 0.02 hr^{-1} in LJW and 0.43 ± 0.01 hr^{-1} in Minnesota River water. These observations again show that indirect photolysis is an insignificant process in natural waters. When solutions of SMX were exposed to the same conditions in WWTP effluents, the degradation rate increased to 0.71 ± 0.12 hr^{-1} for BLE and 0.60 ± 0.06 hr^{-1} for MPE, which demonstrated that indirect photolysis was important in these waters. To elucidate the specific species responsible for the indirect photolysis, solutions of

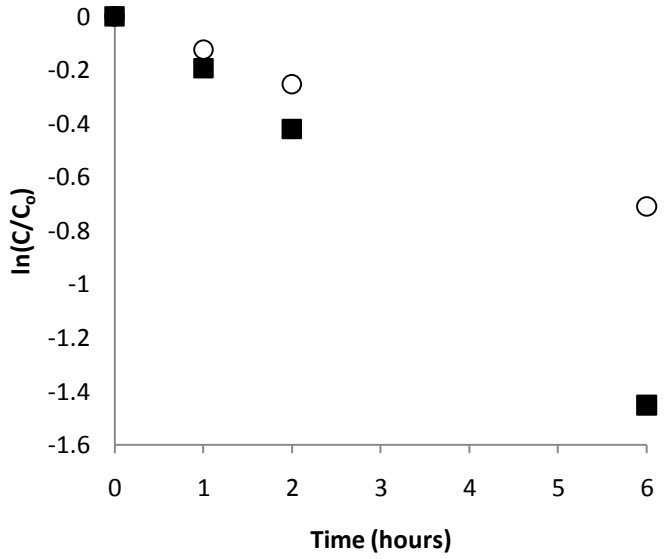


Figure 4-1. Photolysis of sulfamethoxazole. Conditions are as follows: ■ = substrate in effluent from the Blue Lake wastewater treatment plant, ○ = Milli-Q water.

SMX were exposed to various chemicals and conditions while under light from the solar simulator. Adding IPA, a known radical quencher, with SMX in MQW gave a rate constant of 0.40 ± 0.01 hr^{-1}, and in BLE the rate constant was 0.43 ± 0.02 hr^{-1}. IPA appeared to not affect the direct photolysis of SMX as the degradation rates in solutions of MQW are statistically the same whether or not IPA is present. The SMX degradation rate in BLE decreased so that it was statistically the same as for the MQW solution. This observation points to indirect photolysis being reduced by IPA and infers that hydroxyl radicals are important for the indirect photolysis of SMX. Adding azide, a singlet oxygen quencher, gave rate constants of 0.43 ± 0.02 hr^{-1} in MQW and 0.38 ± 0.03 hr^{-1} in BLE. Azide did not seem to affect direct photolysis, as the degradation rates again are the same in MQW with or without this compound being present. When

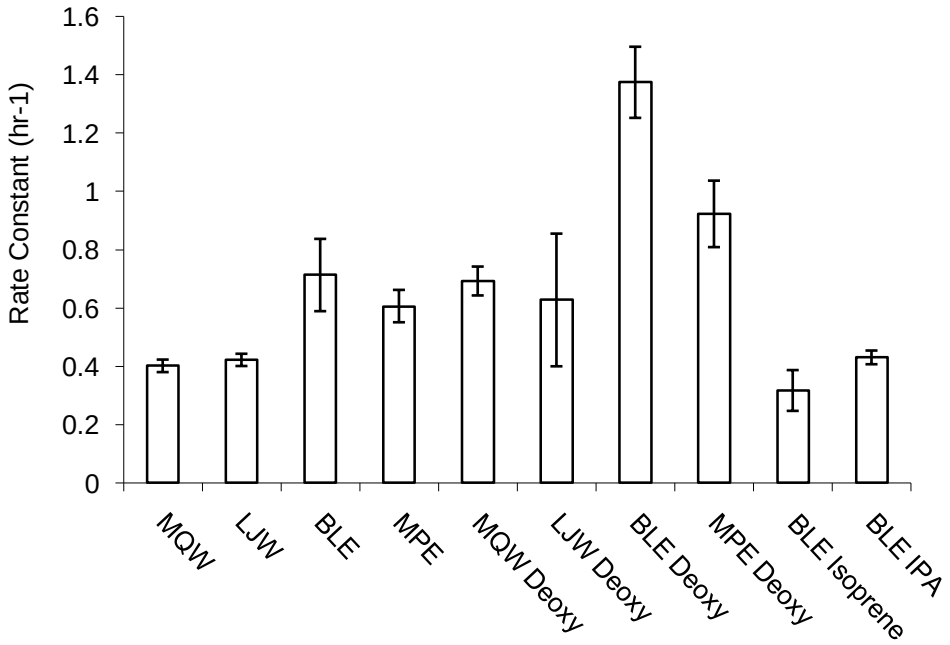


Figure 4-2. First-order rate constant for loss of sulfamethoxazole via photolysis in various waters. Error bars indicate the 95% confidence intervals based on fitting the data to a first-order decay model.

the BLE samples were spiked with the azide ion, however, the degradation rate decreased to match that found in MQW, indicating a possible involvement of singlet oxygen in the indirect photolysis of SMX. Azide, however, is also known to react with hydroxyl radicals. The addition of perinaphthenone, a triplet sensitizer, dramatically increased the degradation rate in MQW to 45 ± 12 hr^{-1}. Adding isoprene, a triplet scavenger, decreased the degradation rate in BLE to 0.32 ± 0.07 hr^{-1}. These results taken together provide strong evidence for the involvement of triplet-excited species in the indirect photolysis of SMX. When solutions of SMX were deoxygenated by having nitrogen or argon gas bubbled through them for five minutes, degradation rates increased. The degradation rates in the deoxygenated solutions were 0.69 ± 0.05 hr^{-1} for MQW, 1.38 ± 0.12 hr^{-1} for BLE, 0.92 ± 0.11 hr^{-1} for MPE, and 0.63 ± 0.23 hr^{-1} for LJW.

Deoxygenating solutions can affect multiple reactive species. When dissolved oxygen is absent, the route of degradation by singlet oxygen is removed. If singlet oxygen were the principal reactive species responsible for the indirect photolysis, deoxygenating samples would dramatically decrease degradation rates. This was not observed. Dissolved oxygen also acts as a quencher of triplet-excited states. Removing dissolved oxygen will promote degradation routes that involve triplet-excited species. Because the results show degradation increases when oxygen was removed from all of the solutions, further evidence is provided for the involvement of triplet-excited species in the degradation of SMX.

The data presented in this study point to triplet-excited states and hydroxyl radicals being important for the indirect photolysis of SMX in WWTP effluent. A schematic for the hypothesized pathway for the degradation is given in Figure 4-3. For direct photolysis the proposed route proceeds as follows, absorbed sunlight excites SMX to a singlet-excited state, which intersystem crosses to a triplet-excited state. From this point the molecule either breaks apart to form products or the dissolved oxygen present in the solution quenches the triplet-excited state, returning the molecule to its ground state. This hypothesis is consistent with the observation that the rate of photodegradation increases when dissolved oxygen is removed from MQW solutions. There are two important degradation pathways for indirect photolysis. The first involves DOM that is excited to a triplet-state by sunlight. The excited DOM can interact with SMX to bring it directly to a triplet-excited state. Again, the excited molecule can then be quenched by dissolved oxygen, causing it to return to the ground state, or it can proceed to break apart to form products. The second pathway involves

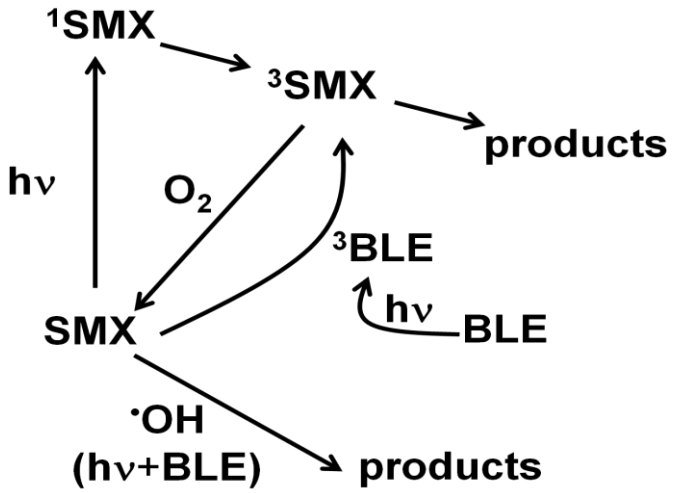


Figure 4-3. Proposed reaction pathway for the direct and indirect photolysis of sulfamethoxazole in effluent from the Blue Lake wastewater treatment plant.

hydroxyl radicals that are produced by sunlight interacting with dissolved species, principally nitrate, in the effluent. The hydroxyl radicals can react directly with the SMX by adding a hydroxyl group or by abstracting a hydrogen atom from the molecule.

4.3.2 Indirect Photolysis of Trimethoprim

In MQW water, TMP does not experience significant degradation when exposed to light from the solar simulator, indicating direct photolysis does not occur to an appreciable extent for this compound. As shown in Figure 4-4, when TMP is dissolved in BLE, degradation takes place, which can be attributed to indirect photolysis.

To investigate the processes by which indirect photolysis occurs, experiments were conducted with TMP that were similar to the experiments performed with SMX. The results of these experiments are displayed in Figure 4-5. In the solar simulator, when TMP was dissolved in BLE the degradation rate constant was 0.19 ± 0.02 hr^{-1}, in

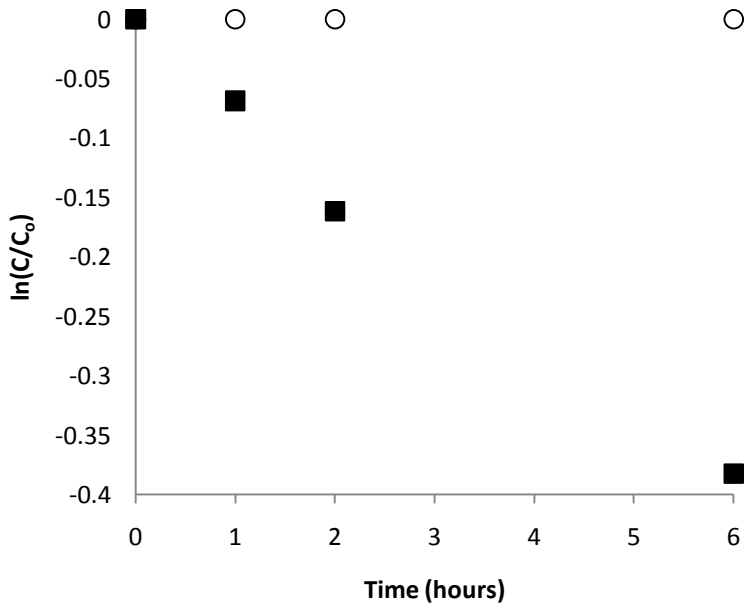


Figure 4-4. Photolysis of trimethoprim. Conditions are as follows: ■ = substrate in effluent from the Blue Lake wastewater treatment plant, ○ = Milli-Q water.

MQW no degradation occurred, and in LJW the degradation rate was 0.05 ± 0.02 hr^{-1}. The degradation in the LJW indicates TMP degrades by indirect photolysis in some natural waters. The degradation rate in BLE, however, is approximately three times greater than in LJW, indicating that the effluent water more readily promotes indirect photolysis. Deoxygenating the BLE and MQW solutions increased the degradation rates to 0.23 ± 0.02 hr^{-1} and 0.37 ± 0.03 hr^{-1} respectively. As with SMX, this gives evidence for triplet-excited states being important for the photodegradation of TMP. Adding perinaphthenone to a MQW solution produced a degradation rate constant of 1.2 ± 0.2 hr^{-1}, also pointing to the involvement of triplet-excited states. Other triplet

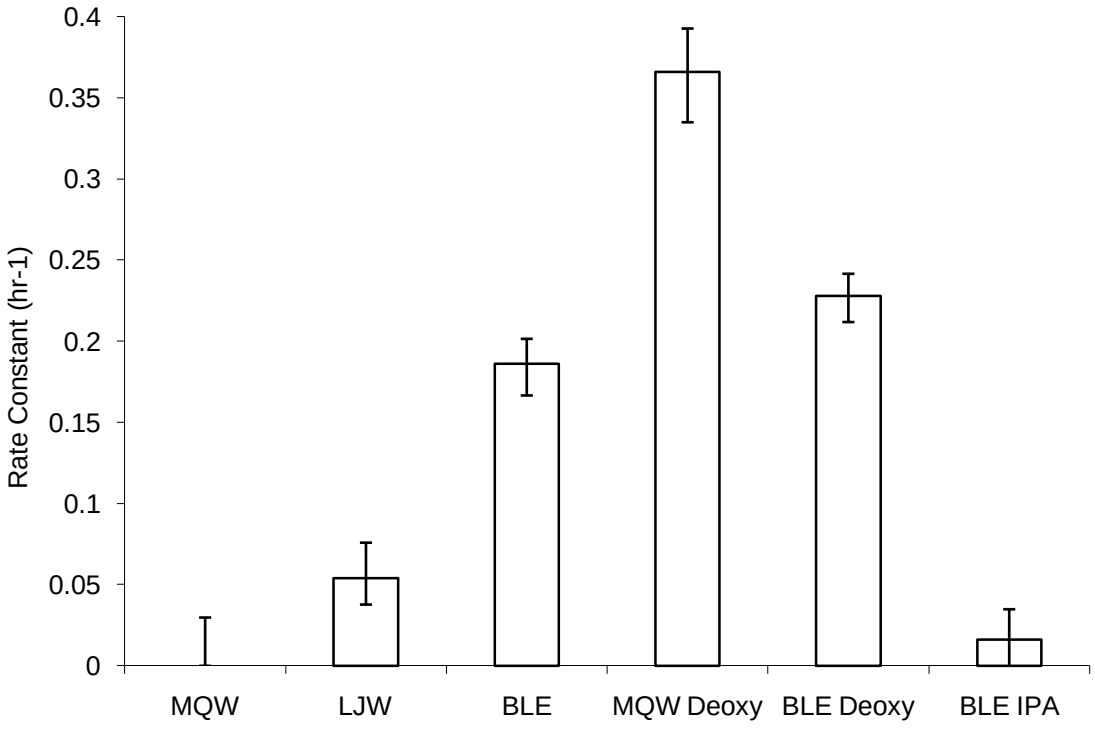


Figure 4-5. First-order rate constant for loss of trimethoprim via photolysis in various waters. Error bars indicate 95% confidence intervals based on fitting the data to a first-order decay model.

sensitizers in MQW solutions, specifically 2'-acetonaphthone and 3'-methoxyacetophenone, also caused degradation to occur with rate constants of 0.023 ± 0.007 hr^{-1} and 0.12 ± 0.01hr^{-1} respectively. With perinaphthenone present in solution, TMP degradation took place at a much faster rate than when 2'-acetonaphthone or 3'-methoxyacetophenone were present, which indicates that the triplet energy of TMP is near the triplet energy of perinaphthenone. When azide was added to BLE, no TMP degradation took place in the solar simulator, indicating singlet oxygen may cause TMP destruction. When solutions were deoxygenated, however, the degradation rate of TMP increased, which gives evidence that singlet oxygen is of minor importance for TMP. When IPA was added to BLE solutions, no TMP degradation occurred. This observation provides evidence for the involvement of radical species in the degradation of TMP.

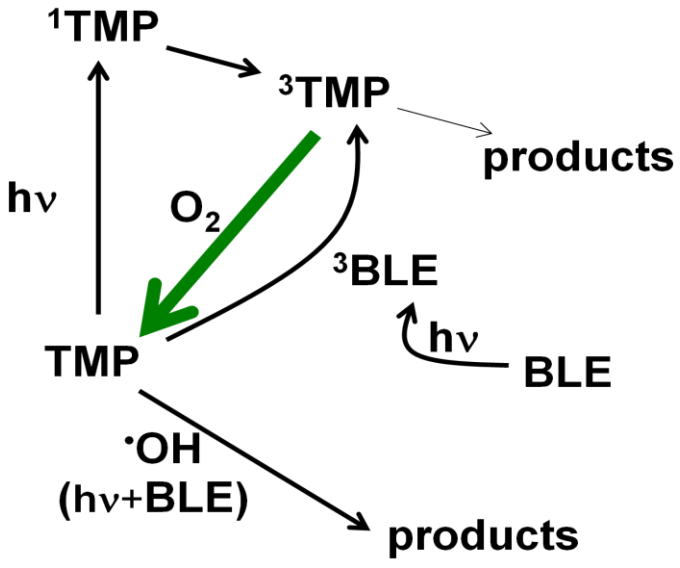


Figure 4-6. Proposed reaction pathway for the direct and indirect photolysis of trimethoprim in effluent from the Blue Lake wastewater treatment plant.

The experiments conducted in the solar simulator point to hydroxyl radicals and triplet-excited states being involved in the degradation. The proposed pathway for TMP degradation is shown in Figure 4-6. In a manner similar to SMX, it is believed that sunlight is absorbed by TMP and excites it to a singlet-excited state, which then inter-system crosses to a triplet-excited state. For MQW solutions, no TMP degradation occurred in the solar simulator. When the MQW solution was deoxygenated, however, substantial degradation occurred by direct photolysis. This observation suggests that when dissolved oxygen is present it is able to quench the triplet-excited TMP effectively enough so that no appreciable direct photodegradation can occur. The degradation of furfuryl alcohol, a chemical known to degrade only from singlet oxygen and direct photolysis (70), was investigated in solutions with and without TMP present. Singlet oxygen would be generated if triplet-states of TMP were being quenched by dissolved oxygen and would lead to furfuryl alcohol degradation. In solutions with TMP present, furfuryl alcohol degraded faster than in solutions without TMP present. The difference, however, could not be determined to be statistically significant. Indirect photolysis was observed in the BLE and MPE solutions. Again, based on the previously mentioned experiments two pathways are predicted for indirect photolysis. One involving triplet-excited DOM, which would interact with TMP to promote it to a triplet-excite state efficiently enough so that it could degrade, and the other involving hydroxyl radicals, which would interact directly with TMP by adding to the molecule or abstracting a hydrogen atom from the molecule.

4.3.3 Predicted Contribution of Excited Species in Indirect Photolysis

To determine the extent to which each photolysis pathway occurs in natural systems, it is necessary to determine the steady-state concentration of hydroxyl radicals produced in the effluent solution under natural sunlight. To do this, 4-chlorobenzoic acid was used to find the steady-state hydroxyl radical concentration in BLE under sunlight. 4-chlorobenzoic acid degrades exclusively by interaction with hydroxyl radicals, with a known second order rate constant of 5×10^{9} $M^{-1}s^{-1}$ (71). When exposed to natural sunlight in a BLE solution, the compound was found to degrade with a first-order rate constant of 7.3×10^{-6} s^{-1}. By dividing the observed first-order rate constant by the second-order rate constant for interaction with hydroxyl radicals, the steady state hydroxyl radical concentration in BLE is determined to be 1.5×10^{-15} M, which is higher than normally reported for natural waters (72).

For SMX, Boreen et al. found the compound to react with hydroxyl radicals with second-order rate constant of 5.8×10^{9} $M^{-1}s^{-1}$ (18). Multiplying this rate constant by the steady-state hydroxyl rate constant found for BLE, gives a pseudo first-order reaction rate constant of 8.5×10^{-6} s^{-1}, which would account for 36% of the degradation of SMX. Direct photolysis of SMX was found to proceed at a rate constant of 1.1×10^{-5} s^{-1} in MQW, which should be the same in BLE, assuming the effect of screening by DOM is negligible. Using this rate, direct photolysis would then account for 48% of the degradation of SMX in BLE. It would be assumed then that triplet-excited DOM is responsible for the rest of SMX degradation. This would result in a pseudo first-order rate constant of 3.7×10^{-6} s^{-1} for the reaction mediated by triplet-excited states, which accounts for 16% of the degradation.

TMP was found by Dodd et al (73) to a have second-order reaction rate constant with hydroxyl radicals of 6.9×10^{9} $M^{-1}s^{-1}$. Multiplying this value by the steady-state hydroxyl radical concentration gives a pseudo first-order rate constant of 1.0×10^{-5} s^{-1} for the hydroxyl radical contribution to TMP degradation in BLE, which accounts for 62% of the observed degradation. A slight amount of direct photolysis was observed to occur for TMP under natural sunlight during the outdoor experiments. The first-order rate constant in the MQW solution was found to be 2.8×10^{-6} s^{-1}, which would account for 18% of the degradation observed in the BLE solution. The remaining 20% of the degradation can be attributed to TMP reacting with triplet-excited DOM with a pseudo first-order rate constant of 3.3×10^{-6} s^{-1}.

The photolysis of SMX and TMP was found to take place both by direct and indirect photolysis in effluent from the Blue Lake WWTP. Triplet-excited states of each compound were found to be involved in the direct photolysis pathway. These excited-states were effectively quenched by dissolved oxygen. Hydroxyl radicals and triplet-excited DOM were both found to contribute to the indirect photolysis of both SMX and TMP. Indirect photolysis of SMX only occurred in the effluent water samples and not in the natural water samples used in this study. For TMP, indirect photolysis was much more rapid in effluent waters than in natural waters. The differences in the photochemical behavior of the drugs in each of the water samples is most likely attributable to unique properties of the effluent waters, including higher nitrate levels and, possibly, DOM of different reactivity than that present in natural waters. The difference between the chemistry of DOM in natural waters and DOM in WWTP effluent may arise from their sources. The source of DOM in natural waters is

often organic material, such as tree leaves, that have been washed into the water bodies. Effluent DOM is generated by human waste that has been microbially processed, first, during digestion in humans and, second, during the wastewater treatment process.

The findings of this study indicate that photolysis in WWTP effluent waters may be a more important loss process than expected. When considering how to remove compounds like SMX and TMP from the municipal waste stream, the use of sunlight may be an important tool. In situations where effluent waters are concentrated and exposed to sunlight, such as in holding ponds following the treatment process or in streams whose flow is dominated by WWTP effluent, the removal of some PPCPs could be greatly accelerated. In these instances, sunlight could be used a cheap and easily accessible tool for treating certain PPCPs.

Chapter 5:

Degradation of Three Antibiotic Compounds and Triclosan During Anaerobic and Aerobic Sludge Digestion

5.1 Introduction

Pharmaceuticals and personal care products (PPCPs) are an emerging class of pollutants, which are made up of hundreds of chemicals found in household products. Often, their use ends with the compounds being flushed into the municipal sewage system and, eventually, entering a wastewater treatment plant (WWTP). While traveling through the treatment process, different compounds behave in vastly different ways. Some are dissolved in the liquid portion of the waste, while others sorb to solids and settle out of the waste stream.

Many WWTPs dispose of solids removed from the waste stream by first concentrating them and then spreading them over agricultural land. If pollutants are sorbed to these solids there is potential that they will also be distributed in the environment. One strategy used to degrade solids and reduce the amount that needs to be transported away from a WWTP is digestion. In additional to degrading the solids, there is also the potential that the digestion process will destroy PPCPs which are sorbed to them.

The purpose of this study is the examine the fate of four PPCPs, tylosin (TYS), sulfamethoxazole (SMX), trimethoprim (TMP), and triclosan (TCS), during lab-scale anaerobic and aerobic digestion. These compounds were spiked into digesters set to various operating temperatures. The digesters were sampled on a regular basis, and the

concentrations of the compounds in the samples were quantified to monitor their degradation during the digestion process.

5.2 Materials and Methods

5.2.1 Materials

Sulfamethoxazole (98%), tylosin tartrate (95%), triclosan (97%), and trimethoprim (98%) were purchased from Sigma-Aldrich (Milwaukee, WI, USA). All solvents were high-performance liquid chromatography (HPLC) grade. Chemicals were used as received.

5.2.2 Instrumental Analysis

Target compound concentrations were quantified using a Hewlett Packard LC-MS (Santa Clara, CA) (1050/1100 MSD). All compounds were analyzed on a Supelco (Park Bellefonte, PA, USA) Ascentis RP Amide 150 mm × 4.6 mm, 5 μm column, using a 10 μL injection volume. For sulfamethoxazole and trimethoprim a methanol: pH 5 acetate-buffer gradient method was used, initially at 20:80, going to 50:50 in one minute, and then held at 50:50 for four minutes, with a 1 ml/min flow rate. The compounds were detected with the mass spectrometer set to electrospray positive ionization mode, at a fragmentor voltage of 70 V, and using selective-ion-monitoring at mass-to-charge ratios of 291.1 and 292.1 for TMP and 254.1 and 255.1 for SMX. For TYS and TCS, a methanol:pH 5 acetate buffer gradient method was used, initially at 30:70, going to 70 percent methanol in 4 minutes, 100 percent methanol in 8 minutes total, and held at 100 percent for 2 minutes. Electrospray negative ionization was used at a fragmentor voltage of 70 V. Detection was done using selective-ion-monitoring at mass-to-charge ratios of 287.0 and 288.9 for TCS, and 1006.8 and 974.7 for TYS. The

drying gas flow rate was 10 L/min at a temperature of 300 °C. The nebulizer gas pressure was 30 psig and the capillary voltage set to 3000 V for all compounds.

5.2.3 Aerobic and Anaerobic Digestion

Anaerobic digesters were constructed using 15 L beer fermenters. The vessels were sealed to prevent interaction with the atmosphere. Gas was recirculated from the top of the reactor through the bottom of the reactor to ensure mixing. Excess gas that was produced by microbial activity was collected in plastic bags and later quantified. The temperature of each reactor was controlled using pipe heating tapes. Duplicate reactors were set to temperatures of 35 °C, 45 °C, 55 °C, and room temperature. The volume of sludge in the reactors was maintained at 15 L. A 15 day residence time was set by replacing five liters of digester sludge with digester feed from the Empire wastewater treatment plant (Empire, MN) every five days. For a detailed explanation of the digester set-up, see the master's thesis of David Diehl (74).

After the microbial communities in the anaerobic digesters had been determined to reach a steady state, SMX, TMP, TYS, and TCS were spiked into the digesters with 0.5 ml of methanol in each digester. The designed initial concentration of the compounds in the digester sludge was 1.5 μM for SMX, TYS and TMP and 0.15 μM for TCS. Periodic, one liter samples were taken eight times during the 15 day operation of the digesters. The samples were immediately frozen for preservation. For analysis the samples were thawed, mixed, and then poured, 50 ml at a time, into borosilicate glass test tubes. These subsamples were then freeze-dried to eliminate the liquid fraction.

Four aerobic digesters were set up using the same 15 L beer fermentors. The digesters were set to temperatures of 35 °C, 45 °C, 55 °C, and room temperature. The

volume of sludge in the digesters was eight liters. Replacing two liters of sludge with two liters of feed everyday resulted in a solids residence time of four days. Air was continuously pumped through the base of the digesters to ensure proper mixing and aeration.

The same four compounds were spiked into the aerobic digesters in 0.5 ml methanol per digester. Each of digesters was sampled daily during the eight day experiment. Sampling consisted of filling two 100 ml glass jars with the digester solids. The jars were immediately frozen and then were later freeze-dried.

5.2.4 Drug Extraction

To analyze the samples taken from the digesters for the compounds of interest, 0.5 g of freeze dried solid were weighed out on an analytical balance and placed into a glass fiber Soxhlet thimble, which was placed inside the extraction chamber. Methanol (300 mL) was placed in a 500 ml round bottom flask, which was attached to the Soxhlet apparatus and heated until the methanol boiling point was reached to begin the extraction process. Each Soxhlet apparatus was run continuously for 16 hours.

Following Soxhlet extraction, using the method of Göbel et al. (75), the methanol solution was reduced by rotary evaporation to less than 20 ml. Milli-Q water was subsequently added until a final volume of 400 ml was reached. Clean up of the resulting solution and concentration of the compounds of interest was conducted through solid-phase extraction, using 30 cc Oasis HLB cartridges. Each cartridge was preconditioned with 2 × 1.5 ml of a 1:1 methanol:ethyl acetate solution, 2 × 1.5 ml of methanol with 1% by volume ammonium hydroxide and finally, 2 × 1.5 ml of Milli-Q water adjusted to pH 4 with sulfuric acid. The sample was then left overnight to run

through the solid-phase extraction cartridge under vacuum. The amount of solution that had run through the cartridges was quantified by subtracting the final sample volume that had not run through the cartridges prior to clogging from the initial sample volume. The solid-phase extraction cartridges were cleaned prior to final elution with 2 × 1.5 ml of 10:90 methanol: Milli-Q water solution and 2 × 1.5 ml of a 30:70 methanol:Milli-Q water solution, and then allowed to dry by running air through the cartridges for one hour. Compounds were eluted off of the cartridges using 2 × 1.5 ml of a solution of 1:1 methanol: ethyl acetate and 2 × 1.5 ml of methanol with 1% by volume ammonium hydroxide. The resulting samples were blown down under a gentle stream of nitrogen to a volume of approximately 2.0 ml and was subsequently raised to a volume of 6.0 ml with Milli-Q water for analysis on LC-MS.

5.3 Results and Discussion

Analyzing samples from the digesters was a difficult and complicated process. Because the this, data sets were not expected have the same neatness as seen in data from photolysis experiments. Although four compounds were spiked into both the anaerobic digesters, the degradation of only two compounds, TYS and TMP, was able to be quantified. SMX was unable to be detected during sample analysis on the LC/MS, most likely because of the high detection limits associated with the sample matrix. TCS was detected in all samples. Its concentration, however, was found to be an order of magnitude higher than what would be expected based on the amount of the compound that was spiked into the digesters. It is likely that the amount of TCS sorbed to the feed sludge for the digesters was greater than the amount spiked in. If the compound had been present only from the amount spiked, the expected solids

concentration would have been 0.5 to 1 µg/g. The typical concentrations measured for TCS in the digested sludge solids, however, ranged from 10 to 40 µg/g. Other studies have found TCS levels in the solids from digested sludge to be approximately 30 µg/g (76). While there was likely more TCS present in the sludge initially than was spiked, the fact that the concentrations did not decrease (Figures A-13 and A-14), indicates that TCS was not removed during the digestion process.

Data for the concentrations of TYS and TMP in each digester is given in Figures 5-1 to 5-3. The tylosin concentration in the 45 °C aerobic digester is not shown in this figure because it was highly erratic. Instead, the data is given in Figure A-15. For the anaerobic digesters, samples have only been analyzed for the 35 °C digester.

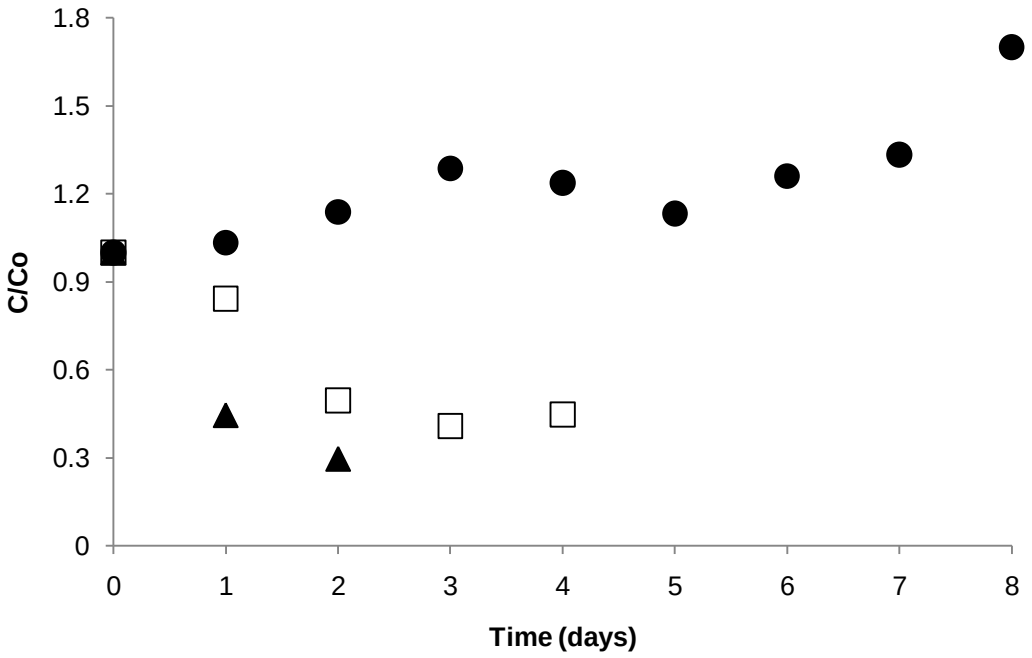


Figure 5-1. Degradation of tylosin during aerobic digestion. All values are adjusted to account for new solids being added periodically to feed the digesters. Digester temperatures are as follows: ● = room temperature, ▲= 35 °C, □ = 55 °C. Reliable data from the 45 °C digester was not obtained.

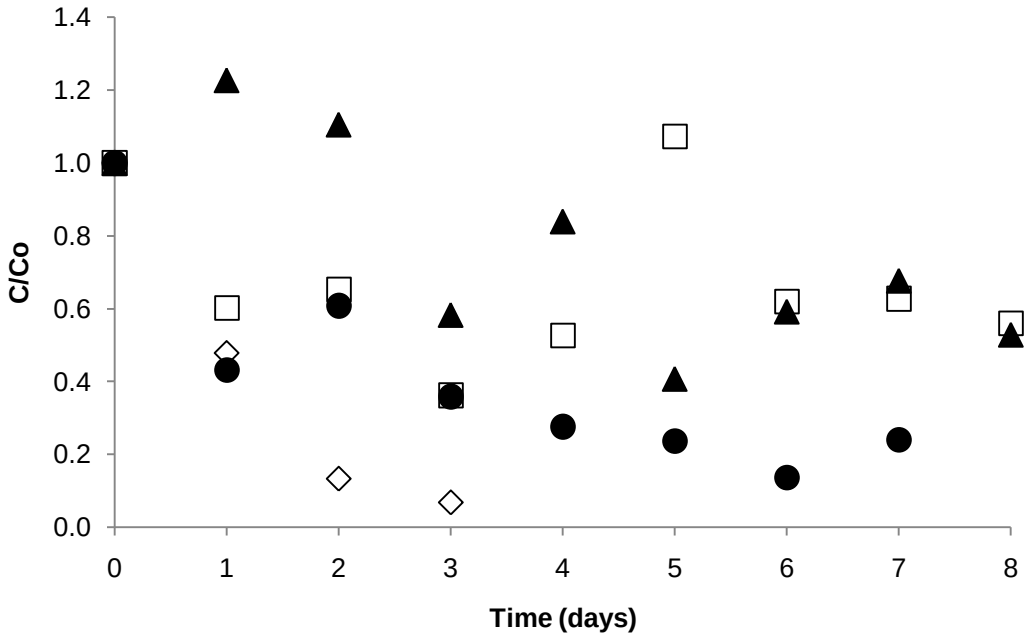


Figure 5-2. Degradation of trimethoprim during aerobic digestion. All values are adjusted to account for new solids being added periodically to feed the digesters. Digester temperatures are as follows: ▲= room temperature, □ = 35 °C, ◇= 45 °C, ● = 55 °C.

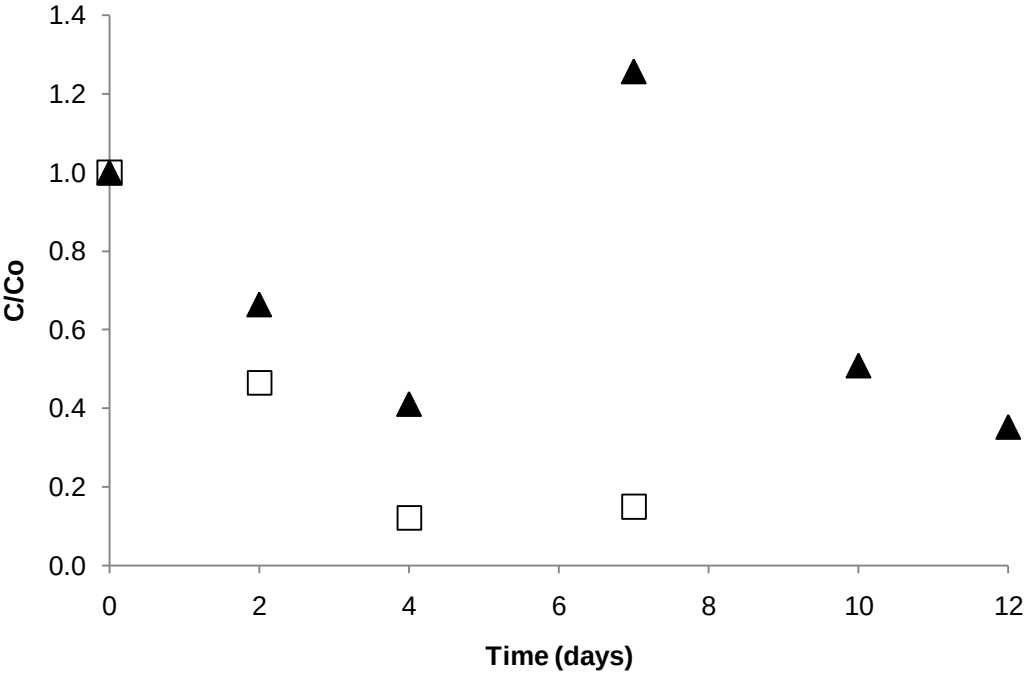


Figure 5-3. Degradation of compounds in the 35 °C anaerobic digester. Compounds are as follows: ▲= tylosin, □ = trimethoprim.

Examining Figure 5-1, it is apparent that no TYS degradation took place in the aerobic, room temperature digester. Degradation appeared to take place in the 35 °C and 55 °C digesters. Although there was no trend connecting degradation rates and digester temperature for TYS, each digester displayed different behavior towards the compound.

The data for the degradation of TMP during aerobic digestion is displayed in Figure 5-2. This figure shows that the compound experienced the fastest degradation in the 45 °C digester. The room temperature and 55 °C digesters also appeared to degrade TMP. In the 35 °C digester the concentration of TMP drops going from the starting concentration to the day one concentration. After this point, however, the concentration remains nearly constant, which suggests that little TMP degradation may have taken place in the 35 °C digester. Again, no clear trend between degradation rate and temperature is apparent even though the behavior of the different digesters towards TMP varied greatly.

For the 35 °C anaerobic digester, the monitored concentrations of TYS and TMP are given in Figure 3-3. Clear trends in the data appear to emerge. TYS does not seem to undergo degradation in this digester, while TMP appears to experience exponential decay. Without data for additional anaerobic digesters, it is not possible to attribute the lack of TYS degradation to anaerobic conditions. It is clear, however, that TMP does degrade under anaerobic conditions.

The findings of this study show that degradation of two antibiotic compounds clearly took place during sludge digestion, presumably as a result of biological

degradation. Although no obvious trend was shown to link temperature and degradation rates, aerobic digesters at varying temperatures clearly displayed varying behavior towards each of the compounds. Degradation for TMP was observed in an anaerobic digester, while degradation for TYS was not observed. This single anaerobic digester, however, was not able to provide enough information to compare the degradation of the compounds during aerobic digestion versus anaerobic digestion.

Chapter 6: Conclusions

This study has investigated the possible fates of five biologically active compounds during the wastewater treatment process, tetracycline, triclosan, tylosin, sulfamethoxazole and trimethoprim. The photodegradation of the compounds in effluent from wastewater treatment plants was compared to their degradation in natural and distilled waters. For two compounds, sulfamethoxazole and trimethoprim, detailed analysis was completed examining the differences in their degradation in each of these waters. Using the data gathered from this work, a lab-scale solar reactor was constructed analyzed to evaluate the possibility of using sunlight as a final treatment step to remove these chemicals from wastewater treatment plant effluent. This idea was also tested in a full-scale situation by monitoring the concentration of triclosan in a holding pond at the Blue Lake wastewater treatment plant. The fate of these compounds in solids from wastewater treatment plants was also studied. Anaerobic and aerobic digesters were constructed to evaluate their potential to degrade compounds during the digestion process.

All of the compounds examined in this study exhibited some type of photodegradation. Direct photolysis was shown to occur rapidly for tetracycline, triclosan, and tylosin. These compounds were observed to appreciably degrade while in the lab-scale solar reactor. Additionally, data on triclosan concentrations in a full-scale holding pond suggest that the compound degraded appreciably in the pond, presumably as a result of exposure to sunlight. For sulfamethoxazole and trimethoprim, direct photolysis occurred at a much slower rate. Indirect photolysis, however, was found to be important for these compounds in wastewater treatment plant effluent. This finding

was interesting because sulfamethoxazole had previous been found to not undergo indirect photolysis in natural surface waters (18). The cause of the indirect photolysis was attributed to interaction with hydroxyl radicals and triplet-excited dissolved organic matter, which were generated by sunlight interacting with nitrate and dissolved organic matter in the effluent.

Experiments involving the fate of the antibiotic compounds during the sludge digestion process showed that tylosin and trimethoprim degraded under certain circumstances. Varying the digester temperature appeared to have an impact on degradation rates, although no clear trends emerged. The behavior of the compounds under aerobic versus anaerobic conditions was not able to be compared.

The results of the study imply that photolysis can be an important loss process for some compounds present in wastewater treatment plant effluent. Evidence was also uncovered suggesting that effluent waters possess unique properties, compared to natural waters, that promote the photodegradation of some compounds. Taken together these findings show that it may be advantageous to hold the effluent from a wastewater treatment plant in a retention pond prior to final discharge. Doing so, would hypothetically allow sunlight to degrade many trace-level chemicals that are often present in the water. Digestion was shown to be effective at degrading several compounds. This result shows that digestion may be important for treating certain pharmaceuticals and personal care products in waste solids before the solids are spread onto agricultural lands.

Further work is necessary to better understand the role of various dissolved species present in effluent waters in the photolysis of pharmaceutical chemicals. Work

to characterize and compare dissolved organic matter in effluent waters to dissolved organic matter in natural waters would provide some insight on this front. It would also be beneficial to study how the photolysis of additional compounds is altered in effluent waters versus natural waters. Additional research on the impact of temperature on the removal of antibiotic compounds during sludge digestion is also needed.

This study has provided insight into possible methods for the treatment of pharmaceuticals and personal care products during the wastewater treatment process. Findings from this study raise new and interesting questions about the composition of wastewater treatment plant effluent waters.

Appendix

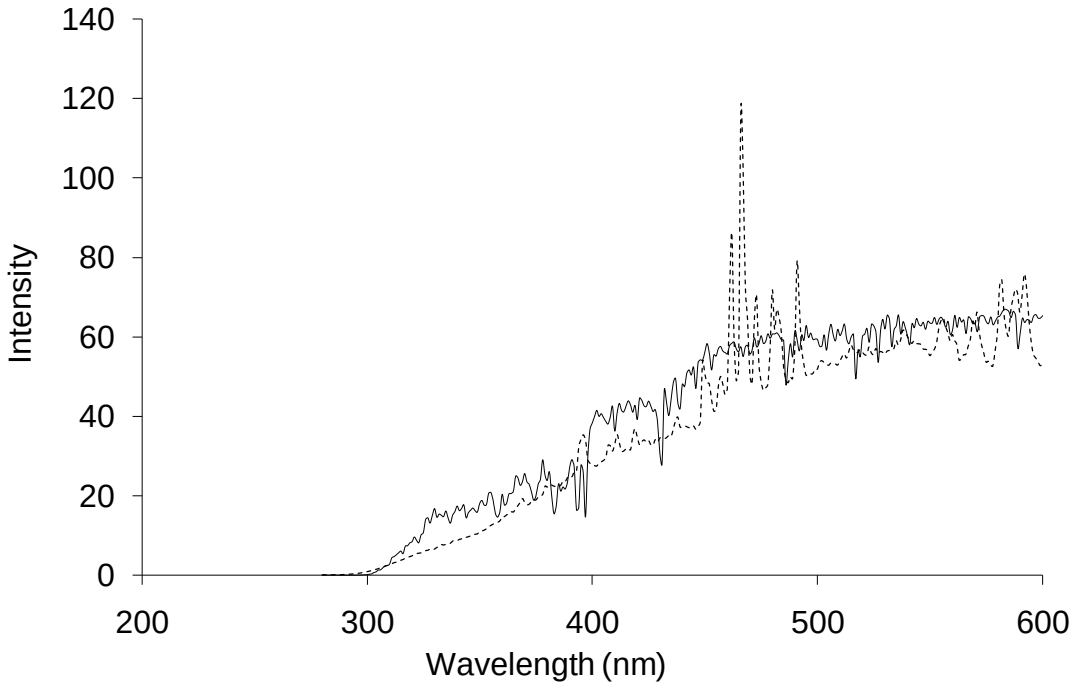


Figure A-1. Emission spectra of sunlight and solar simulator. The solid line is the sunlight spectrum. The dashed line is the solar simulator spectrum.

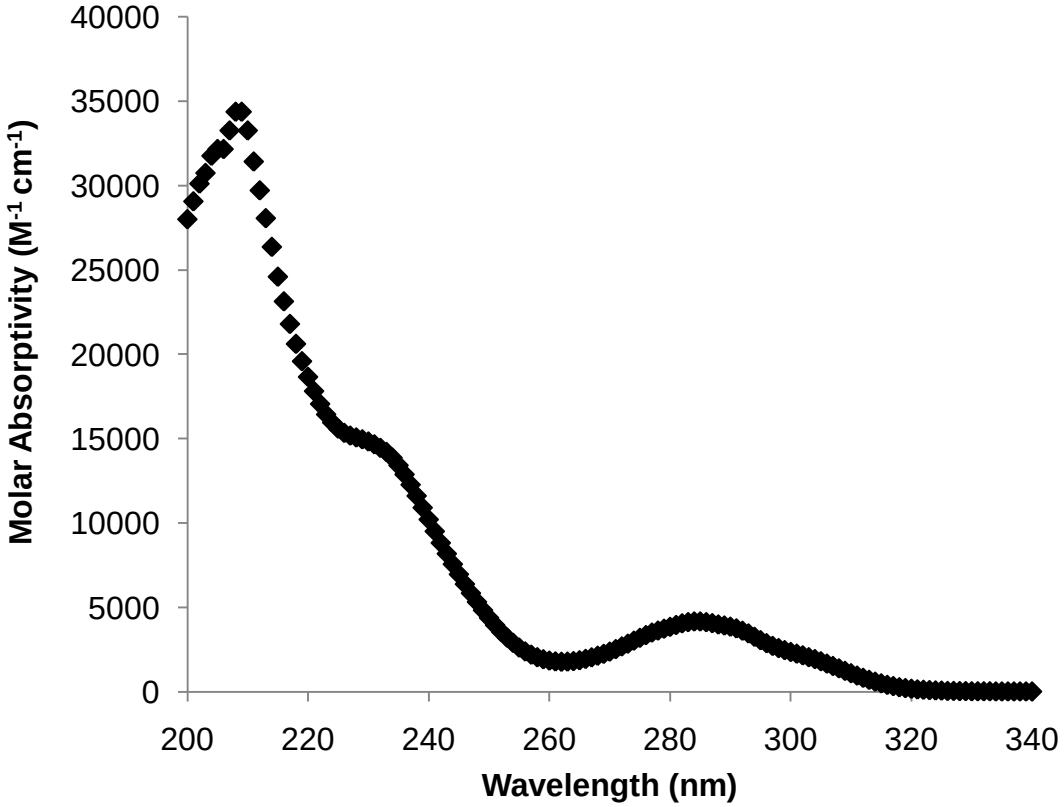


Figure A-2. Triclosan absorbance spectrum in 95% water, 5% methanol at pH 8.

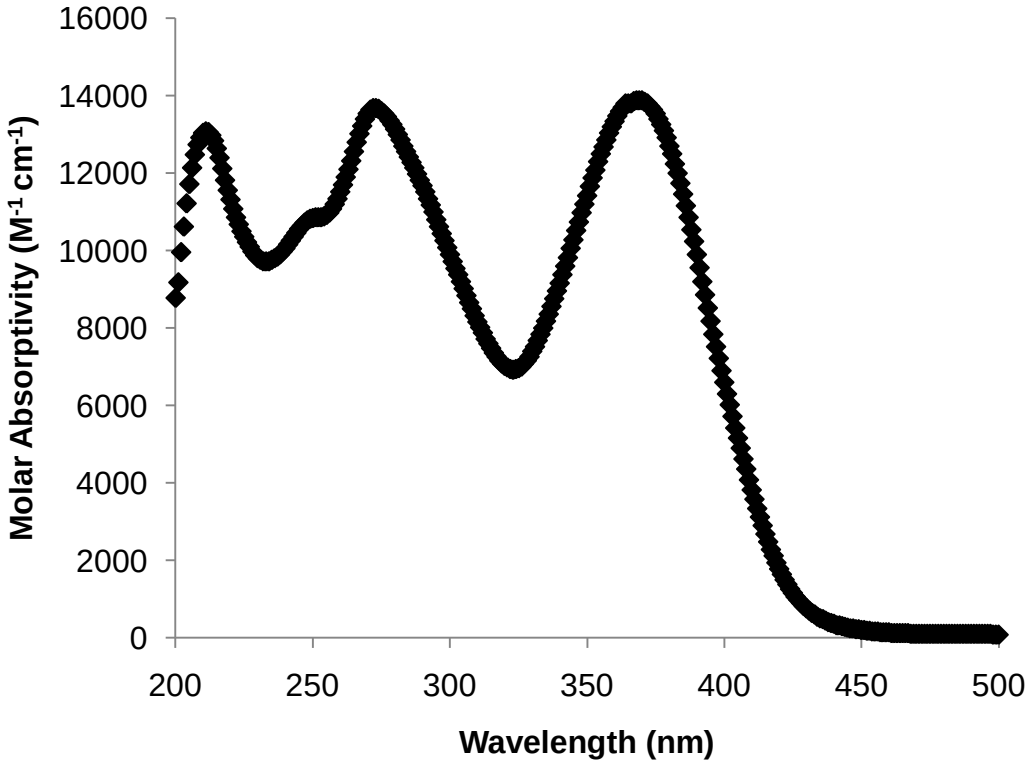


Figure A-3. Tetracycline absorbance spectrum in ultrapure, Milli-Q water at pH 8.

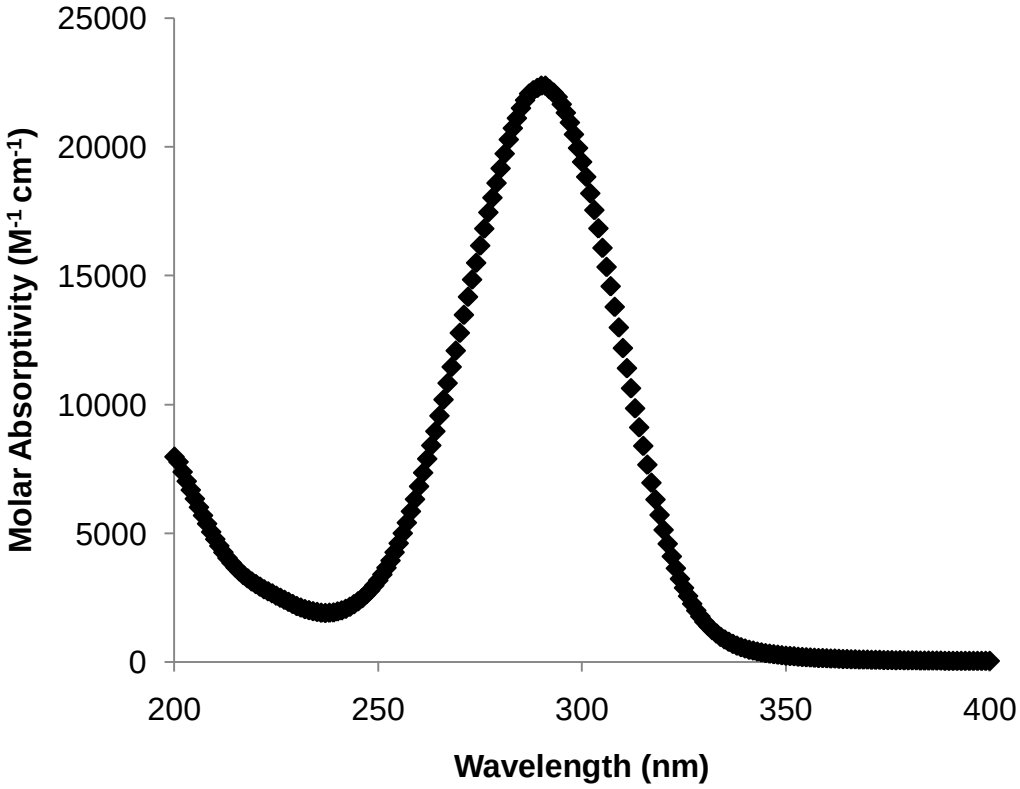


Figure A-4. Tylosin absorbance spectrum in 98% water, 2% methanol at pH 8.

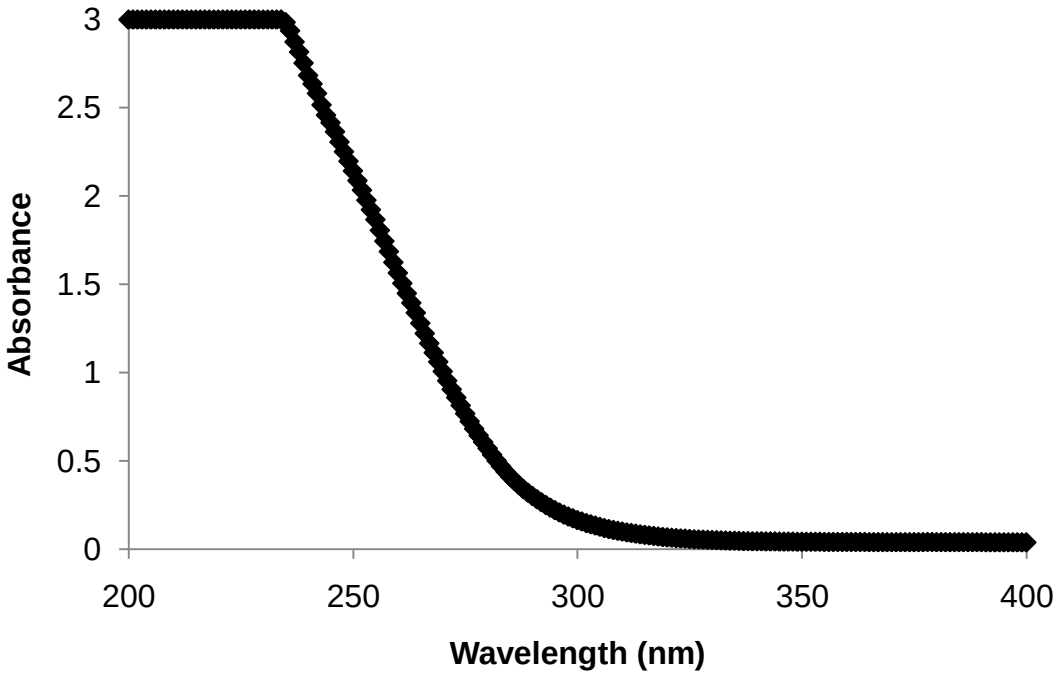


Figure A-5. Absorbance spectrum of 1/4" thick borosilicate glass plate.

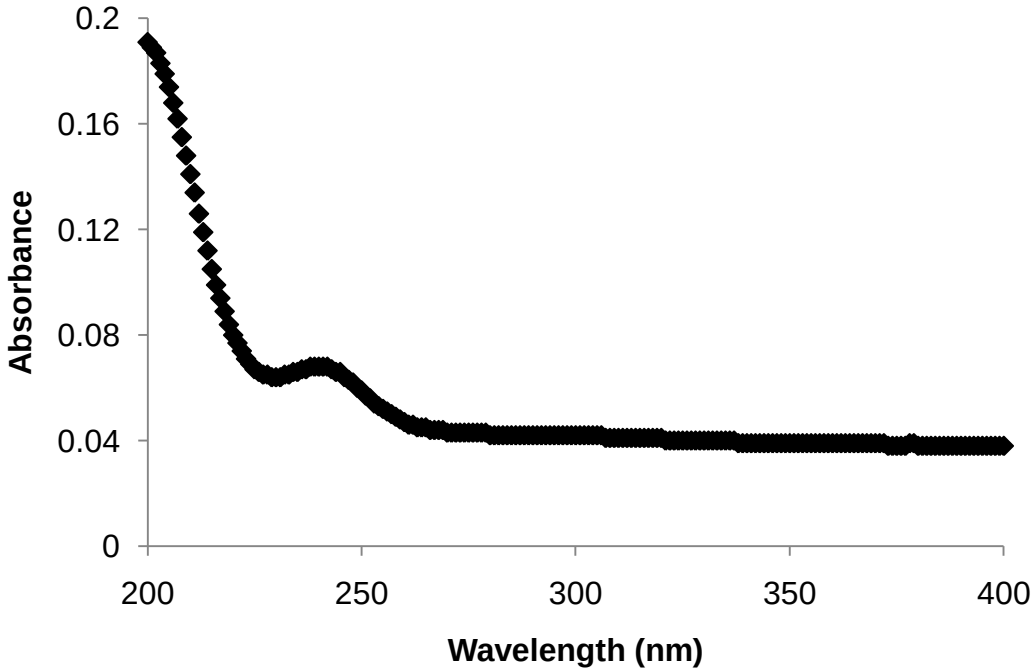


Figure A-6. Absorbance spectrum of 1/8" thick quartz glass plate.

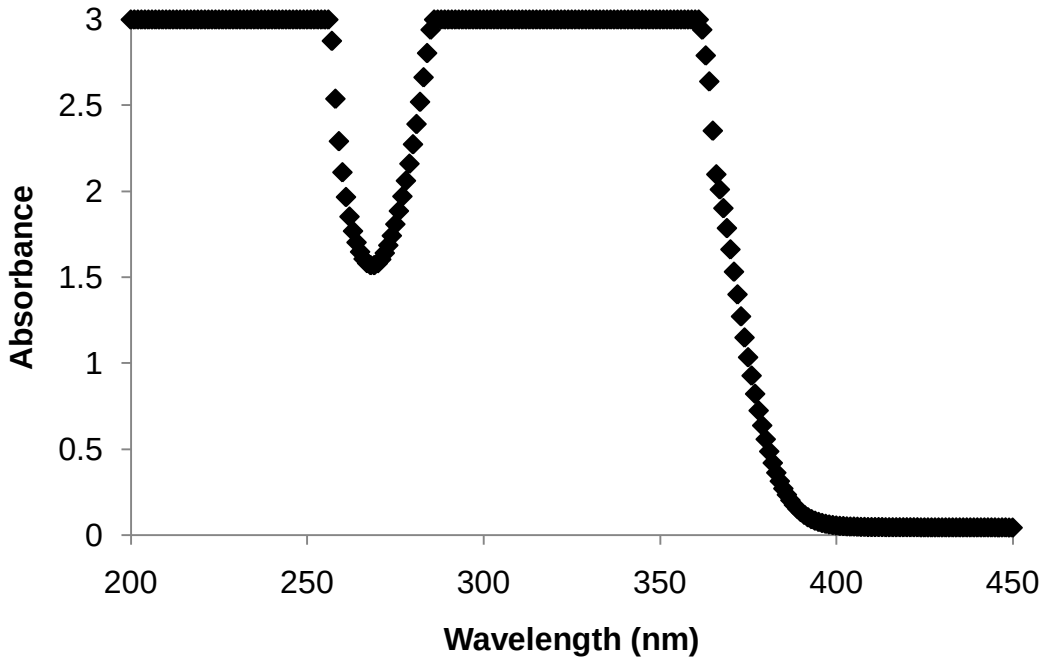


Figure A-7. Absorbance spectrum of 1/8" thick acrylic plate.

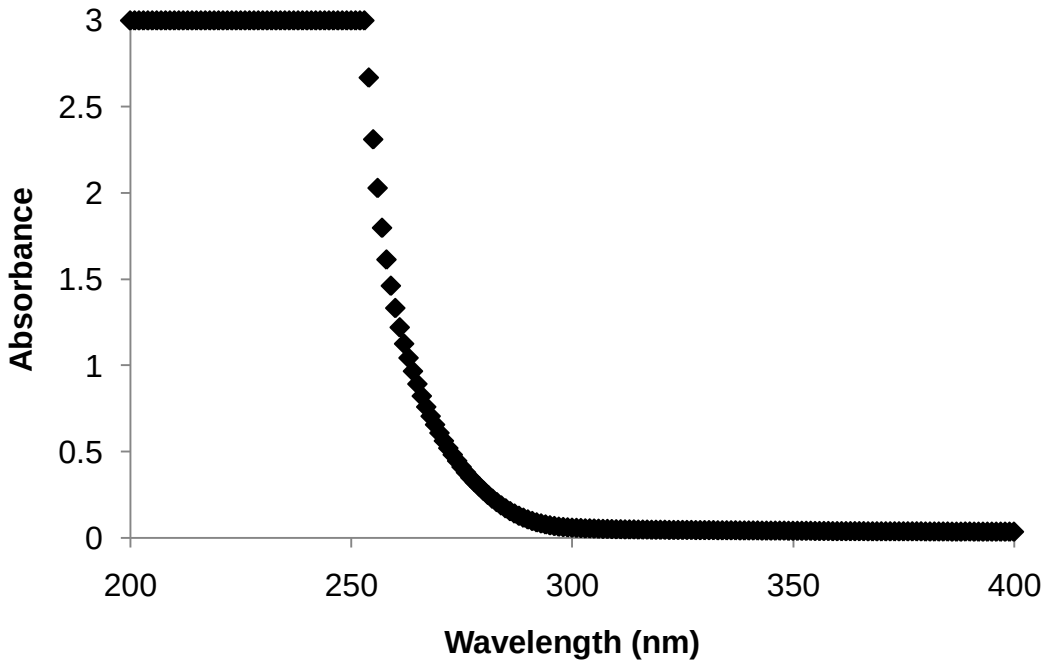


Figure A-8. Absorbance spectrum of 1/8" thick UVT acrylic plate.

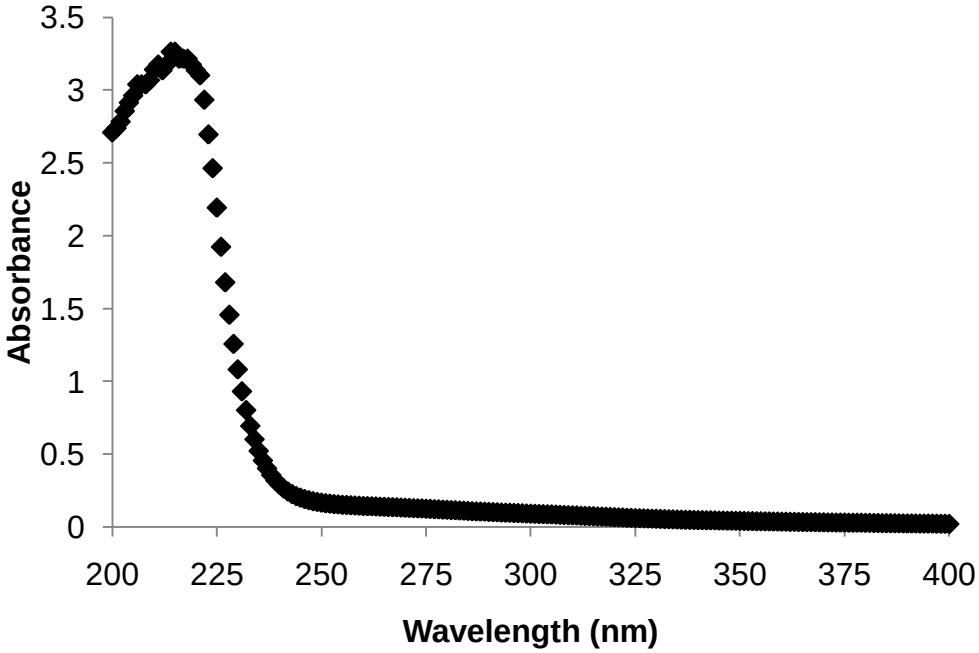


Figure A-9. Absorbance spectrum of prechlorination effluent from Blue Lake wastewater treatment plant (Shakopee, MN).

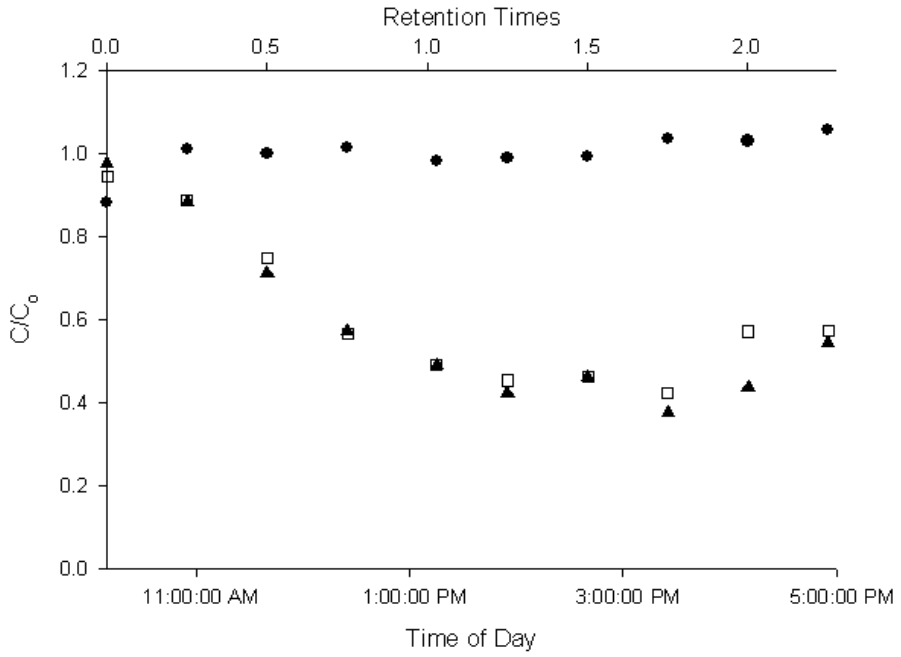


Figure A-10. Degradation of tetracycline during a solar reactor run on October 29, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

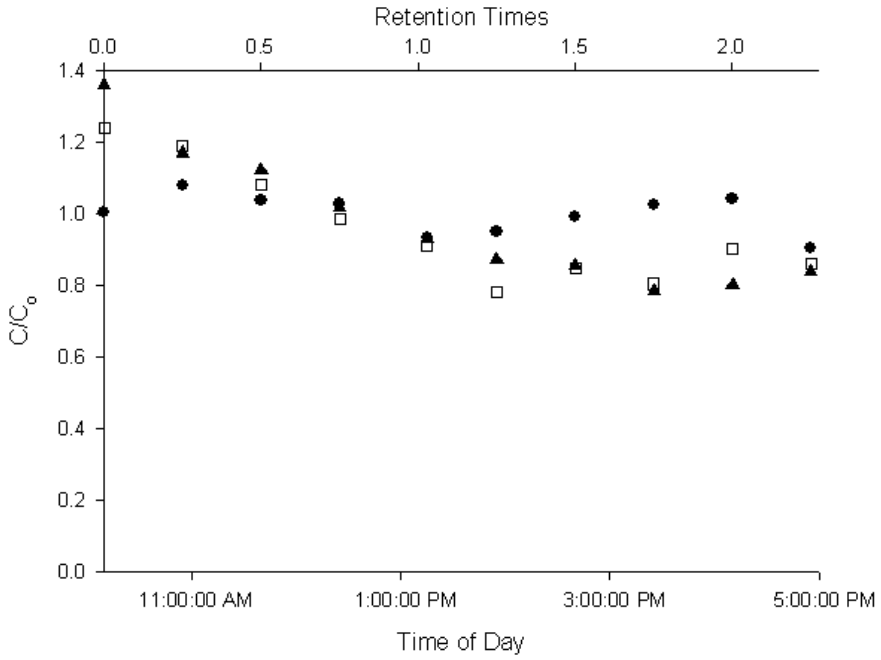


Figure A-11. Degradation of triclosan during a solar reactor run on October 29, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

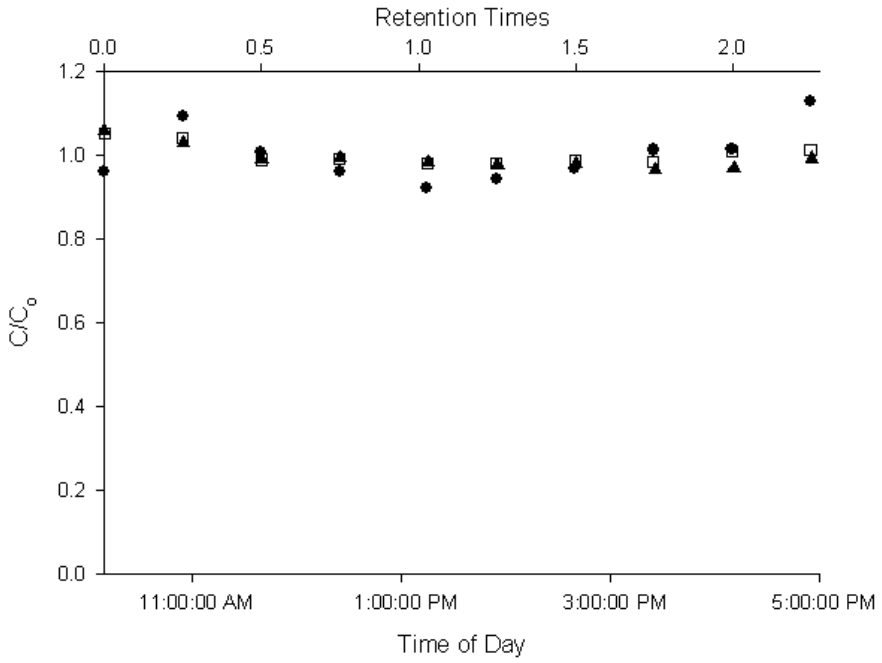


Figure A-12. Degradation of tylosin during a solar reactor run on October 29, 2008. Conditions are as follows: ● = influent concentration, □ = middle, ▲= effluent.

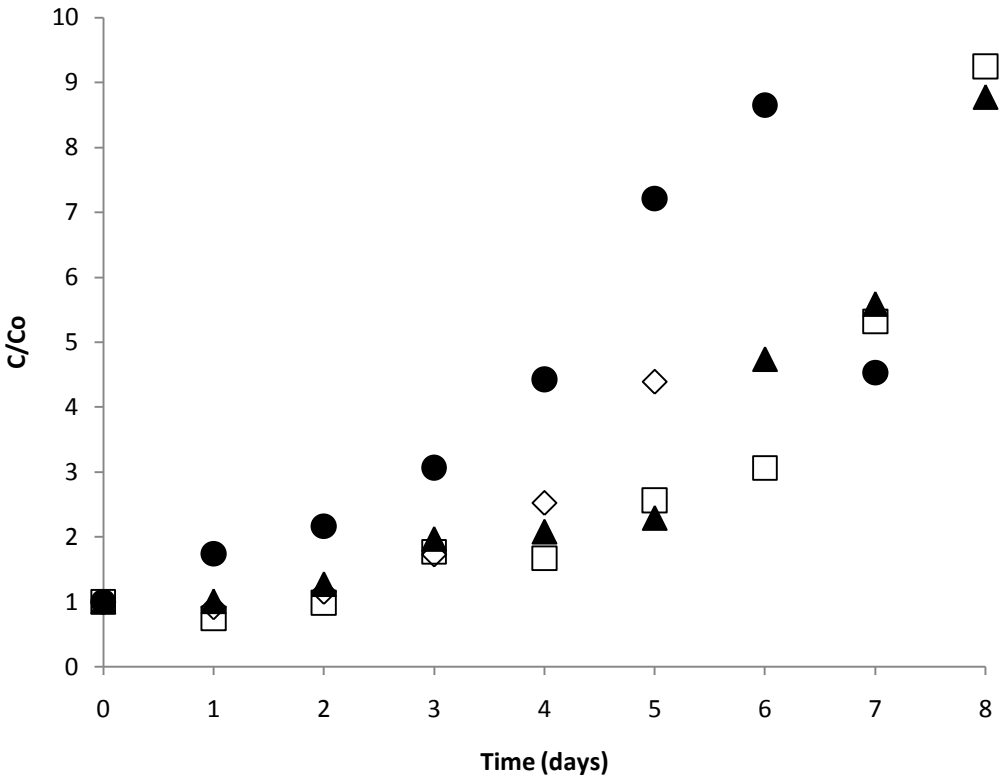


Figure A-13. Concentration of triclosan during aerobic digestion. All values are adjusted to account for new solids being added periodically to feed the digesters. A point for the 55 °C digester on day 8 with a C/C_o value of 23.2 is excluded. Digester temperatures are as follows: ▲= room temperature, □ = 35 °C, ◇= 45 °C, ● = 55 °C.

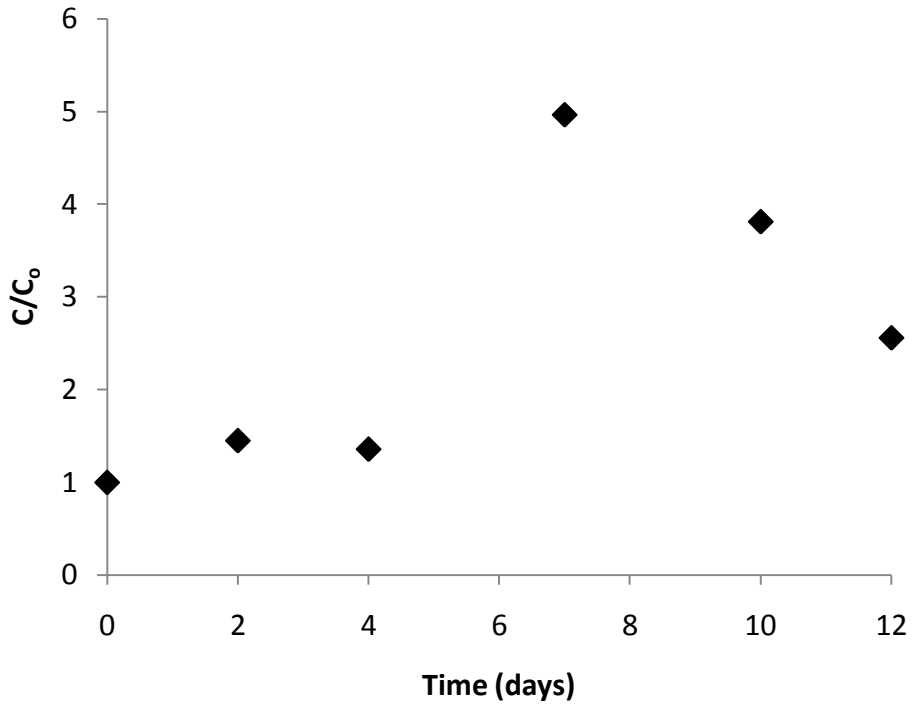


Figure A-14. Concentration of triclosan in the 35 °C anaerobic digester.

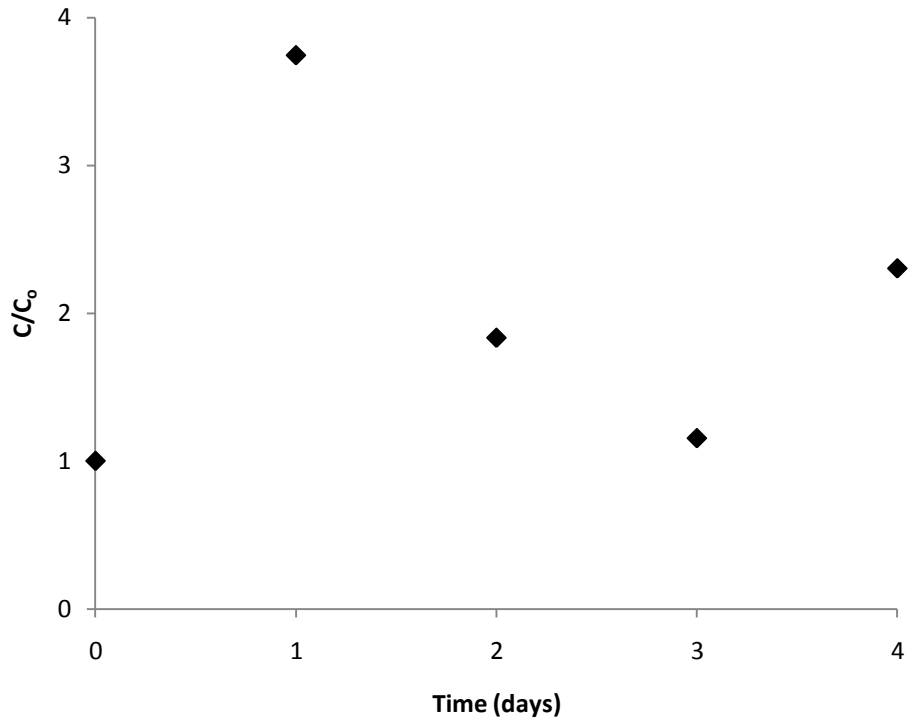


Figure A-15. Measured tylosin concentrations in the 45 °C aerobic digester.

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