ABSTRACT

Title of Dissertation: INFLUENCE OF VARIOUS WASTEWATER TREATMENT PROCESSES ON CONCENTRATIONS OF ANTHROPOGENIC POLLUTANTS AND THEIR TRANSFORMATION PRODUCTS

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Whether the use be in household, industrial, medicinal, or agricultural situations, modern society relies heavily on the use of chemicals. Unsurprisingly, many of these compounds are washed down the drain and have been detected in the wastewater treatment system. Compounds such as pharmaceuticals and personal care products (PPCPs), flame retardants, surfactants, and plasticizers have all been consistently detected in samples collected from wastewater treatment plants (WWTPs). Wastewater treatment is not designed specifically to remove these pollutants so they are oftentimes released into the environment via the discharge of WWTP effluent to local water bodies or the land application of treated sludge, also known as biosolids. Once released into the environment, chemicals can influence environmental health due to toxicity, bioaccumulation, microbial resistance issues, etc. Additionally, when degradation of these chemicals during treatment does take

place, they are often not fully mineralized, leading to concerns regarding the environmental effects of transformation products.

This research focuses on the impact that individual treatment systems have on concentrations of the antimicrobials triclosan (TCS) and triclocarban (TCC), 4 phthalate plasticizers, and their transformation products. The primary compounds studied have been shown to possess endocrine disrupting capabilities and to be present in biosolids at high concentrations due to extensive use. Treatment systems studied included activated sludge, nitrification, anaerobic digestion, and Cambi Thermal Hydrolysis Process (CambiTHP) pretreatment. Experiments were carried out in-lab using bioreactors to simulate treatment in a controlled manner as well as on-site at local WWTPs. The final goal of this research was the development of an extraction/analytical method for the detection of 27 compounds of concern in wastewater solids samples. Experimental results indicate that aerobic, anaerobic, and physical treatment processes can have mixed impacts on compound degradation and transformation product formation.

INFLUENCE OF VARIOUS WASTEWATER TREATMENT PROCESSES ON CONCENTRATIONS OF ANTHROPOGENIC POLLUTANTS AND THEIR TRANSFORMATION PRODUCTS

By

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1.1 Background

1.1.1 Organic Compounds in Wastewater Treatment

Modern society relies heavily on chemical use; whether the consumption is industrial, medicinal, agricultural, or household, the utilization of chemicals has become prolific in everyday human life. Due to this heavy reliance, chemical use worldwide is expected to increase well beyond human population growth, as outlined in Figure 1-1 [1]. Unsurprisingly, this has resulted in the presence of numerous compounds within the wastewater treatment system.

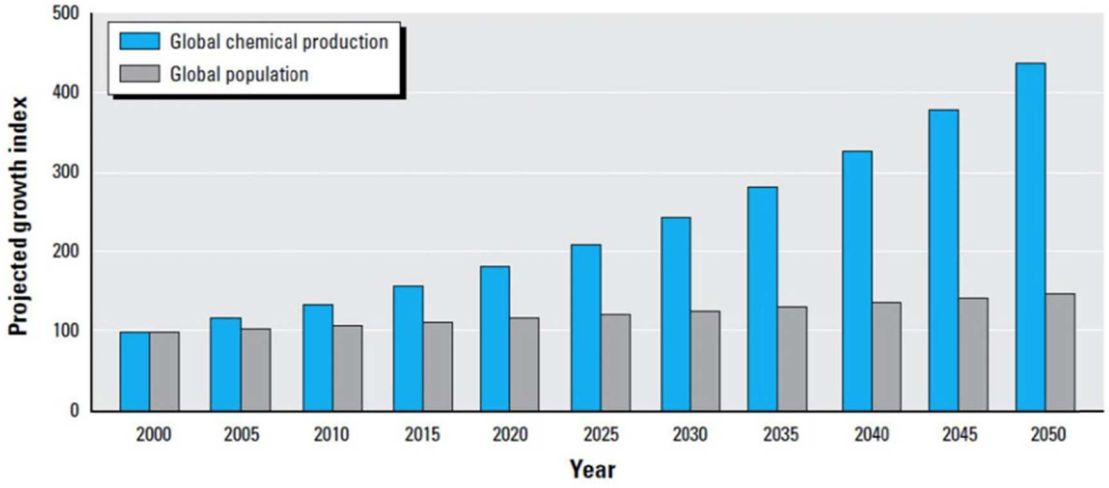

Figure 1-1: Worldwide projected population growth and chemical consumption [1]

The primary objective of wastewater treatment is the reduction nutrients, pathogens, turbidity, and organics from raw sewage prior to release into local water bodies. Because treatment systems are not focused on removal of anthropogenic chemicals, their removal efficiencies can be varied depending on the treatment systems employed by individual wastewater treatment plants (WWTPs) [2].

Pharmaceutical compounds, both prescribed and over the counter, can reach wastewater treatment systems through numerous sources, including consumer excretion via urine or feces, improper disposal (i.e. flushing of substances), manufacturing facility wastewater, hospital wastewater, and landfill leachate [3,4]. Excreted compounds can take the form of untransformed pharmaceuticals or as various metabolites of the parent compound. The rates of excretion can vary depending on the chemistry of the compound but are estimated to be as high as 70 - 90% for antibiotics. However, even pharmaceuticals with low excretion rates can be found at relatively high levels in wastewater if consumption of the compound is high [5]. Chemicals associated with personal care products, such as antimicrobials in soaps, fragrances, UV blockers in sunscreens, etc. [6,7], are typically washed down drains and into the wastewater treatment system due to the nature of their consumption [8]. Due to frequent daily use of pharmaceuticals and personal care products (PPCPs), compounds within this classification are commonly detected in wastewater samples. A study of a Portuguese WWTP found a number of pharmaceuticals in influent samples, with anti-diabetics, analgesics/antipyretics, and psycho-stimulants detected at the highest concentrations. Removal efficiency of detected compounds varied and was determined to rely on the efficiency of the secondary treatment method [9]. Furthermore, research focusing on 56 pharmaceuticals and pharmaceutical metabolites at a German WWTP found that while significant aqueous phase removal occurred for 20 of the analyzed compounds, only 5 were removed more than 50%. Additionally, concentrations of 5 different compounds were found to increase during treatment [10]. Levels of the antimicrobials triclosan and triclocarban were found to decrease by over 97% during

sewage treatment, but a majority of this removal was due to association with solids rather than actual degradation of the compounds [11].

Phthalic acid esters (PAEs), also known as phthalate plasticizers can enter wastewater systems through the use of plastics (leaching), such as PVC piping, and urban runoff [12-14]. These compounds are produced in high volumes worldwide - over 4 million metric tons per year and > 90% of worldwide plasticizer production [13,15,16] and have been detected in wastewater treatment at high concentrations. During the treatment process, high molecular weight PAEs tend to sorb to solids, causing them to be present at notable levels in biosolids. Dargnat et al. (2009) found that of the 78% removal of bis(2-ethylhexyl) phthalate (DEHP) during treatment, a significant portion of that was due to association with sludge rather than actual degradation of the compound [13]. A study of six PAEs in Austrian WWTPs, showed that DEHP, benzyl butyl phthalate (BBP), and dibutyl phthalate (DBP) predominantly associated with wastewater sludge rather than undergo biotransformation or discharge via liquid effluent. Concentrations of DEHP were approximately two orders of magnitude higher in sludge samples than the other compounds analyzed and ranged from 20,00 to 29,000 µg/kg [17].

Numerous compounds fall into the “flame retardant” category. As with other discussed chemicals, their prolific use has caused them to be present in the wastewater treatment system. Polybrominated diphenyl ethers (PBDEs), for example, can be released into waste treatment via the washing of textiles treated with the compounds or exposed to such compounds via indoor dust [18]. PBDE and polychlorinated biphenyl (PCB) congeners have been found to overwhelmingly associate with wastewater sludge during treatment, while flame retardants such as triphenyl phosphate (TPP) tend to associate with the aqueous faction [19]. In a

study focused on Swedish sewage sludge, Olofsson et al (2013) found that concentrations of 7 PCB compounds ranges from 23 – 100 µg/kg while PBDEs ranged from 390 – 870 µg/kg. Both compound classifications were detected with 100% frequency in collected sludge samples. Additionally, organo phosphorus compounds, including TPP, ranged from 310 – 2800 µg/kg [20]. A study on the trends of PBDEs in limed biosolids over a 7-year period demonstrated that while concentrations of the congeners BDE-47 and BDE-99 decreased by 42%, likely due to phase-out, concentrations of BDE-209 generally remained steady during the sample period [21].

The presence of numerous anthropogenic compounds in the wastewater treatment system and the inability of treatment to consistently degrade compounds have drawn concern regarding the environmental health impacts. Specifically since effects due to long-term exposure at trace concentrations is not well understood for many chemicals.

1.1.2 Health and Environmental Concerns of Organic Pollutants

Varied removal efficiencies of anthropogenic organic pollutants by wastewater treatment leads to their presence in the environment primarily due to discharge of effluent into local water bodies or the land application of treated sewage sludge, known as biosolids [22]. Detection of pharmaceutical compounds in water bodies downstream of WWTPs is common. A two-year study of the Huangpu River system in China detected ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid at various sample sites, with average concentrations ranging from 1.6 – 28.6 ng/L. A cause for concern not just for environmental health, but human health as well since the river system acts as a source of raw drinking water for Shanghai [23]. Numerous PPCP compounds have been detected in tissue (brain, liver, kidney, and

muscle) and plasma samples of Japanese wild cyprinoid fish whose habitat entailed streams impacted by wastewater treatment [24]. Triclosan and its metabolite methyl triclosan have been demonstrated to persist in soils amended with wastewater biosolids, with half lives estimated to be 104 and 443 days, respectively [25]. Both compounds have been shown to possess endocrine disrupting capabilities [26,27]. Uptake of carbamazepine by zucchini plants has been demonstrated to reduce plant biomass, both above and below ground, reduction of photosynthetic pigment in plant leaves, shifts in hormone concentrations within the plants, and leaf nutrient levels [28].

Once present in environmental samples, PAEs have been found to migrate to living organisms. For instance, studies have found that the compounds can transfer from soils to vegetation. A cultivation study conducted using lettuce, strawberry, and carrots determined that DEHP, DBP, and their primary metabolites could be translocated into plant tissues, with bioconcentration factors ranging from 0.16 to 4.78 [29]. A study regarding anthropogenic compounds and their concentrations in soils and earthworms after biosolids amendments to soils found soils levels for diethylhexyl phthalate to range from non detect (ND) to 2251 ng/g while earthworm concentrations ranged from ND to 288 ng/g [30]. While some compounds within the PAE classification may be rapidly degraded under the proper conditions [31], they still are of health concern. The developmental effects of DEHP have been classified as “a serious concern” to critically ill children while the compound’s reproductive effects are classified as a “concern” to children under one year old and “some concern” for male fetuses during pregnancy and children over the age of one exposed to high doses [32]. Furthermore, it has been demonstrated that PAE metabolites can posses toxicological properties [31].

Flame retardants have been found in environmental and biotic samples worldwide. PBDEswere found in dolphin samples from Brazil ranging from 3 – 5960 ng/, in US osprey eggs at concentrations of 98 – 897 ng/g, in United Kingdom (UK) harbor porpoises at 54.6 – 913 µg/kg, Korean blue mussels at 6.6 – 44 ng/g, etc. Polychlorinated biphenyls have been detected in Canadian sea lion samples from 272 – 14280 µg/kg, Greenland polar bears at 10537 ng, UK otter livers from 1.8 – 140000 ng/g, from 0.1 – 0.28 ng/g in clam samples collected in Fiji, etc. [33]. Andrade et al (2010) found that after application of biosolids, average concentrations of PBDEs in soil ranged from 15.2 µg/kg in fields that had received one application to 53.0 µg/kg in fields with multiple biosolids applications. Of the congeners analyzed, BDE-209 was detected at the highest rate [34]. Flame retardants such as the PBDEs have demonstrated the ability to disrupt thyroid function (distorted functionality and reduction of hormone levels) and cause neurodevelopmental effects and cancer [35].

The environmental and health impacts of anthropogenic compounds, whether they are released into the environment via wastewater treatment or other mechanism, are still being understood. However, current research indicates that the impacts of the vast numbers of compounds can have numerous effects, including bioconcentration/bioaccumulation, endocrine disruption, growth limitations, etc. Limiting the use of these compounds, where able, and understanding how wastewater treatment impacts these compounds and what treatments and changes to treatments help to degrade them is vital in preventing their discharge into the environment.

1.1.3 Overview of Study Wastewater Treatment Plant (DC Water, Blue Plains)

DC Water's Blue Plains Advanced WWTP serves a population of over 2 million residents from Washington DC, Maryland, and Virginia. A large-scale WWTP, Blue Plains treats an average of 1.14 million m^3 of raw wastewater per day. Maximum treatment capacity is 1.4 million m^3. The Blue Plains facility achieves waste treatment via sedimentation, activated sludge, nitrification-denitrification, filtration, and disinfection. Sludge is treated via the pretreatment by the Cambi Thermal Hydrolysis Process™ (CambiTHP) and mesophilic anaerobic digestion. Biosolids produced by this method are considered Class A biosolids by the US EPA. Prior to 2015, sludge waste treated by the addition of lime, on a 15 – 20% by weight basis. Class B biosolids were produced from this method. A majority of the biosolids produced by Blue Plains are land applied for agricultural use. Figure 1-2 outlines the current treatment system at Blue Plains.

1.2 Research Objectives

WWTPs can employ an assortment of treatment methods, and as such, degradation and removal of anthropogenic organic pollutants can vary widely. Even variations of the same treatment method can have significant influence on compound removal. For instance, Badia-Fabregat et al. (2015) found that pharmaceutically active compounds were better removed via fungal degradation when external nutrients (glucose and ammonia tartrate) were added to the treatment process while an increase in aeration led to an increase in levels of several compounds, including salicylic acid and ketoprofen, among others [36]. Moreover, factors such as sludge "age", hydraulic retention times, and ambient temperatures can influence the effectiveness of biological treatment processes in regards to

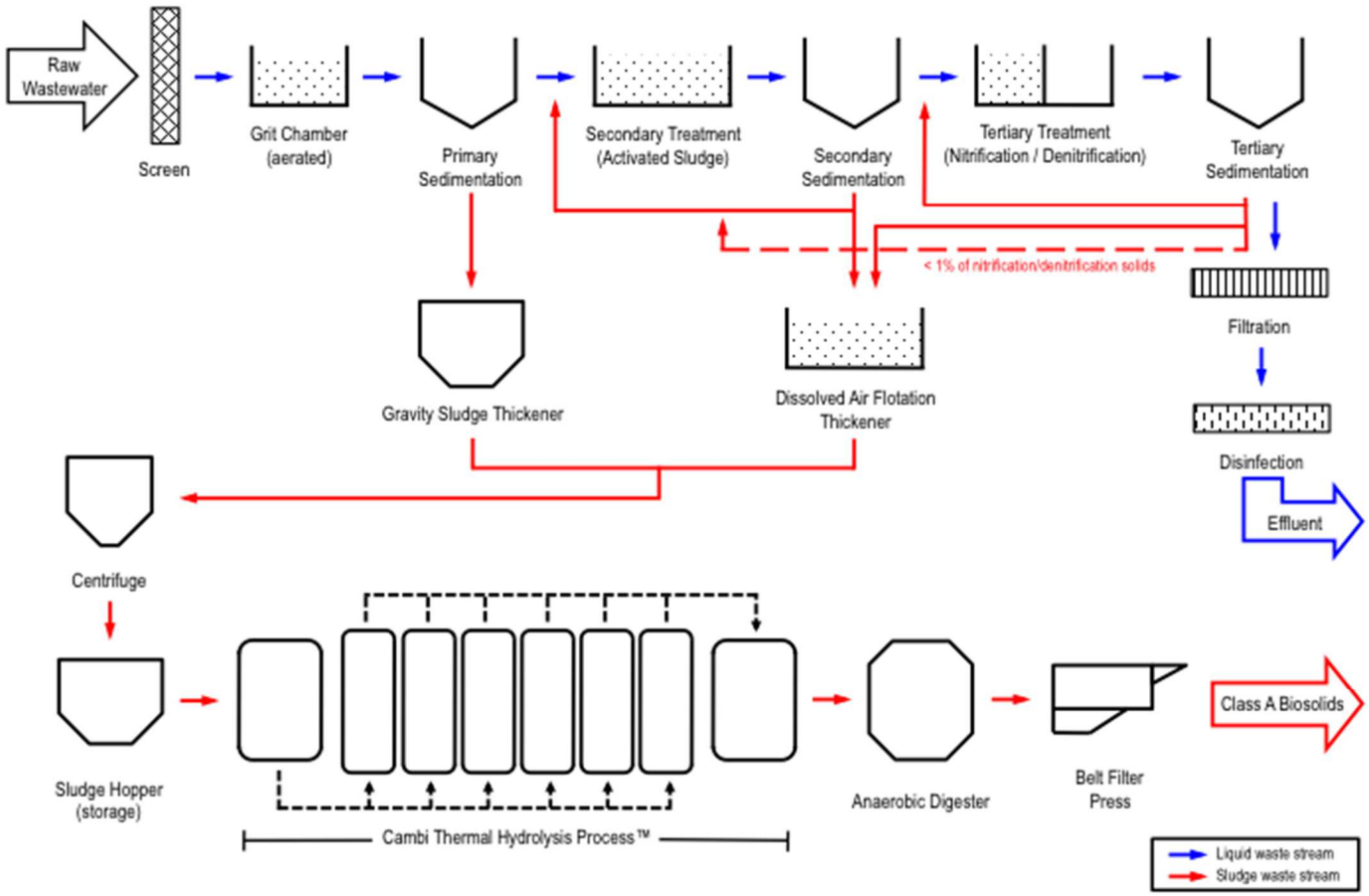

Figure 1-2: Process Flow Diagram of DC Water's Blue Plains WWTP

compound degradation [2]. Additionally, degradation effectiveness is influenced by the chemical properties of individual compounds [2].

Furthermore, degradation of a parent compound of concern does not guarantee that the pollutant is being fully mineralized. Quite often the chemicals is transformed into numerous degradation products that may have environmental and health implications of its own. Transformation products and pathways are often poorly characterized [2] and represent a gap in the research regarding the fate of organic pollutants in the wastewater treatment process. A survey of scientists in the environmental field identified research priorities of PPCPs to include the questions "what effluent treatment methods are effective in reducing the effects of PPCPs in the environment whereas at the same time not increasing the toxicity of whole effluents?" and "how can the environmental risks of metabolites and environmental transformation products of PPCPs be assed?" [37]. Considering all this, the objective of this research project focus on the impact that amending in-place treatment can have on compounds of concern and their transformation products, how new treatment technologies effect compound and transformation product concentrations, and analysis of samples using a method that can detect for a large number of compounds at once, allowing for a more efficient analysis and more readily broadens the understanding of the fate of anthropogenic compounds in wastewater treatment.

1.2.1 Objective #1: Study the influence that aerobic treatment processes (activated sludge and nitrification) have on degradation of the antimicrobials TCS and TCC as well as the formation of their transformation products using benchtop bioreactors.

Hypothesis 1-1: TCS will be degraded by both processes, resulting in formation of degradation products, but more effectively by activated sludge. As TCS concentrations decrease, we expect higher concentrations of MeTCS.

Hypothesis 1-2: Degradation of TCC will be more limited than TCS but will occur; degradation will be more effective during nitrification.

Experimental Approach/Tasks:

Study the influence that aerobic treatment processes (activated sludge and nitrification) have on degradation of the antimicrobials TCS and TCC as well as the formation of their transformation products using benchtop bioreactors.

New Brunswick BioFlo® 115 benchtop bioreactors will be used to simulate the activated sludge and nitrification processes. Utilization of these bioreactors will allow for the simulation of the wastewater treatment processes (activated sludge and nitrification) under controlled laboratory conditions.

Activated Sludge Experiments

Activated sludge (AS) bioreactor experiments will be run for 122 hours to simulate extended hydraulic retention time. Two bioreactors will be run simultaneously (under duplicate conditions) and experiments will be performed with 5 L (per reactor) of mixed liquor collected from DC Water's AS reactors (near reactor input, in an area where sufficient mixing was occurs). Two reactor "runs" will be performed at different temperatures to understand temperature effects on compound degradation. Reactor conditions are as follows:

- Temperature: 21°C (Run #1) and 30°C (Run #2).
- pH: 6.5 – 7.5
- DO: 4 mg/L

Samples (160 mL) will be collected periodically from the reactors for TCS, TCC, and MeTCS analysis of both the aqueous and solid phase. Additionally, activated sludge samples will be scanned (qualitatively) for the TCS and TCC transformation products: 2,4-DCP, TCS-o-sulfate (TCS-o-sulf), 3,4-DCA, 4-CA, 4,4'-dichlorocarbanilide (DCC), 1-(3-chlorophenyl)-3-phenylurea (MCC), and carbanilide (NCC).

Nitrification Experiments

Nitrification bioreactor experiments will be run for 171 hours to, again, simulate extended hydraulic retention time. As with the AS experiments, two bioreactors will be run concurrently under matching conditions with 5L of sample collected from DC Water's nitrification reactors (in a well mixed area close to reactor input). The two reactor "runs" will be performed at different pH ranges as this can influence the activity of nitrifying bacteria species. Reactor conditions are as follows:

- Temperature: 21°C
- pH: 6.5 – 7.5 (Run #1) and 8.5 – 9.5 (Run #2)
- DO: 2.5 mg/L

Samples (200 mL) will be collected from the reactors periodically and analyzed quantitatively for TCS, TCC, MeTCS 2,4-DCP, TCS-o-sulf, 3,4-DCA, 4-CA, DCC, MCC, and NCC. This study will allow for the determination of TCS and TCC degradation rates as well as formation rates of detected transformation products.

1.2.2 Objective #2: Determine how the TH-AD influences concentrations of TCC, TCS, and their transformation products as well as phthalic acid esters in-plant.

Hypothesis 2-1: The thermal hydrolysis process will have the ability to partially degrade TCS, TCC, and MeTCS and will result in the formation of 4-CA and 3,4-

DCA due to hydrolysis of TCC. Concentrations of DEHP, DiNP, DiDP, and BBP will also degrade during treatment.

Hypothesis 2-2: Remaining concentrations of TCS and TCC will be dechlorinated during anaerobic digestion and will result in the formation of the dechlorination products of TCC. Concentrations of the phthalates DEHP, DiNP, DiDP, and BBP will be unaffected by anaerobic digestion treatment.

Experimental Approach/Tasks:

The second objective of this research project is to determine the influence that the TH-AD process has on concentrations of TCS, TCC and their degradation products as well as four phthalic acid ester compounds when compared to concentrations in limed (Class B) biosolids. Class B biosolids have been collected routinely from the DC Water facility since 2005 as part of previous experiments on temporal trends of organic compounds in biosolids. Samples were collected directly after the liming/mixing process (prior to on-site storage) and have been archived at -20°C for future use. Implementation of the TH-AD process began in 2014 and Class A biosolids from this process have also been collected regularly and archived at -20°C for future analysis. Class A biosolids (collected after January 2015) and Class B biosolids (collected between August 2011 and January 2015) will be analyzed for TCS, TCC, TCC/TCS transformation products, DEHP, DiNP, DiDP, and BBP to compare how concentrations of these compounds in the final biosolids product differs with change in sludge treatment processes. Additionally, sludge samples will be collected throughout the TH-AD process, including the final cake (Class A biosolids) for compound analysis. This will help to demonstrate the degree of degradation and transformation compound formation, if any, that occurs in each step of the TH-AD process. This study will give insight into the ability of the TH-AD to

degrade these endocrine disrupting compounds as well as the influence that a major change in solids treatment has on final compound concentrations in biosolids prior to land-application.

1.2.3 Objective #3: Influence of Aerobic and Anaerobic Treatment Processes on Concentrations of Phthalate Acid Esters

Hypothesis 3-1: Anaerobic digestion processes will have no effect on concentrations of DEHP, DiNP, DiDP, and BBP while aerobic processes will degrade the compounds.

Experimental Approach/Tasks:

Research from this task will focus on the fate of four PAEs during solids treatment at six different WWTPs. Grab samples will be collected prior to treatment, after treatment (including individual treatment steps), and from final solids processes in order to understand the impact that the individual solids treatment has on compound concentrations. Samples will be collected from DC Water's Blue Plains facility in Washington, DC, and the following Maryland WWTPs: Marlay-Taylor, Sod Run, Back River, Tolchester, and Worton. Samples will be analyzed for DEHP, DiNP, DiDP, and BBP. The study WWTPs represent a mixture of large and small WWTPs located in urban and rural areas. Four of the facilities employ anaerobic processes while the remaining two utilize aerobic processes. This research will allow for a straightforward comparison of 1) how different treatment processes (aerobic and anaerobic) impact concentrations of PAEs and 2) how similar processes at different facilities can impact compound concentrations.

1.2.4 Objective #4: Simulate the anaerobic digestion process with and without thermal hydrolysis pretreatment using bioreactors to gauge the degradation of TCS, TCC, and PAEs and the formation of transformation products.

Hypothesis 4-1: Concentrations of TCS, TCC will not readily degrade while transformation product formation will be minimal in anaerobically digested sludge pretreated with the thermal hydrolysis process.

Hypothesis 4-2: Concentrations of PAEs will increase while concentrations of transformation products will decrease in anaerobically digested sludge pretreated with the thermal hydrolysis process.

Experimental Approach/Tasks:

Degradation studies will be conducted using sealed serum bottles shaken continuously in a dark incubation chamber under mesophilic conditions. Destructive samples techniques will be utilized. Solids material will be collected before thermal hydrolysis treatment, after the flash tank of the process, and from the anaerobic digestion tanks at DC Water's Blue Plains facility. Reactors will setup to achieve a inoculum to substrate volatile solids ratio of 1.5:1. Experiments will be run for 22 days and samples will be collected periodically from for analysis of TCS, TCC, and 5 transformation products as well as DEHP, DiNP, BBP, and 4 metabolites. Samples will also be analyzed for total solids, total volatile solids, chemical oxygen demand, and volatile fatty acids. Finally, gas will be collected, the flow measured, and analyzed for methane via a gas chromatograph. The reactors will be covered with aluminum foil to prevent any potential compound loss through photolysis. This study will allow for the calculation or degradation rates as well as metabolite formation rates.

1.2.5 Objective #5: : Analyze for 27 compounds of concern in sludge samples and determine the influence of wastewater treatment processes on concentrations of compounds.

Hypothesis 5-1: Degradation via aerobic and anaerobic digestion will be compound specific.

Hypothesis 5-2: Compounds not degraded during treatment will be concentrated due to the reduction in mass of solids during anaerobic digestion.

Experimental Approach/Tasks:

The first task of this objective is to fine-tune a previously established extraction methods for wastewater solids and analyze up to 53 chemical pollutants (list to be narrowed down based on recovery percentages) in sludge samples collected throughout the TH-AD process. The compound list, provided in Table 1-1, a) represents a mix of compounds that are commonly found in the WWT process, b) consists of an variety of use classifications, and c) is made-up of chemicals with varying physical and chemical properties. A Shimadzu ultra high performance liquid chromatograph, triple quadrupole mass spectrometer (UPHLC MS/MS) will be utilized for analysis. Common extraction methods (sonication, microwave, and pressurized liquid extraction), in conjunction with solid phase extraction (SPE) cleanup, will be compared to determine the most efficient mode of extraction. While the development of a full extraction and analytical method for all 53 compounds may not be possible, the goal is to establish a working method for as many of the compounds as possible.

Once the final analytical/extraction method is determined, samples collected from Objective #3 will be analyzed for the adjusted compound list. This allows for comparison of the impact that different treatment (i.e. anaerobic digestion, aerobic digestion) methods have on compound concentrations in sludges and biosolids. Sludge samples from each WWTP will be collected pre-treatment, post-treatment, and as final solids (biosolids). Collecting and analyzing samples from different facilities will give insight into trends of compound concentrations among different regions as well as the influence of treatment technologies on compound degradation.

Table 1-1: Compounds for Method Development

Chemical Use	Compound
Angiotensin II receptor blocker	Irbesartan
Anti-acne product / aspirin metabolite	Salicylic acid
Antibiotic	Ciprofloxacin
	Sulfamethoxazole
	Trimethoprim
Anticonvulsant	Primidone
Anticonvulsant/Mood Stabilizer	Carbamazepine
Antidepressant	Fluoxetine
	Venlafaxine
Antifungal	Fluconazole
Antihistamine	Diphenhydramine
Antimicrobial	Triclocarban
	Triclosan
Antipsychotic	Risperidone
Artificial Sweetener	Acesulfame Potassium
	Aspartame
	Saccharin
	Sucralose
Beta Blocker	Betaxolol
	Bisoprolol
Corticosteroid	Hydrocortisone
	Prednisone
	Triamcinolone
Fire retardant	TCPP, mixture of isomers
	Triphenyl phosphate
	Tris(2-butoxyethyl) phosphate
	Tris(2-chloroethyl) phosphate
Herbicide	Atrazine
	Dichlobenil
	Pendimethalin
	Simazine
Hormone	Testosterone
Insect repellent	N,N-Diethyl-3-methylbenzamide [DEET]
Insecticide	Chlorpyrifos
	Emamectin benzoate
	Flubendiamide
Nondihydropyridine (non-DHP)	Diltiazem hydrochloride
Nonsteroidal Anti-inflammatory Drug (NSAID)	Diclofenac sodium salt
	Ibuprofen
	Naproxen
Plastics	Bis(2-ethylhexyl) phthalate [DEHP]
	Bisphenol A
	Diisodecyl phthalate [DIDP]
	Diisononyl phthalate [DINP]
Steroid	Dexamethasone
Steroid Hormone	Norethindrone
	Norgestrel
Stimulant	Caffeine
Surfactant	Perfluorohexanoic acid [PFHxA]
	Perfluorononanoic acid [PFNA]
	Perfluorooctanoic acid [PFOA]
	Perfluorooctanesulfonic acid [PFOS]
UV Absorber	Oxybenzone

Chapter 2: Degradation of Triclosan and Triclocarban and Formation of Transformation Products in Activated Sludge Using Benchtop Bioreactors

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2.1 Abstract

Benchtop bioreactors were run aerobically with activated sludge samples collected from a large municipal wastewater treatment plant (WWTP) to understand how increased hydraulic retention time (HRT), sludge retention time (SRT), and varying treatment temperatures (21°C and 30°C) impact concentrations of the endocrine disrupting antimicrobials triclosan (TCS), triclocarban (TCC), and their transformation products. Samples from the reactors were collected periodically over a 122 to 196 h period and the solid and liquid fraction were separately quantitated for TCS, TCC, and methyltriclosan (MeTCS) and scanned qualitatively for six other transformation products. Results indicated that TCS, TCC and MeTCS were predominately associated with the solids fraction of the activated sludge with only nominal concentrations in the liquids fraction. TCS was degraded in the solids fraction, with increased rates at 30°C (-0.0224 ± 0.007 h^{-1}) when compared to reactors run at 21°C (- 0.0170 ± 0.003 h^{-1}). Conversely, TCC concentrations did not significantly change in solids samples from reactors run at 21°C, while an increase in reactor temperature to 30°C resulted in TCC degradation at an average rate of -0.0158 ± 0.012 h^{-1}. Additionally, MeTCS formation in the solids fraction was

observed in three out of four reactors run - indicating a notable transformation of TCS. Qualitative appearance of 2,4-dichlorophenol and 4-chloroanaline was observed in the liquids fraction of all reactor samples. The remaining four qualitatively scanned compounds were not detected. These experiments demonstrate that increased HRT, SRT, and temperature result in enhanced removal of TCS and TCC from wastewater during the activated sludge process. Furthermore, a substantial formation of TCS into MeTCS was observed.

2.2 Introduction

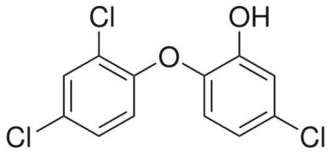
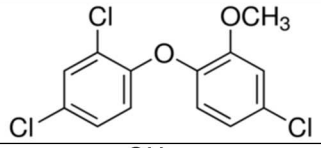
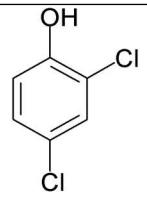
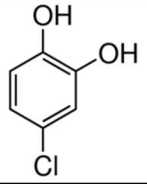
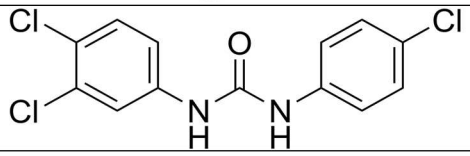
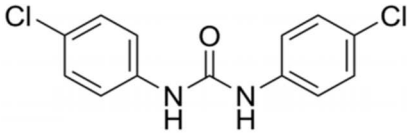
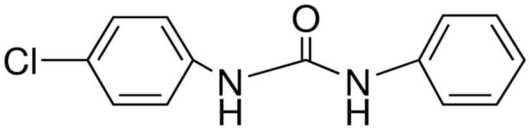
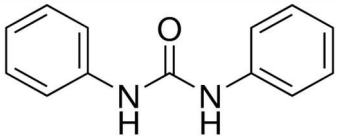
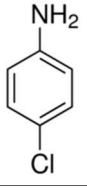
Triclosan [5-chloro-2-(2,4-dichlorophenoxy) phenol] (TCS) and triclocarban [1-(4-chlorophenyl)-3-(3,4-dichlorophenyl) urea] (TCC) are antimicrobial organic chemicals utilized in a variety of consumer products, including personal care products, plastics, paints, and textiles [38]. Heavy use of these compounds has resulted in their presence in both environmental [39,40] and biotic samples [41-44], which has drawn concern due to the toxicological properties of TCS and TCC, most notably endocrine disruption [26,27,45,46]. These environmental and toxicological concerns have lead the United States Food and Drug Administration to issue a ruling restricting the use of these compounds in consumer products [47]. One such source of these antimicrobials to the environment is the wastewater treatment (WWT) process, where they are typically only partially degraded and, therefore, present in wastewater effluent [11], untreated sludge [48], and treated sludge or biosolids [21,48,49]. While degradation of TCS and TCC can occur during WWT, the extent to which this takes place is dependent on the treatment methods utilized by the WWT plant [49,50].

TCS has been demonstrated to transform biologically into several compounds, including methyltriclosan (MeTCS), 2,4-dichlorophenol (2,4-DCP), and

4-chlorocatechol [11,51-53]. TCC, meanwhile, can be biologically transformed into carbanilides, including 4,4'-dichlorocarbanilide (DCC), 1-(3-chlorophenyl)-3-phenylurea (MCC), and carbanilide (NCC), or either biologically or abiotically into 4-chloroaniline (4-CA) [54]. Like their parent compounds, TCS and TCC transformation products, provided in Table 2-1, have themselves demonstrated undesirable environmental and health characteristics. MeTCS has been demonstrated to be more persistent than TCS [25] and, like TCS, possesses endocrine disrupting capabilities [26]. Additionally the carbanilide analogs of TCC have also demonstrated the ability to impact the endocrine system [45].

TCS and TCC degradation and the formation of transformation products have been shown to occur in the wastewater treatment process [11,50]. This research aims to simulate the activated sludge process in a controlled environment by using benchtop bioreactors, allowing for degradation rates and formation of degradation products to be determined under varying operational conditions. Changes in concentrations of TCC, TCS, and MeTCS were determined in two reactors run simultaneously over a 122 to 196 hour time period at fixed-temperatures of 21°C and 30°C and degradation or formation rates were calculated. Additionally, samples collected from the reactors were monitored qualitatively for the presence of 2,4-DCP, DCC, MCC, NCC, 4-chlorocatechol, and 4-CA. The study was performed using in situ compound concentrations and microbial populations (no spiking or augmentation). This research gives insight into how an individual, and common, treatment process can influence concentrations of antimicrobials and their transformation products in the wastewater treatment process and, thus, the amount emitted to the environment by these facilities.

Table 2-1: Compounds Analyzed and Their Structures

Compound	Structure
Triclosan (TCS)	
Methyl triclosan (MeTCS)	
2,4-Dichlorophenol (2,4-DCP)	
4-Chlorocatechol	
Triclocarban (TCC)	
4,4'-Dichlorocarbanilide (DCC)	
1-(3-Chlorophenyl)-3-phenylurea (MCC)	
Carbanilide (NCC)	
4-Chloroaniline (4-CA)	

2.3 Materials and Methods

2.3.1 Standards and Reagents

TCC (>97%), TCS (>97%), MeTCS (>97%) and isotopically labeled $^{13}C_{13}$-TCC (≥99%), $^{13}C_{12}$-TCS (≥99%), and $^{13}C_{12}$-MeTCS (≥99%) were obtained from Wellington Laboratories (Guelph, ON, Canada). MCC (N/A), NCC (98%), 3-CA (99%), 2,4-DCP (≥97%), and 4-chlorocatechol (97%) were obtained by Sigma-Aldrich (St. Louis, MO, USA). DCC (N/A) was acquired through Oakwood Chemicals (West Columbia, SC, USA). Laboratory-grade sand was obtained from J.T. Baker® (Avantor Performance Materials, Center Valley, PA, USA). Compound structures and properties are provided in Table 2-1.

All organic solvents used were of high performance liquid chromatography (HPLC) grade (Burdick and Jackson; Fisher Scientific). Potassium phosphate (monobasic) (99.2%), potassium phosphate (dibasic, anhydrous) (99.6%), ammonium acetate (99%), sulfuric acid, and acetic acid (both certified ACS grade) were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Organic-free, UV-treated water was obtained using a Picosystem UV Plus treatment system (Hydro Service &Supplies, Inc.; Durham, NC, USA).

2.3.2 Wastewater Treatment Plant Background and Sample Collection

Samples were collected directly from an activated sludge reactor at a large municipal WWT plant located in the Mid-Atlantic region of the United States (US). The facility serves a population greater than 2 million residents and treats approximately 1.25 million cubic meters (m^3) of raw wastewater per day. The raw wastewater at this facility is treated via preliminary treatment, primary treatment, aerobic secondary treatment (“activated sludge”), tertiary nitrification-denitrification treatment, filtration, and disinfection. At the time of sampling, final solids were

handled by dewatering via centrifugation and treated with lime (~15% dry weight basis) to form Class B biosolids. Solids treatment at the facility has since changed to the Cambi Thermal Hydrolysis Process™/anaerobic digestion [49,55].

Mixed liquor samples were collected near the input of an activated sludge reactor, in an area where sufficient mixing was occurring. All samples were grab samples. After collection, samples were transferred to the laboratory rapidly for use within the bioreactors.

2.3.3 Benchtop Bioreactors

Two BioFlo® 115 Benchtop Bioreactors (New Brunswick Scientific, Edison, NJ, USA) were utilized to simulate the activated sludge process (Figure SI-A1, supplemental information). Reactors were run concurrently on two occasions, once at a temperature of 21°C and then at 30°C to observe the influence of temperature on concentrations of TCS and TCC and their transformation products. Dissolved oxygen (DO) in each reactor was monitored via a DO probe and maintained at an approximate concentration of 4 mg/L via aeration and mixing. pH was also monitored by probes and maintained in a neutral range of 6.5 to 7.5 by the addition of 3M sodium hydroxide (NaOH) or 1.5M sulfuric acid (H_2SO_4), as needed. Mixing rates for DO concentration maintenance and the addition of acid/base to the reactors was controlled automatically by the BioFlow 115® control panel according to settings entered into the program prior to the start of each run. Each reactor was covered in aluminum foil to avoid any photolysis.

Each reactor run lasted for at least 122 hours to simulate an extended hydraulic retention time (HRT) and extended sludge retention time (SRT). The typical HRT is 6 - 8 h and the typical SRT is approximately 55 h at the facility. These time periods may be much longer during low flow periods and much shorter during

high flow events at the facility. Temperature ranges at the study WWTP are typically 14°C to 23°C. At the start of each run 5 L of mixed liquor was added to each reactor. No additional mixed liquor was added once the experiments had begun. 160 mL samples were collected from the reactors via a sample port at hours 0, 2, 4, 6, 10, 12, 24, 28, 32, 36, 48, 54, 60, 72, 84, 96, 106, and 122. Due to an observation of remaining solids in reactor #1 at 30˚C, this reactor was run for an additional 76 hours, with added sampling points at hours 150 and 198. After sample collection, a portion of each sample was frozen at -20°C for later extraction and analysis of TCS, TCC, and transformation products while the remainder of the sample was utilized for the determination of solids, soluble chemical oxygen demand, and microbial activity.

Three airtight jars were set-up as control reactors. Each control reactor was filled with 0.5 L mixed liquor collected in the same activated sludge tank of the WWTP used for the degradation experiments. Mercuric chloride (1 g) was added to each control reactor to eliminate any bacterial activity. The control bioreactors were agitated and maintained at 30°C for 122 h. TCC and TCS were analyzed at the beginning and at the end of the experiment. Additionally, samples from the control reactors at hours 0 and 122 were collected and plated to verify that bacteria within the reactors did not survive the mercury treatment.

2.3.4 Solids Analysis

Total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), and total volatile solids (TVS) were processed as outlined by the US Environmental Protection Agency's (US EPA) standard methods [56].

2.3.5 Microbial Activity

Each sample collected from the reactors was analyzed for total microbial activity using a modified version of a previously published method [57]. This method utilizes fluorescein diacetate (FDA), which can be hydrolyzed by microbial enzymes to fluorescein, to determine relative microbial activity. FDA methods have been shown to be effective in determining microbial activity in activated sludge samples.

Briefly, 1 to 4 mL of mixed liquor from each reactor was placed into 50 mL Erlenmeyer flasks. Fifteen (15) milliliters of a 60 mM potassium phosphate buffer (pH 7.6) and 0.2 mL of a 1000 µg/mL FDA solution was added to each flask and shaken briefly by hand. Samples were incubated and agitated in a water bath at 30°C. After incubation, 15 mL of a chloroform:methanol solution (2:1 v/v) was added to each flask. The samples were shaken by hand, transferred to disposable centrifuge tubes and centrifuged at 3000 x g for 3 minutes. Samples were filtered into ultraviolet-visible (UV-Vis) spectrophotometer cells. Fluorescein concentrations in the filtrates were determined using a UV-1800 UV-Vis spectrophotometer (Shimadzu North America, Columbia, MD, USA) at a wavelength of 490 nm. The UV-Vis was previously calibrated using fluorescein standards of 0.1, 0.5, 1, and 5 µg/mL. All samples were extracted and analyzed in duplicate.

2.3.6 Chemical Oxygen Demand

Soluble chemical oxygen demand (sCOD) was determined by filtering mixed liquor samples through a Whatman GA/F 0.7 µm filter (GE Healthcare Life Sciences, Pittsburgh, PA, USA). Filtrates were acidified with H_2SO_4 and refrigerated at 4°C until analysis. Low range dichromate COD vials (0-150 ppm) (Chemetrics, Midland, VA, USA), in conjunction with a digester block and photometer (Hach Company, Loveland, CO, USA) were employed according to US EPA standard methods [56].

2.3.7 Triclosan, Triclocarban, & Transformation Product Extraction and Analysis

2.3.7.1 Sample Extraction

After collection from the benchtop bioreactors, samples for TCC, TCS, and byproduct analysis were frozen at -20°C until extraction and analysis. The liquid and solids fraction of the samples (40 mL aliquots) were separated via centrifugation at 3000 x g for 30 minutes. The liquid fraction was extracted as outlined previously [11,58]. In brief, the samples, acidified to pH ~2, were loaded onto Oasis® HLB solid phase extraction (SPE) cartridges (6 mL, 200 mg) (Waters Corporation, Milford, MA, USA) that were previously conditioned with 10 mL of organic-free deionized water. Analytes were eluted from the cartridges with 10 mL of a 10 mM acetic acid in methanol:acetone (50:50 v/v) solution. Eluates were then evaporated under a gentle stream of nitrogen and reconstituted in 1.5 mL methanol (MeOH) for instrumental analysis.

Solids extraction was performed using accelerated solvent extraction (ASE) [59]. Briefly, solids samples were placed into ASE cells and packed with laboratory-grade sand. Extraction was accomplished using a Dionex ASE #300 extraction apparatus (Dionex Corporation, Sunnyvale, CA, USA) with a solvent mixture of isopropyl alcohol (IPA) and organic-free water (80:20 v/v). Oasis® HLB solid phase extraction (SPE) cartridges (6 mL, 200 mg) (Waters Corporation, Milford, MA, USA) were again utilized for sample clean-up. Samples were loaded onto the cartridges in conjunction with a phosphate buffer (pH = 7) and analytes were eluted using a dichloromethane (DCM):diethyl ether (DEE) solution (80:20 v/v). Samples were evaporated using a rotary evaporator and reconstituted in 1.5 mL MeOH for analysis.

2.3.7.2 Instrumental Analysis

2.3.7.2.1 Triclosan and Triclocarban

TCS and TCC analysis was performed using HPLC, tandem mass spectrometry (HPLC-MS/MS) via a Waters 2690XE (Waters Corporation, Milford, MA, USA) in conjunction Quattro Ultima triple quadrupole MS (Micromass Limited, Manchester, UK) using an electrospray negative spray ionization (ESI-) source. Chromatographic separation was achieved by an Xterra C18 reverse phase column (5 µm, 150 x 2.1 mm) (Waters Corp., Milford, MA, USA) and 1% formic acid:MeOH (70:30 v/v) (solvent A) and MeOH (solvent B). MS acquisition was achieved in selected ion recording (SIR) mode. MassLynx 4.0 (Micromass Limited, Manchester, UK) was used for peak integration and quantitation. Specifics regarding HPLC-MS/MS conditions are provided in the supplemental information.

2.3.7.2.2 Transformation Products (excluding MeTCS)

After analysis for TCC and TCS via HPLC, samples were analyzed for 2,4-DCP, DCC, MCC, NCC, 4-chlorocatechol, and 4-CA by ultra high performance liquid chromatography, tandem mass spectrometry (UHPLC-MS/MS) using a Shimadzu Nexera X2 UPLC coupled with a Shimadzu 8040 triple quadrupole MS (Shimadzu North America, Columbia, MD, USA) equipped with an ESI source. A Supelco Ascentis® Express C18 reverse phase UHPLC/HPLC column (2.7 µm, 50 x 2.1 mm) (Sigma-Aldrich, St. Louis, MO, USA) with an isocratically run mobile phase of 10 mM ammonium acetate in a solution of MeOH:acetonitrile:water (60:15:25 v/v) were used to achieve chromatographic separation [49]. Multiple reaction monitoring (MRM) mode was used for MS acquisition for all compounds except 4-CA and 4-chlorocatechol, where selected-ion monitoring was used. Specifics regarding UHPLC-MS/MS conditions are provided in the supplemental information.

2.3.7.2.3 Methyltriclosan

Following HPLC-MS/MS and UHPLC-MS/MS analysis, samples were evaporated and reconstituted in 1 mL of hexane for measurement of MeTCS via an Agilent 6890 gas chromatograph (GC) in conjunction with an Agilent 5975 mass selective detector (MSD) run in positive electron impact ionization mode [25]. Compound separation was accomplished by a capillary column (DB-5-MS) with a length of 15 m, diameter of 0.25 mm, and film thickness of 0.1 µm (J&W Scientific, Folsom, CA, USA). GC-MS setting details are provided in the supplemental information.

2.3.8 Quality Assurance and Quality Control

Method Detection Limits (MDLs) were determined using the procedure established by U.S EPA (EPA, 1984). The Limit of Quantification (LOQ) was set at 2 times the MDL values. All samples were fortified with labeled $^{13}C_{12}$-TCC, $^{13}C_{12}$-TCS $^{13}C_{12}$-MeTCS internal standard for analyte quantification and to correct for possible matrix interactions and any losses during sample extraction. At least seven standards at concentrations other than zero were run for each set of analyses and linearity correlations were required to yield r-squared values ≥ 0.99. Standards were injected every ten samples in order to verify stability of the instrument during the analyses. A laboratory blank, duplicate and spike were included in each batch that always included less than 15 samples. Statistical analysis was performed using GraphPad Prism 7.0 Software, Inc., San Diego, CA. Table 2-2 shows the TCC, TCS and MeTCS MDLs, LOQs, recoveries and Relative Standard Deviation (RSDs) for water, sludge and filter samples. To validate the method, 7 replicates samples plus two procedural blanks were spiked and processed for each compound following USEPA protocols (EPA, 1984). Concentrations below the LOQ are not reported.

Table 2-2 shows the TCC, TCS, and MeTCS MDLs, LOQs, recoveries and Relative Standard Deviation (RSDs) for water, sludge, and filter samples. To validate the method, 7 replicates samples plus two procedural blanks were spiked and processed for each compound following USEPA protocols (EPA, 1984). Concentrations below the LOQ are not reported.

Table 2-2: Method detection limit (MDL), Relative Standard Deviation (RSD), Recoveries (Rec) and Quantification Limits (LOQ) (n=7)

Sample	TCC			
	MDL ng L^{-1}	**RSD %**	**Rec %**	**LOQ ng L^{-1}**
Water	1.2	2.8	70.6 ± 2.0	2.4
Filter	2.1	11.5	91.5 ± 5.4	4.2
[a]Sludge	7.9	5.9	92.0 ± 6.8	15.8
	TCS			
Water	8.3	16	88.8 ± 14.3	16.6
Filter	19.4	3	99.2 ± 3.0	38.5
[a]Sludge	13.9	7	88.9 ± 2.9	27.8
	MeTCS			
Water	2.5	7.6	111.6 ± 8.4	5
Filter	2.5	5.2	108.0 ± 5.6	5
[a]Sludge	13.3	9.5	94.8 ± 3.0	26.6

[a]MDL and LOQ are presented on ng g^{-1} dry wt

2.4 Results and Discussion

Results for secondary analyses (microbial activity, sCOD, and solids analyses) are provided in Figure SI-A2 through SI-A7.

2.4.1 Triclosan, Triclocarban, and Methyltriclosan

For TCS, TCC, and MeTCS, degradation or formation rates (Table 2-3) were calculated using pseudo-first-order kinetics via equation #1:

$$\ln\frac{C}{C_0} = -kt \qquad (1)$$

where C is the concentration at any given time, C_0 is the initial concentration, t is time, and k is the pseudo-first order degradation rate.

Table 2-3: Calculated TCS, TCC, and MeTCS Rates of Change During Activated Sludge Treatment

Compound	Temperature	Rate of Change in Solids ($hour^{-1}$)	Rate of Change in Liquids ($hour^{-1}$)
TCS	21°C	- 0.0170 ± 0.003	- 0.0737 ± 0.015 [a]
	30°C	- 0.0224 ± 0.007	- 0.0191 ± 0.002
TCC	21°C	N/A	N/A
	30°C	- 0.0158 ± 0.012	N/A
MeTCS	21°C	+ 0.0415 [b]	N/A
	30°C	+ 0.0071 [c]; + 0.0191 [d]	N/A

[a] Degradation began after 60 hours of the reactor running, prior to that, TCS concentrations were unchanging.

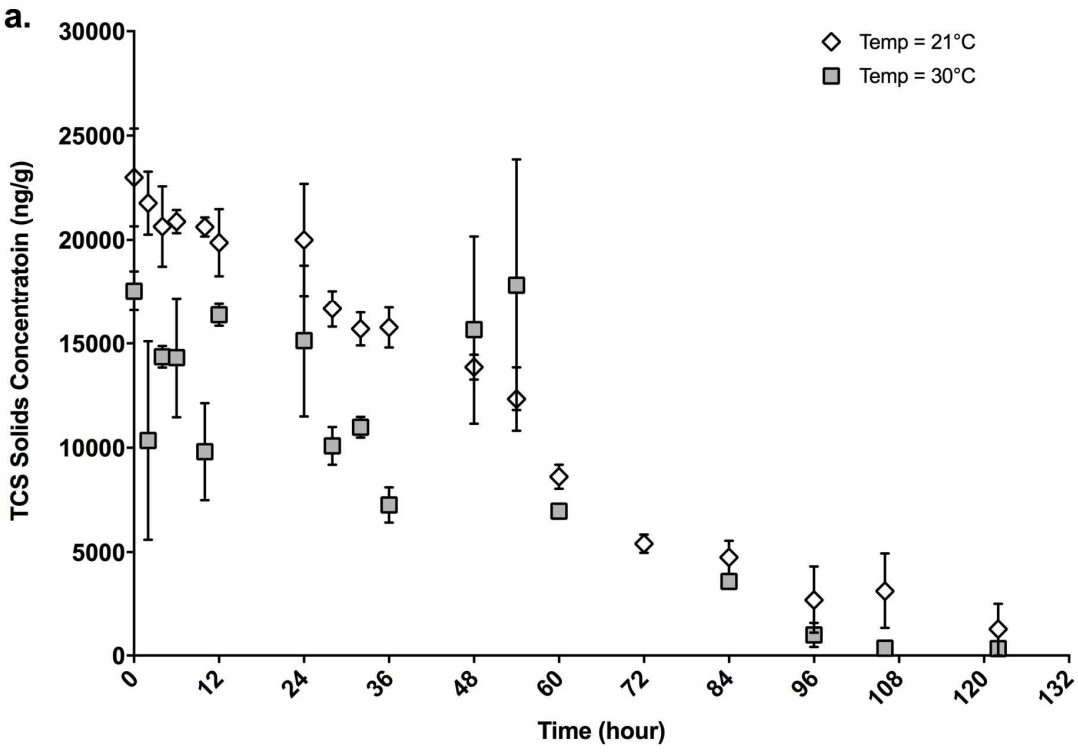
[b] Bioreactor #1 only, no MeTCS formation was observed in bioreactor #2 at 21°C.

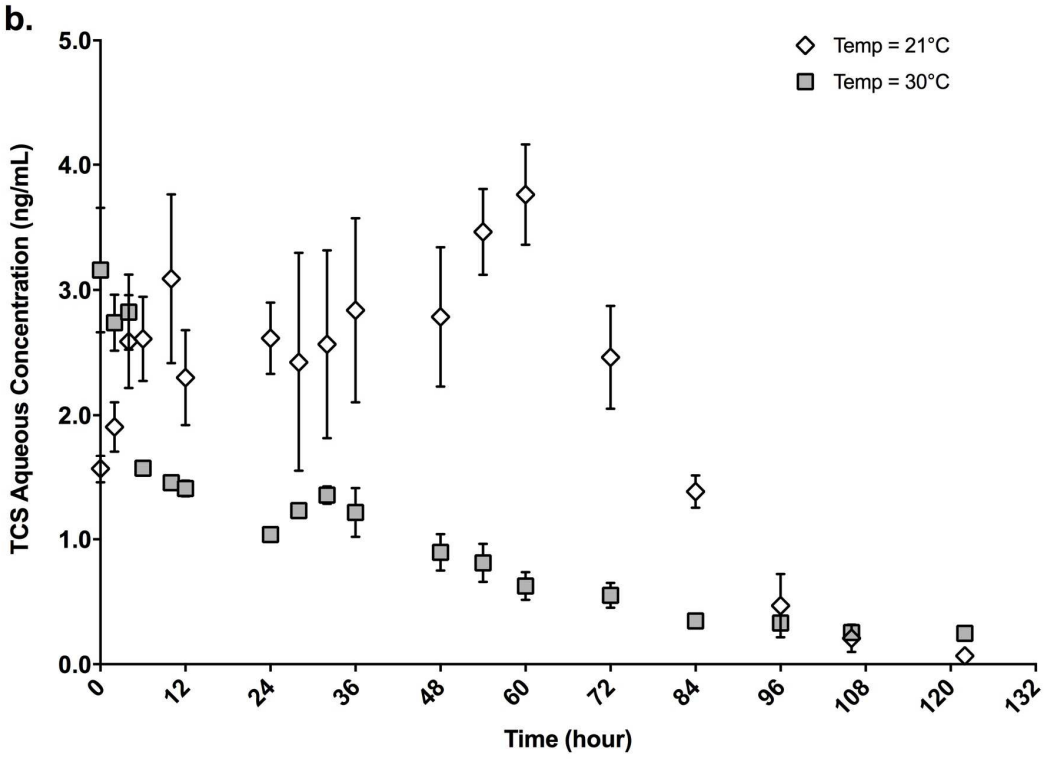
[c] Bioreactor #1 only.

[d] Bioreactor #2 only.

2.4.1.1 Triclosan

The majority of the total TCS concentration in the reactors was associated with the solids fraction (Figure 2-1a). This is unsurprising given its hydrophobic log K_{ow} value of 4.8 [60]. Overall, TCS concentrations in activated sludge solids decreased during the course of the experiments at 0.0170 ± 0.003 $hour^{-1}$ (21°) and 0.0224 ± 0.007 $hour^{-1}$ (30˚C). TCS concentrations in the solids fraction were reduced to 50% of the initial concentrations between hours 48 – 60 at 21°C and hours 28 – 36 at 30°C, demonstrating that TCS reduction is the solids matrix of activated sludge treatment is more effective at a higher temperature. The activated sludge HRT for the studied WWTP is 6-8 h and the SRT is ~55 hours. After six hours of treatment, approximately 9.14 ± 4.89% of the initial TCS was removed at 21ºC. Removal in the solids fraction after the first six hours of treatment was increased in reactors run at 30˚C: 18.8 ± 11.1% removal. At 21ºC, about 56.7% of


a.
Temp = 21°C
Temp = 30°C
TCS Solids Concentratoin (ng/g)
30000
25000
20000
15000
10000
5000
0
0
12
24
36
48
60
72
84
96
108
120
132
Time (hour)


b.
Temp = 21°C
Temp = 30°C
TCS Aqueous Concentration (ng/mL)
5.0
4.0
3.0
2.0
1.0
0.0
0
12
24
36
48
60
72
84
96
108
120
132
Time (hour)

TCS was removed after 55 hours, while approximately 70.3% was removed by 55 hours at a temperature of 30°C. This implies that TCS removal from activated sludge reactors at WWTPs will be increased during weather periods of increased temperature as well as with increased HRT and SRT. In a study focusing on seasonal variations of 40 organic contaminants in a membrane bioreactor (MBR) from an Australian WWTP, Trinh et al. (2016) found that TCS removal via biodegradation was increased during summer sampling (24˚C) when compared to winter sampling (15˚C). Conversely, removal of TCS via sorption to sludge increased in the winter, concurrent with the decrease in biodegradation removal rates [61]. Guerra et al. (2015) studied the concentrations of 41 organic pollutants in various stages of treatment in five different Canadian WWTPs. The study, which also compared seasonal differences, found that TCS concentrations in waste activated sludge and aerobically digested biosolids were significantly higher in samples collected in colder temperatures when compared to those collected in warmer temperatures. This difference in TCS concentrations in samples collected between the different sampling temperatures was attributed to an increased microbial degradation rate during warmer conditions. [62] Thus, the present study agrees with previous studies concluding that increased temperature results in an increase in TCS degradation.

In addition to temperature influences on TCS degradation, factors like HRT and SRT are also important in impacting TCS concentrations during wastewater treatment. As evidenced in the present study, extending the HRT beyond the 6-8 hours and the SRT beyond the 55 hours that typically occur at the study WWTP leads to a greater overall TCS degradation at both 21˚C and 30˚C. Furthermore, in a study focusing on the degradation of the pharmaceuticals atenolol, gemfibrozil, and

ciprofloxacin from laboratory bioreactor studies simulating activated sludge, a MBR, and submerged attached biofilter, it was determined that a reduction in HRT from 10 hours to 5 hours resulted in reduced pharmaceutical removal efficiencies due to a reduction in the exposure time of the compounds to microbial populations. In the same study, a decrease in the SRT for the activated sludge and MBR treatments also led to a decrease in atenolol, gemfibrozil, and ciprofloxacin degradation [63]. Clara et al. (2005), found that the degradation of pharmaceuticals, musks, and endocrine disrupting chemicals is increased (for compounds that can be biologically degraded under aerobic conditions) when SRT in both laboratory MBRs and full-scale WWTPs is increased. An increased SRT allows for a more diverse microbial population with more diverse physiological capabilities to develop since the increased residence time allows for slow-growing microbes, that would not have time to establish populations during reduced time periods, to proliferate. [64]

Concentrations of TCS in the aqueous fraction (Figure 2-1b) of all reactors (21°C and 30°C) were below 5 ng/mL. These concentrations were much lower than those associated with solids and represent a negligible (less than 0.01%) fraction of the total mass of TCS in the reactors. Trends of TCS in the aqueous fraction differed between the reactors run at 21°C and those run at 30°C. The aqueous phase concentrations in reactors run at 21°C (Figure 2-1b) fluctuated until hour 60, when TCS then decreased significantly at a rate of 0.0737 ± 0.015 $hour^{-1}$. Bioreactors run at 30°C, on the other hand (Figure 1b), demonstrated a steady decrease in TCS concentrations during the course of the experiments, with a degradation rate of 0.0191 ± 0.002 $hour^{-1}$. Fifty percent (50%) reduction of TCS concentrations occurred between hours 84 and 96 at 21°C and 12 to 36 hours at 30°C, indicating that the increased temperature was more effective at reducing TCS

concentrations in the aqueous fraction during activated sludge treatment, as was observed with solids samples. No TCS was removed from the aqueous fraction after 6 hours, which is the facility HRT, at 21°C and at 30°C 36.4 ± 7.78% removal occurred after 6 hours. Again, this indicates that increases in temperature and HRT help to further degrade TCS in activated sludge.

2.4.1.2 Triclocarban

As observed with TCS, concentrations of TCC in the activated sludge predominantly associated with the solids fraction. Concentrations in the solid phase, however, did not decrease over the course of the experiments run at 21°C until hour 96 (Figure 2-2a). The decrease at these time points was likely due to the reduced solids impacting the amount of sample available for analysis (not enough sample volume for detection above analytical limits). Conversely, reactors run at 30°C (Figure 2-2a) demonstrated an overall decreasing trend (0.0158 ± 0.012 $hour^{-1}$) in the solids fraction throughout the course of the experiments. While the increased temperature did improve upon TCC degradation, 50% removal did not occur until between hours 48 – 54, indicating reduced removal efficiency at this temperature, when compared to TCS, and the importance of an increase in HRT and SRT for improved degradation.

Average concentrations of TCC in the aqueous fraction was less than 2.0 ng/mL for all bioreactors at both 21°C and 30°C (Figure 2-2b). While some variation occurred, concentrations of TCC generally remained stable during experiments at or just above the LOQ. Overall, meaningful reduction did not occur in aqueous samples.

Low percentages of TCC removal in WWTPs have been found within the literature. For instance, Heidler et al. (2006) observed that even though 97% of TCC

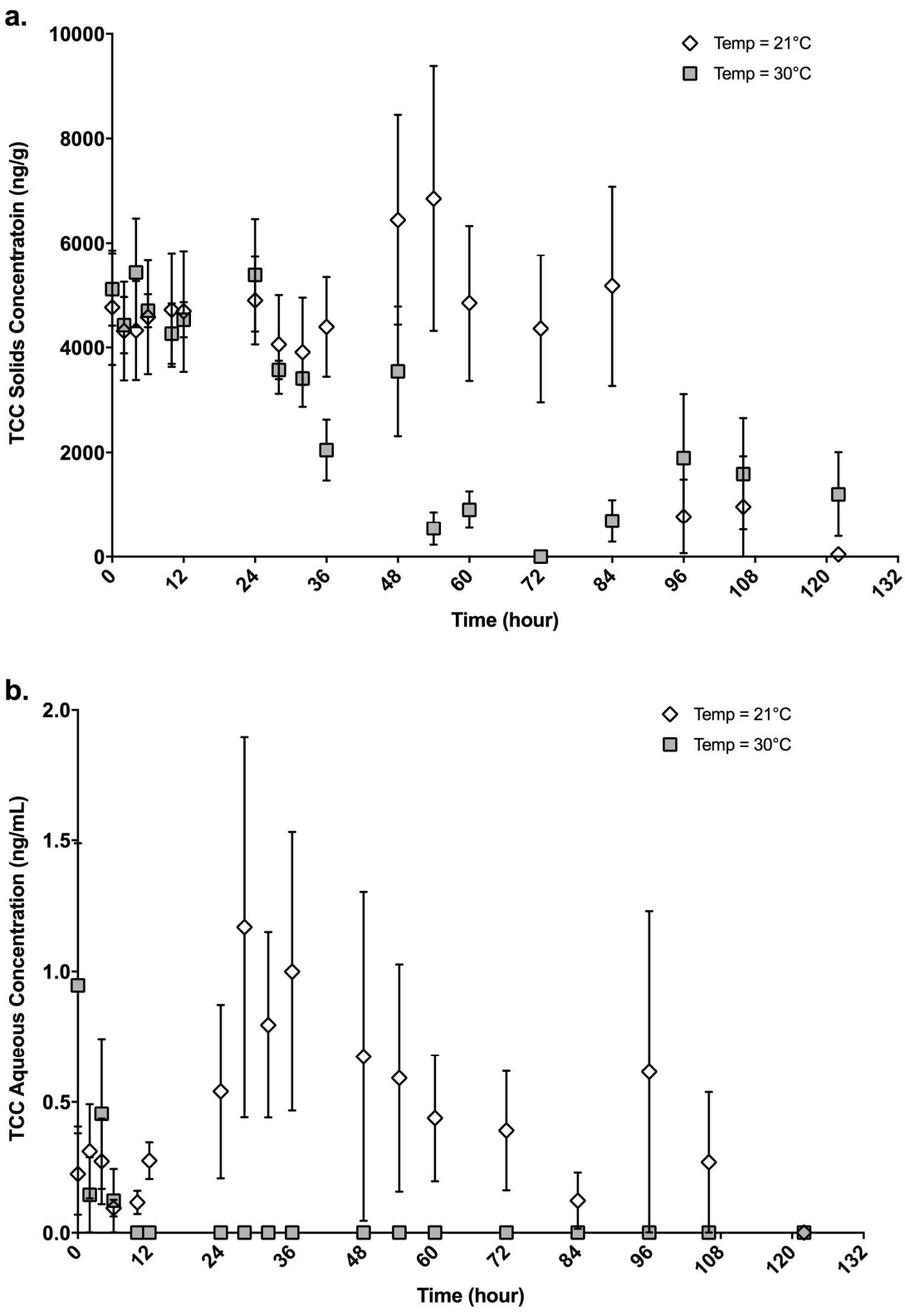


Figure: 2-2 Concentrations of TCC in a) Solids and b) Aqueous Factions of Activated Sludge Samples Over Time

present in the liquid fraction was removed from a WWTP with a secondary treatment followed by tertiary treatment of disinfection, 76% of this TCC removed from the water line was still present in the solids fraction. It was concluded that no more than 21% of the influent TCC was lost to bio-, chemical, or physical transformation. [65] Additionally, Lozano et al. (2013) studied all treatment stages of the WWTP where samples were collected for this current study and no TCC removal was observed during activated sludge process and only 18% of total TCC removal was obtained along the whole WWTP [11]. A study by Blair et al. (2015) using a 190 L batch reactor and focusing on the fate of 57 hormones, pharmaceuticals, and personal care products within mixed liquor suspended solids collected from a conventional activated sludge tank in Wisconsin, USA found that the removal efficiency of TCC was 11.4%. It was also observed that for several compounds degradation plateaued, where despite what is expected from first-order kinetics, degradation slows/stops for TCC. The degradation plateau for TCC was determined to be ~50 ng/L and degradation was not observed when concentrations were near this value but did occur for concentrations much greater than 50 ng/L, indicating the relevance of initial concentration in degradation efficiency. [66] While TCC degradation has been shown to occur under aerobic conditions in WWTPs, degradation occurs at slower rates than TCS. Studies of these two compounds in soils under aerobic conditions also demonstrate this trend [67,68] further indicating the capacity for TCC to be biologically degraded with sufficient time. This further indicates that increases in wastewater treatment parameters such as HRT and SRT, in an attempt to increase the contact time between microbes and TCC, are vital in improving degradation.

2.4.1.3 Methyltriclosan

MeTCS, like TCS and TCC, is predominantly associated with solids fraction of reactor samples. Concentrations of the compound also demonstrated the ability to increase during activated sludge treatment, although this was not observed uniformly within each reactor. Due to the varying difference in MeTCS formation during experiments, MeTCS data for each individual reactor is presented separately. At 21°C, bioreactor #1 MeTCS concentrations were relatively stable until hour 72 and 84, when concentrations increased at a rate of 0.0415 $hour^{-1}$, after which concentrations were not detected above the MDL, likely due to a low amount of solids available for analysis at this point in the experiment (not enough sample volume for detection above analytical limits) rather than an actual decrease in concentrations (Figure 2-3a). This MeTCS formation represents approximately 42% of initial TCS in the bioreactor. Alternatively, concentrations remained stable in bioreactor #2 until hour 96, when, again, concentrations were below the MDL. Experiments run at 30°C (Figure 2-3b) resulted in formation of MeTCS in both bioreactors. Concentrations increased in bioreactor #1 at a rate of 0.0071 $hour^{-1}$ beginning at hour 96 while MeTCS increased at a rate of 0.0191 $hour^{-1}$ beginning at hour 48 in bioreactor #2. The production of MeTCS at 30°C represents approximately 81% of the initial TCS concentrations in bioreactor #1 and 176% of the initial concentrations in bioreactor #2. Elevated concentrations of MeTCS in solids are of concern as the compound has higher endocrine disrupting capabilities than TCS [26]. These high rates of MeTCS production may be due to not only direct transformation of TCS to MeTCS, but the transformation of other TCS byproducts not analyzed in this study as well. Complex transformation pathways with numerous intermediate products have been demonstrated in the literature [52,69,70]. Further

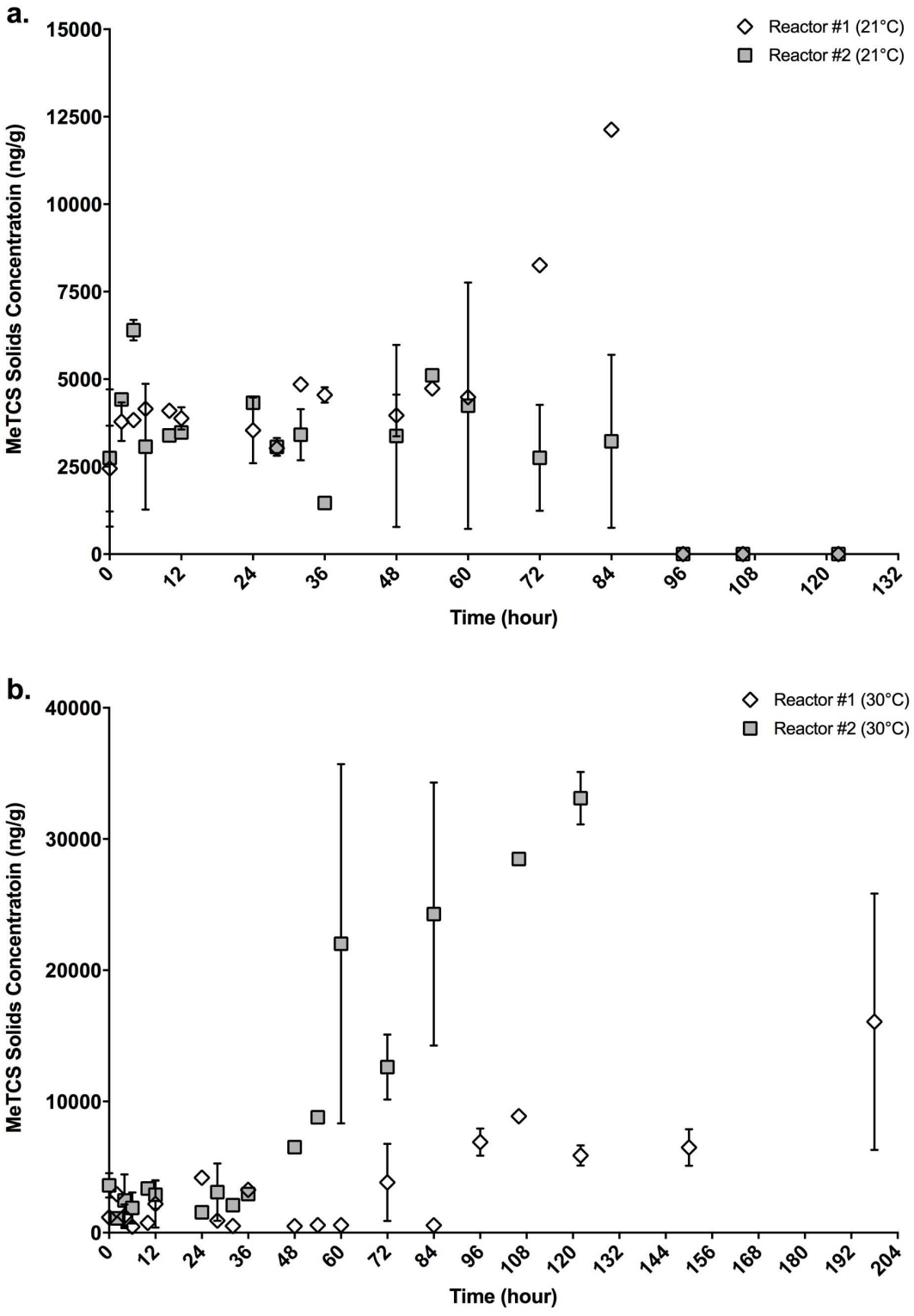


Figure 2-3: Concentrations of MeTCS in the Solids Faction of Activated Sludge Samples at a) 21°C and b) 30°C Over Time

investigations into the presence of these intermediates in activated sludge and their influence on MeTCS formation have not yet been conducted. These results indicate that an increase in HRT and temperature may influence the production of MeTCS from TCS. Additionally, because MeTCS production was reactor specific, the results indicated that microbial communities formed in an activated sludge reactor would also likely have a significant influence on the amount of MeTCS produced. Further research into the identification of microbial communities in different activated sludge treatment systems is needed.

Conversely, MeTCS concentrations in the aqueous fraction of activated sludge (Figure SI-A8) remained stable at both 21°C and 30°C. Additionally, average concentrations were below 3.5 ng/mL for both temperatures, indicating that the aqueous fraction represented only a very small portion of the total MeTCS in the activated sludge.

Studies have shown that MeTCS can be formed by the biological degradation of TCS under aerobic conditions [71,72]. Many of these studies are done at lab scale and with TCS spiked at the beginning of experiments, with concentrations that favor MeTCS formation and detection. Chen et al. (2011) observed that high TCS concentrations (1,000 and 2,000 µg/L) were necessary to observe MeTCS at detectable concentrations after aerobic treatment of activated sludge. These TCS concentrations were much higher than the initial TCS concentrations detected in this study. For initial concentrations of 20 µg/l (unspiked), Chen et al. did not observe MeTCS formation. However, it is important to note that in the studies performed by Chen et al., only the liquid fraction of reactor samples was analyzed for MeTCS. Given the propensity of MeTCS to associate with solids (log K_{ow} = 5), it can be expected that much of the MeTCS formed would be found within the sludge phase of

a wastewater sample rather than the aqueous phase, as was observed in the present study. Additionally, in the same study performed by Chen et al., it was observed in preliminary reactor experiments that MeTCS significantly increased by 16% during aerobic experiments after 80 hours with an initial MeTCS concentration of 30 µg/L [71]. Furthermore, Tohidi & Cai (2017) noted MeTCS formation from TCS to be as high as 25.5% during secondary biological treatment in a study focusing on three WWTPs with different treatment streams [73]. Overall, these results are in concordance with Lozano et al. (2013), who observed an increase in MeTCS in the activated sludge process at the typical TCS concentrations found in the wastewater of the current study facility.

2.4.1.4 TCS and TCC degradation products

The TCS and TCC byproducts 2,4-DCP, 4-chlorocatechol, DCC, MCC, NCC and 4-CA were monitored qualitatively to determine their presence in the bioreactors. 4-Chlorocatechol, DCC, MCC and NCC were not detected in any solid or liquid samples collected from reactors run at 21 and 30ºC. However 2,4-DCP and 4-CA were clearly detected in the liquids samples but not in the solids. Both compounds were confirmed using authentic standards. While this study was not designed to quantify the byproducts, a preliminary qualitative scan was performed (no internal standards were used, QA/QC was not determined).

2,4-DCP is known to form from aerobic biodegradation of TCS [74,75] and photolysis [69]. No formation of 2,4-DCP was observed during the present study at either of the temperatures studied. However, decreases were observed at both 21 and 30ºC. All concentrations of 2,4-DCP observed in this study were below 3.5 ng/mL and represent a very small portion of the total TCS detected. The ability of 2,4-DCP to be biodegraded aerobically has been demonstrated in the literature. For

instance, Matafonova et al. (2006) found that *Bacillus* sp. isolated from an aeration basin at a Russian pulp and paper mill were able to degrade 2,4-DCP. The *Bacillus* sp. were effective in biodegradation for concentrations of 2,4-DCP 400 μM and below; higher concentrations inhibited cell growth and efficient degradation did not occur [76]. 2,4-DCP is listed as a priority pollutant by the US Environmental Protection Agency and has demonstrated toxicological properties including endocrine disruption [69,77] and its removal during treatment prior to release by wastewater treatment liquid effluent is important for environmental health. Further studies involving quantitation with QA/QC protocols should be conducted to further understand the fate of 2,4-DCP in activated sludge.

Chloroanilines, such as 4-CA, can be formed from TCC biodegradation [54]. In this study, 4-CA was detected in liquid samples from bioreactors run at both 21˚C and 30˚C. The concentrations were found to be very variable during the reactor runs. Because 4-CA was analyzed qualitatively in this study, variation of the compound may have been due to the fact that the extraction method was not optimized for this compound. Also, 4-CA can be biologically degraded to 4-chlorocatechol [54,78] but has also been found to be present in wastewaters due to their utilization in the production of products such as pharmaceuticals, dyes, and pesticides [79], further complicating concentrations in the wastewater treatment system. 4-CA concentrations in this study were below 40 ng/mL and represent a small portion of total TCC concentrations observed within the bioreactors. Again, further studies regarding the fate of 4-CA conducted under QA/QC protocols should be conducted to better understand the fate of 4-CA in activated sludge.

2.5 Conclusions

The antimicrobials TCS and TCC have demonstrated various environmental and health concerns, notably endocrine disruption. Prolific use of these compounds has resulted in their presence in the wastewater treatment process, where they are only partially degraded and often released into the environment via wastewater effluent or the land application of biosolids. Understanding how wastewater treatment processes influence degradation of these compounds is vital to reducing their discharge from the wastewater process. While TCS was predominantly associated with the solids fraction of the samples, activated sludge treatment demonstrated the ability to degrade TCS from both the liquid and solids fraction. Solids fraction degradation rates were increased in reactors run at 30°C and the increased HRT and SRT resulted in more efficient TCS removal. MeTCS increased in concentration in the solids fraction of three of the four reactors run, indicating a noteworthy formation of the compound from degraded TCS or other TCS byproducts not analyzed in the present study. Further research would need to be conducted to further understand compound sources for MeTCS formation during activated sludge treatment. TCC concentrations were only observed to decrease in the solids fraction in reactors run at 30°C, emphasizing the importance of increased temperature during anaerobic digestion for efficient TCC removal. Additionally, as with TCS, the increased HRT and SRT resulted in improved TCC removal from the system. Finally, 2,4-DCP and 4-CA were observed in the liquids fraction of the bioreactors while 4-chlorocatechol, DCC, MCC and NCC were not detected. Further studies involving quantitation with QA/QC protocols should be conducted to further understand the fate of these additional TCS/TCC transformation products in activated sludge.

Chapter 3: Fate of Triclosan, Triclocarban, and Their Transformation Products in Wastewater Under Nitrifying Conditions

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3.1 Abstract

The nitrification process was simulated using benchtop bioreactors to gain insight into the fate of the antimicrobials triclosan (TCS) and triclocarban (TCC), as well their transformation products, during wastewater treatment. Currently, little information exists on the impact of nitrification treatment on concentrations of TCC, TCC degradation products, and TCS degradation products. Reactors were run using samples collected from a large municipal wastewater treatment plant at two pH ranges (6.5 – 7.5 and 8.5 – 9.5) for 171 hours to simulate an extended hydraulic retention time (HRT). TCS was degraded under both pH conditions, with a 28.5% overall reduction in solids samples when the pH range was 6.5 – 7.5 and an overall reduction of 83.2% in solids samples when the pH ranged 8.5 – 9.5. Methyltriclosan (MeTCS) was formed in solids samples during both treatment conditions. MeTCS formed the most rapidly during the first 25 hours of treatment at pH 8.5 – 9.5. Levels of 2,4-dichlorophenol, a TCS photolysis product, and TCC did not change over the 171 h treatment period, indicating that nitrification is not an effective treatment for reduction of these compounds. Three TCC dechlorination products and triclosan-O-sulfate were not observed at or above the limit of quantitation in any bioreactor samples.

3.2 Introduction

The land application of treated sludge (biosolids) and discharge of liquid effluent to local water bodies from wastewater treatment plants (WWTPs) can act as environmental sources of emerging contaminants (ECs) not fully removed within treatment systems. With worldwide chemical production projected to increase beyond population growth [1], the inevitable reliance on chemicals by industry, medicine, agriculture, households, etc. can result in the presence of a variety of ECs within wastewater treatment processes. Such chemicals can include pharmaceuticals and personal care products (PPCPs), flame retardants, surfactants, and food additives, among others [80].

One group of PPCPs that have garnered interest from regulatory agencies due to health and environmental concerns are antimicrobials – specifically, triclosan (TCS) and triclocarban (TCC). In 2016, the United States Food and Drug Administration issued a phase-out of TCS and TCC from consumer antiseptic washes [7], while in the same year TCS was not approved for use in product-type 1 items (human hygiene biocidal products for skin or scalps) by the European Commission [81]. In the United States these antimicrobials can still remain in products used in healthcare situations [7]. Concerns regarding TCS and TCC in the environment primarily center around their endocrine disrupting capabilities [27,45,46] as well as microbial resistance issues [82,83]. Furthermore, TCS and TCC transformation products, such as methyltriclosan (MeTCS) and carbanilides, can also induce endocrine disruption [45].

TCS and TCC have been shown to be prolific in the wastewater treatment system [73,84,85] and, as such, present in the environment due to wastewater effluent discharge and biosolids application. Field studies have estimated the half-

lives of TCS and TCC in soils after biosolids applications to be 104 days and 288 days, respectively, while MeTCS is more persistent, with a half-life of approximately 443 days after biosolids application [25,86]. Additionally, it has been demonstrated that the long-term application of biosoids to agricultural soils can result in the migration of both TCS and TCC through a terrestrial food web, from primary consumers to secondary and tertiary consumers [87] as well as the phytoaccumulation of the compounds in different vegetative species, including switch grass and squash [88]. In surface waters, concentrations of the antimicrobials have been detected in aqueous, suspended particulate, and sediment samples, with the highest concentrations occurring near WWTP discharge points [89]. TCS, TCC, and MeTCS can bioaccumulate in freshwater snails and algae [90] and, furthermore, TCS and TCC can also inhibit the growth of freshwater algae [91].

One way to reduce the presence of TCS and TCC in the environment via wastewater treatment discharge is by altering common wastewater treatment processes to improve compound degradation. Nitrification treatment biologically converts ammonia/ammonium in waste streams to nitrate and the process has demonstrated the ability to degrade TCS [92,93]. Little information exists on the impact of nitrification treatment on concentrations of TCC, TCC degradation products, and TCS degradation products. This study focuses on the fate of TCS, TCC, and their transformation products during nitrification treatment in a laboratory setting. Benchtop bioreactors were used to simulate the nitrification process and allowed for conditions such as hydraulic retention time (HRT) and pH to be altered in order to understand how these changes impact compound concentration over time. Research has demonstrated that increasing the HRT during activated sludge treatment improves TCS and TCC degradation [94] and that the optimum pH for

nitrification in waste streams can vary [95,96]. The US EPA reports that while the ideal pH range for *Nitrosomonas* and *Nitrobacter* is between 7.0 and 8.0, nitrification can occur at pH concentrations ranging from 6.6 to 9.7 due to additional environmental factors that can impact nitrifying bacterial populations [97]. Two reactors were run simultaneously at 21°C for 171 hours at a pH range of 6.5 – 7.5 and then again at a range of 8.5 – 9.5 with sample collected from a nitrification reactor from a large WWTP located in the Mid-Atlantic region of the United States. No spiking of the target compounds into the bioreactors took place. Samples were collected periodically from each reactor so that TCS and TCC degradation rates and transformation product formation rates could be calculated over the 171-hour period. Results from this study demonstrate how altering a somewhat common treatment process can impact concentrations of antimicrobials and their transformation products in wastewater, which, in turn, can influence the amount of each compound emitted to the environment.

3.3 Materials and Methods

3.3.1 Target Analytes

All samples were analyzed for concentrations of TCS and its transformation products: MeTCS, 2,4-dichlorophenol (2,4-DCP), and triclosan-O-sulfate (TCS-O-sulf). Additionally, samples were analyzed for TCC and its dechlorination products: 4,4'-dichlorocarbanilide (DCC), 1-(3-chlorophenyl)-3-phenylurea (MCC), and carbanilide (NCC). Further information regarding the suppliers of analytical standards for the target analytes and the compound purities is located in Table SI-B1 of the Supplemental Information.

3.3.2 Wastewater Treatment Plant Background

The study facility is located in the Mid-Atlantic region of the United States and serves a population of over 2 million, with a daily treatment load of approximately 1.14 million m^3 of raw sewage per day. The facility treats incoming wastewater by preliminary treatment, primary treatment, aerobic secondary treatment ("activated sludge"), tertiary nitrification-denitrification treatment, filtration, and disinfection. Sludge is treated by the Cambi Thermal Hydrolysis Process™ in conjunction with mesophilic anaerobic digestion. A process flow diagram of treatment stages at the facility is provided in Figure SI-1 in the Supplemental Information.

The average HRT for the nitrification-denitrification reactors combined is 8 hours while the average sludge retention time for the two reactors is 20 days. These time periods, however, can vary with flow. The target dissolved oxygen (DO) concentration for the nitrification reactors is 2.0 mg/L but can range from 1.2 to 4.8 mg/L. The target pH of the nitrification process is 6.5. The on-site reactors are outdoors, exposed to the elements, and without heating elements. As such, typical temperatures within the reactors can range from 14 to 23°C, depending on seasonal ambient temperatures.

3.3.3 Benchtop Bioreactor Experimental Setup

The nitrification treatment process was simulated at a laboratory-scale using two BioFlo® 115 Benchtop Bioreactors (New Brunswick Scientific, Edison, NJ, USA). Samples were collected from the study WWTP near the input of a nitrification reactor, in an area of sufficient mixing, and immediately transported to the laboratory for use within the bioreactors. Five (5) liters of nitrification sample was placed in each bioreactor. No spiking of target compounds into the bioreactors took place. No additional wastewater was added to the reactors after the start of the experiments.

The two reactors were operated simultaneously, as duplicates, for two separate runs. Experimental conditions for each run are provided in Table 1. The BioFlow 115® control panel allowed, as needed, for the automatic addition of 3M sodium hydroxide or 1.5M sulfuric acid to constantly maintain the pH within the allowable range provided in Table 1 as well as the automatic adjustment of mixing rates to maintain a target dissolved oxygen (DO) concentration of 2 mg/L.

The bioreactors were operated for 171 hours during each run – well beyond the 8-hour HRT for the nitrification reactors at the study WWTP. In order to gauge trends of TCS, TCC, and transformation product concentrations, 200 mL samples were collected at hours 0, 2, 4, 6, 8, 12, 25, 29, 33, 37, 49 (Run #1 only), 55, 59, 73, 85, 98, 122, 146, and 171 from the sample port of each bioreactor. Samples were analyzed immediately for solids, soluble chemical oxygen demand, and microbial activity. The remainder was frozen at -20°C for eventual analysis of TCS, TCC, and transformation products. Additionally, 1L airtight jars were filled with 0.5 L of nitrification sample and 1g of mercuric chloride to act as control reactors. The control reactors were placed in a water bath, maintained at 30°C, and agitated continuously for 171 hours. Initial samples (hour 0) and final samples (hour 171) were analyzed for all target compounds. Furthermore, final samples were plated to confirm that microbial populations within the control reactors did not survive treatment with mercuric chloride.

Table 3-1: Experimental Conditions for Bioreactor Runs

	Run #1	Run #2
Temperature (°C)	21	21
pH (target)	7.0	9.0
pH (range allowed)	6.5 - 7.5	8.5 - 9.5
DO (mg/L)	2.0	2.0
Total run time (hours)	171	171

3.3.4 Extraction and Analysis of Target Analytes

3.3.4.1 Sample Extraction

The aqueous and solid fractions of each sample collected from the bioreactor were analyzed separately. To achieve this, each sample was filtered through a Whatman GA/F 0.7 µm filter (GE Healthcare Life Sciences, Pittsburgh, PA, USA) prior to extraction. All samples were extracted in duplicate.

The aqueous matrix was extracted according to a previously established method [11,58]. In short, Oasis® HLB solid phase extraction (SPE) cartridges (6 mL, 200 mg) (Waters Corporation, Milford, MA, USA) were utilized to separate the target compounds from acidified (pH ~2) liquid samples. A solution of 10 mM acetic acid in methanol:acetone (50:50 v/v) was used for compound elution from the cartridges prior to eluate evaporation and reconstitution for instrumental analysis in 1.5mL methanol.

The filters containing the solids fraction of the bioreactor samples were baked at 100°C for at least 24 hours so that the samples could be extracted on a dry weight basis using a method published previously [59]. Briefly, dried filters and solids samples were packed in accelerated solvent extraction (ASE) cells along with laboratory-grade sand. A Dionex ASE #300 instrument (Dionex Corporation,

Sunnyvale, CA, USA) was run with an isopropyl alcohol and organic-free water (80:20 v/v) mixture to extract the target analytes from the sludge matrix. Extract clean-up was achieved with Oasis® HLB solid phase extraction (SPE) cartridges (6 mL, 200 mg) (Waters Corporation, Milford, MA, USA). SPE eluates were evaporated and reconstituted in 1.5 mL methanol.

3.3.4.2 Instrumental Analysis

Analysis of all compounds except MeTCS took place using a Shimadzu Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) coupled with a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA) with an electrospray ionization source run in negative mode. To achieve chromatographic separation of TCS, TCC, 2,4-DCP, DCC, MCC and NCC, a solution of 10 mM ammonium acetate in methanol:acetonitrile:water (60:15:25 v/v) was run isocratically through a Supelco Ascentis® Express C18 reverse phase column (2.7 µm, 50 x 2.1 mm) (Sigma-Aldrich, St. Louis, MO, USA) at a rate of 0.5 mL/min [49]. For TCS-O-sulf, an isocratically run mobile phase of 0.2 % formic acid in methanol along with a Supelco Ascentis® Express C18 reverse phase column (2.7 µm, 50 x 2.1 mm) (Sigma-Aldrich, St. Louis, MO, USA) was run at a rate of 0.55 mL/min [49]. For all compounds, multiple reaction monitoring was used for MS acquisition. Further details regarding UHPLC-MS/MS conditions are provided in elsewhere [49].

After all UHPLC-MS/MS analyses were completed, samples were evaporated and reconstituted in 1 mL hexane for MeTCS analysis via an Agilent 7890B gas chromatograph (GC) in conjunction with an Agilent 5977A mass selective detector (MSD) run in positive electron impact ionization mode [25]. For this method, chromatographic separation was achieved using a 15 m capillary column (DB-5-MS)

with diameter of 0.25 mm, and film thickness of 0.1 μm (J&W Scientific, Folsom, CA, USA). Further details regarding the GC-MS analytical methodology are provided in a previous publication [49].

3.3.5 Secondary Analyses

3.3.5.1 Solids Analysis

All samples collected from the bioreactors were analyzed for total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS), and total volatile solids (TVS) via the standard methods established by the American Public Health Association [56]

3.3.5.2 Soluble Chemical Oxygen Demand (sCOD)

Soluble chemical oxygen demand (sCOD) was determined according to standard methods [56]. Nitrification samples collected at each time point were filtered through a Whatman GA/F 0.7 μm filter (GE Healthcare Life Sciences, Pittsburgh, PA, USA), acidified with H_2SO_4, and refrigerated at 4°C until analysis. Dichromate COD vials (low range, 0-150 ppm) (Chemetrics, Midland, VA, USA), a digester block, and a photometer (Hach Company, Loveland, CO, USA) were used for the measurement sCOD in each sample.

3.3.5.3 Microbial Activity

A previously established fluorescein diacetate (FDA) method was utilized for total microbial activity [94]. In short, for each time point, 1 to 2 mL of sample from each reactor was shaken with 15 mL of a potassium phosphate buffer and 0.2 mL of a FDA solution. The mixtures were then incubated at 30°C after which 15 mL of a chloroform/MeOH solution was added to each flask. Finally, samples were shaken, centrifuged, and filtered for analysis of fluorescein concentrations in the filtrates via a

UV-1800 UV-Vis spectrophotometer (Shimadzu North America, Columbia, MD, USA) at a wavelength of 490 nm.

3.3.6 Quality Assurance/Quality Control

The method detection limits (MDLs) for all target analytes were calculated using a United States Environmental Protection Agency established method [98] and the limits of quantitation (LOQs) for each compound were defined as two times the MDL. MDLs and LOQs for all compounds are provided in Table SI-B2 in the Supplemental Information.

Prior to the extraction process, all samples were spiked with $^{13}C_{13}$-TCC, $^{13}C_{12}$-TCS $^{13}C_{12}$-MeTCS, and d_3-2,4-DCP to account for extraction efficiency. All extractions were carried out in batches of 12 or less and all samples were extracted in duplicate. Each extraction batch consisted of a blank (either organic-free water or laboratory-grade sand) and a spiked sample for the determination of compound recoveries. Average compound recovery for target analytes in each sample matrix are provided in Table SI-B2 in the Supplemental Information.

For UHPLC-MS/MS and GC-MS analysis, calibration curve consisting of a minimum of seven standards for each compound at concentrations other than zero was run. Linear calibrations curves yield r-squared values ≥ 0.99. Additionally, two standards and two solvent blanks were injected every 10 samples to verify instrument stability.

GraphPad 7 was used for statistical analyses and figure creation (GraphPad Software Inc., San Diego, CA, USA).

3.4 Results and Discussion

Microbial activity, sCOD, and solids analyses results are provided in Figures SI-B2 through SI-B4 in the Supplemental Information. Microbial activity generally

decreased during the experiments with the most rapid decrease occurring between hours 0 and 36 (Figure SI-B2). sCOD (Figure SI-B3), on the other hand, steadily increased in all reactors during the course of nitrification experiments. Finally, solids concentrations (in all forms) decreased overall (Figure SI-B4).

Pseudo-first-order kinetics were used to determine compound degradation or formation rates, where applicable, via equation #1:

$$\ln \frac{C}{C_o} = -kt \qquad (1)$$

where C is the target compound concentration at a given time point, C_o is the initial concentrations of the target compound, t is time, and k is the degradation rate.

3.4.1 Triclosan and Its Transformation Products

3.4.1.1 Triclosan

The majority of the TCS mass in the nitrification reactors was sorbed to the solids matrix at both experimental pH ranges (Figure 1a). At a pH range of 6.5 – 7.5, TCS concentrations decreased under nitrifying conditions at an average rate of 0.0195 ± 0.006 h^{-1} until approximately 37 – 49 hours, after which concentrations leveled out and did not change significantly. Conversely, when the reactors were run at a pH range of 8.5 – 9.5, TCS was consistently degraded over the 171-hour period at a rate of 0.0101 ± 0.005 h^{-1}. After 171 hours, concentrations of TCS were reduced by 28.5 ± 0.09 % at a pH range of 6.5 – 7.5 and 83.2 ± 0.05 % at a pH range of 8.5 – 9.5, demonstrating an increased efficiency over the course of the experiments of compound degradation during nitrification at a higher pH range. Additionally, TCS degradation continued to occur beyond the 8-hour mark under both treatment conditions, demonstrating that extending treatment beyond facility HRT led to increased degradation of TCS.

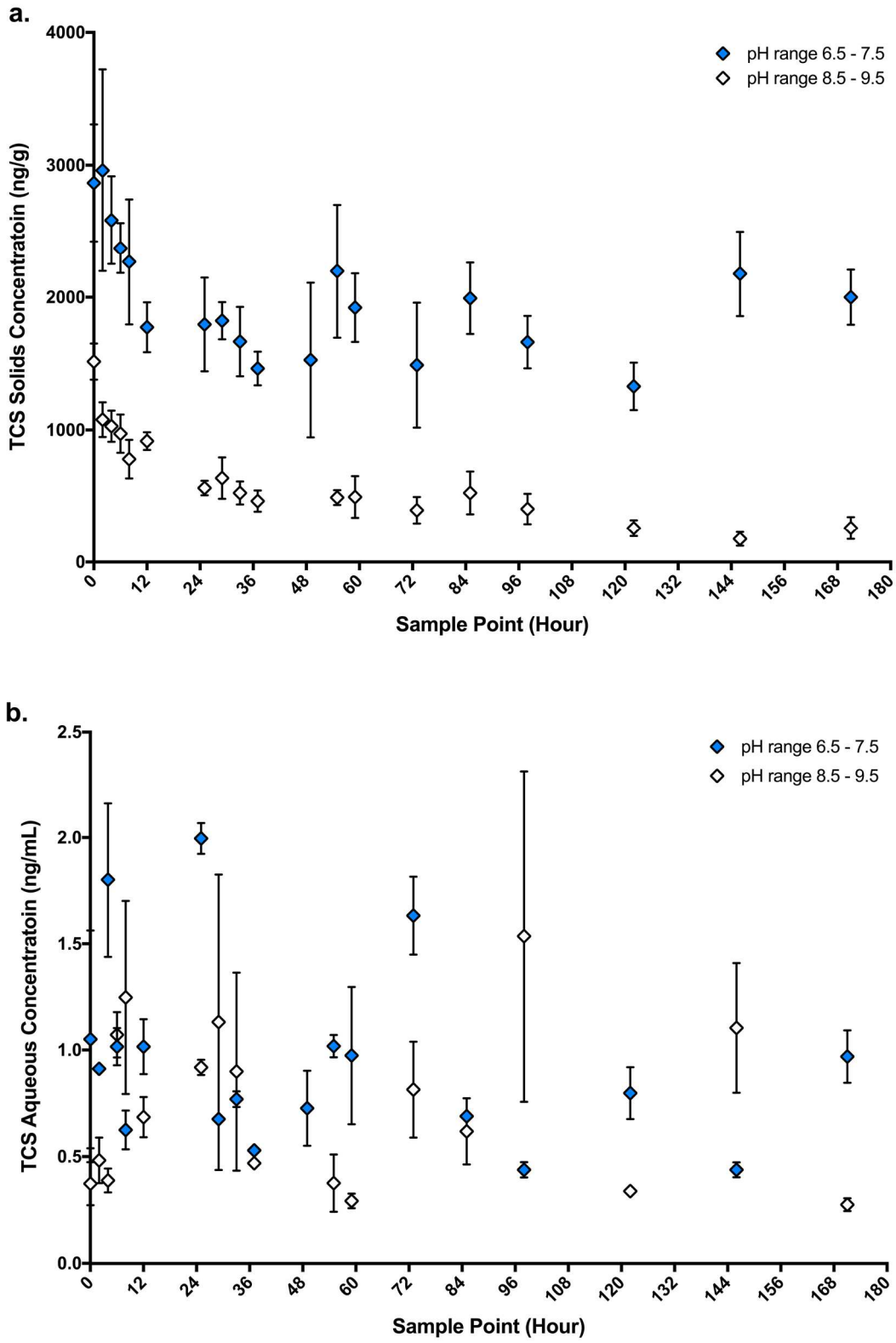

Figure 3-1: Concentrations of TCS in a) Solids and b) Aqueous Factions of Nitrification Samples Over Time

Concentrations of TCS in the aqueous fraction (Figure 1b) of nitrification samples did not significantly change over the 171-hour period when the bioreactors were run at a pH range of 6.5 – 7.5 (Linear regression slope test, P = 0.2034) and 8.5 – 9.5 (Linear regression slope test, P = 0.8329). Average concentrations in aqueous samples were below 2.5 ng/mL under reactor conditions.

Observations within this study that demonstrate that TCS can be degraded under nitrifying conditions are consistent with those within the literature. Roh et al. (2009) found that a pure culture of the ammonia oxidizing bacteria (AOB) *Nitrosomonas europaea* has the ability to biodegrade the antimicrobial via cometabolic reactions. In experiments carried out in 1-L flasks, the bacterial strain reduced TCS concentrations by over 60%. Additionally, the authors demonstrated the TCS could be significantly removed by nitrifying activated sludge samples over a 5-day period, concluding that both AOB and heterotrophic microbes possess the ability to biodegrade the compound [92]. Laboratory-scale simultaneous nitrification and denitrification (SND) reactors operated with synthetic wastewater resulted in high TCS removal rates. Seventy seven percent (77%) of TCS removal from the SNDs was attributed to various processes which included biodegradation but not sorption or removal via effluent [99]. However, while these experiments demonstrate the ability of microbial populations associated with nitrification-denitrification can degrade TCS, they do not specifically focus on actual treatment of wastewater by either nitrification, as in the present study, or nitrification-denitrification.

In a mass balance performed at the same WWTP utilized for the present study, Lozano et al. (2013) found that the combined nitrification-denitrification process at the facility was able to remove 2.29 kg TCS per day. It was also observed that this removal was higher than that observed during secondary

activated sludge treatment at the facility [11]. Conversely, in a similar study conducted using benchtop bioreactors and activated sludge from the same facility, TCS degradation rates in the solids fraction for reactors run at 21°C and 30°C (- 0.0170 ± 0.003 and - 0.0224 ± 0.007 h-1, respectively) were fairly similar to those observed in the course of this study (Table 2) [94], indicating that a variety of aerobic microbial populations can degrade TCS in wastewater. Results from these studies, in conjunction with the present study, demonstrate the complexity of the treatment process and, based on these results, future work should focus on the impact of the denitrification process and identification of microbial species present during nitrification treatment.

Table 3-2: TCS. TCC, MeTCS Rates of Change in Nitrification and Activated Sludge

	Present Study Nitrification		**Armstrong et al. (2018) Activated Sludge**	
	pH: 6.5 - 7.5	**pH: 8.5 - 9.5**	**Temp: 21°C**	**Temp: 30°C**
TCS	- 0.0195 ± 0.006	- 0.0101 ± 0.005	- 0.0170 ± 0.003	- 0.0224 ± 0.007
TCC	N/A	N/A	N/A	- 0.0158 ± 0.012
MeTCS	+ 0.00985 ± 0.007	+ 0.0023 ± 0.004	+ 0.0415 [a]	+ 0.0071 [b]; + 0.0191 [c]

[a] Bioreactor #1 only, no MeTCS formation was observed in bioreactor #2 at 21°C.

[b] Bioreactor #1 only.

[c] Bioreactor #2 only.

3.4.1.2 Methyltriclosan

As was observed for TCS, the MeTCS present within the systems was overwhelmingly associated with the solids fraction (Figure 2), while concentrations within the aqueous fraction were not detected above the LOQ. MeTCS formation began right from the start of the experiments under both pH conditions. In reactors run at the pH range of 6.5 – 7.5, concentrations increased slowly, at a rate of

0.00985 ± 0.007 h^{-1} until hour 98. MeTCS concentrations then leveled out for the remainder of the experiments. However, when run at a pH range of 8.5 – 9.5, MeTCS solids concentrations within the bioreactors increased more rapidly, at a rate of 0.0174 ± 0.005 h^{-1} until hour 25 and then continued to increase more slowly, at a rate of 0.00190 ± 0.002 h^{-1}. The overall rate of MeTCS formation for reactors run with a pH range of 8.5 – 9.5 was 0.0023 ± 0.004 h^{-1}. While MeTCS is considered one of the main TCS transformation products, its formation in solids samples was associated with 19.5% and 22.2% of observed TCS loss in solids for pH ranges of 6.5 – 7.5 and 8.5 – 9.5, respectively, indicating that more mechanistic studies focusing on both TCS degradation and MeTCS formation are needed.

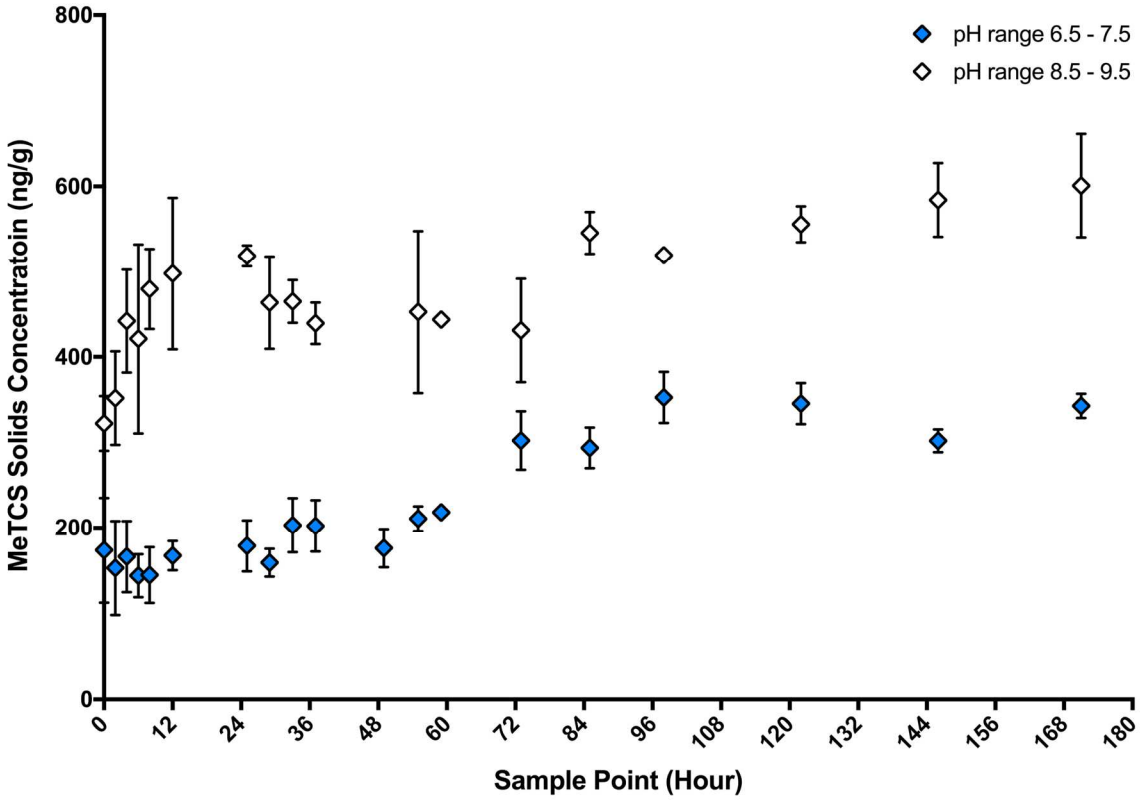

Figure 3-2: Concentrations of MeTCS in the Solids Fraction of Nitrification Samples

Little information exists regarding the formation of MeTCS during the nitrification treatment process. Lozano et al. (2013) observed that significant MeTCS formation occurred during nitrification-denitrification treatment at the same WWTP used for the present study. A mass balance of the facility revealed that 0.03 kg MeTCS per day was produced and was associated with TCS degradation [11]. Formation rates of MeTCS in the present study differed from those observed by Armstrong et al. (2018) in bioreactors run using activated sludge from the same WWTP (Table 2). In the previous study, formation rates of MeTCS appeared to be more variable – different reactors run under the same treatment conditions were shown to result in dissimilar final concentrations of MeTCS during the course of experiments. This demonstrates the complexity of the degradation/formation process during aerobic wastewater treatment.

3.4.1.3 2,4-Dichlorophenol

2,4-DCP was only detected in solids samples from all bioreactors (Figure 3). Over the course of the experiments, concentrations did not significantly change in bioreactors run at a pH range of 6.5 – 7.5 (Linear regression slope test, $P = 0.8632$) or 8.5 – 9.5 (Linear regression slope test, $P = 0.3472$). The presence of 2,4-DCP in the reactors could be due to a combination of TCS photolysis [69] upstream and the degradation of other organic compounds that result in 2,4-DCP formation. Given that 2,4-DCP can be both formed by the degradation of other compounds, such as dichlorprop (2,4-DP) and 2,4-dichlorophenoxyacetic acid (2,4-D) [100-102] and degraded aerobically [103], it may be that concentrations remain relatively steady over the course of the experiments due to continuous formation/degradation.

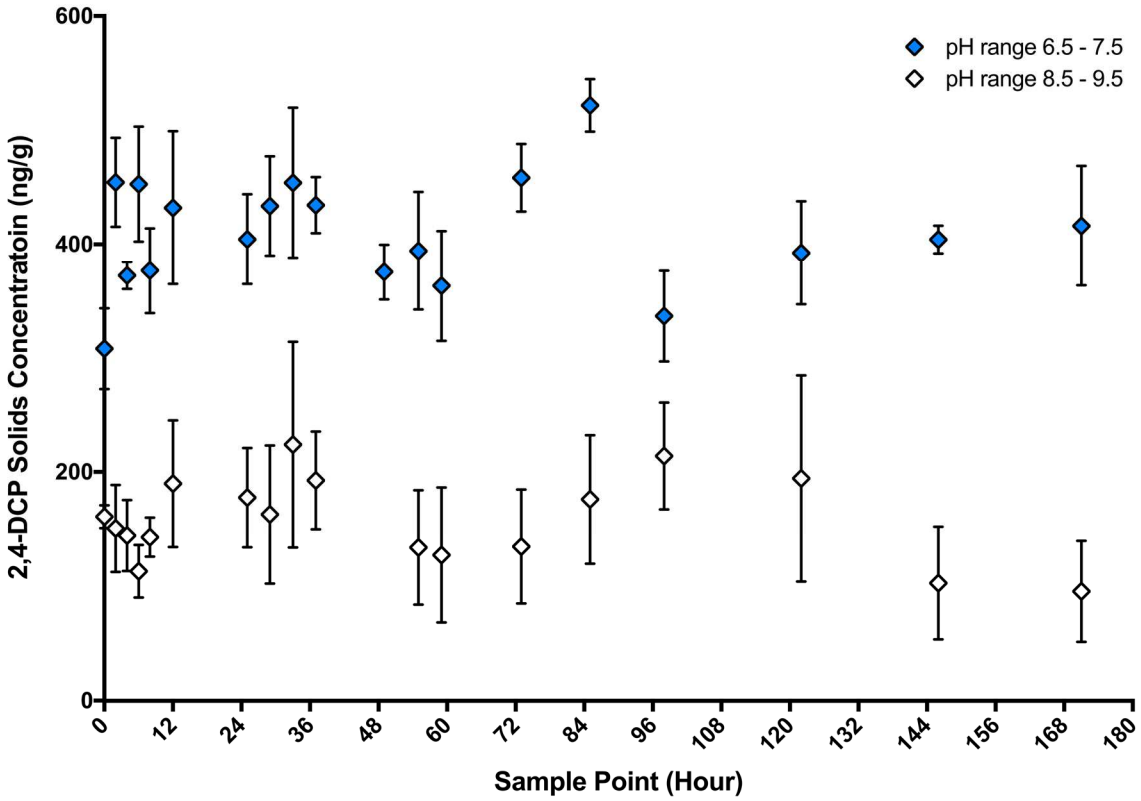

Figure 3-3: Concentrations of 2,4-DCP in the Solids Fraction of Nitrification Samples

3.4.1.4 Triclosan-O-Sulfate

TCS-O-sulf was not detected at or above the LOQ in any samples, aqueous or solids fraction, collected from the bioreactors.

3.4.2 Triclocarban and Its Transformation Products

3.4.2.1 Triclocarban

TCC was predominantly affiliated with the solids fraction of nitrification samples (Figure 4a). Concentrations within the solids matrix were unchanged under both experimental pH ranges [Linear regression slope test, P = 0.1209 (pH: 6.5 – 7.5); P = 0.1151 (pH: 8.5 – 9.5)]. Furthermore, TCC was not detected above 2.5 ng/mL in any aqueous samples (Figure 4b). Results show that the nitrification process was unable to degrade TCC significantly. Conversely, Lozano et al. (2013)

observed that at the same study facility, significant amounts of TCC was degraded during nitrification-denitrification treatment. However, removal of TCC during nitrification-denitrification, as observed by Lozano et al., may have been due predominantly to the denitrification process. The bacterial strain *Ochrobactrum* sp. TCC-1 has been demonstrated to degrade TCC under anoxic conditions and could do such during wastewater denitrification [104]. Under aerobic conditions, activated sludge was demonstrated to degrade TCC at a rate of - 0.0158 ± 0.012 h^{-1} at a temperature of 30°C but was not removed at all at 21°C (Table 2) [94]. Further research into the separate impacts of wastewater nitrification and denitrification on concentrations of TCC, as well as the microbial populations involved in the processes needs to be conducted to gain further insight into the degradability of the antimicrobial during treatment.

3.4.2.2 Triclocarban Dechlorination Products

Concentrations of DCC, MCC, and NCC were not detected at or above the LOQ during the course of the experiments.

3.5 Conclusions

TCS and TCC are commonly detected in the wastewater treatment process. Concerns exist regarding their environmental impacts due to release via wastewater effluent discharge and the land application of biosolids. Simulation of nitrification process allows for a better understanding of how the treatment can influence concentrations of TCS, TCC, and their antimicrobials. The majority of TCS was associated with the solids fraction of nitrification samples and concentrations decreased when treatment was operated at both experimental pH ranges: 6.5 – 7.5

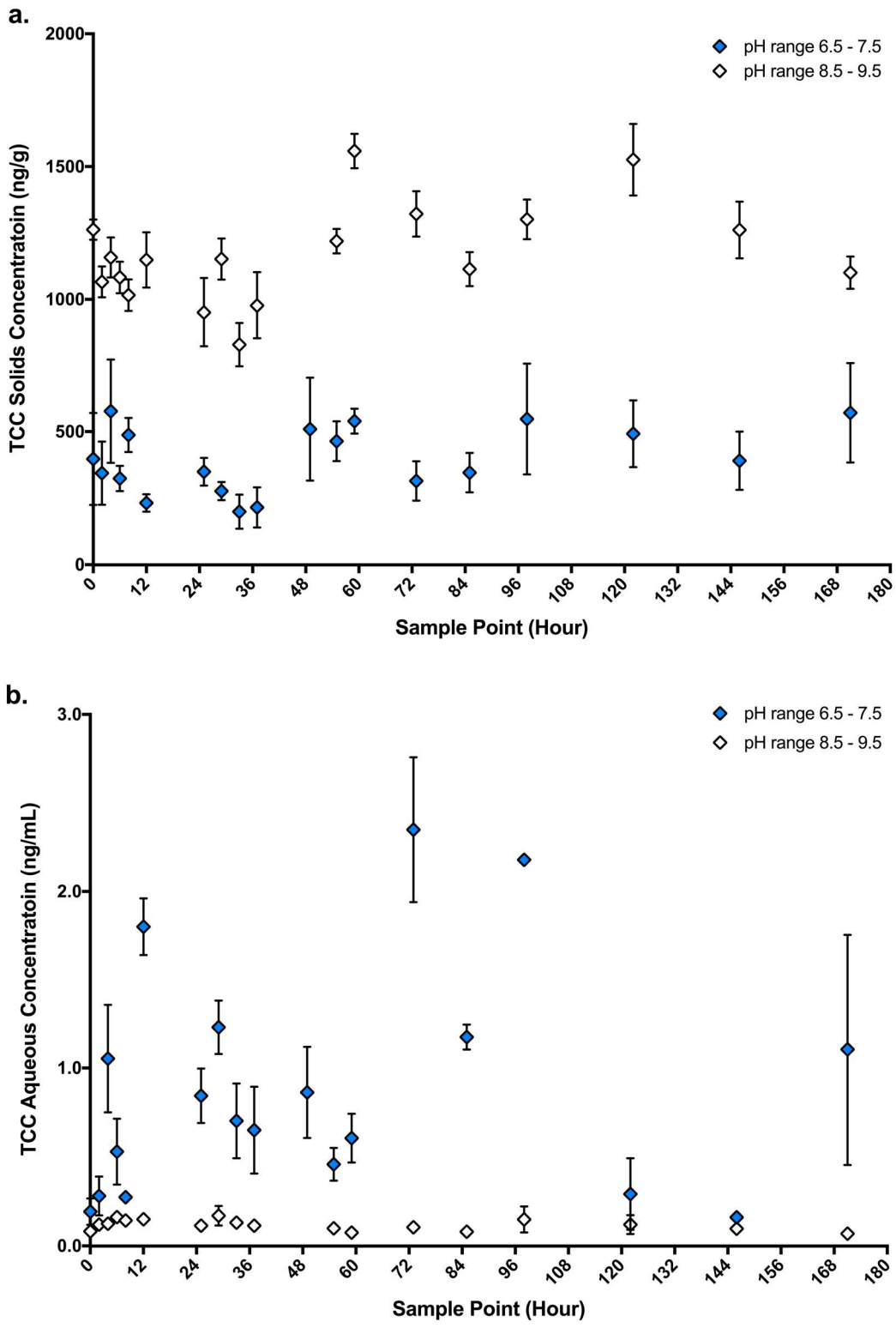

Figure 3-4: Concentrations of TCC in a) Solids and b) Aqueous Factions of Nitrification Samples Over Time

and 8.5 – 9.5. A greater reduction of TCS levels in solids samples occurred at the higher pH range. MeTCS was formed in solids samples under both pH conditions, with formation rates being the most rapid under pH conditions ranging 8.5 – 9.5 ($0.0174 \pm 0.005\ h^{-1}$, first 25 hours). TCC and 2,4-DCP concentrations, however, did not change during either nitrification treatment. Overall, the impact of nitrification treatment on the antimicrobials and their transformation products was varied.

3.6 Acknowledgements

Funding for this research was provided by the District of Columbia Water and Sewer Authority (DC Water). The authors would like to thank Taylor Lachance of the USDA for her assistance with sample analysis.

Chapter 4: Influence of Thermal Hydrolysis-Anaerobic Digestion Treatment of Wastewater Solids on Concentrations of Triclosan, Triclocarban, and their Transformation Products in Biosolids

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4.1 Abstract

The growing concern worldwide regarding the presence of emerging contaminants in biosolids calls for a better understanding of how different treatment technologies at water resource recovery facilities (WRRFs) can influence concentrations prior to biosolids land application. This study focuses on the influence of solids treatment via the Cambi Thermal Hydrolysis Process™ in conjunction with anaerobic digestion (TH-AD) on concentrations of triclosan (TCS), triclocarban (TCC), and their transformation products in biosolids and sludge. Concentrations of the target analytes in biosolids from the TH-AD process (Class A), sludge from the individual TH-AD treatment steps, and limed biosolids (Class B) from the same WRRF were compared. TCC concentrations were significantly lower in Class A biosolids than those in the Class B product - a removal that occurred during thermal hydrolysis. Concentrations of TCS, methyl triclosan, and 2,4-dichlorophenol, conversely, increased during anaerobic digestion, leading to significantly higher concentrations of these compounds in Class A biosolids when compared to Class B biosolids. Implementation of the TH-AD process had mixed effect on contaminant concentrations.

4.2 Introduction

Extensive use of pharmaceutical and personal care products (PPCPs) by society has led to their presence in the wastewater treatment (WWT) process, including wastewater effluent and biosolids [11,105]. Two such compounds are the antimicrobials triclosan [5-chloro-2-(2,4-dichlorophenoxy)-phenol] (TCS) and triclocarban [N-(4-chlorophenyl)-N-(3,4-dichlorophenyl) urea] (TCC), both of which have been demonstrated to show endocrine disrupting capabilities [26,27,45,46] and, due to concerns regarding their ecological impact, are currently under phase-out regulations in consumer antiseptic wash products in the United States (U.S.) [47]. TCC and TCS have been detected in all stages of the WWT process and most notably concentrate in the solids fraction [11,65,106].

In the US, treated wastewater solids (biosolids) are commonly land-applied as a means of nutrient recovery/soil reclamation [107] allowing for this material to become a potential source of organic pollutants to the environment. TCC and TCS have been detected in biosolids from wastewater treatment facilities throughout the U.S. [21,50] and studies have shown the ability of TCC and TCS to persist in agricultural soils after the land-application of biosolids, with estimated half-lives of 191 and 107 - 258 d, respectively [59,108]. Furthermore, these antimicrobials can accumulate in the roots of plants grown in biosolids-amended soils [109,110] and earthworms living in treated soils [41,42], indicating the potential for ecological risk.

TCC and TCS can also be partially transformed both biotically and abiotically during the WWT process. For instance, methyl triclosan (MeTCS) can form during aerobic treatment [11], has higher endocrine disrupting capabilities [26] than its parent compound TCS, and is more persistent in biosolids amended soils than TCS [25]. Furthermore, the carbanilide analogs of TCC associate primarily with solids

within the WWT process [50] and have also been linked to endocrine system disruption [45]. The amount of TCC and TCS degradation and the compounds formed is highly dependent on the treatment processes employed by the WWT plant [50].

Currently, WWT facilities within the United States (US) are focusing efforts into the beneficial recovery of resources throughout the treatment process and many have begun changing their designation from WWT plant to water resource reclamation facility (WRRF) (WEF, 2014). This emphasis on resource recovery not only includes the land-application of biosolids for beneficial uses, but changes in treatment processes as well. One such process is the innovative Cambi Thermal Hydrolysis Process™ (CambiTHP™), a pretreatment for anaerobic digestion of wastewater sludge. Amongst other benefits, the CambiTHP™ allows for a reduction in the volume of final solids as well as increases the biodegrability of sludge – leading to an increase in biogas production during anaerobic digestion, which can be captured and beneficially used as an energy source. Limited studies have dealt with the fate of organic microconstituents in the CambiTHP™ process. Previous experiments on the fate of TCS, bisphenol-A, and nonylphenol ethoxylates in spiked water samples and nonlyphenol in spiked sludge treated via high temperature and pressure by a lab-scale chemical digestion bomb found that these compounds were not degraded during the treatment. Furthermore, the study found that laboratory-scale anaerobic digesters treating sludge with conventional mesophilic anaerobic digestion (MAD) more readily transformed nonylphenol ethoxylates to nonylphenol than sludge that had been pretreated with thermal hydrolysis prior to MAD. [111] The present study examines concentrations of TCC and TCS as well as three TCS and five TCC transformation products in biosolids from a single WRRF in the Mid

Atlantic region of the US that has recently changed its solids handling process. In October 2014, the facility began transitioning from lime-stabilizing final solids (Class B biosolids) to treating solids via the CambiTHP™, in conjunction with anaerobic digestion (TH-AD) (Class A biosolids), the first of its kind in the US and currently the largest in the world. Class A biosolids have severely reduced pathogen levels as well as increased vector attraction reduction when compared to Class B biosolids, allowing for increased options for land application. The study goal was to expand upon a previous study of historical trends of TCC and TCS in limed biosolids from this WRRF [21] by examining how advances in solids handling processes within this same facility can influence the concentrations of these antimicrobials as well as their degradation products (not examined in the previous study) in biosolids prior to their application onto agricultural soils. Additionally, the study examines the influence of individual stages of the TH-AD process itself on the concentrations of these antimicrobials and their transformation products.

4.3 Materials and Methods

4.3.1 Target Analytes

Class A and Class B biosolids, as well as sludge samples collected throughout the TH-AD process, were analyzed for the antimicrobials TCS and TCC. Additionally, all samples were analyzed for three TCS transformation products: MeTCS, 2,4-dichlorophenol (2,4-DCP), and triclosan-o-sulfate (TCS-O-Sulf); and five TCC degradation products: 4,4'-dichlorocarbanilide (DCC), 1-(3-chlorophenyl)-3-phenylurea (MCC), carbanilide (NCC), 4-chloroaniline (4-CA), and 3,4-dichloraniline (3,4-DCA). These compounds have been identified previously in the literature as TCS and TCC byproducts [50,51,69,71,112]. Compound structures are provided in Figure 4-1.

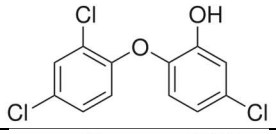
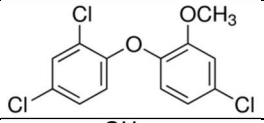
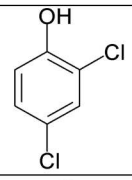
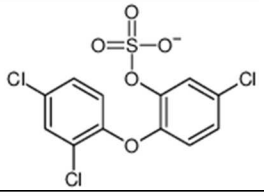
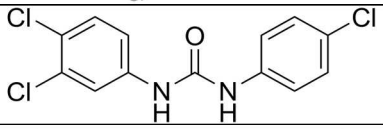
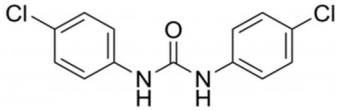
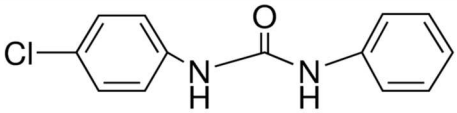
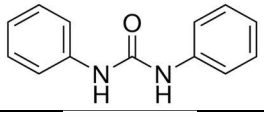
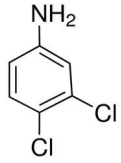
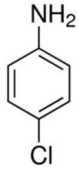
Compound	Structure
Triclosan	
Methyl triclosan	
2,4-Dichlorophenol	
Triclosan-o-sulfate	
Triclocarban	
4,4'-Dichlorocarbanilide	
1-(3-Chlorophenyl)-3-phenylurea	
Carbanilide	
3,4-Dichloroaniline	
4-Chloroaniline	

Figure 4-1: Structures of Triclosan, Triclocarban, and their Transformation Products

4.3.2 Wastewater Treatment Plant

The current study focuses on sludge and biosolids samples collected from an east coast U.S. municipal WRRF serving a highly populated region. Approximately 1.25 million cubic meters (m^3) of raw sewage is treated daily by the facility via open-air primary sedimentation, activated sludge, and tertiary treatment (including nitrification-denitrification, filtration, and disinfection). Prior to full phase-out in February 2015, treated sludge from the facility was classified as Class B biosolids and the final product achieved by thickening, centrifugation, and addition of lime (15 – 20% on a dry weight basis) to solids from primary, secondary, and nitrification-denitrification treatment. Solids treated with lime did not go through an anaerobic digestion treatment. Class B biosolids from the WRRF were typically land-applied to agricultural fields for resource recovery. This solids treatment process may be utilized in in the future during situations requiring the processing of short-term peak loads due to lack of TH-AD treatment capacity.

Implementation of the TH-AD processes began in October 2014, with solids meeting US Environmental Protection Agency (US EPA) Exceptional Quality Class A requirements by February 2015. Solids from primary, secondary, nitrification-denitrification, and filtration are currently treated by TH-AD. Primary solids are thickened by gravity and secondary solids by dissolved air floatation thickening process, blended, and dewatered by one of four centrifuges yielding approximately 15 – 19 % dry solids. The separated water is returned back into the wastewater treatment process. The dewatered sludge is then sent to a storage hopper prior for processing via the CambiTHP™. The WWTP employs four (4) TH process streams. Each stream consists of:

a) One pulper, which preheats the sludge to approximately 60 – 99 °C via recycled steam from the TH process.

b) Six digesters, where hydrolysis occurs via heat (150 – 180 °C) and pressure (0.37 - 0.95 MPa). Retention time is 30+ minutes.

c) One flash tank, where heat and pressure are rapidly decreased by flashing steam back into the pulper. The sludge is released at a temperature of approximately 70 – 115 °C and consists of 8 – 12 % total solids.

Prior to anaerobic digestion (AD), the sludge is diluted via service water to decrease the sludge temperature and the percentage of dry solids to approximately 9.5 %. The TH streams feed into four AD tanks that can each accommodate approximately 14,500 m^2 of sludge. After a retention time of approximately 22 d at 37 °C, the digested solids are dewatered via belt presses. The final solids are classified by the US EPA as Exceptional Quality Class A biosolids and predominantly land-applied for agricultural purposes. A diagram of the TH-AD process is provided in Figure 4-2.

4.3.3 Sample Collection and Handling

4.3.3.1 Biosolids Collection

Class B biosolids were collected routinely (approximately every 2 – 3 months) from the WRRF treatment line (post liming) between 2005 and 2015 for previous studies on trends of persistent organic pollutants (POPs), including TCC and TCC, in biosolids [21,113]. Liquid streams from the dewatering of solids were not sampled. Samples were archived and stored at -20 °C until analysis. For this study, a subset of the samples collected between 2011 and 2015 were analyzed. More information regarding long-term temporal trends of TCS and TCC between 2005 and 2011 in Class B biosolids from this facility can be found elsewhere [21].

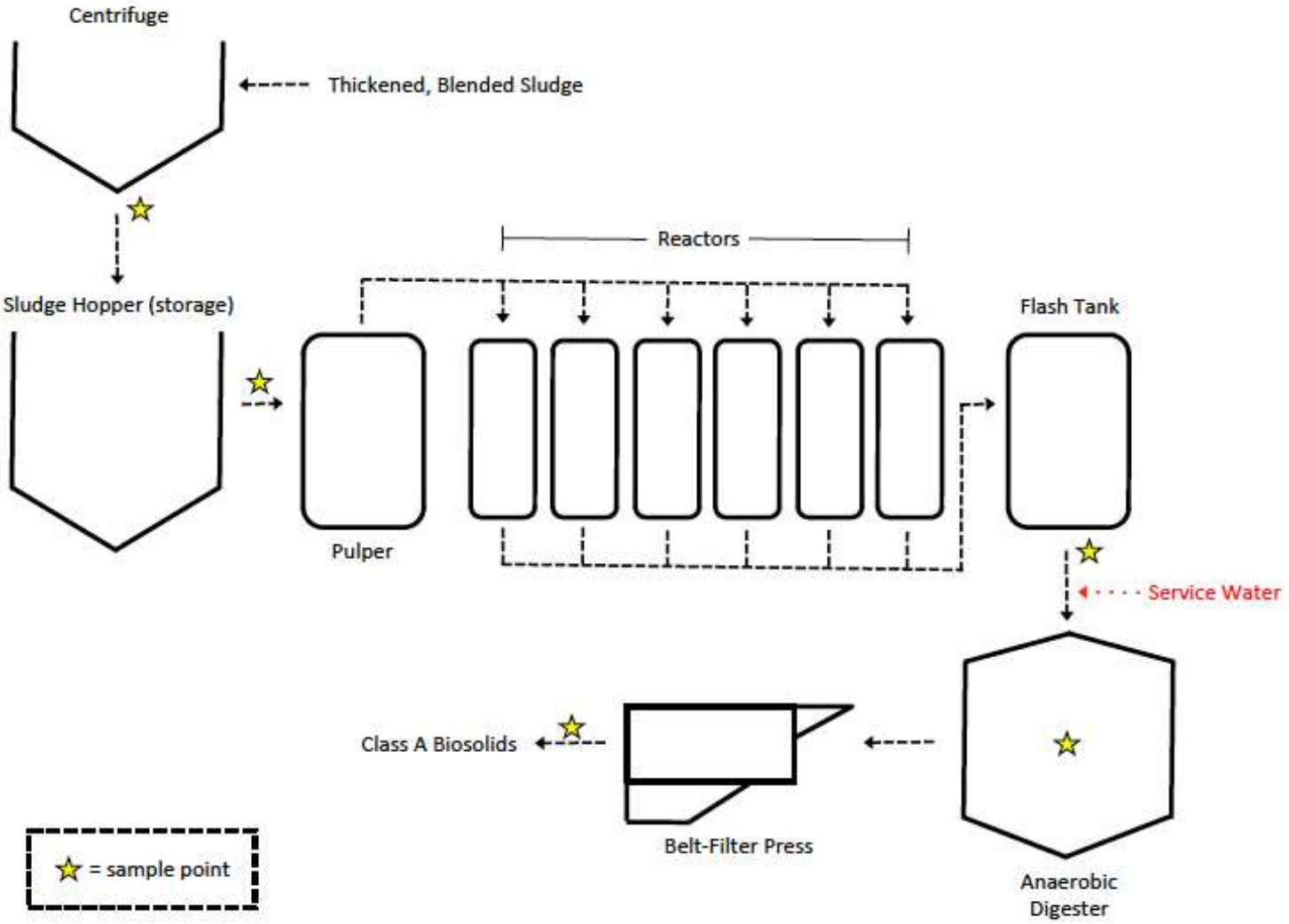


Figure 4-2: Diagram of the CambiTHP™ - Anaerobic Digestion Solids Treatment Process and Sampling Locations

Grab samples of Class A biosolids produced from the TH-AD process were collected beginning in November 2014. Samples were collected on a weekly basis while the TH-AH process was still in the beginning stages of full-scale implementation (between the end of November 2014 and the beginning of March 2015). Samples were collected on a monthly basis thereafter. Samples were stored at -20 °C until analysis. Again, a subset of the samples was analyzed for this study.

4.3.3.2 TH-AD Process Sample Collection

In June 2015, grab samples were collected from various stages within the TH-AD process: a) after centrifugation; b) along one of the four CambTHP™ streams (input and post-flash tank); c) from one AD tank (via the digestion solids recycle line); and d) from the belt press dewatering process (final solids). All sampling locations are provided in Figure 4-2. After collection, samples were placed on ice until laboratory arrival, where they were stored at -20 °C until analysis.

4.3.4 Standards and Reagents

TCC (>97 %), TCS (>97 %), and MeTCS (>97 %) were obtained from Wellington Laboratories (Guelph, ON, Canada). MCC (N/A), NCC (98 %), 2,4-DCP (≥97 %), and 4-CA (98 %) were acquired by Sigma-Aldrich (St. Louis, MO, USA). DCC (>97 %) was acquired through Oakwood Chemicals (West Columbia, SC, USA). TCS-O-Sulf (N/A) was obtained from Toronto Research Chemicals (Toronto, Canada). Isotopically-labeled $^{13}C_{13}$-TCC (≥99 %), $^{13}C_{12}$-TCS (≥99 %), and $^{13}C_{12}$-MeTCS (≥99 %) were obtained from Wellington Laboratories (Guelph, ON, Canada), d_2-3,4-DCA (98 %) from Santa Cruz Biotechnology (Dallas, TX, USA), and d_3-2,4-DCP (98 %) through Cambridge Isotope Laboratories (Andover, MA, USA).

All organic solvents used were high performance liquid chromatography (HPLC) or ultra high performance liquid chromatography (UHPLC) grade (Burdick

and Jackson; Fisher Scientific). Laboratory-grade sand was obtained from J.T. Baker® (Avantor Performance Materials, Center Valley, PA, USA). Potassium phosphate (monobasic and dibasic), ammonium acetate, sulfuric acid, and acetic acid were acquired through Fisher Scientific (Fair Lawn, NJ, USA). A Picosystem UV Plus treatment system (Hydro Service & Supplies, Inc.; Durham, NC, USA) provided organic-free, UV-treated water.

4.3.5 Triclosan, Triclocarban, & Transformation Product Extraction and Analysis

4.3.5.1 Sample Extraction

Biosolids samples were extracted on a wet weight basis. However, due to the high liquid content and high amount of solubilized organics in the liquid faction of the sludges collected from the TH-AD process (specifically post-flash tank and anaerobic digestion samples), sludge collected from this process were lyophilized prior to extraction.

Samples for analysis of all compounds except 4-CA and 3,4-DCA were processed using a previously published method [25]. Briefly, samples were extracted using a Dionex Accelerated Solvent Extraction (ASE) #300 system (Dionex Corporation, Sunnyvale, CA, USA) with a 20:80 (v/v) blend of water:isopropyl alcohol (IPA). Oasis® HLB solid phase extraction (SPE) cartridges (6 mL, 200 mg) (Waters Corporation, Milford, MA, USA) were employed for extract clean-up and analytes were eluted from the cartridges with a dichloromethane (DCM):diethyl ether (DEE) solution (80:20 v/v). Eluates were evaporated using a rotary evaporator and reconstituted in 1.5 mL methanol (MeOH) for instrumental analysis. Further details regarding the extraction method are provided in the supplemental information.

For 4-CA and 3,4-DCA extraction, samples were placed in a 15 mL centrifuge tube and vortexed for 5 minutes in a solution of acetonitrile (ACN):MeOH (50:50 v/v). Samples were then centrifuged for 3 minutes and the supernatant decanted. The extraction process was repeated two additional times and the supernatants for each sample combined. The samples were then evaporated under nitrogen at 35 ˚C and reconstituted in 1 mL ACN for instrumental analysis.

4.3.5.2 Instrumental Analysis

A Shimadzu Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) coupled with a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA) equipped with an ESI-source was utilized for the analysis of TCC, TCS, as well as the transformation products 2,4-DCP, DCC, MCC, and NCC. The UHPLC was run isocratically using a mobile phase of 10 mM ammonium acetate in a solution of MeOH:ACN:water (60:15:25 v/v) at a flow of 0.5 mL/min and equipped with a Supelco Ascentis® Express C18 reverse phase column (2.7 µm, 50 x 2.1 mm) (Sigma-Aldrich, St. Louis, MO, USA). The total run time per sample was 1.45 minutes. MS acquisition occurred in multiple reaction monitoring (MRM). TCS-O-Sulf was also analyzed via UHPLC-MS/MS with chromatographic separation occurring via 0.2 % formic acid in MeOH run isocratically through a Supelco Ascentis® Express C18 reverse phase column (2.7 µm, 50 x 2.1 mm) at a rate of 0.55 mL/min for 0.9 min. MS acquisition was achieved in MRM. Additional details regarding all UHPLC-MS/MS conditions are provided in the supplemental information.

Following UHPLC-MS/MS analysis, samples from the ASE extraction process were evaporated and reconstituted in 1 mL of hexane for measurement of MeTCS. Samples were analyzed by an Agilent 7890B gas chromatograph (GC) in

conjunction with an Agilent 5977A mass selective detector (MSD) run in positive electron impact ionization mode [25]. A capillary column (DB-5-MS) with a length of 15 m, diameter of 0.25 mm, and film thickness of 0.1 µm (J&W Scientific, Folsom, CA, USA) was used for compound separation. 4-CA and 3,4-DCA were also analyzed via the same GC-MS set-up and details for both analytical methods are provided in the supplemental information.

Table 4-1: MDLs and Recoveries of Target Analytes

	TCS	**TCC**	**MeTCS**	**DCC**	**MCC**
MDL (ng L-1)	16.2	8.5	15.8	75.2	88.1
Recovery (%)	85.4 ± 3.9	91.1 ± 7.0	83.1 ± 4.2	87.5 ± 8.0	90.9 ± 6.5
	NCC	**TCS-O-Sulf**	**2,4-DCP**	**4-CA**	**3,4-DCA**
MDL (ng L-1)	81.7	55.3	90.4	100.3	112.9
Recovery (%)	90.3 ± 3.7	71.8 ± 22.3	75.2 ± 13.9	62.3 ± 26.1	67.0 ± 19.4

4.3.5.3 Quality Assurance/Quality Control

All samples were spiked with 150 ng of $^{13}C_{13}$-TCC, $^{13}C_{12}$-TCS, $^{13}C_{12}$-MeTCS and d_2-3,4-DCA as surrogate standards prior to extraction. Samples were extracted in duplicate and extractions were performed in batches containing less than 20 samples, including a blank of laboratory-grade sand a sample spiked with all compounds analyzed for recovery determination. A standard curve of eight standards at concentrations other than zero was run for each compound, with linearity correlations yielding $r^2 \geq 0.99$. Instrument stability was verified via injection of standards and solvent blanks every 10 samples. Method detection limits (MDLs) were determined using United States Environmental Protection Agency guidelines [98]. Average compound recoveries and MDLs are provided in Table 4-1. Statistical

analysis was performed using GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, USA).

4.4 Results and Discussion

4.4.1 Concentrations in the TH-AD Process

Concentrations of TCC, TCS, MeTCS, and 2,4-DCP throughout the TH-AD process are presented in Figure 4-3. 4-CA, 3,4-DCA, DCC, MCC, NCC, and TCS-O-sulfate were not detected in samples collected within the TH-AD process.

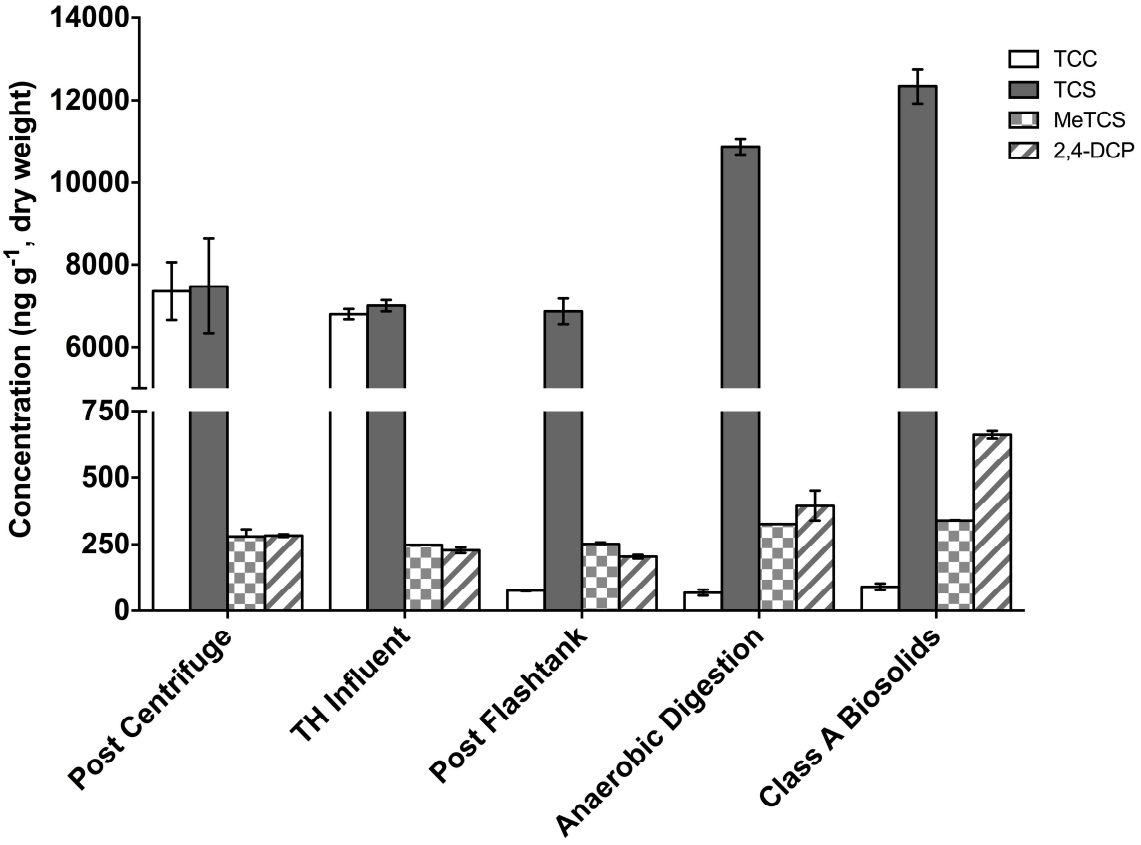


Figure 4-3: Concentrations of TCC, TCS, MeTCS, and 2,4-DCP in Individual Stages of the TH-AD Treatment Process (error bars represent the standard error of the mean)

Levels of TCC significantly decreased during the thermal hydrolysis step of the TH-AD process (Tukey's multiple comparisons test, $P<0.01$). Average concentrations of TCC prior to TH ranged from 6,816 – 7,368 ng g-1 dry weight (dw)

while concentrations collected after TH treatment ranged from 67.5 – 89.9 ng g-1 dw. While degradation pathways were not determined in this study, research has shown that TCC can be hydrolyzed to 4-CA and 3,4-DCA [54,112]. 4-CA and 3,4-DCA were also not detected in this study, but this may be due to their ability to be degraded by reactive oxygen species [114]. Free radicals have been shown to form during heat treatment of biomass [115]. Pyrolysis of biosolids has been demonstrated to degrade TCC to 1-(3,4-dichlorophenyl)-3-phenylurea and 1-(4-chlorophenyl)-3-phenylurea [116], two TCC dechlorination compounds not analyzed in this study.

Conversely, concentrations of TCS increased during the anaerobic digestion stage of the TH-AD process. Average concentrations of TCS before and after AD treatment ranged from 6,884 – 7,489 ng g-1 dw and 10,872 – 12,345 ng g-1 dw, respectively. The increase in average TCS concentration between TH and AD was statistically significant (Tukey's multiple comparisons test, $P<0.05$). Like TCS, MeTCS concentrations also increased significantly during the AD phase of treatment (Tukey's multiple comparisons test, $P<0.05$) with average concentrations prior to AD ranging from 248 – 280 ng g-1 dw and after ranging from 326 – 340 ng g-1 dw. Finally, concentrations of 2,4-DCP increased significantly (Tukey's multiple comparisons test, $P<0.05$) during AD as well. Average 2,4-DCP concentrations before and after AD ranged from 204 – 283 ng g-1 dw and 396 – 661 ng g-1 dw, respectively. Concentrations of 2,4-DCP also increased significantly between the AD stage and final cake samples (Tukey's multiple comparisons test, $P<0.01$), likely due to the dewatering process. These results indicate that the increase of TCS, MeTCS, and 2,4-DCP can be explained by the biological activity of the AD treatment step. If the compounds cannot be degraded by either TH or AD, they will instead

become concentrated during AD as 60 – 70 % of the sludge volumes are reduced during microbial anaerobic respiration. The addition of a thermal hydrolysis pretreatment step allows for more efficient sludge reduction during AD, causing an even further reduction in sludge volumes over conventional AD. [117] Therefor, the combination of efficient sludge removal during AD and the lack of target compound degradation causes TCS, MeTCS, and 2,4-DCP to become concentrated in the remaining sludge, thus increasing concentrations associated with sludge and biosolids. Additionally, concentrations of 2,4-DCP appeared to increase (approximately 95 %) more than TCS and MeTCS (~58 % and ~30 %, respectively) during anaerobic digestion (when compared to concentrations during TH). This may be due to the degradation of other organic compounds that can form 2,4-DCP as a degradation product. For instance, compounds such as the herbicides 2,4-dichlorophenoxyacetic acid and 2-(2,4-dichloro-phenoxy)propionic acid have been demonstrated to form 2,4-DCP during degradation [100,101], amongst others.

4.4.2 Concentrations in Biosolids

4.4.2.1 TCC & TCC Degradation Products

Concentrations of TCC in biosolids after both treatment processes are provided in Figure 4-4a. Average concentrations of TCC in Class A biosolids samples ranged from 102 – 3,006 ng g-1 dw with an average concentration over the November 2014 – August 2015 sampling period of 630 [standard deviation (SD) = 974] ng g-1 dw. Average concentrations in Class B biosolids ranged from 2,627 – 9,483 ng g-1 dw and the overall average between August 2011 – January 2015 was 5,734 (SD = 2,182) ng g-1 dw. Statistical analysis (Kolmogorov-Smirnov test) showed that the overall mean concentration of TCC in Class A solids was

significantly lower than that of Class B biosolids (P<0.01), indicating that the TH-AD process was effective in decreasing TCC levels in final solids. It is important to note

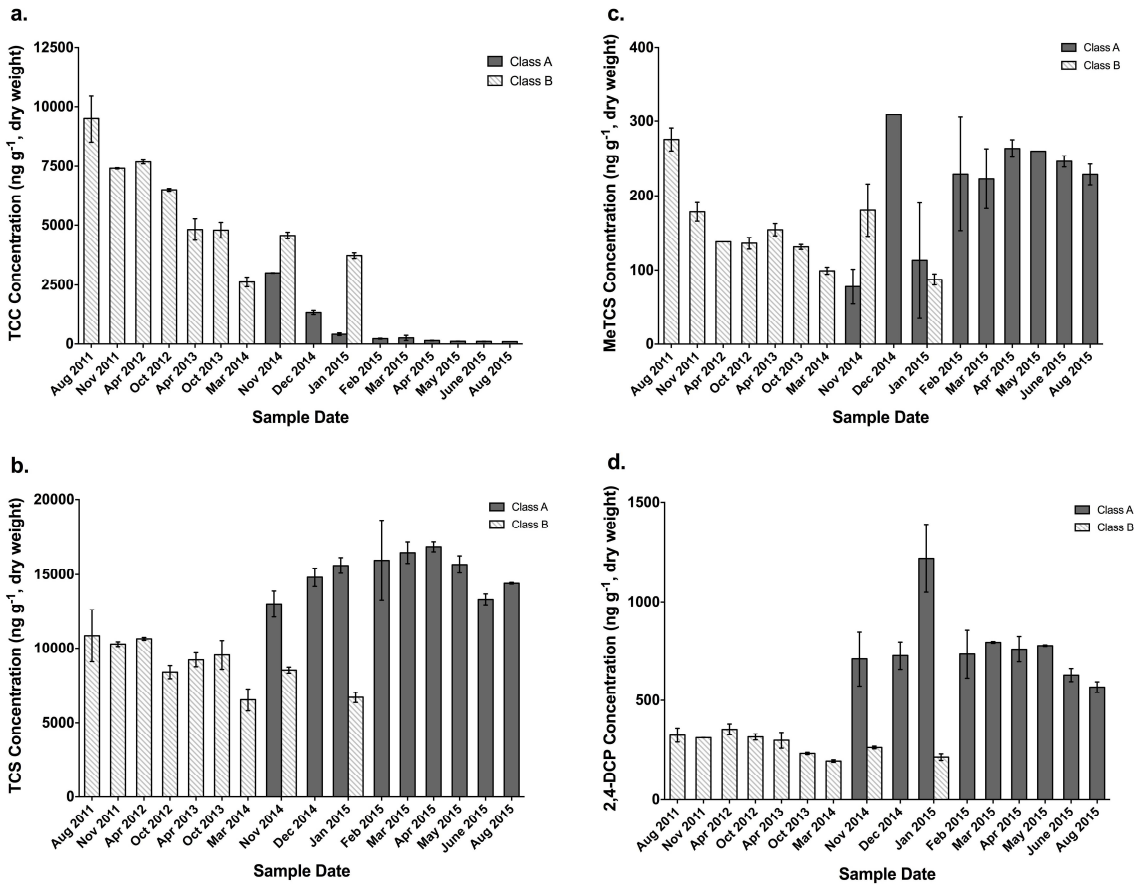


Figure 4-4: Concentrations of a) TCC, b) TCS, c) MeTCS, and d) 2,4-DCP in Class A and Class B Biosolids (error bars represent the standard error of the mean)

that during the start-up period of the TH-AD process TCC concentrations in Class A biosolids continuously decreased until the TH-AD process was stabilized and implemented full time in February 2015, after which concentrations remained relatively steady with a range of 102 – 294 ng g-1 dw. The implementation of the TH-AD process at the study facility allowed for a significant decrease in TCC concentrations in Class A biosolids, stabilizing after full-scale system operation at levels over 95 % lower (when comparing overall mean concentrations) than those

present in Class B biosolids from the same facility. Temporal trends of TCC at the same facility (prior to the employment of the TH-AD process) demonstrated that while TCC concentrations in Class B biosolids (n = 31) were decreasing between 2005 and 2011, the concentrations were still generally greater than 10,000 ng g-1 dw [21]. Additionally, a study of TCS, TCC, and their transformation products in biosolids in WRRFs throughout the United States showed that TCC concentrations did not decrease in samples collected monthly between March 2009 and April 2010 [50]. Therefore, this study demonstrates that treating solids via TH-AD can significantly reduce concentrations of TCC in biosolids and, thus, the amount of TCC being introduced to the environment via the beneficial reuse of biosolids.

Although 4-CA and 3,4-DCA can be formed via hydrolysis of TCC [54,112], they were not detected in the biosolids samples from this study. This lack of detection may be due to their propensity to associate in the liquid phase over the solids phase (log K_{ow} = 1.72 and 2.37, respectively) and the ability of these chloroanilines to be degraded biologically [112,118] and in the presence of reactive oxygen species [114].

The formation of the TCC dechlorination products DCC, MCC, and NCC has been demonstrated in anaerobic sediment communities [119,120] and some dechlorination products have been detected in biosolids samples throughout the United States [50]. These compounds, however, were not detected in biosolids samples from this study.

4.4.2.2 TCS & TCS Degradation Products

Levels of TCS in Class A & B biosolids are provided in Figure 4-4b. Average TCS concentrations in Class A biosolids ranged from 13,008 – 16,839 ng g-1 dw with an overall average of 15,119 (SD = 1,334) ng g-1 dw over the sampling period.

Concentrations ranged from 6,514 – 10,180 ng g-1 dw in Class B biosolids and the overall average was 8,955 (SD = 1,594) ng g-1 dw between August 2011 and January 2015. Comparisons of overall average concentrations in Class A and Class B biosolids indicate that TCS is significantly higher in Class A biosolids (Kolmogorov-Smirnov test; $P<0.01$). This signifies that the TH-AD process is concentrating TCS in final solids. Temporal studies of TCS have shown that TCS concentrations are remaining relatively steady in biosolids collected from WRRFs the United States [21,50]. Implementation of the TH-AD process significantly concentrates levels in biosolids (due, specifically to anaerobic digestion), demonstrating that without an increase in input of TCS into the wastewater treatment system, the use of this new process could increase the discharge of TCS into the environment via land-application of biosolids assuming equivalent application rates.

While less pronounced, MeTCS concentrations (provided in Figure 4-4c) exhibited similar trends to its parent compound TCS. Concentrations ranged from 77.9 – 309 ng g-1 dw in Class A biosolids and 87.6 – 276 ng g-1 dw in Class B biosolids. Overall average MeTCS concentrations over the sampling periods [216.9 (SD = 74.1) ng g-1 dw for Class A solids; 153.2 (SD = 55.6) ng g-1 dw for Class B solids] were significantly different (Kolmogorov-Smirnov test; $P<0.05$), demonstrating that the TH-AD process increased concentrations over lime treatment of solids and, as with TCS, the new treatment process, specifically the AD step, will increase the amount of MeTCS being introduced to the environment via the beneficial reuse of biosolids.

Increases in 2,4-DCP concentrations, shown in Figure 4-4d, also demonstrated a notable increase after the change from lime treatment to the TH-AD process. Average concentrations in Class A biosolids between November 2014 and

August 2015 ranged from 566 – 1,220 ng g-1 dw while average concentrations of samples of Class B biosolids collected between August 2011 and January 2015 ranged from 196 – 356 ng g-1 dw. Overall average concentrations of 2,4-DCP were 768 (SD = 184) ng g-1 dw in Class A solids and 281 (SD = 55.5) ng g-1 dw in Class B solids. The overall average for 2,4-DCP in Class A biosolids were significantly higher than that of Class B biosolids (Kolmogorov-Smirnov test; $P<0.01$). This again demonstrates that the implementation of the anaerobic digestion can increase the concentration of organic pollutants in final biosolids.

Liming of biosolids at the facility for production of Class B biosolids was executed at a target rate of 15 – 20 % on a dry weight basis, which could, at times, vary between 10 – 25 %, depending on processing loads. However, given that average TCS, MeTCS, and 2,4-DCP concentrations increased by approximately 68 %, 41 %, and 173 %, respectively, dilution of concentrations in biosolids via liming cannot be the only reason for the increase in compound levels in the Class A product. Another explanation is reduction of total solids by the AD process. Solids reduction at the facility during AD treatment ranges from 60 – 70 % indicating that if compound degradation is not occurring, TCS, MeTCS, and 2,4-DCP could effectively be concentrated during this process. Figure 4-5 presents average concentrations of the detected target analytes in Class A biosolids (n = 9) compared with concentrations in Class B biosolids (n = 9) corrected for an 80 % change in solids amount (assuming a 15% dilution from liming and 65% reduction of solids from AD treatment). t-tests were performed to determine whether the average concentrations in Class A biosolids and corrected Class B biosolids were significantly different (via the Holm-Sidak method). Results indicated that the averages for TCS and MeTCS were not significantly different ($P>0.05$), further supporting the notion that increases

in concentrations of these two compounds in biosolids due to TH-AD treatment was due solids reduction. Conversely, the averages for 2,4-DCP were significantly

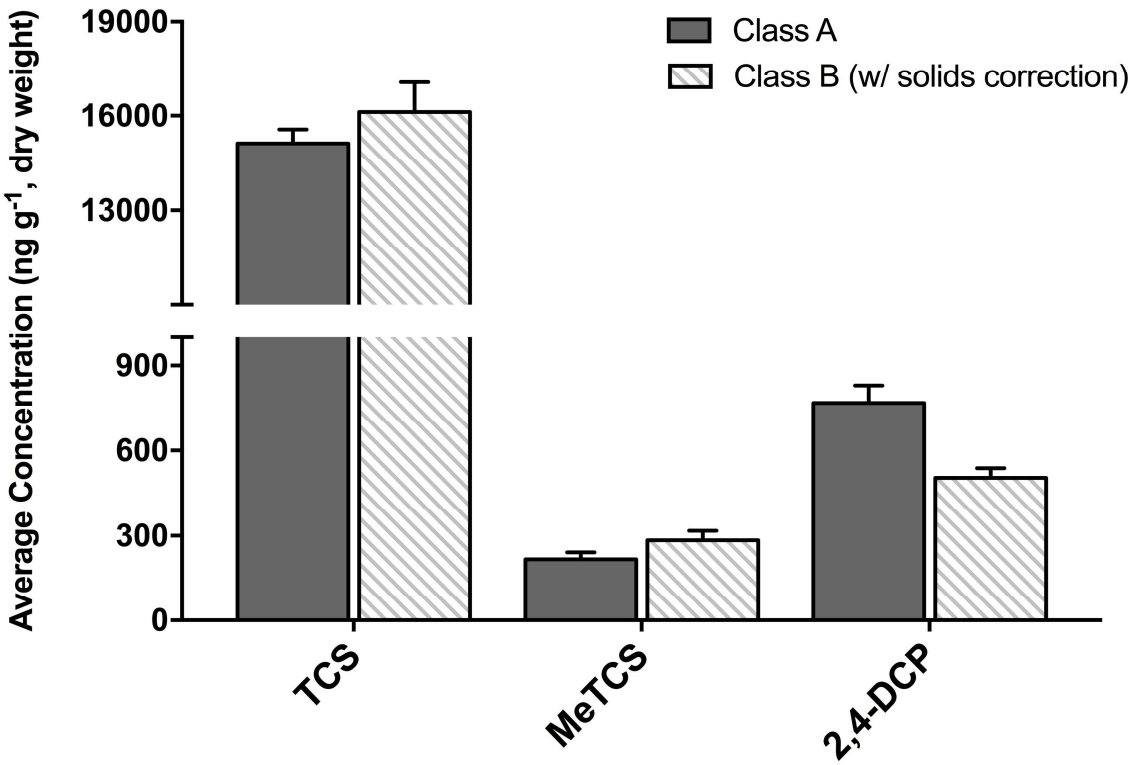


Figure 4-5: Concentrations of TCS, MeTCS, and 2,4-DCP in Class A Biosolids and Class B Biosolids (Corrected for Solids Concentration Losses) (error bars represent the standard error of the mean; n = 9)

different ($P<0.05$) with concentrations in the Class A product remaining higher than those in Class B, even after factoring in the change in solids, showing that increases in concentrations of this compound were not due solely to solids reduction. 2,4-DCP has been shown to be a degradation product of other chlorinated organic compounds not analyzed in this study [100,101] and its increase may be due to the breakdown of such compounds during TH-AH treatment. Biosolids from this facility are typically land-applied at a rate that meets each individual farm's nutrient management needs. Typical concentrations of total nitrogen (TN), ammonium as N

(NH_4-N), and total phosphorus (TP) are provided in Table 4-2. Given that concentrations of TN, NH_4-N, and TP are all higher in Class A biosolids, the application rates may be lower at farms when compared to rates used for the Class B product, indicating that input of TCS, MeTCS, and 2,4-DCP into the environment via land-application of Class A biosolids may not be as high as the concentrations in the final product indicate.

TCS-O-sulfate was not detected in any biosolids samples at or above the MDL.

Table 4-2: Typical Nutrient Concentrations in Class A and Class B Biosolids

	Total Nitrogen (mg kg-1, dw)	Ammonium as N (mg kg-1, dw)	Total Phosphorus (mg kg-1, dw)
Class A Biosolids	48000	7500	33000
Class B Biosolids	37000	1400	13000

4.5 Conclusions

The implementation of the TH-AD solids treatment process allows for the WRRF to utilize a more environmentally friendly technology – namely, via the creation of Class A biosolids (more opportunities for land application over Class B biosolids due to reduced pathogen concentrations and increased vector attraction reduction), reduction in volume of final solids created, and efficient methane production for the WRRF's energy needs. The growing awareness and concern regarding emerging contaminants in biosolids brings forth the need to assess how new treatment technologies influence contaminant concentrations. This study demonstrated a significant decrease in TCC concentrations in US EPA Exceptional Quality Class A biosolids, when compared to Class B biosolids from the same WRRF; the removal occurred during the thermal hydrolysis stage of treatment.

Conversely, TCS, MeTCS, and 2,4-DCP concentrations increased during TH-AD treatment, with concentrations in Class A biosolids significantly higher than those in Class B biosolids. The concentration of these compounds occurred during the anaerobic digestion stage of treatment. The degradation compounds TCS-o-sulf, DCC, MCC, NCC, 4-CA, and 3,4-DCA were not detected in sludge or biosolids samples. This research shows that implementation of TH-AD treatment can have mixed results on emerging contaminant concentrations and further research exploring the influence of the treatment on other compounds should be performed. Additionally, further research should be conducted to compare the TH-AD process on concentrations of emerging contaminants with AD processes without TH treatment as well as other AD treatments.

4.6 Acknowledgements

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Chapter 5: Effect of Cambi Thermal Hydrolysis Process-Anaerobic Digestion Treatment on Concentrations of Phthalate Plasticizers in Wastewater Sludge

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5.1 Abstract

The impact of the recently implemented Cambi Thermal Hydrolysis Process™-Anaerobic Digestion (TH-AD) solids treatment method on concentrations of 4 phthalate plasticizers in wastewater sludge samples was explored in this study. Samples were analyzed for diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), di(2-ethylhexyl) phthalate (DEHP), and benzyl butyl phthalate (BBP) concentrations during individual stages of the TH-AD treatment process, in biosolids produced by the TH-AD process (Class A biosolids), and Class B biosolids from the same facility produced via the liming of sludges. Results showed significantly higher concentrations of all 4 compounds in Class A biosolids when compared to concentrations in Class B biosolids. For DEHP, DiNP, and DiDP, this increase occurred during the anaerobic stage of treatment. Calculations indicate that increases of the four study compounds in Class A biosolids, when compared to Class B biosolids, was not solely due to solids reduction during anaerobic digestion and dilution during liming. Overall, implementation of the TH-AD process increased concentrations of phthalate plasticizers in biosolids.

5.2 Introduction

The presence of phthalate plasticizers in biota and the environment is of concern due to their toxicological properties, chiefly their endocrine disrupting capabilities [121,122]. Phthalate plasticizers are commonly used to increase the flexibility of products such as polyvinyl chloride (PVC), rubber, cellulose, and styrene and are also frequently used as additives in paints/varnishes, lubricants, propellants, adhesives, and cosmetics [15,17,123,124]. Production of these compounds is estimated to be over 4 million metric tons per year, with the compounds diisononyl phthalate (DiNP), diisodecyl phthalate (DiDP), and di(2-ethylhexyl) phthalate (DEHP) being the most highly produced [13,15]. Phthalates are not chemically bonded to the plastics and polymers in which they are used, allowing the compounds to leach from consumer and commercial products [13,14]. For instance, DEHP is frequently used in food packaging and, thus, has been detected in various food samples, such as dairy products, meats, fish, and breads, among others. [14,125,126]. Tubing and storage bags containing DEHP and other plasticizers are frequently used in medical settings and extensive leaching of the compound from this equipment has been observed as well [125,127]. Leaching of these compounds from consumer and industrial products has led to the detection of these compounds and their metabolites in human [32,128] and environmental samples [129,130].

Phthalate plasticizers make up over 90% of worldwide plasticizer production [16] and their content in plastics is typically 20 – 40% [35]. The extensive use of these compounds in piping and consumer products has led to the detection of these compounds in the wastewater treatment (WWT) process, including wastewater effluent and final solids [13,16,35]. High molecular weight phthalate plasticizers have been demonstrated to predominantly adsorb to wastewater solids [17,131,132],

with approximately 80% of DEHP [log K_{ow} = 8.39 [18]] in the WWT process accumulating in sludges [17]. In the United States an estimated 60% of biosolids produced are land-applied for beneficial reuse [133], leading to concern over the introduction of toxic organic pollutants to the environment via the application of biosolids. A study concerning plasticizers in agricultural fields in France found the sum of nine phthalate plasticizers to be 407 ng/g in the surface soil horizon (0 – 20 cm) of an agricultural soil, with DEHP accounting for over 50% of the total [131]. In China, samples collected from agricultural soils in 123 different regions were analyzed for 15 phthalate plasticizer compounds. The mean sum of the plasticizers was determined to be 1,088 ng/g, again with DEHP demonstrating the highest concentrations of the compounds analyzed [134]. Phthalate plasticizers in soils have been shown to transfer into vegetation [29,135], leach into deeper soil horizons [131,136], accumulate in earthworms [30], and to migrate via water run-off from solid waste dumps [137].

Due to toxicity concerns and the ability of phthalate plasticizers to travel in the environment and into biota, it is important to understand how different wastewater treatment technologies influence concentrations of these compounds in biosolids prior to their land-application. Given the desire of WWTPs within the United States to increase resource recovery throughout treatment [138], studying how different technologies that improve resource recovery, such as Cambi THP™, impact concentrations of organic pollutants is vital. When used as a pretreatment for anaerobic digestion, the Cambi THP™ results in both increased solids reduction as well as an increase in biogas production, a renewable resource, when compared to anaerobic digestion treatment alone. However, despite its use since 1995, few studies have explored the influence of the Cambi THP™ in conjunction with

anaerobic digestion (TH-AD) on concentrations of organic pollutants. In the only research conducted to date on the influence of a full-scale TH-AD treatment facility, Armstrong et al. (2017) observed that the ability of the process to degrade organic pollutants was very compound specific [49], indicating the need to further understand the impact that TH-AD treatment has on a variety of compounds. This study focuses on the influence of the Cambi Thermal Hydrolysis Process™ (Cambi THP™) in conjunction with anaerobic digestion (TH-AD) on concentrations of four common phthalate plasticizers in wastewater sludges and biosolids. The objectives of the present study are two-fold: 1) to understand the influence that individual stages in the TH-AD process have on concentrations of phthalate plasticizers and 2) compare concentrations of phthalate plasticizers in biosolids produced by the TH-AD process (Class A biosolids) to those in limed biosolids (Class B biosolids) produced at the same facility since the liming of biosolids is a treatment still frequently used in WWTPs located within the USA [139].

5.3 Materials and Methods

5.3.1 Target Analytes

Sludge and biosolids samples were analyzed for the phthalate plasticizers: benzyl butyl phthalate (BBP), DEHP, DiNP, and DiDP. Compound structures and log K_{ow} values are provided in Table SI-D1 of the Supplemental Information.

5.3.2 Sample Collection and Handling

Biosolids and sludge samples were collected from a municipal wastewater treatment plant (WWTP) located in the Mid-Atlantic, USA. The facility, which serves a population of over 2 million residents, treats an average of 1.14 million m^3 of raw sewage daily (300 million gallons per day). The facility employs primary sedimentation, activated sludge, nitrification-denitrification, filtration, and disinfection

for waste treatment. Sludge at the facility is currently treated via TH-AD (Figure 5-1) to produce Exceptional Quality Class A biosolids, as designated by the United States Environmental Protection Agency (US EPA). Briefly, the TH-AH process consists of:

1. Centrifugation of primary and secondary solids, resulting in a sludge consisting of 15 – 19% dry solids.
2. Preheating of sludge at 60 – 99 °C in pulper.
3. Hydrolysis of sludge under high heat (150 – 180 °C) and high pressure (0.37 - 0.95 MPa) conditions.
4. Rapid reduction of heat and pressure in flash tank to 70 – 115 °C. Here the sludge consists of 8 – 12 % total solids.
5. Anaerobic digestion of sludge at 37 °C for 22 days.
6. Dewatering of sludge by belt presses.

Wastewater sludge was previously treated via liming (approximately 15% by weight) to produce Class B biosolids prior to the phase-out of the process in February 2015. Liming is still commonly used in most US facilities [139]. Further details regarding past and present solids treatment at the study facility can be found elsewhere [49]. Biosolids from this WWTP are land-applied on local agricultural fields as a soil amendment.

5.3.2.1 TH-AD In-line Sampling

In October 2016, sludge samples were collected in-line at various locations throughout the TH-AD process, including: post centrifuge, Cambi THP™ input and effluent (post flashtank), anaerobic digestion, and final solids (Class A biosolids). Sampling locations are provided in Figure 5-1. Samples were immediately transferred to the lab and stored at -20°C until analysis.

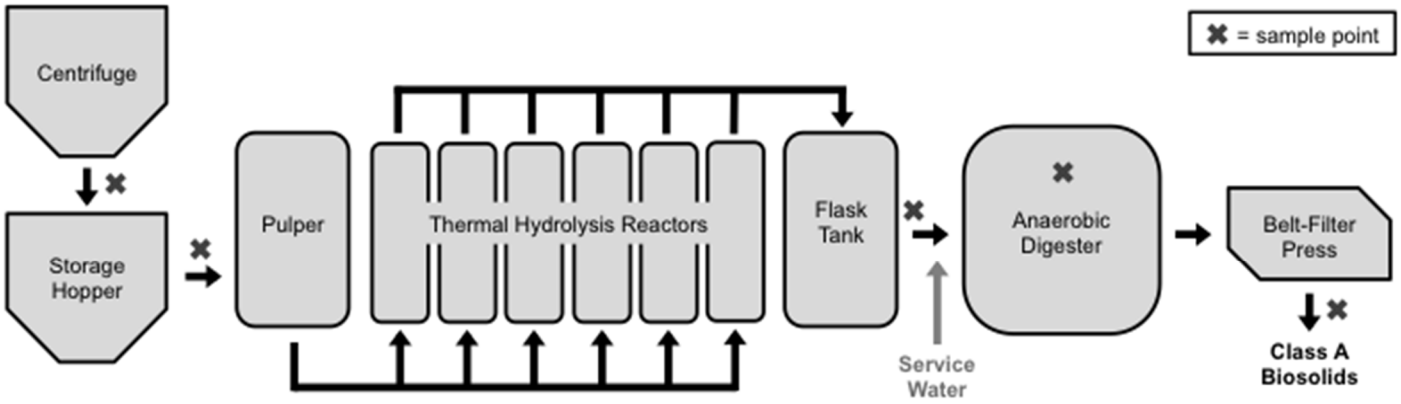

Figure 5-1: Diagram of the Cambi THP™-Anaerobic Treatment Process

5.3.2.2 Biosolids Sampling

Routine sampling of Class B biosolids from the WWTP occurred on a bimonthly basis beginning in 2005 and continuing until phase-out of this treatment process (liming of sludge) in 2015. The samples were collected from the treatment line, immediately after the liming process and stored at -20°C until analysis. Routine sampling of Class A biosolids from the facility began in 2014 during start-up of the TH-AD treatment process and continues through present day. Samples were collected after TH-AD treatment and dewatering and stored at -20°C until analysis. A subset of the Class A and Class B biosolids samples collected during these sampling campaigns was used for this study.

5.3.3 Standards and Reagents

DEHP (99.7%), DiNP (≥ 99%), DiDP (≥ 99%), BBP (98%), d_4-DEHP (99.7%), and d_4-BBP (98%) were acquired from Sigma Aldrich (St. Louis, MO, USA). Solvents were purchased from Burdick and Jackson® (Morris Plains, NJ, USA) and Fisher Scientific (Hampton, NJ, USA) and were of ultra high performance liquid chromatography (UHPLC) grade. Laboratory-grade sand was procured from Fisher Scientific (Hampton, NJ, USA).

5.3.4 Extraction Method

Sludge and biosolids produced by TH-AD treatment contain a high volume of solubilized organics in the liquid faction. Because of this, samples were lyophilized and the solid and liquid faction of all samples were extracted and analyzed together. Compound extraction from lyophilized samples was achieved using a Dionex Accelerated Solvent Extraction (ASE) #300 system (Dionex Corporation, Sunnyvale, CA, USA) according to a previously published method [140]. Briefly, a solvent mixture of dichloromethane (DCM):acetone (50:50 v/v) was used for compound

extraction at a temperature of 110°C and pressure of 1500 psi. Three extraction sequences in total were performed. Extracts were cleaned-up via the modification of an established method [131]. ASE extracts were evaporated gently under nitrogen at a temperature of 55°C and reconstituted in 2 mL of DCM. Extracts were then loaded onto HyperSep™ Florisil (1 g, 6 mL) solid phase extraction (SPE) cartridges (Thermo Fisher Scientific, Waltham, MA, USA) and the eluates collected. The vials previously housing the ASE extracts were rinsed with 2 mL of DCM and the solvent was, again, loaded onto the SPE cartridges and the eluates collected. Compounds were eluted from the cartridges via 2 x 5 mL of hexane:diethyl ether (80:20 v/v). The combined eluates were evaporated under nitrogen at 55°C and reconstituted in 2 mL methanol (MeOH) for instrumental analysis.

5.3.5 Instrument Analysis

Samples were analyzed for BBP, DEHP, DiNP, and DiDP, via a Shimadzu Nexera X2 UHPLC system paired with a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA). A mobile phase of 5 mM of ammonium formate in MeOH and a Waters Acquity UPLC® HSS T3 column (1.8 µm, 2.1 x 100 mm) were utilized for compound separation. The mobile phase was run isocratically at a rate of 0.55 mL/min for 1.7 min. The MS was equipped with an electrospray ionization source in negative mode and acquisition was achieved by multiple reaction monitoring. Further details regarding UHPLC-MS/MS settings are available in the Supplemental Information.

5.3.6 Quality Assurance and Quality Control

Materials containing plastics were avoided during all sampling campaigns and glass jars were used for sample storage. d_4-DEHP and d_4-BBP were used as surrogate standards and, prior to extraction, all biosolids and sludge samples were

spiked with 150 ng of each. All samples were extracted in duplicate. Extractions were performed in batches containing 12 samples or less and each extraction batch included a blank consisting of laboratory-grade sand a sample spiked with all 4 plasticizer compounds for the determination of recovery percentages. In instances where target analytes were detected in extraction blanks above the method detection limit (MDL), this value was subtracted from all samples within that extraction batch.

During instrumental analysis, a standard curve of six standards at concentrations of 50, 100, 250, 500, 1000, 2500 ng/mL was run for each compound. Linearity correlations of the standard curve yielded $r^2 \geq 0.99$. Instrument stability was verified via injection of standards and MeOH blanks every 10 samples. MDLs were determined using US EPA guidelines [98]. Average compound MDLs and recoveries ranged from 14.6 – 27.2 ng/L and 87.1 – 100.8%, respectively. Individual values are provided in Table SI-2. GraphPad Prism 7 (GraphPad Software, Inc., San Diego, CA, USA) was used for figure creation and statistical analysis of data.

5.4 Results and Discussion

5.4.1 Influence of TH-AD Treatment

Analyses of plasticizer concentrations throughout the TH-AD process show concentrations of DEHP, DiNP, and DiDP increasing during anaerobic digestion (Figure 5-2). Average DEHP concentrations were 29.2 ± 0.886 [standard error of the mean (SEM)] mg/kg post flashtank and 51.8 ± 2.17 mg/kg during anaerobic digestion. Similarly, DiNP concentrations post flashtank were 20.3 ± 0.114 mg/kg and 39.2 ± 4.30 mg/kg in anaerobic digestion. DiDP was detected post flashtank at 11.5 ± 0.405 mg/kg and 19.7 ± 1.93 mg/kg during anaerobic digestion. The increase in DEHP, DiNP, and DiDP between the post flashtank samples and anaerobic

digestion samples was statistically significant for all three compounds [Tukey's multiple comparisons test; $P < 0.0001$ (DEHP), $P < 0.0001$ (DiNP), $P = 0.0355$ (DiDP)].

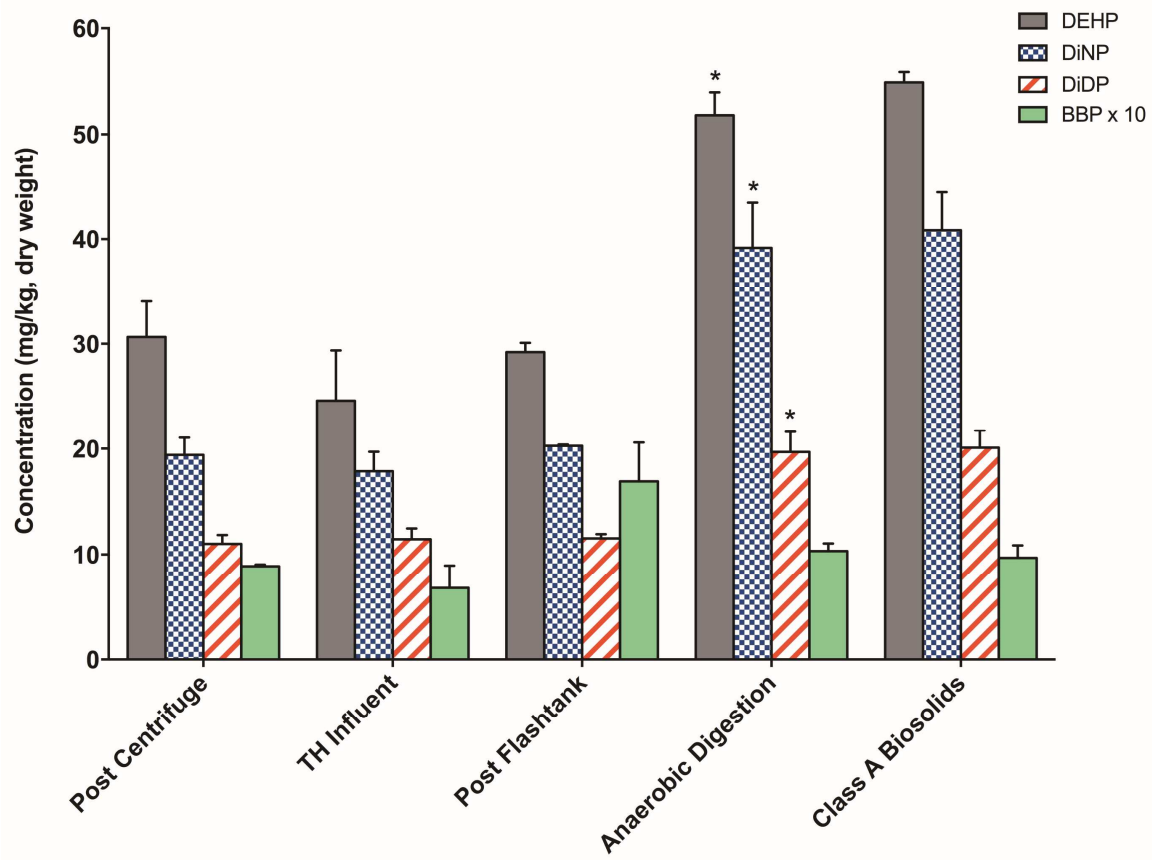

Figure 5-2: Concentrations of DEHP, DiNP, and DiDP and BBP (x10) in Individual Stages of TH-AD Treatment. BBP concentrations were multiplied by 10 for ease of viewing. (* designates concentrations are statistically different than those in the previous treatment step; error bars are standard error of the mean, n=2)

Results from this study indicate that concentrations of DEHP, DiNP, and DiDP were not reduced during treatment. This observation is consistent with studies focusing on the impact of anaerobic digestion on phthalate plasticizers. For instance, DEHP in landfill waste incubated under anaerobic conditions was shown to degrade only 15% after a 278 day period [141]. Additionally, microcosms run under anaerobic methanogenic conditions with BBP-degrading enrichment culture

demonstrated only an 8% decrease in DEHP concentrations after approximately 90 days. The authors concluded that phthalate plasticizers with low solubility were more recalcitrant and that it may be due to the three-dimensional structure of the hydrophobic moieties. [142] Given that the retention time of the anaerobic digesters at the study facility is approximately 22 days, it is likely that there was not a sufficient amount of time for DEHP degradation to occur. This is consistent with the observation in a previous study that DEHP is not removed during anaerobic digestion of wastewater sludge [132]. While little information exists on the degradation of DiNP and DiDP under anaerobic conditions, Lertsirisopon et al. (2006) observed that in natural sediment microcosms run under anaerobic condition, approximately only 10% of DiNP was degraded after 90 days – less than was observed for DEHP [143]. DiDP has a lower solubility and higher log K_{ow} than DEHP and DiNP, indicating that substantial degradation during anaerobic digestion is unlikely due to decreased bioavailability, based on observations regarding the fate of DEHP and DiNP [144]. In a previous study of antimicrobials in the TH-AD process, Armstrong et al. (2017) noted that triclosan and two of its transformation compounds were not degraded but rather, as observed here, concentrations increased during the AD stage of treatment. This was attributed to the solids reduction of the anaerobic digestion process, which essentially concentrated the compounds on the remaining solids [49].

Conversely, concentrations of BBP appeared to increase during the thermal hydrolysis treatment stage and decrease again during anaerobic digestion (Figure 2). While the increase of BBP during TH and decrease during AD was not statistically significant [Tukey's multiple comparisons test; P = 0.995 (TH increase), P = 0.999 (AD decrease)], it is still important to note. The increase in BBP during TH

treatment could not be explained but BBP has been shown to be degradable under anaerobic conditions, which may explain the decrease in concentrations during AD. Ejlertsson et al. (1997) found in that BBP was fully degraded in anaerobic methanogenic microcosms after approximately 30 days [142]. BBP in landfill waste was degraded by 70% over a 278 day period when incubated under anaerobic conditions [141].

5.4.2 Comparison of plasticizers in Class A and Class B biosolids

Overall, concentrations of DEHP, DiNP, DiDP, and BBP in Class A biosolids were higher than those in Class B biosolids. Concentrations of DEHP in Class A biosolids produced by the TH-AD ranged from 54.3 to 87.5 mg/kg with an overall average of 66.7 mg/kg (SEM = 2.47 mg/kg). Concentrations in the limed Class B biosolids ranged from 3.28 to 50.0 mg/kg with an overall average of 25.0 (SEM = 3.78) mg/kg. Differences in average concentrations of DEHP between the two biosolids classifications were statistically different (Kolmogorov-Smirnov test, $P < 0.0001$). DEHP values in Class A and Class B biosolids are provided in Figure 5-3a. Concentrations of DEHP in Class A and Class B biosolids are within range of those from other studies focusing on sewage sludge [16,130,132]. While the US EPA has not issued maximum concentrations for organic pollutants in biosolids, concentrations in Class A and Class B biosolids from this study were below the 100 mg/kg proposed, and later withdrawn, by the European Commission. However, those associated with Class A biosolids were higher than the 50 mg/kg instituted by Denmark. [145]

The overall average DiNP concentration in Class A biosolids was 48.2 (SEM = 1.56) mg/kg (range of 38.6 to 57.7 mg/kg) while the overall average in Class B biosolids was 18.6 (SEM = 1.60) mg/kg (range of 11.5 to 28.1 mg/kg) (Figure 5-3b).

Concentrations of DiNP in Class A biosolids were statistically higher than those in Class B biosolids (Kolmogorov-Smirnov test, $P < 0.0001$). A previous study of DiNP in sludge from a WWTP with secondary treatment and a WWTP with tertiary treatment found concentrations to range from 2.60 – 31.3 mg/kg and below detection limit to 37.1 mg/kg, respectively [140]. DiNP in sludge samples collected from seven Swedish WWTPs ranged from 21 – 78 mg/kg [20]. Concentrations of DiNP biosolids from the present study are within these ranges.

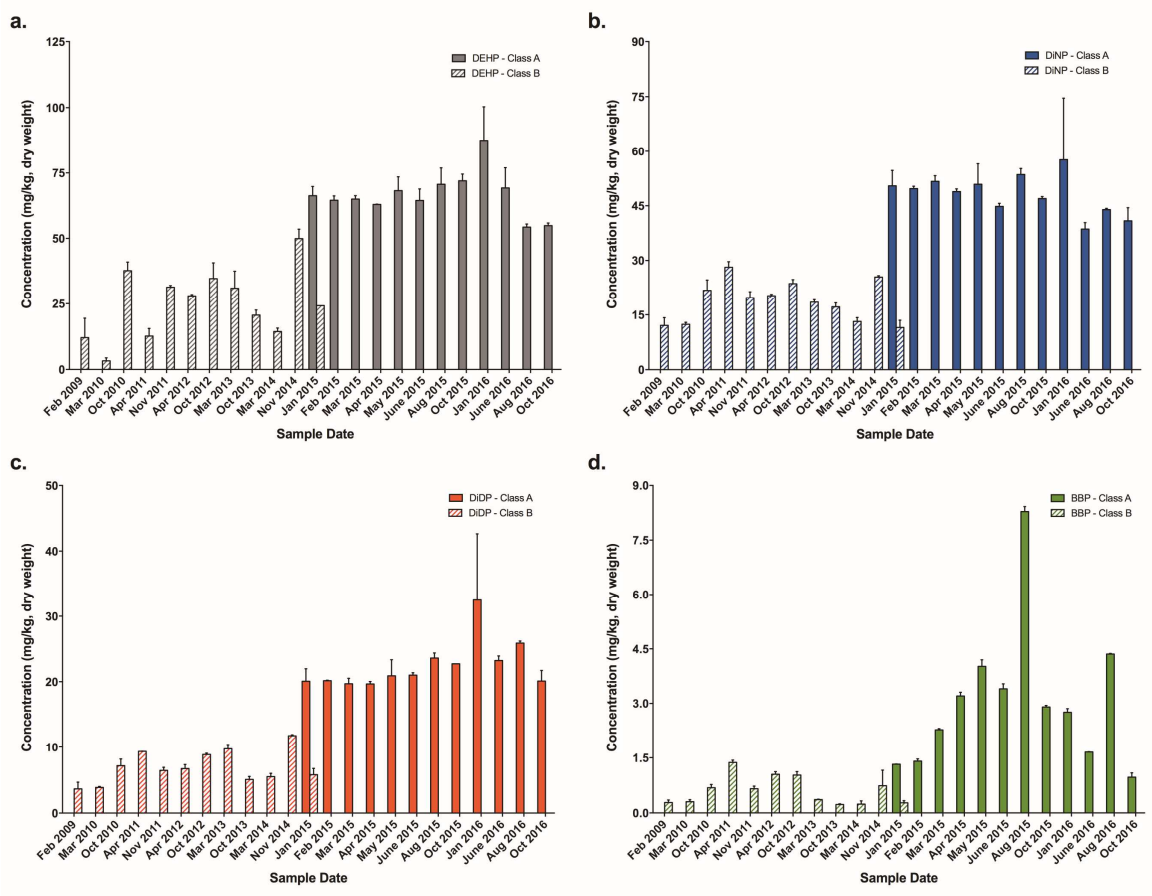

Figure 5-3: Concentrations of a) DEHP, b) DiNP, c) DiDP, and d) BBP in Class A and Class B Biosolids (error bars represent the standard error of the mean)

DiDP concentrations (Figure 5-3c) ranged from 19.7 to 32.5 mg/kg in Class A biosolids and 3.64 to 11.7 mg/kg in Class B biosolids. Overall averages in Class A and Class B biosolids were 22.5 (SEM = 1.07) mg/kg and 6.98 (SEM = 0.717) mg/kg, respectively. Again, concentrations were significantly higher in Class A biosolids when compared to Class B biosolids (Kolmogorov-Smirnov test, $P < 0.0001$). However, as demonstrated with DEHP and DiNP, these concentrations are in accordance with those from other WWTPs [20].

Concentrations of BBP in Class A biosolids ranged from 0.969 to 8.28 mg/kg with an overall average of 3.05 mg/kg (SEM = 0.570 mg/kg). Concentrations in the limed Class B biosolids ranged from 0.229 to 1.37 mg/kg with an overall average of 0.601 (SEM = 0.111) mg/kg. Differences in average concentrations of BBP between the two biosolids classifications were statistically different (Kolmogorov-Smirnov test, $P = 0.0005$). BBP values in Class A and Class B biosolids are provided in Figure 5-3d. Concentrations from Turkish WWTPs had sludges with BBP concentrations ranging from 2.8 to 6.2 mg/kg [132]. Class B biosolids from the present study possessed BBP concentrations that were lower than this range while BBP in Class A biosolids were within or above this range.

These results indicate that utilization of the TH-AD resulted in higher concentrations of the four phthalate plasticizers in biosolids when compared to the liming process. As indicated in Figure 5-2, an increase occurs during the anaerobic digestion process for DEHP, DiNP, and DiDP. On the other hand, despite the difference in BBP concentrations within Class A and Class B biosolids, levels of BBP did not change significantly throughout the TH-AD process. Overall, this implies that the 4 phthalate plasticizers studied did not degrade during TH-AD treatment. One explanation for this difference between the two biosolids types is the combination of

solids reduction that occurs during anaerobic digestion of the TH-AD process and addition of lime to produce the Class B biosolids. To produce Class B biosolids, lime was applied to sludge at a rate of 15 – 20% on a dry weight basis. Based on processing loads, this percentage could vary between 10 – 25%. During the anaerobic digestion stage of TH-AD treatment, solids are reduced by 60 – 70%, a higher reduction than conventional AD due to the TH pretreatment [117]. The average concentrations of each target phthalate plasticizer in Class A biosolids were compared to concentrations in Class B biosolids adjusted for an 80% change in solids (15% for liming and 65% for loss of solids during AD treatment) and are provided in Figure 5-4. For all compounds, concentrations in Class A biosolids were significantly higher than the adjusted Class B concentrations (multiple t-tests; Holm-Sidak method; $P < 0.01$). This demonstrates that differences in concentrations of DEHP, DiNP, DiDP, and BBP between Class A and Class B biosolids were not solely due to liming dilutions and/or solids reduction. As discussed in Section 3.1, compounds such as DEHP and DiNP degrade very little during anaerobic digestion. However, unlike what was noted in this study, significant increases in concentrations of these compounds during anaerobic treatment were not observed in previous studies. BBP was shown to be degradable under anaerobic conditions but, again, no explanation or observations of increases in BBP were seen in the literature. One possible explanation for the difference in compound concentrations between treatment technologies is deconjugation of phthalate plasticizer metabolites. Research has indicated that when phthalate plasticizers are consumed and metabolized by the human body they can form conjugates, which can be excreted into the WWT system [146-149]. The transformation of conjugated pharmaceuticals back to the original compound (deconjugation) has been demonstrated to occur

during WWT [36,150]. Further research would have to be conducted to confirm whether deconjugation is a viable explanation for the observations of the current study.

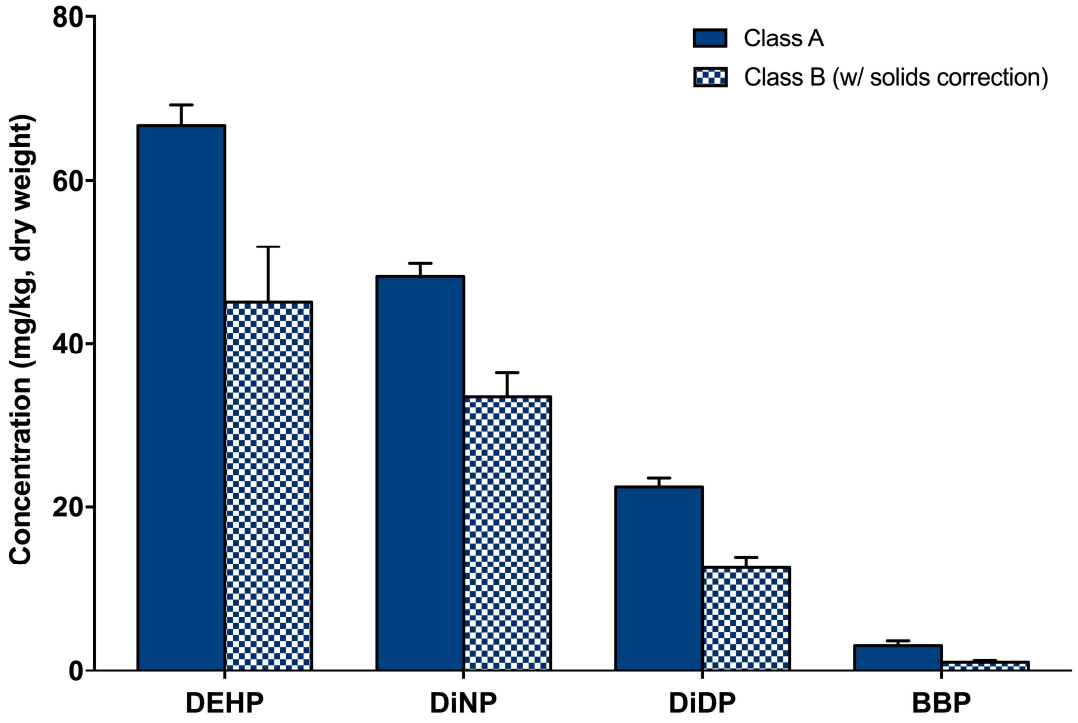

Figure 5-4: Concentrations of Phthalate Plasticizers in Class A Biosolids and Class B Biosolids (Corrected for Solids Concentration Losses) (error bars represent the standard error of the mean)

Another reason for the difference in phthalate plasticizer concentrations between Class A and Class B biosolids may be due to the hydrolysis of these compounds due to the liming of sludge. Previous research has indicated that phthalate plasticizers may be hydrolyzed to monoester and diacid products in aqueous samples under alkaline conditions, with hydrolysis rates decreasing with an increase in chain length [151,152]. Given that after the liming process sludge pH in Class B biosolids is ~12, this may be an explanation for the variation in

concentrations between the two biosolids types studied. However, estimated hydrolysis half lives for phthalate plasticizers can be quite high [152], indicating that this process may only be a partial explanation for concentrations differences – further research exploring hydrolysis in sludge via liming would need to be conducted.

5.4.3 Predicted Concentrations of Phthalate Plasticizers in Soil

Given that the land-application of biosolids to agricultural fields would constitute a major source of phthalate plasticizers to the soils, the predicted environmental concentration (PEC) was calculated for DEHP, DiNP, DiDP, and BBP for the application of the two different types of biosolids. The PEC for each compound and biosolids type was calculated using:

$$PEC = C_{soil} + \frac{C_{biosolids} \times AR}{D \times SD \times CF}$$

The initial soil concentrations (C_{soil}) was assumed to be zero, the concentration of the phthalate plasticizer in the biosolids ($C_{biosolids}$) was assumed to be the average concentration outlined in Section 3.2 and provided in Table 5-1, the density (D) was assumed as 1.35 kg/cm^3 [25,34], the soil depth (SD) was assumed as 7.6 cm to represent no till biosolids application [34], and CF is a units conversion factor. The application rate (AR) was based on the average rate employed for application of biosolids from the study WWTP onto agricultural fields. The ARs used are provided in Table 5-1 and differ between Class A and Class B biosolids due to higher nutrient concentrations in Class A biosolids [49].

Most toxicity studies regarding phthalate plasticizers in soil ecosystems revolve around DEHP (of those analyzed in the present study). Hulzebos et al. (1993) determined the DEHP EC_{50} for the lettuce *Lactuca sativa* to be > 1000 µg/g

[153]. A study conducted on juvenile and adult collembolans (*Folsomia fimetarra*) found that juvenile mortality, growth, and development of the organisms were unaffected at DEHP concentrations up to 5000 mg/kg [154]. Additionally, Cartwright et al. (2000) observed that soil microbial populations were unaffected by DEHP at concentrations of 0.1 mg/g (representative on nonindustrial environmental) and 100

Table 5-1: Average Concentrations of Phthalate Plasticizers in Biosolids and their Predicted Environmental Concentrations

		DEHP		DiNP	
Biosolids Type	**Application Rate (dry kg/ha)**	**Avg Conc in Biosolids (mg/kg)**	**PEC in Soils (mg/kg)**	**Avg Conc in Biosolids (mg/kg)**	**PEC in Soils (mg/kg)**
Class A Biosolids	6.95×10^3	66.7	0.452	48.2	0.327
Class B Biosolids	9.42×10^3	25.0	0.230	18.6	0.171
		DiDP		**BBP**	
Biosolids Type	**Application Rate (dry kg/ha)**	**Avg Conc in Biosolids (mg/kg)**	**PEC in Soils (mg/kg)**	**Avg Conc in Biosolids (mg/kg)**	**PEC in Soils (mg/kg)**
Class A Biosolids	6.95×10^3	22.5	0.152	3.05	0.0207
Class B Biosolids	9.42×10^3	6.98	0.0641	0.601	0.00552

mg/g (representative of a spill environment) [155]. However, Hu et al. (2005) noted that uptake of the compound by earthworms was rapid during the initial 10 day contact period and estimated the biota-to-soil accumulation factor to be 0.17 ± 0.03 in soils with a DEHP concentration of 5 mg/kg [156]. Furthermore, an environmental risk limit emphasizing endocrine disrupting properties for DEHP was determined to be 1 mg/kg for fresh soil and sediments [157]. These determined toxicity values, and the environmental risk limit of 1 mg/kg are all above the PEC for DEHP in soils applied with Class A or Class B biosolids. Further studies regarding the impact of

DEHP on ecological risks as well as the impact of other phthalate plasticizers are needed.

5.5 Conclusions

Concentrations of DEHP, DiNP, and DiDP in wastewater sludge increased significantly during the anaerobic digestion stage of the TH-AD process. No change in concentration was observed during the thermal hydrolysis stage of treatment for these three compounds. An increase in BBP was observed during thermal hydrolysis with a decrease in concentrations occurring during anaerobic digestion. These changes in BBP concentrations during TH-AD, however, were not statistically significant. Implementation of the TH-AD process led to higher concentrations of DEHP, DiNP, DiDP, and BBP in biosolids, when comparing Class A and Class B biosolids from the same facility. Adjusting concentrations for solids reduction during anaerobic digestion and dilution via liming did not fully account for the differences in concentrations of phthalate plasticizers in Class A and Class B biosolids. Further research should be conducted to determine the reason for differences in plasticizer concentrations between Class A and Class B biosolids. Calculation of PECs for all 4 compounds indicate that concentrations in soils land-applied with biosolids may not be above toxic levels, but more information regarding the ecological impact of these compounds needs to be explored.

5.6 Acknowledgements

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Chapter 6: Fate of Four Phthalate Plasticizers Under Various Wastewater Treatment Processes

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6.1 Abstract

The fate of four phthalate plasticizers during wastewater treatment processes at six different wastewater treatment plants (WWTPs) was investigated. Concentrations of benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), diisononyl phthalate (DiNP), and diisodecyl phthalate (DiDP) were determined prior to either aerobic or anaerobic (conventional and advanced) treatment, after treatment, and in final, dewatered solids. Despite their elevated use worldwide, the fate of DiNP and DiDP during wastewater treatment have not been well characterized. DEHP was readily degraded during aerobic treatments while anaerobic digestion resulted in either no significant change in concentrations or an increase in concentration, in the case of more advanced anaerobic processes (thermal hydrolysis pretreatment and a two-phase acid/gas process). Impacts of the various treatment systems on DiNP, DiDP, and BBP concentrations were more varied – anaerobic digestion led to significant decreases, increases, or no significant change for these compounds, depending on the treatment facility, while aerobic treatment was generally effective at degrading the compounds. Additionally, thermal hydrolysis pretreatment of sludge prior to anaerobic digestion resulted in increases in DiNP, DiDP, and BBP concentrations. The predicted environmental

concentrations for all four compounds in soils after a single biosolids application were calculated and the risk quotients for DEHP in soils were determined. The estimated toxicity risk for DEHP in soils treated with a single application of sludge from any of the six studied WWTPs is lower than the level of concern for acute and chronic risk, as defined by the US EPA.

6.2 Introduction

Phthalate plasticizers, also known as phthalic acid esters (PAEs), are typically used to improve upon the flexibility of plastics [[124]], among other uses, and have been detected extensively in the environment, such as in air [129,130,158], dust [159], sea water [130,160], fresh water [129,130], sediments [129,130,160,161], and soil samples [129,130]. These compounds are not chemically bound to the polymer they are associated with, allowing for leaching of these compounds to occur [13,14,162]. This leaching, in conjunction with their high production volumes - over 4 million tons worldwide [13], has led to concerns regarding environmental and human exposure. PAEs have been demonstrated to induce various negative health effects, including reproductive toxicity [162-164], changes in hormone levels [165], birth defects [165], tumor formation [162], disturbance of thyroid function [122], and metabolic disorders [162]. Several PAEs, including benzyl butyl phthalate (BBP), di(2-ethylhexyl) phthalate (DEHP), diisodecyl phthalate (DiDP), and diisononyl phthalate (DiNP), have been listed in Proposition 65 by the State of California as chemicals known to cause cancer or reproductive toxicity [166].

Various PAE compounds have been detected throughout the wastewater treatment (WWT) process, including wastewater effluent and biosolids, indicating that WWT can act as a source of these compounds to the environment. Armstrong et al. (2018) determined concentrations of DEHP, DiNP, DiDP, and BBP to be as high

as 87.5, 57.7, 32.5, and 8.28 mg/kg dry weight (dw), respectively, in Class A biosolids produced by thermal hydrolysis/anaerobic digestion. These concentrations were higher than those in limed Class B biosolids from the same facility [55], indicating the importance of treatment processes on PAE concentrations. A study of plasticizers in sludges from Canadian WWT plants demonstrated concentrations of DEHP in dewatered final sludge ranging from non detect to 119 mg/kg [16]. In a 2017 study performed in Saudi Arabia, concentrations of 6 PAEs and bisphenol A were measured from the effluent of 5 WWT facilities. PAEs such as dibutyl phthalate (DBP), BBP, and DEHP were analyzed and concentrations ranged from 0.408 – 1.037, 0.108 – 0.660, and 0.340 – 0.935 µg/L, respectively [167]. Relatively high concentrations of PAEs in biosolids lead to concern over the environmental contamination of soils due to biosolids land application. It is estimated that in the United States approximately 60% of biosolids are land applied for soil reclamation [133]. In a cultivation study conducted with lettuce, strawberry, and carrot, Sun et al. (2015) observed the ability of all three hydroponically grown plant types to uptake DBP, DEHP, and two principal metabolites [29]. Similarly, Zhao et al. (2015) demonstrated the ability of a variety of cabbage cultivars to accumulate DBP and DEHP from soils contaminated with the PAE compounds [135]. In both studies, the DBP and DEHP accumulation occurred predominantly in the roots faction of the vegetation studied. Regarding biota, a study of both live and deceased ants showed the ability of DBP, diisobutyl phthalate, DEHP, and BBP to be absorbed by cuticles of living ants [168]. Additionally, earthworm DEHP bioaccumulation factors from soils were determined to be 0.1 – 0.3 [30].

Indications of the potential of PAEs to transfer to vegetation and biota demonstrates the need to better understand how to reduce concentrations of these

compounds in biosolids prior to their land application. The present study focuses on the impact of various wastewater treatment processes on concentrations of four high molecular weight PAEs from six municipal WWT plants in the Mid Atlantic region of the US. Treatments within the six facilities vary between aerobic and anaerobic (both conventional and advanced) and the facilities themselves are located in both rural and developed areas. Samples were collected prior to treatment, post-treatment, and after final solids dewatering and were analyzed for DEHP, DiNP, DiDP, and BBP. While DEHP and BBP have been well studied due to extensive use, few investigations have focused on the fate of DiNP and DiDP during wastewater treatment and in biosolids, despite the fact that they among the most highly produced PAEs [15]. Results from this study allow for increased insight into how anaerobic digestion, including two infrequently characterized advanced processes (thermal hydrolysis pretreatment and a two-phase acid/gas process), or aerobic digestion impact concentrations of commonly utilized PAEs in wastewater and final solids. Finally, the predicted environmental concentrations of the four compounds in soils after a single biosolids application and the risk quotients for DEHP in these soils were determined as a means of gauging the risk that these PAEs pose to the environment due to the beneficial reuse of biosolids.

6.3 Materials and Methods

6.3.1 Sample Collection and Handling

The focus of this study is on concentrations of BBP, DEHP, DiNP, and DiDP in final solids and the influence that varying treatment processes can have on the fate of these compounds in final solids. Because of this objective, the solids treatment processes at each facility were the primary sampling focus. In the case of WWTPs #5 and 6, which are very small facilities that employ a single treatment

process, the system utilized was the sampling focus since a separate solids treatment process does not exist at these facilities. All wastewater and sludge samples were lyophilized prior to extraction and analyzed for the four target PAEs. For wastewater samples, the solids and liquids factions were not separated prior to analysis but, rather, analyzed together.

6.3.2 Study treatment facilities

Six different WWTPs located in the Mid-Atlantic region of the United States (Maryland and Washington, D.C.) were sampled for PAE analysis. Four (4) facilities (WWTPs #1 – 4) utilized anaerobic digestion for sludge treatment while two employed aerobic processes (WWTPs #5 and 6). Further details regarding specific treatment processes, average and maximum flows, and solids disposal practices are provided in Table 6-1.

6.3.3 Standards and Reagents

PAE analytical standards were purchased from Sigma Aldrich (St. Louis, MO, USA): DEHP (99.7%), d_4-DEHP (99.7%), DiNP (≥99.0%), DiDP (≥99.0%), BBP (98.0%), and d_4-BBP (98.0%). All organic solvents were of at least high performance liquid chromatography grade and were obtained from Fisher Scientific (Hampton, NJ, USA). Laboratory-grade sand for sample extraction was also obtained from Fisher Scientific.

6.3.4 Extraction Method

All samples were extracted in duplicate. Prior to extraction, samples were spiked with 300 ng of d_4-DEHP and d_4-BBP as surrogate standards. PAE compounds were extracted from lyophilized sludge samples using a Dionex Accelerated Solvent Extraction (ASE) #300 System (Dionex Corporation, Sunnyvale, CA, USA). Extraction via the ASE system were performed at a pressure 1500 psi

Table 6-1: Treatment Details for the Six Study WWTPs

	Average Flow (m^3/day)	Maximum Capacity (m^3/day)	Treatment System Configuration	Final Solids Disposal	Sample Date
WWTP #1	14,400	22,700	- Primary treatment; Bardenpho biological nutrient removal (w/ Evoqua Biomag® system) - Anaerobic solids digestion (37°C, RT 8 days); Belt filter dewatering	Biosolids land application (Class B biosolids[1])	January 2017
WWTP #2	34,000	196,800	- Primary treatment; 3-pass process reactor (anaerobic-anoxic-oxic); Denitrification (Leopold® Deep Bed Filtration) - Fermentation of primary solids; anaerobic solids digestion (35°C, RT 23 days); Belt filter dewatering	Biosolids land application (Class B biosolids[1])	January 2017
WWTP #3	1,140,000	1,400,000	- Primary treatment; Conventional activated sludge; Nitrification-denitrification - Anaerobic solids digestion (37°C, RT 22 days) w/ Cambi Thermal Hydrolysis Process™ pretreatment; Belt filter dewatering	Biosolids land application (Class A biosolids[1])	January 2017
WWTP #4	511,000	1,500,000	- Primary treatment; Activated sludge (w/ tetrafilter post treatment); Effluent re-aeration by step-dam cascade - Anaerobic solids digestion (30-38-37°C, RT 25 days[2]) by 2-phase acid/gas process; Centrifuge dewatering; Heat drying (pelletized fertilizer produced) (2/3 of solids) and composting (1/3 of solids) treatment	Land application of fertilizer pellets & compost	February 2017
WWTP #5	305	1,005	- Aerobic sequencing batch reactor (HRT 6 hours, SRT 20 - 30 days); Asphalt pad solids dewatering	Landfill	January 2017
WWTP #6	285	945	- Aerobic membrane bioreactor system (HRT 6 hours, SRT 20 - 25 days); Gravity system dewatering	Landfill	January 2017

RT = residence time; HRT = hydraulic retention time; SRT = solids retention time

[1] biosolids classifications designated by the US Environmental Protection Agency

[2] anaerobic digestion is a 2-phase, 3-stage process: one acid phase reactor run at 30°C, two egg-shaped digesters run at 38°C, and six in-ground digesters run at 37°C. RT is for mesophilic processes.

and temperature of 110°C [[140]]. A solvent mixture of acetone:2-isopropanol (50:50 v/v) was utilized for the three extraction sequences. Clean up of extracts was achieved via HyperSep™ Florisil (1 g, 6 mL) solid phase extraction cartridges (Thermo Scientific, Waltham, MA, USA) using a previously published method [55]. All samples were extracted in batches of 12 or less. Each extraction batch consisted of a blank and a spiked sample for recovery calculations.

6.3.5 Instrumental Analysis

A Shimadzu Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) coupled with a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) was used for DEHP, DiNP, DiDP, and BBP analysis. Chromatographic separation was achieved via 5 mM ammonium formate in methanol run isocratically through a Waters Acquity UPLC® HSS T3 column (1.8 µm, 2.1 x 100 mm). The MS source was an electrospray ionization source run in negative mode. Acquisition occurred in multiple reaction monitoring. Further details regarding the UHPLC-MS/MS method, as well as the compound method detection limits (MDLs), can be found in a previous publication [55].

6.4 Results and Discussion

6.4.1 Di(2-ethylhexyl) Phthalate (DEHP)

Concentrations of DEHP throughout the treatment processes of the six WWTPs sampled are provided in Figure 6-1a and Table 6-2. DEHP concentrations demonstrated three distinct trends. In WWTPs #1 and #2, DEHP was observed to decrease during anaerobic digestion treatment and then increase in final solids. For WWTP #1, the decrease during anaerobic digestion was not significant while the increased concentrations in final solids were significantly higher (130%) than those

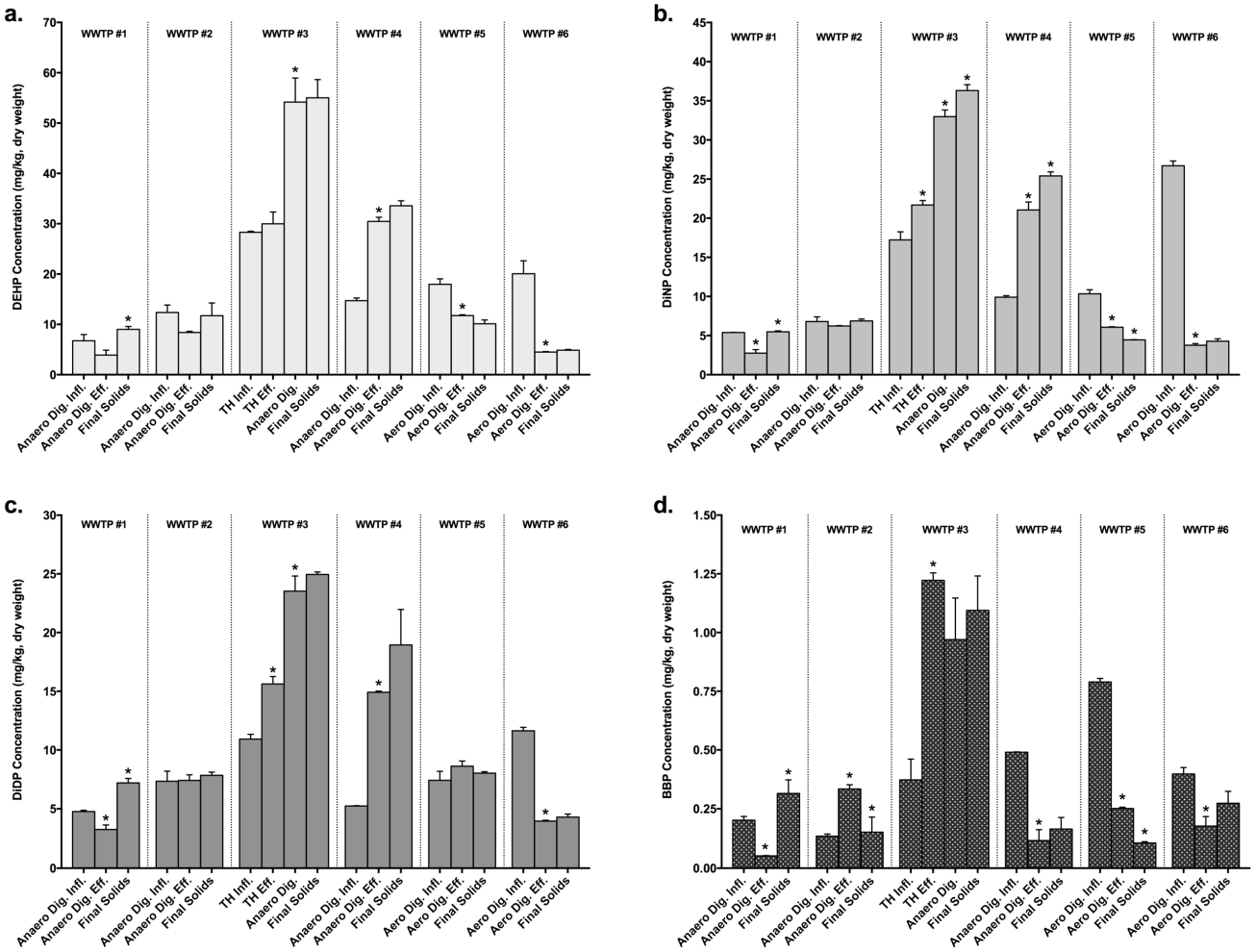

Figure 6-1: Concentrations of a) DEHP, b) DiNP, c) DiDP, and d) BBP during each stage of treatment in WWTPs #1 – 6. * denotes concentrations are significantly different than those in the preceding stage of treatment. Error bars represent the standard deviation.

Table 6-2: Concentrations and calculated percent changes of PAEs during each stage of treatment in WWTPs #1 – 6.

		DEHP		DiNP		DiDP		BBP	
		Conc ± SD (mg/kg dw)	**% Change***	**Conc ± SD (mg/kg dw)**	**% Change***	**Conc ± SD (mg/kg dw)**	**% Change***	**Conc ± SD (mg/kg dw)**	**% Change***
WWTP #1	Anaerobic Digestion Influent	6.74 ± 1.24	---	5.38 ± 0.0377	---	4.76 ± 0.109	---	0.202 ± 0.0157	---
	Anaerobic Digestion Effluent	3.91 ± 0.967	NS	2.76 ± 0.447	- 48.7%	3.25 ± 0.380	- 31.7%	0.0520 ± 0.00128	- 74.3%
	Final Solids	9.02 ± 0.561	+ 130%	5.49 ± 0.123	+ 98.9%	7.19 ± 0.376	+ 121%	0.315 ± 0.0580	+ 506%
WWTP #2	Anaerobic Digestion Influent	12.4 ± 1.47	---	6.80 ± 0.592	---	7.34 ± 0.857	---	0.135 ± 0.00892	---
	Anaerobic Digestion Effluent	8.39 ± 0.227	NS	6.23 ± 0.0498	NS	6.90 ± 0.242	NS	0.334 ± 0.0180	+ 147%
	Final Solids	11.7 ± 2.53	NS	6.87 ± 0.234	NS	7.84 ± 0.276	NS	0.152 ± 0.0635	- 54.5%
WWTP #3	Thermal Hydrolysis Influent	28.3 ± 0.207	---	17.2 ± 1.03	---	10.9 ± 0.408	---	0.373 ± 0.0881	---
	Thermal Hydrolysis Effluent	30.0 ± 2.35	NS	21.7 ± 0.572	+ 26.2%	15.6 ± 0.637	+ 43.1 %	1.22 ± 0.0316	+ 227%
	Anaerobic Digestion	54.2 ± 4.78	+ 80.7%	33.0 ± 0.842	+ 52.1%	23.5 ± 1.29	+ 50.6%	0.970 ± 0.177	NS
	Final Solids	55.0 ± 3.63	NS	36.3 ± 0.746	+ 10.0%	24.9 ± 0.214	NS	1.09 ± 0.146	NS
WWTP #4	Anaerobic Digestion Influent	14.7 ± 0.539	---	9.90 ± 0.197	---	5.24 ± 0.0221	---	0.491 ± 0.00108	---
	Anaerobic Digestion Effluent	30.5 ± 0.830	+ 107%	21.1 ± 1.01	+ 113%	14.9 ± 0.0933	+ 184%	0.116 ± 0.0458	- 76.4%
	Final Solids	33.6 ± 0.982	NS	25.4 ± 0.513	+ 20.4%	18.9 ± 3.02	NS	0.165 ± 0.0485	NS
WWTP #5	Aerobic Digestion Influent	18.0 ± 1.09	---	10.3 ± 0.515	---	7.42 ± 0.761	---	0.790 ± 0.0146	---
	Aerobic Digestion Effluent	11.7 ± 0.171	- 35.0%	6.07 ± 0.0590	- 41.1%	8.62 ± 0.443	NS	0.251 ± 0.00506	- 68.2%
	Final Solids	10.1 ± 0.753	NS	4.45 ± 0.0336	- 26.7%	8.03 ± 0.115	NS	0.106 ± 0.00529	- 57.8%
WWTP #6	Aerobic Digestion Influent	20.1 ± 2.57	---	26.7 ± 0.604	---	11.6 ± 0.295	---	0.398 ± 0.0271	---
	Aerobic Digestion Effluent	4.50 ± 0.105	- 77.6%	3.76 ± 0.219	- 85.9%	3.97 ± 0.0746	- 65.8%	0.177 ± 0.0398	- 55.5%
	Final Solids	4.87 ± 0.141	NS	4.28 ± 0.308	NS	4.30 ± 0.253	NS	0.273 ± 0.0504	NS

NS = change in concentration not significant and, thus, not calculated

SD = standard deviation

* calculated change from previous treatment step

in the anaerobic effluent (Tukey's multiple comparisons test, P = 0.027), which may be due to sampling from biosolids stockpiles on-site rather than directly after the dewatering process. Likewise, the decrease in DEHP concentrations during anaerobic digestion was not significant during treatment at WWTP #2. Concentrations again increased in final solids, but the increase was not significant. Conversely, an increase in DEHP concentrations during anaerobic digestion was observed in WWTPs #3 and #4. In WWTP #3, concentrations of the compound remained steady during thermal hydrolysis treatment while anaerobic digestion resulted in a significant increase of 80.7% (Tukey's multiple comparisons test, P = 0.0059). There was no significant change in DEHP concentrations between anaerobic digestion and final solids. Similarly, a significant (107%) increase in DEHP (Tukey's multiple comparisons test, P = 0.0007) was observed during anaerobic digestion at WWTP #4. Additionally, concentrations of DEHP were, again, not significantly different between samples collected from anaerobic digestion and final solids post dewatering.

Previous studies have demonstrated that DEHP is not well degraded under anaerobic conditions. For instance, in a study of a Turkish WWTP, Çifci et al. (2013) observed that during anaerobic digestion treatment, only 17% of DEHP was degraded while the remaining amount was either recirculated through the treatment process or sorbed to dewatered sludge [132]. Additionally, anaerobic methanogenic microcosms enriched with a BBP-degrading culture showed that PAEs with low solubilities were not readily degraded, resulting in a reduction of DEHP concentrations by just 8% after 90 days [142]. During previous research conducted on the influence of the TH-AD treatment process at WWTP #3 on concentrations of PAEs in sludge, DEHP concentrations did not significantly change during thermal

hydrolysis treatment but were significantly higher during anaerobic digestion [55]. Furthermore, laboratory experiments comparing the influence of sludge thermally pretreated at 70°C prior to anaerobic digestion and sludge digested without pretreatment demonstrated that pretreatment had negative impacts on PAEs, including DEHP [169]. Overall, the current research supports prior studies that DEHP is not readily degraded anaerobically, an occurrence that can be exasperated by pretreatment prior to digestion.

Conversely, aerobic processes at the study WWTPs demonstrated the ability to greatly reduce DEHP concentrations. The aerobic SBR of WWTP #5 significantly reduced DEHP concentrations by 35% (Tukey's multiple comparisons test, $P = 0.0081$) while WWTP #6's MBR caused an even larger (77.6%), and still significant, reduction of DEHP during treatment (Tukey's multiple comparisons test, $P < 0.0038$). Concentrations in samples collected after aerobic treatment and from final solids were not significantly different for either WWTP #5 or WWTP #6.

Observations of DEHP degradation during aerobic treatment are consistent with those from previous studies. For instance, microbial degradation of DEHP in activated sludge from a Danish WWTP was estimated to be 81% [170]. A study concentrating on Indian WWTPs found that treatment via either a SBR or conventional activated sludge was effective at degrading DEHP by 61 – 70% [171]. Thus indicating that aerobic treatments appear to be more effective in degrading DEHP than anaerobic.

6.4.2 Diisononyl Phthalate (DiNP)

Treatment effects of the various WWTPs on concentrations of DiNP (Figure 6-1b, Table 6-2) were similar to those observed for DEHP. Concentrations of DiNP in WWTP #1 significantly decreased by 48.7% (Tukey's multiple comparisons test, P =

0.0047) during anaerobic digestion and then significantly increased by 98.9% (Tukey's multiple comparisons test, P = 0.0042) in final solids samples. This increase, again, is likely due to sampling from biosolids stockpiles on-site rather than directly after the dewatering process. Conversely, DiNP concentrations did not significantly change across all treatments in WWTP #2. WWTPs #3 and #4 demonstrated an increase in DiNP concentrations during treatment. Thermal hydrolysis pretreatment in WWTP #3 resulted in a small, but significant increase (26.2%) in concentrations (Tukey's multiple comparisons test, P < 0.018) as well as significant increases of DiNP during anaerobic digestion (52.1%) (Tukey's multiple comparisons test, P < 0.0005). Additionally, concentrations of DiNP in the final solids of WWTP #3 were significantly higher than those in samples collected from the anaerobic digestion reactor by 10.0% (Tukey's multiple comparisons test, P < 0.049). Treatment at WWTP #4 also resulted a significant increase in concentrations by 113% during anaerobic digestion (Tukey's multiple comparisons test, P < 0.0010) and in final solids by 20.4% (Tukey's multiple comparisons test, P < 0.015).

Few studies exist regarding the fate of DiNP under anaerobic processes during wastewater treatment. Armstrong et al. (2018) observed that, similar to the present study, concentrations of DiNP greatly increased during the anaerobic stage of treatment at WWTP #3, increasing from approximately 30 mg/kg to over 50 mg/kg [55]. While not conducted with wastewater, a microcosm study performed with natural sediments under anaerobic conditions resulted in a DiNP degradation of only ~10% after a 90 day period [143], demonstrating that DiNP is not readily degraded under anaerobic conditions. The increase in DiNP concentrations in WWTPs #3 and #4 are similar to what was observed for DEHP and may indicate that more advanced

anaerobic treatment processes, such as the thermal hydrolysis pretreatment or the two-phase acid/gas process, may have inhibitory effect on DiNP degradation.

As with DEHP, aerobic processes resulted in decreased concentrations of DiNP. Treatment of wastewater via SBR at WWTP #5 resulted in significantly decreased (41.1%) concentrations of DiNP in aerobic effluent samples (Tukey's multiple comparisons test, $P = 0.0016$) and final cake samples (26.7%) (Tukey's multiple comparisons test, $P = 0.025$). Concentrations of DiNP also decreased significantly (85.9%) during aerobic wastewater treatment (Tukey's multiple comparisons test, $P < 0.0001$) at WWTP #6. Little research regarding the degradability of DiNP under aerobic conditions at WWTPs is available. Nevertheless, it has been demonstrated that bacteria such as *Sphingobium chungbukense* can effectively degrade the compound aerobically under various starting concentrations. Full degradation of DiNP by this bacterium strain, however, can take from 54 to 228 hours for initial concentrations ranging from 50 to 500 mg/L and a lag phase was required for higher initial concentrations [172]. This, along with the present study, indicates that aerobic treatment can be an effective means for removing DiNP from wastewater, but factors such as duration of treatment and initial concentration, along with microbial populations present, will influence degradation of the compound.

6.4.3 Diisodecyl Phthalate (DiDP)

DiDP concentrations during treatment at the six studied treatment plants are provided in Figure 6-1c and Table 6-2. The treatment system employed by WWTP #1 resulted in a significant decrease of DiDP concentrations by 31.7% during anaerobic digestion (Tukey's multiple comparisons test, $P = 0.035$). However, DiDP was significantly higher (121%) in final solids samples (Tukey's multiple comparisons test, $P = 0.0023$), when compared to anaerobic digestion effluent samples – an

occurrence likely due to final solids sampling locations and timing. The anaerobic treatment system at WWTP #2 had no significant impact on DiDP; concentrations remained stable in all samples. Conversely, the TH-AD treatment process utilized by WWTP #3 resulted in significant increases of DiDP during both the thermal hydrolysis (43.1%) and anaerobic digestion (50.6%) stages of treatment [Tukey's multiple comparisons test, P = 0.012 (thermal hydrolysis), P = 0.0016 (anaerobic digestion)], as was observed for DEHP and DiNP. Similarly, WWTP #4 treatment systems resulted in a significant increase of 184% in DiDP during anaerobic treatment (Tukey's multiple comparisons test, P = 0.0234). While higher than those in anaerobic digestion effluent samples, concentrations of DiDP were not significantly higher in final solids collected from WWTP #4.

As with DiNP, there is little information regarding the impact that anaerobic treatment has on concentrations of DiDP in the wastewater treatment process. While little information exists on the fate of this compound during anaerobic digestion, the fact that DiDP has a very low solubility (< 0.001 g/L) and its log K_{ow} values are higher than those of DEHP and DiNP [173] support the observation that DiDP would not be readily degraded during anaerobic treatment. Furthermore, increases in DiDP concentrations were observed during anaerobic digestion during a previous study conducted at WWTP #3 [55], indicating that, along with the results in the present study, advanced anaerobic treatment processes may have a negative impact on DiDP degradation, as was observed for DEHP and DiNP.

While the SBR at WWTP #5 was able to decrease DEHP and DiNP concentrations, levels of DiDP did not change significantly throughout the treatment. The MBR employed by WWTP #6, however, was able to significantly decrease DiDP concentrations by 65.8% (Tukey's multiple comparisons test, P < 0.0001). Again,

there is little information available regarding the fate of DiDP during aerobic wastewater treatment. A study focusing on *Bacillus* sp. SB-007 found that this bacteria species was able to efficiently degrade DiDP under aerobic conditions, with degradation rates decreasing with increased starting concentrations. Furthermore, degradation of initial DiDP concentrations between 100 and 500 mg/L did not begin until after a lag time of 12 to 60 hours and complete removal could take up to 234 hours for starting concentrations of 500 mg/L [174]. The mixed effectiveness of aerobic treatment for degrading DiDP observed in the present study, along with the results presented by Park et al. (2009), indicate that aerobic processes may be a useful tool in reducing concentrations, but factors such as treatment time, microbial populations, and initial concentrations would likely have a large impact on treatment efficiency.

6.4.4 Benzyl Butyl Phthalate (BBP)

BBP concentrations throughout the treatment processes of the six study WWTPs are provided in Figure 6-1d and Table 6-2. The anaerobic digestion process at WWTP #1 resulted in a significant decrease of BBP concentrations by 74.3% (Tukey's multiple comparisons test, P = 0.046), similar to what was observed for DiNP and DiDP. Additionally, as was observed with the other study PAEs, the final solids samples demonstrated a significant increase (506%) in BPP concentrations (Tukey's multiple comparisons test, P = 0.0098), which may be due to sampling of final solids from stockpiles on-site. Conversely, BBP exhibited different trends in WWTP #2 than was previously observed for DEHP, DiNP, and DiDP. Concentrations were significantly increased by 147% during anaerobic digestion (Tukey's multiple comparisons test, P = 0.028) while levels observed in final solids were significantly lower by 54.5% than those in anaerobic digestion effluent (Tukey's multiple

comparisons test, P = 0.036). Thermal hydrolysis pretreatment at WWTP #3 resulted in significantly increased concentrations of BBP (227%) (Tukey's multiple comparisons test, P = 0.0082). Concentrations at the facility did not significantly change during anaerobic digestion or in final solids. Anaerobic digestion treatment at WWTP #4, however, resulted in significantly decreased BBP concentrations (76.4%) (Tukey's multiple comparisons test, P = 0.0048) while concentrations in the final solids were not significantly different than those observed in the anaerobic digestion effluent.

Anaerobic conditions have been demonstrated to degrade BBP in previous studies. For instance, anaerobic microcosms run under methanogenic conditions resulted in full BBP degradation after 30 days of treatment [142]. Furthermore, landfill waste containing BBP incubated under anaerobic conditions resulted in 70% degradation after 278 days [141]. The large increase in BBP concentrations at WWTP #2 during anaerobic treatment, however, could not be explained by a literature search. An increase in BBP during thermal hydrolysis was observed in a previous study conducted at WWTP #3 by Armstrong et al. (2018). While the detected increase during TH treatment in the previous study was not significant, it is still important to note, as it is consistent with observations from this current study.

Aerobic treatment at the study treatment facilities decreased BBP concentrations, with both the SBR at WWTP #5 and the MBR at WWTP #6 significantly reducing concentrations of the compound by 68.2% and 55.5%, respectively, during treatment [Tukey's multiple comparisons test, P < 0.0001 (WWTP #5), P = 0.024 (WWTP #6)]. Additionally, concentrations of BBP in final solids from WWTP #5 were significantly lower (57.8%) than those from aerobic digestion effluent concentrations (Tukey's multiple comparisons test, P = 0.0013)

whereas BBP concentrations in final solids collected from WWTP #6 were not significantly different than those in aerobic treatment effluent from the facility.

BBP has been well documented to be degradable under aerobic conditions. Roslev et al. (2007) found that 90% of BBP was degraded microbially during activated sludge treatment in a Danish WWTP [170]. A study of Indian WWTPs showed that the compound was biologically removed by 88% and 38% in a SBR and during activated sludge treatment, respectively [171]. Results from this study indicate that both SBRs and MBRs can be an effective tool for degrading BBP during wastewater treatment.

6.4.5 Predicted Environmental Concentrations of PAEs in Soil and DEHP Risk

A majority of the WWTPs sampled in this study land apply their biosolids as at least one of their means of disposal. Given the high concentrations in final solids, this land application can act as a source of PAEs into the environment. Predicted environmental concentrations (PECs) of the 4 PAEs analyzed were calculated as a means of estimating soil concentrations after a single biosolids land application. The PEC for each compound from each WWTP was determined according to Equation 1:

$$PEC = C_{soil} + \frac{C_{biosolids} \times AR}{D \times SD} \qquad (1)$$

where C_{soil} is the initial concentration in soils, prior to biosolids application, assumed to be zero; $C_{biosolids}$ is the concentration in final solids, provided in Table 6-2; D is the soil density, assumed to be 1.35 g/cm^3 [55]; and SD is the soil depth, assumed to be 7.6 cm to represent no till application [34]. The application rate (AR) was assumed to be 9.42 x 10^3 dry kg/ha for Class B biosolids and landfilled solids (WWTPs #1, #2, & #4 - #6) and 6.95 x 10^3 dry kg/ha for Class A biosolids (WWTP #3) due to differences in nutrient content of the products [49,55].

The PECs of the analyzed PAEs in soil after land application of final solids from WWTPs #1 - #6, provided in Table 6-3, ranged from 0.0447 – 0.373 mg/kg for

Table 6-3: Predicted environmental concentrations of PAEs in soils for each WWTP after a single biosolids application.

	PEC in Soils (mg/kg)			
	DEHP	**DiNP**	**DiDP**	**BBP**
WWTP #1	0.0828	0.0504	0.0660	0.00289
WWTP #2	0.107	0.0631	0.0720	0.00140
WWTP #3	0.373	0.246	0.169	0.00738
WWTP #4	0.308	0.233	0.174	0.00151
WWTP #5	0.0927	0.0409	0.0737	0.000973
WWTP #6	0.0447	0.0393	0.0395	0.00251

DEHP, 0.0393 – 0.246 mg/kg for DiNP, 0.0395 – 0.174 mg/kg for DiDP, and 0.000973 – 0.00738 mg/kg for BBP. These predicted concentrations are consistent with those observed in various studies of PAEs in soils [129]. A majority of toxicity studies concerning PAE compounds in soils focus on DEHP [55]. van Wezel et al. (2000) calculated the environmental risk limit (ERL) for DEHP in soils and sediments, in regards to endocrine or reproductive endpoints, to be 1.0 mg/kg [157]. This ERL is appreciably higher than the PECs calculated for DEHP from all six study WWTPs. Furthermore, the lethal concentration 50 (LC50), the effective concentration 50 (EC_{50}), and the inhibitory concentration 50 (IC_{50}) of DEHP for a variety of species have been determined [130,153,154,175]. The risk quotient (RQ) of DEHP in soils to these species could be estimated using Equation 2 [176]:

$$RQ = \frac{PEC}{\text{Toxicity Value}} \qquad (2)$$

where the toxicity value is the determined LC_{50}, EC_{50}, or IC_{50}. The RQs for the DEHP PEC from all six WWTPs, presented in Table 6-4, were several orders of magnitude

lower than 0.5, the level of concern for acute risk, and 1.0, the level of concern for chronic risk [176]. The ERL determined by van Wezel et al. and the calculated RQs presented in Table 6-4 all show that the PECs for DEHP in soil are lower than these indicators of concern, signifying that the estimated concentrations in soils are lower than the amount needed to induce toxicological concern after a single biosolids application. Further research regarding PAE concentrations in soil and toxicity to terrestrial species needs to be conducted to further understand the environmental and health risk of these compounds – specifically, further research into the various PAE compounds in biosolids (including metabolites), impacts on different terrestrial species, the concentrations in soils and their toxicological effects after several biosolids applications, varying application rates and frequencies, etc. – as RQs are simply conservative screening-level estimates.

6.5 Conclusions

Impacts of treatment processes on concentrations of DEHP, DiNP, DiDP, and BBP varied between treatment facilities. Overall, aerobic treatment resulted in significantly reduced concentrations of all compounds except for the inability of the sequencing batch reactor to influence DiDP concentrations. Conversely, standard anaerobic digestion of wastewater sludge could result in an increase, decrease, or no significant change, dependent on the facility and PAE compound. The more advanced anaerobic digestion processes generally resulted in concentration increases. Anaerobically digested sludge pretreated via thermal hydrolysis resulted in significant increases of DEHP, DiNP, and DiDP while the thermal hydrolysis pretreatment process itself increased DiNP, DiDP, and BBP concentrations in sludge. Additionally, sludge treated anaerobically via a two-phase acid/gas system also significantly increased DEHP, DiNP, and DiDP concentrations. Further research

Table 6-4: DEHP risk quotients for terrestrial organisms for each WWTP after a single biosolids application.

	Risk Quotients						
Organism:	Rodents	Collembolan	Collembolan	Lettuce	Rape Seed	Rape Seed	Rape Seed
Toxicity Test:	EC_{50}	EC/LC_{50} (adult)	EC/LC_{50} (juvenile)	EC_{50}	IC_{50} (shoot elongation)	IC_{50} (root elongation)	IC_{50} (biomass)
Toxicity Value:	40 g/kg[a]	>5,000 mg/kg[b]	>1,000 mg/kg[b]	1000 ug/g[c]	2,014 mg/kg[d]	1,426 mg/kg[d]	3,767 mg/kg[d]
WWTP #1	2.07E-06	1.66E-05	8.28E-05	8.28E-05	4.11E-05	5.81E-05	2.20E-05
WWTP #2	2.69E-06	2.15E-05	1.07E-04	1.07E-04	5.33E-05	7.53E-05	2.85E-05
WWTP #3	9.31E-06	7.45E-05	3.73E-04	3.73E-04	1.85E-04	2.61E-04	9.89E-05
WWTP #4	7.71E-06	6.17E-05	3.08E-04	3.08E-04	1.53E-04	2.16E-04	8.19E-05
WWTP #5	2.32E-06	1.85E-05	9.27E-05	9.27E-05	4.60E-05	6.50E-05	2.46E-05
WWTP #6	1.12E-06	8.94E-06	4.47E-05	4.47E-05	2.22E-05	3.14E-05	1.19E-05

[a] Net et al. (2015); [b] Jensen et al. (2001); [c] Hulzebos et al. (1993); [d] Ma et al. (2013)

as to why these more advanced processes result in compound increases during treatment should be investigated, as factors such as an increase in solids reduction over conventional activated sludge and transformation of PAE metabolites may play a role in this phenomenon.

Risk quotients for estimated DEHP concentrations in soils after a single biosolids application were determined and indicated that the acute and chronic risk of this compound is low – well below the levels of concern indicated by the US EPA. However, further research into the impacts of multiple application rates, additional PAE compounds, etc. on toxicity to various terrestrial species should be conducted.

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Chapter 7: Influence of Anaerobic Digestion With and Without Thermal Hydrolysis Pretreatment on Concentrations of Endocrine Disrupting Compounds and Their Transformation Products in Wastewater Sludge

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Research presented in this chapter is one part of a collaborative effort with S.J. Fischer of UMD (focus on microbial aspects of anaerobic digestion) and R. Lupitskyy of the USDA (focus on biogas production during digestion). Only the research performed by D.L. Armstrong (focus on fate of chemicals) is included in this chapter. All three research aspects will be combined for eventual publication in a peer reviewed journal.

7.1 Abstract

Mesophilic anaerobic digestion of wastewater sludge with and without pretreatment by the Cambi Thermal Hydrolysis Process™ (CambiTHP™) was simulated using 250 mL serum bottles. The fate of two antimicrobials, triclosan (TCS) and triclocarban, three phthalic acid esters (PAEs), and their transformation products during treatment was explored over a 22-day period. Results show that TCS and its metabolite methyl triclosan increased during anaerobic digestion with no significant impact when pretreated via CambiTHP™. However, concentrations of TCC increased more rapidly when sludge was not pretreated while 2,4-dichlorophenol levels increased at a faster rate with CambiTHP™ pretreatment. Anaerobic digestion and CambiTHP™ pretreatment had a more varying effect on PAEs with di(2-ethylhexyl) phthalate decreasing, diisononyl phthalate levels remaining steady, and benzyl butyl phthalate concentrations increasing with CambiTHP™ pretreatment and decreasing with out pretreatment. PAE metabolites generally increased during the course of the experiments.

7.2 Introduction

The land application of treated wastewater sludge, or biosolids, as a means to improve soil quality [133,177] has become common practice in various regions worldwide. For instance, over 50% of treated sludge produced within the United States, Europe, and Canada is land applied [178,179]. While this practice can help to increase soil nutrient concentrations, organic matter composition and water holding capacity [133,177], the land application of biosolids can also act as an environmental source of organic pollutants, including endocrine disrupting compounds (EDCs). Such compounds are not always fully removed during the wastewater treatment process and normally accumulate in wastewater sludge [11,55,180].

Compounds such as phthalate plasticizers (PAEs) and the antimicrobials triclosan (TCS) and triclocarban (TCC) are considered to be EDCs [26,45,122] and have been detected in biosolids at relatively high concentrations [16,35,49,55]. Concerns regarding the health and environmental impacts of these compounds have garnered the attention of regulatory agencies. In 2016, TCS was rejected for use in the European Union in human hygiene biocidal products for skin and scalps [81]. Also in 2016, the United States Food and Drug Administration instituted a phase-out of both TCS and TCC from over the counter antiseptic washes, such as hand soaps, bar soaps, and body washes [7]. Additionally, both the United States and European Union regulate the amount of several PAE compounds allowed for use in toys and children's products [181]. Moreover, some transformation products of these EDCs can also display toxicological properties [26,31,45,182], further emphasizing the negative impacts these compounds may have when released into the environment.

The individual solids treatment processes utilized by wastewater treatment plants (WWTPs) can have a varying effect on TCS, TCC, and PAE concentrations in biosolids [49,183](Chapter 6 - results). As more WWTPs move to employ treatment methods that enhance resource recovery [138], it is important to understand how these newer systems influence these antimicrobial and PAE concentrations in biosolids. The Cambi Thermal Hydrolysis Process™ (CambiTHP™) is an anaerobic digestion pretreatment that can both improve sludge biodegrability and reduce the volume of final sludge solids. A major benefit to the enhanced biodegradation provided by the CambiTHP™ is the increase in the production of biogas – a renewable energy source [49]. However, when Cambi THP™ is coupled with anaerobic digestion, the overall process can have a varying effect on EDCs concentrations. The treatment of wastewater sludge with CambiTHP™ has been demonstrated to significantly reduce TCC concentrations while TCS and its transformation products were unaffected. Furthermore, concentrations of TCS and its transformation products increased during the anaerobic digestion of sludge pretreated with CambiTHP™ [49]. A similar study concentrating on four PAEs throughout CambiTHP™/anaerobic digestion treatment demonstrated that the treatment system resulted in significantly higher concentrations of all four compounds during anaerobic digestion while the CambiTHP™ itself did not have any impact on compound concentrations [55].

The objective of this study was to simulate the anaerobic digestion of wastewater sludge with and without CambiTHP™ pretreatment at a laboratory scale in an effort to gain further insight into how the innovative pretreatment process influences concentrations of TCS, TCC, PAEs, and their metabolites during the digestion process. Furthermore, microbial populations between the two treatments

were assessed as a means to 1) understand how CambiTHP™ can influence populations during anaerobic digestion and 2) correlate populations to changes in target compound concentrations.

7.3 Materials and Methods

7.3.1 Wastewater Treatment Plant Background

The present study centered on sludge samples collected from a large municipal WWTP. The facility, located in the Mid-Atlantic region of the United States, provides sewage treatment to over 2 million customers – approximately 1.14 million m^3 of raw sewage per day. Treatment at the facility is achieved via primary sedimentation, activated sludge treatment, nitrification-denitrification treatment, filtration, and disinfection. Sludge is pretreated by CambiTHP™ as follows: centrifugation of solids, preheating of sludge to 60 - 99°C using recycled stream, hydrolysis of sludge using heat (150 - 180°C) and pressure (0.37 – 0.95 MPa), and a rapid decrease of heat and pressure in a flash tank. After CambiTHP™, sludge is anaerobically digested at 37°C for approximately 22 days prior to dewatering by belt presses. Treated solids from the WWTP are classified as Exceptional Quality Class A biosolids by the United States Environmental Protection Agency (US EPA) and are typically land-applied to agricultural fields. Further details regarding the CambiTHP™/anaerobic digestion process at the facility can be found elsewhere [49,55].

7.3.2 Sample Collection and Experimental Setup

Sludge from the study WWTP was collected 1) after centrifugation but prior to CambiTHP™ treatment [henceforth classified as “without thermal hydrolysis (TH) pretreatment”] and 2) after CambiTHP™ treatment, post flash tank (considered “with TH pretreatment”). Anaerobic digestion sample used as inoculant for the

experiments was collected from an anaerobic digestion solids recycle line. Use of plastic was avoided during the sampling campaign to avoid contamination of samples with phthalates.

Anaerobic digestion experiments were carried out using 250 mL serum bottles. Each bottle was filled with 20 mL of sludge without TH pretreatment and 130 mL of anaerobic digestion inoculum or 22 mL of sludge with TH pretreatment and 128 mL of anaerobic digestion inoculum to maintain an inoculum to substrate volatile solids ratio of 1.5:1 [184,185]. Treatment controls were setup in the same manner as the experimental bottles, but were treated with 1 g of mercuric chloride while inoculum control bottles consisted of 150 mL of anaerobic digestion inoculum only. Prior to sealing with rubber stoppers, each bottle was purged with N_2 gas. Additionally, each bottle was covered with aluminum foil to prevent light exposure of the samples. Bottles were then placed on tabletop shakers for consistent agitation and maintained at 37°C in a dark incubation room for 22 days.

A sufficient number of serum bottles were prepared so the destructive sampling at predetermined time points could take place, allowing for the fate of TCS, TCC, PAEs, and their transformation products over the course of experiments to be determined. Destructive sampling dates occurred on days 0, 1, 3, 5, 7, 10, 13, 17, 20, and 22. Two duplicate bottles were sampled on each specified date. In addition to organic compound analysis, these samples were also analyzed for total solids (TS), volatile solids (VS), soluble chemical oxygen demand (sCOD), volatile fatty acids (VFAs), and biogas composition. Additionally, microbial analyses were performed on samples collected at days 0, 10, and 22.

7.3.3 Target Analytes

Experimental target analytes consisted of the antimicrobials TCS and TCC, several PAEs, and at least one commonly detected transformation product for each compound. Compounds analyzed are provided in Table 7-1. Details regarding suppliers of analytical standards and standard purities are provided in Table SI-E1 of the Supplemental Information.

Table 7-1: Compounds Analyzed and Their Descriptions, Recoveries, and LOQs

Compound	Description	Recovery (%)	LOQ (ng/g)
Triclosan (TCS)	Antimicrobial	79.3 ± 4.4	18.9
Methyl triclosan (MeTCS)	TCS metabolite	71.8 ± 6.1	22.7
2,4-Dichlorophenol (2,4-DCP)	TCS photolysis product	77.0 ± 9.6	85.1
Triclocarban (TCC)	Antimicrobial	88.3 ± 5.9	11.0
4,4'-Dichlorocarbanilide (DCC)	TCC dechlorination product	82.2 ± 9.2	79.2
1-(3-Chlorophenyl)-3-phenylurea (MCC)	TCC dechlorination product	89.7 ± 5.0	94.3
Carbanilide (NCC)	TCC dechlorination product	84.1 ± 4.8	73.9
Bis(2-ethylhexyl) phthalate (DEHP)	Phthalate plasticizer	88.5 ± 4.3	50.8
Mono-(2-ethylhexyl) phthalate (MEHP)	DEHP metabolite	68.4 ± 12.5	88.8
Diisononyl phthalate (DiNP)	Phthalate plasticizer	97.6 ± 11.1	53.2
Monoisononyl phthalate (MNP)	DiNP metabolite	62.9 ± 10.7	84.4
Benzyl butyl phthalate (BBP)	Phthalate plasticizer	93.8 ± 6.4	35.3
Monobutyl phthalate (MBuP)	BBP metabolite	60.3 ± 6.6	49.0
Monobenzyl phthalate (MBeP)	BBP metabolite	70.1 ± 9.4	42.5

7.3.3.1 Extraction Methods and Instrumental Analyses

Prior to extraction and analysis for the compounds outlined in Table 7-1, all samples were lyophilized, the liquids and solids fraction of each sample were not separated prior to lyophilization. Samples were extracted for TCS, TCC, and their transformation products using a Dionex Accelerated Solvent Extraction (ASE) system in conjunction with Oasis® HLB solid phase extraction (SPE) cartridges, as previously published [25], with the exception of one slight modification: a solution of

ethyl acetate:diethyl ether (65:35 v/v) was used for SPE conditioning and elution rather than the previously published dichloromethane:diethyl ether (80:20 v/v) solution. After extraction, samples were analyzed for TCS, TCC, and all transformation products except MeTCS using a Shimadzu Nexera X2 Ultra High Performance Liquid Chromatograph coupled to a Shimadzu 8040 triple quadrupole mass spectrometer (UHPLC-MS/MS) using an electrospray negative source and a mobile phase of 10 mM ammonium acetate in methanol:acetonitrile:water (60:15:25 v/v). Afterwards, all samples were evaporated and reconstituted in hexane for MeTCS analysis by an Agilent 7890B gas chromatograph (GC) with an Agilent 5977A mass selective detector (MSD) run in positive electron impact ionization mode. Further details regarding UHPLC-MS/MS and GC-MS analytical conditions are provided in a previous publication [49].

For extraction of PAEs and their metabolites, the Dionex ASE system was also used, along with HyperSep™ Florisil SPE cartridges. Further details regarding this extraction method can be found elsewhere (Chapter 6). PAE compound analysis occurred using the previously mentioned Shimadzu UHPLC-MS/MS instrument with a mobile phase of 5 mM ammonium formate in methanol and the MS electrospray ionization source also run in negative mode, as previously published [55].

7.3.4 Substrate Characterization

Samples were analyzed for total solids (TS) and volatile solids (VS) using the standard methods established by the American Public Health Association [56]. Soluble chemical oxygen demand (sCOD) was determined using Chemetrics dichromate COD vials and a Hach Company digester block with filtered samples [94].

7.3.5 Volatile Fatty Acid Analysis

Anaerobic digestion samples were analyzed for four volatile fatty acids (VFAs): acetic acid, propionic acid, n-butyric acid, and n-valeric acid. VFA analysis was conducted via acidification to a pH of 2 using sulfuric acid and filtering of samples with 0.45 µm and 0.22 µm filters. An HP 7890A gas chromatograph was used for VFA analysis [186].

7.3.6 Methane Biogas Characterization

The compositions of the methane produced via sludge anaerobic digestion was assessed on the same day that antimicrobial and PAE samples were analyzed. A 50 mL gas-tight glass syringe was used to collect a gas sample from the serum bottles. Gas samples were injected into an Agilent 6890 gas chromatograph joined with a thermal conductivity detector. Due to some leaks in bottle caps, total biogas production could not be accurately assessed.

7.3.7 Quality Assurance/Quality Control

Each destructive time point consisted of two serum bottles, run as duplicates. Additionally, sampling from each serum bottle for target analyte analysis was done in duplicate. Prior to extraction, samples were spiked with the surrogate standards $^{13}C_{13}$-TCC, $^{13}C_{12}$-TCS, $^{13}C_{12}$-MeTCS, d_3-2,4-DCP, d_4-DEHP, d_4-MNP, d_4-BBP, and d_4-MBuP. All extraction batches included a blank (laboratory-grade sand) and a sample spiked with unlabeled target analytes for determination of recovery percentages (provided in Table 7-1). Extraction batches consisted of no more than 12 samples. Method detection limits for each compound were determined as outlined by the US EPA [98] and the limits of quantitation (LOQs), provided in Table 7-1, were set as two times the MDL. Calibration curves for instrumental analysis consisted of at least six non-zero standards and run before and after sample

analysis, yielding r^2 values ≥ 0.99. Two standards and two solvent blanks were also analyzed every 10 samples as a verification of instrument stability.

7.4 Results and Discussion

Results for secondary analyses, including solids, methane production, VFAs, and sCOD, are provided in Figures SI-E1 through SI-E4 of the supplemental information.

7.4.1 Antimicrobial Compounds

7.4.1.1 TCS and TCS Transformation Products

Concentrations of TCS, MeTCS, and 2,4-DCP increased over the course of the experiments both with and without TH pretreatment (Figures 7-1a – 1c). Linear regression analysis was applied to the datasets for all three compounds. Concentrations of TCS significantly increased during the course of the experiment, both with (R^2 = 0.8862, P < 0.0001) and without (R^2 = 0.7881, P = 0.0006) TH pretreatment (Figure 7-1a). Furthermore, an extra-sum-of-squares F test indicated that the slopes of each regression model fit to TCS concentrations with and without TH pretreatment (*m* = 221.9 ± 28.11 ng/g/day and 242.6 ± 44.47 ng/g/day, respectively) were not significantly different (P = 0.7107). Similarly, linear regression analysis also indicated concentrations of MeTCS increased in pretreated (R^2 = 0.8069, P = 0.0004) and non-pretreated (R^2 = 0.9153, P < 0.0001) anaerobically digested sludge (Figure 7-1b). As was observed with TCS, the slopes of the MeTCS linear models were not significantly different (P = 0.2250) for anaerobic reactors run with TH pretreatment (*m* = 6.673 ± 1.154 ng/g/day) and without TH pretreatment (*m* = 8.225 ± 0.8847 ng/g/day), as determined with an extra-sum-of-squares F test. These results demonstrate that the CambiTHP™ does not appear to influence the

fate of TCS and MeTCS during the anaerobic digestion process, when compared to sludge digested without pretreatment.

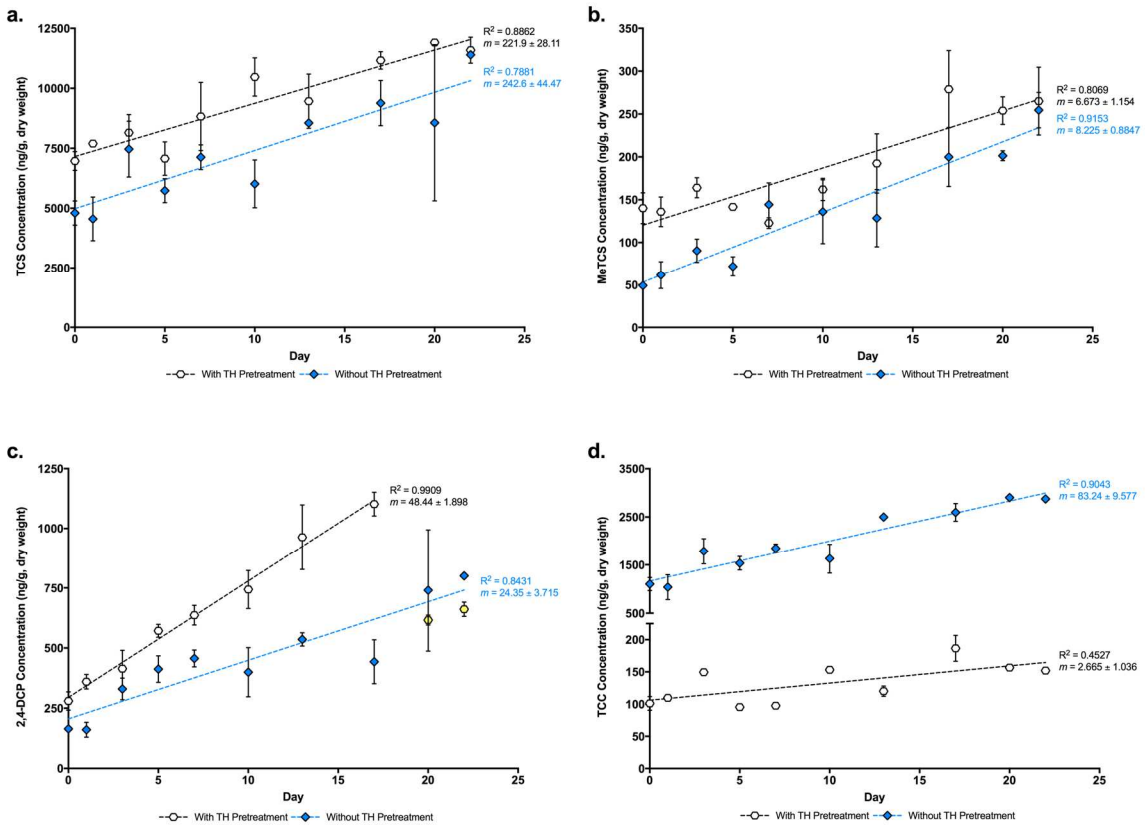

Figure 7-1: Concentrations and Trends of a) TCS, b) MeTCS, c) 2,4-DCP, and d) TCC in Anaerobic Digestion Samples With and Without TH Pretreatment

A previous study focusing on the fate of antimicrobials throughout the CambiTHP™/anaerobic digestion process also found that concentrations of TCS and MeTCS increased during anaerobic digestion treatment, as was observed in the present study, but concluded that the increase was due to a reduction in total solids due to the anaerobic digestion process rather than a direct result of CambiTHP™ [49]. However, given that TCS concentrations during the present experiment increased by approximately 166% and 238% for anaerobically digested sludge with and without TH pretreatment, respectively, and MeTCS concentrations by

approximately 189% and 519%, respectively, the reduction of total solids observed over the 22-day treatment (a reduction of less than 1.5 %; Figure SI-E1, supplemental information) is likely not the only reason for compound increase. One such explanation is that despite total solids not decreasing sufficiently over the 22 day experimental time period, the amount of suspended solids have still have decreased enough to effectively concentrate TCS and MeTCS (assuming that the compounds partition predominantly to the suspended particles) while slightly increasing the amount of dissolved solids during the degradation process, thus resulting in a total solids degradation pattern that does not sufficiently explain increases in compound concentrations. Unfortunately, due to the nature of the solids in the system, the quantity of suspended solids versus dissolved solids within samples could not be determined due to filter clogging. Presenting results on a wet weight basis did not change the overall trends of these compounds during the experiments, indicating there is more at play here than just the influence of solids concentrations. Other such explanations for the notable increase in TCS and MeTCS concentrations during the anaerobic digestion experiments could be the formation of these compounds from other metabolites and compound derivatives. TCS degradation has been demonstrated to be complex, with numerous intermediates forming during the process [69,70,75]. Furthermore, higher chlorinated derivatives of TCS may be formed during the transport of waste to a WWTP and within the treatment system itself [187]. Deconjugation of metabolites or the dechlorination of compounds such as tetra(II)closan [4,5-dichloro-2-(2,4-dichloro-phenoxy)-phenol], tetra(III)closan [5,6-dichloro-2-(2,4-dichloro-phenoxy)-phenol], and pentaclosan [4,5,6-trichloro-2-(2,4-dichloro-phenoxy)-phenol] may be responsible, in part, for the formation of TCS during digestion. Further research into the presence

of additional TCS intermediates and derivatives, as well as a more complex study into solids concentrations during treatment need to be explored.

Unlike TCS and MeTCS, concentrations of 2,4-DCP were appreciably impacted by TH pretreatment (Figure 7-1c), as observed in a previous on-site study [49]. Linear regression models demonstrated significant increases of 2,4-DCP both with [R^2 = 0.9909, P < 0.001 (excluding days 20 and 22)] and without (R^2 = 0.8431, P = 0.002) TH pretreatment. However, when excluding days 20 and 22, which demonstrated a break from the trend, concentrations of the compound during anaerobic digestion increased at a much higher rate when pretreated with CambiTHP™ (*m* = 48.44 ± 1.898 ng/g/day) compared to no pretreatment (*m* = 24.35 ± 3.715 ng/g/day). The difference in rate of compound formation between the two treatments was significant (extra-sum-of-squares F test, P = 0.0001). Since TCS degradation was not observed and precautions were taken to prevent photolysis, increases in 2,4-DCP are likely due to, in part, the previously discussed changes in total solids during the digestion process. However, the difference in formation rates between the two treatments may be due to the fact that 2,4-DCP is a degradation product of other compounds besides TCS such as the herbicides 2,4-dichlorophenoxyacetic acid (2,4-D) [101] and dichlorprop [100]. Pretreatment of wastewater sludge by CambiTHP™ may allow for more efficient degradation of other organic compounds, thus causing a greater increase in 2,4-DCP formation during anaerobic digestion.

7.4.1.2 TCC and TCC Transformation Products

Despite the fact that TCC has been observed to be dechlorinated into DCC, MCC, and NCC [120,183,188], this phenomenon was not observed during the experiments as the dechlorination products of TCC were not detected at or above

the LOQ and TCC concentrations rose rather than fell (Figure 7-1d). When linear regression analysis was applied, it was revealed that the increases in TCC levels during anaerobic digestion were significant both with (R^2 = 0.4527, P = 0.0330) and without (R^2 = 0.9043, P < 0.0001) CambiTHP™ pretreatment. Concentrations of the antimicrobial increased at a rate of 83.24 ± 9.577 ng/g/day without TH pretreatment, with an overall increase of approximately 261%. When pretreated with CambiTHP™, TCC levels increased by approximately 150% at a rate of 2.665 ± 1.036 ng/g/day, which was significantly slower than that of sludge digested without pretreatment (extra-sum-of-squares F test, P < 0.0001). Additionally, initial concentrations of TCC were approximately 10 times higher in sludge that had not undergone CambiTHP™. A previous on-site study demonstrated that this is due to the TH process itself significantly reducing TCC concentrations [49]. Reasons for the increase of TCC are likely similar to those for TCS – mainly, reduction of solids, as described above, and transformation of intermediates. Souchier et al. (2016) outlined several proposed dechlorination pathways of TCC that involved a number of intermediates [189]. Furthermore, as with TCS, higher chlorinated congeners of TCC exist and have been observed in environmental samples [190]. The transformation of these compounds into TCC may be taking place during the anaerobic digestion process. Further research into the fate of specific intermediates and the higher chlorinated TCC derivatives needs to be explored in the future.

7.4.2 Phthalic Acid Esters

For all PAE compounds and treatments, rates of change were calculated using pseudo-first-order kinetics according to Equation 1 and are provided in Table 7-2.

$$ln\frac{C}{C_0} = -kt \qquad (1)$$

Table 7-2: Phthalic Acid Ester Rates of Change During Anaerobic Digestion

Compound		Rate of Change (day^{-1})	
		Without TH Pretreatment	With TH Pretreatment
DEHP	Parent compound	-0.0126 ± 0.0039	-0.00360 ± 0.0021
MEHP	DEHP TP	+0.0625 ± 0.0098	+0.0445 ± 0.0047
DiNP	Parent Compound	-0.00220 ± 0.0079	+0.00195 ± 0.0033
MNP	DiNP TP	+0.0889 ± 0.032	+0.0830 ± 0.0061
BBP	Parent Compound	-0.0648 ± 0.030	+0.0171 ± 0.010
MBuP	BBP TP	+0.142 ± 0.015	+0.131 ± 0.013
MBeP	BBP TP	+0.00590 ± 0.0030	+0.0350 ± 0.010

TP = Transformation product

7.4.2.1 DEHP and MEHP

Overall, DEHP concentrations in wastewater sludge decreased during anaerobic digestion while its metabolite MEHP increased during treatment (Figures 7-2a & b). Two separate studies have concluded that concentrations DEHP increase during anaerobic digestion with CambiTHP™ pretreatment [55](Chapter 6 - results), while results from this study appear to slightly deviate from that narrative. In general, DEHP concentrations changed very little between day 0 and day 22 of digestion with pretreatment but did, indeed, decrease slightly (-0.00360 ± 0.0021 day^{-1}) while DEHP in sludge that did not undergo pretreatment degraded more rapidly (-0.0126 ± 0.0039 day^{-1}). MEHP concentrations increased under both treatment types, and the rate of increase was not notably different for either. However, during sludge digestion with and without TH pretreatment, MEHP increased at a more rapid rate than DEHP decreased. One explanation for this may be the formation of MEHP through other DEHP metabolites, such as mono-(2-ethyl-

5-carboxypentyl)phthalate, mono-[2-(carboxymethyl)-hexyl]phthalate, mono-(2-ethyl-5-hydroxyhexyl)phthalate, and mono-(2-ethyl-5-oxy-hexyl)phthalate [128].

7.4.2.2 DiNP and MNP

Concentrations of DiNP changed very little over the course of the experiments (Figures 7-2c & d), with rates of change varying very little from zero both with (+0.00195 ± 0.0033 day^{-1}) and without (0.00220 ± 0.0079 day^{-1}) TH pretreatment. Similar to what was observed with DEHP, concentrations of DiNP were previously observed to increase in sludge anaerobically digested after CambiTHP™ pretreatment [55](Chapter 6 - results), a trend that was not followed during the present study. MNP, on the other hand, increased at similar rates both with and without pretreatment. Concentrations of the metabolite, however, were over an order of magnitude lower than DiNP concentrations. While MNP formation could not be directly correlated to DiNP degradation, given the much lower levels of the metabolite, a relationship to trends with the parent compound could be hidden within the standard error of analysis.

7.4.2.3 BBP, MBuP, and MBeP

Concentrations of BBP decreased steadily in anaerobically digested sludge not treated by CambiTHP™ (-0.0648 ± 0.030 day^{-1}) while concentrations increased when sludge underwent TH pretreatment (+0.0171 ± 0.010 day^{-1}) (Figures 7-2e & f). In a previous study on the fate of BBP at six different WWTPs, it was observed that anaerobic digestion could have a varying impact on concentrations of BBP. Standard anaerobic digestion treatment and more advanced systems were shown possess the capacity to increase or degrade BBP during treatment. Pretreatment with CambiTHP™ in the study was shown to increase BBP levels (Chapter 6 -

results). The BBP metabolite MBeP increased in sludge what had undergone TH pretreatment (+0.0350 ± 0.010 day^{-1}) but showed very little change in sludge that

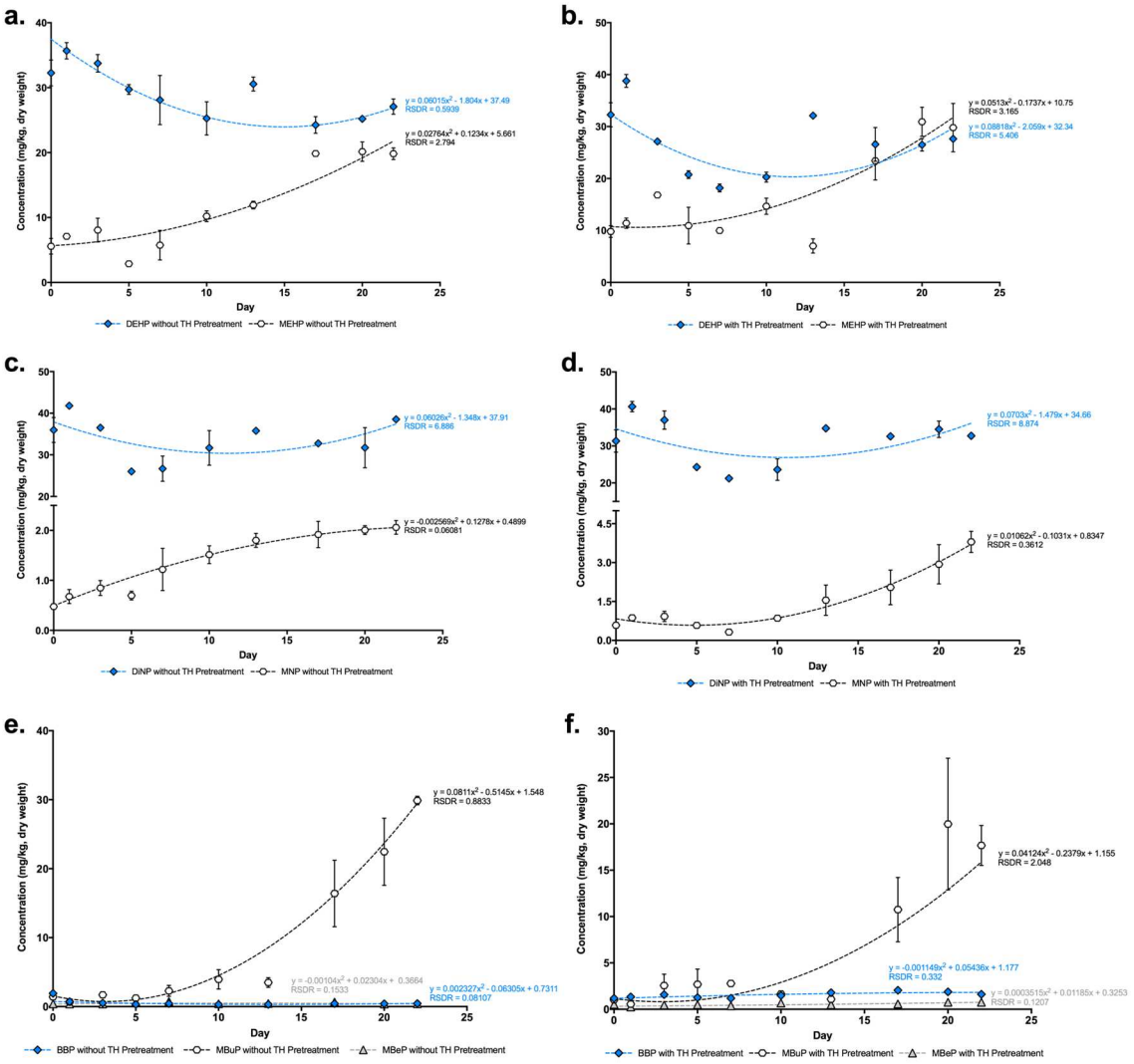

Figure 7-2: Concentrations and Trends of a) DEHP/MEHP Without TH Pretreatment, b) DEHP/MEHP With TH Pretreatment, c) DiNP/MNP Without TH Pretreatment, d) DiNP/MNP With TH Pretreatment, e) BBP/MBuP/MBeP Without TH Pretreatment, and f) BBP/MBuP/MBeP With TH Pretreatment in Anaerobic Digestion Samples

had not been exposed to the CambiTHP™ (+0.00590 ± 0.0030 day^{-1}). MBuP, however, demonstrated rapid increases during the course of the experiment; with final concentrations approximately an order of magnitude higher than starting BBP levels. Since MBuP levels cannot be correlated to BBP degradation, its formation is

likely due to degradation of other PAE compounds. For instance, dibutyl phthalate has been shown to degrade into MBuP under anaerobic conditions [141].

7.5 Conclusions

Results from this study demonstrate the variable impacts an individual treatment system can have on EDCs in sludge. Concentrations of the antimicrobials TCS and TCC and their detected transformation products increased during anaerobic digestion both with and without CambiTHP™. While there was no significant difference in rate of increase of TCS and MeTCS through the two treatment conditions, sludge that underwent pretreatment with CambiTHP™ resulted in a more rapid increase of 2,4-DCP while TCC concentrations increased at a slower rate when compared to sludge digested without pretreatment. The influence of anaerobic digestion and CambiTHP™ on concentrations of PAEs and their metabolites was much more extensive. DEHP revealed decreasing trends during the course of the experiments while DiNP concentrations did not generally change. BBP, on the other hand, was more widely impacted by CambiTHP™ pretreatment, with concentrations increasing with pretreatment and decreasing in sludge digested without pretreatment. Overall, PAE metabolites increased during anaerobic digestion treatment, sometimes despite no degradation of the parent compound, implying a very complex compound transformation process.

7.6 Acknowledgements

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Chapter 8: Impact of Various Wastewater Treatment Conditions on Concentrations of 27 Emerging Contaminants and Their Estimated Concentrations in Soils

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This chapter will be submitted for publication in *Chemosphere*.

8.1 Abstract

The influence of solids treatment processes at six different wastewater treatment plants (WWTPs) on concentrations of 27 emerging contaminants (ECs) was explored. Of the WWTPs sampled, two employed conventional anaerobic digestion for solids treatment, two utilized advanced anaerobic digestion processes, and two employed aerobic processes. Of the 27 compounds analyzed, 16 were detected in at least one WWTP sample: 10 pharmaceutical and personal care products, 2 pesticides, 2 flame retardants, 1 food additive, and 1 perfluorinated compound. For many ECs, the impact of treatments on compound concentrations was wide-ranging, emphasizing just how varied the impact that different WWTPs can have on EC concentrations, even in cases where the same treatment method is applied. Furthermore, predicted environmental concentrations (PECs) of detected ECs in soil were calculated to understand the potential output of these compounds by WWTPs. Ciprofloxacin, diphenhydramine, norethindrone, prednisone, and tris(2-butoxyethyl) phosphate had the highest PECs in soil.

8.2 Introduction

Prolific use of chemicals by modern society has led to the presence of emerging contaminants (ECs) in the wastewater treatment (WWT) process. Such compounds, which can include pharmaceuticals and personal care products (PPCPs), plasticizers, food additives, pesticides, fire retardants, etc., are often not

fully removed by treatment processes [80]. Removal efficiencies of ECs can be dependent on several factors, including the treatment systems employed by individual wastewater treatment plants (WWTPs), the physiochemical properties of individual compounds, as well as influent compound concentrations [9]. This inability to completely remove ECs can result in WWT systems acting as a source of such contaminants to the environment, whether through the discharge of effluent to local water bodies or through the land application of treated sludge, or biosolids.

ECs have been well documented in wastewater biosolids. For example, pharmaceuticals such as diclofenac, a nonsteroidal anti-inflammatory drug, and carbamazepine, an anticonvulsant and mood stabilizer, were detected as high as 4,240 and 11,060 ng/g, respectively, in dewatered sludge from Chinese WWTPs with their estimated discharges to the environment via the disposal of sludge as high as 1,023 and 494 g/day, respectively [191]. The antidepressants citalopram, amitriptyline, and venlafaxine were detected at peak concentrations of 1,033, 768, and 833 ng/g, respectively, in Canadian biosolids [192]. Armstrong et al. (2017) found average concentrations of the plasticizer bis(2-ethylhexyl) phthalate (DEHP) in biosolids from a large municipal WWTP in the Mid-Atlantic region of the United States to be 66,700 ng/g for Class A biosolids and 25,000 ng/g for Class B biosolids [55]. Furthermore, temporal studies have demonstrated that concentrations of some compounds have remained continuous in biosolids over time. For instance, Andrade et al. (2015) found that while the polybrominated diphenyl ether (PBDE) congeners BDE-47 and BDE-99 decreased in limed biosolids samples over a 7-year period, BDE-209 concentrations were unchanged during the study period. Additionally, the same study revealed that the antimicrobial triclosan also did not decrease in

concentration during this time period, further emphasizing that biosolids, if land applied, can act as a steady source of certain ECs to the environment. [21]

While there are numerous benefits to the land application of biosolids, such as increases in nutrient levels, improved water holding capacity, recovery of disturbed landscapes, etc. [133], concerns regarding the practice exist as well. These worries are often related to the ECs associated with the biosolids and include concerns surrounding microbial resistance issues [193,194], endocrine disrupting capabilities of some ECs [195], indirect human exposure via groundwater infiltration and crop or animal uptake [196], toxicity [197], etc. Furthermore, a 3-year study on the fate of numerous PPCPs in outdoor mesocosms demonstrated that compounds can persist in soils well after biosolids application. PPCPs such as diphenhydramine, fluoxetine, and triclocarban demonstrated no observable degradation over the 3-year period, whereas the half-lives of various other compounds could be as high as 2,310 days (ciprofloxacin) [198]. Moreover, the half-lives of five perfluorinated compounds in a similar mesocosm study were determined to be as high as 866 days (perfluoroheptanoic acid) and compound loss was theorized to be due to ecological mobility via leaching, vegetative uptake, and/or volatilization rather than degradation [199]. One such way to prevent or diminish the negative effects of ECs during the beneficial reuse of wastewater sludge via land application is to reduce the concentrations of ECs present in the material in the first place. To do so, further understanding of how different wastewater treatment technologies can influence concentrations of this broad classification of compounds in sludge is needed.

To accomplish this, the presence and fate of 27 ECs during final solids treatment was explored at six WWTPs located in the Mid-Atlantic region of the

United States. Solids treatments at the study facilities include aerobic, such as sequencing batch reactor and membrane bioreactor, or anaerobic (including conventional and advanced) processes. Grab samples from each facility were collected pretreatment, post treatment, and after final solids dewatering to understand how each phase of treatment impacts compound concentrations. Furthermore, predicted environmental concentrations of detected compounds in soils after a single biosolids application, as well as their estimated risk quotient were calculated to gain insight into the environmental implications of detected ECs in final solids from each study WWTP.

8.3 Materials and Methods

8.3.1 Target Analytes

All samples were analyzed for 27 commonly utilized ECs. The compounds analyzed as well as their physiochemical properties and usages are provided in Table 8-1. Analytical standard suppliers and compound purities are provided in Table SI-F1, located in the Supplemental Information.

8.3.2 Sample Collection and Handling

Wastewater and final solids samples were collected from six different municipal WWTPs in the Mid Atlantic region of the United States. The WWTPs sampled employ a variety of different treatment configurations (aerobic and/or anaerobic) and are situated in rural and metropolitan settings. Specifications of the study WWTPs are provided in Table 6-1 [200].

As this study concentrates on EC concentrations in final solids and the influence that treatment has on EC concentrations in final solids, the solids treatment processes at each facility were the sampling focus. At WWTPs #1, 2, 3, and 4, grab

Table 8-1: Compounds Analyzed and Their Physiochemical Properties

Compound	Usage	log K_{ow}	Solubility (mg/L)	log K_{oa}	log K_{oc}
	Pharmaceuticals and Personal Care Products (PPCPs)				
Betaxolol	Beta Blocker	2.98	451	14.0	2.60
Bisoprolol	Beta Blocker	1.84	2240	14.8	1.52
Carbamazipine	Anticonvulsant/Mood Stabilizer	2.25	17.7	10.8	3.59
Ciprofloxacin	Antibiotic	0.00	11,480	17.0	1.55
n,n-Diethyl-3-methylbenzamide (DEET)	Insect repellent	2.26	666	8.25	2.73
Diltiazem	Calcium channel blocker	2.79	12.3	17.2	3.98
Diphenhydramine	Antihistamine	3.11	363	10.1	3.92
Fluconazole	Antifungal	0.25	336	11.6	4.72
Irbesartan	Angiotensin II receptor blocker	5.31	0.0599	18.1	7.91
Norethindrone	Steroid Hormone	2.99	118	10.6	3.43
Oxybenzone	UV Stabilizer/Absorber	3.52	68.6	10.0	3.10
Prednisone	Corticosteroid	1.59	312	9.40	1.56
Resperidone	Antipsychotic	3.49	2.76	17.5	6.65
Testosterone	Hormone	3.27	67.8	10.2	3.15
Venlafaxine	Antidepressant	3.28	267	12.4	3.17
	Pesticides				
Chlorpyrifos	Insecticide	4.66	0.357	8.88	3.83
Emamectin Benzoate	Insecticide	5.00	93.0	NA	5.42
Flubendiamide	Insecticide	NA	NA	NA	NA
Pendimethaline	Herbicide	2.62	89.7	18.8	4.05
	Fire Retardants				
Tris(1-chloro-2-propyl) phosphate (TCPP)	Fire retardant	NA	NA	NA	NA
Triphenyl phosphate	Fire retardant	4.70	1.03	8.46	3.72
Tris(2-butoxyethyl) phosphate	Fire retardant	3.00	1.96	13.1	5.67
	Food Additives				
Aspartame	Artificial Sweetener	0.07	565	16.1	1.79
	Surfactants				
Perfluorohexanoic acid (PFHxA)	Surfactant	NA	NA	NA	NA
Perfluorononanoic acid (PFNA)	Surfactant	7.27	0.00188	5.98	5.09
Perfluorooctanoic acid (PFOA)	Surfactant	6.30	0.260	5.73	4.43
Perfluorooctanesulfonic acid (PFOS)	Surfactant	NA	NA	NA	NA

NA = not available
Source of chemical properties: US EPA EPI Suite

samples were collected prior to anaerobic digestion, after anaerobic digestion, and after final solids dewatering. At WWTP #3, samples were collected prior to thermal hydrolysis, after thermal hydrolysis, from the anaerobic digestion tank (via the digested solids recycle line), and after final solids dewatering. As WWTPs #5 and #6 are very small and employ only a single treatment process (both aerobic), samples were collected prior to aerobic treatment, after aerobic treatment, and after final solids dewatering. Samples were collected in January and February 2017. After collection, all samples were stored at -20°C until analysis. Further descriptions of the collection process can be found elsewhere (Chapter 6).

8.3.3 Extraction Method

Prior to extraction, all samples (wastewater and final solids) were lyophilized. The liquid and solids faction of wastewater samples were extracted together. Each sample was extracted in duplicate. A modification of a previously established method [201] was utilized for compound extraction. Approximately 0.15 g of sample was placed into a 50 mL centrifuge tube. Fifteen (15) milliliters of phosphate buffer (pH ~2) was added to the tubes and vortexed for 5 minutes. Twenty (20) milliliters of acetonitrile (ACN) was added to each centrifuge tube and the mixture vortexed briefly. Next, all tubes were sonicated for 20 minutes then centrifuged for 5 minutes. Supernatants were then decanted into 250 mL round bottom flasks and the extraction process was repeated. Afterwards, 15 mL of an ACN/diethyl ether mixture (80:20 v/v) was added to each centrifuge tube, vortexed briefly, sonicated for 20 minutes and centrifuged for 5 minutes. Supernatants for each sample were combined with those from the previous two extraction steps. This extraction sequence was then repeated once. Combined sample extracts were evaporated

using a rotary evaporator at 40°C until approximately 20 to 30 mL of extract remained. Approximately 500 g of ethylenediaminetetraacetic acid and 200 mL of organic-free water (H_2O) were added to each round bottom flask and swirled to mix.

Sample extracts were then loaded onto Oasis® HLB solid phase extraction (SPE) cartridges (Waters Corporation, Milford, MA, USA) (200 mg, 6 mL) that were previously conditioned with 20 mL methanol (MeOH), 7.5 mL of H_2O, and 7.5 mL of acidified H_2O (pH ~2). Cartridges were washed with 5 mL of H_2O after sample loading and allowed to dry under vacuum. Target compounds were eluted from the SPE cartridges using 15 mL MeOH and 15 mL of a MeOH/acetone mixture (50:50 v/v). Eluates were evaporated gently under nitrogen at 55°C and reconstituted for instrumental analysis in 1.5 mL of a 0.1% formic acid in MeOH solution.

8.3.4 Instrumental Analysis

Sample extracts were analyzed using a Shimadzu Nexera X2 Ultra High Performance Liquid Chromatograph (UHPLC) linked to a Shimadzu 8040 triple quadrupole mass spectrometer (MS/MS) (Shimadzu North America, Columbia, MD, USA). A mobile phase of 0.1% formic acid/0.1 mM ammonium acetate in H_2O:MeOH (95:5 v/v) (Solvent A) and 0.1% formic acid/0.1 mM ammonium acetate in MeOH (Solvent B) [202] was run at a gradient to obtain chromatographic separation of compounds. Gradient conditions began at 65% Solvent B and increased to 90% Solvent B over a 4-minute period. Solvent B was then decreased back to 65% by 4.2 minutes, where it was maintained at this percentage for a minute – total run time of 5.2 minutes. Solvent flow began at 0.42 mL/min and was increased to 0.55 mL/min at 1.5 minutes, where it was maintained for the rest of the run. A Phenomenex Synergi™ Polar-RP column (2.5 µm, 100 Å, 2.0 x 100mm) (Phenomenex Inc., Torrance, CA, USA) was utilized and maintained at a

temperature of 45°C. The mass spectrometer was operated using an electrospray ionization (ESI) source with a source temperature of 350°C, a heat block temperature of 400°C, and desolvation line temperature of 250°C. Multiple reaction monitoring was utilized for compound acquisition. Further details regarding MS/MS parameters, such as precursor/product masses, collision energies, etc., are provided in Table SI-F1 of the Supplemental Information.

8.3.5 Quality Assurance/Quality Control

A single grab sample was collected into amber glass jars from each sample point at each WWTP from an area in each process where sufficient mixing has occurred. Samples were extracted, in duplicate, in batches of 12 samples or less. Each extraction batch consisted of a laboratory-grade sand method blank and a sample spiked with all target compounds for recovery determination. Each sample was spiked with $^{13}C_6$-carbamazipine, d_6-methyl chlorpyrifos, $^{13}C_4$-ciprofloxacin, d_6-DEET, $^{13}C_3$-fluconazole, $^{13}C_6$-oxybenzone, $^{13}C_5$-PFHxA, $^{13}C_9$-PFNA, $^{13}C_8$-PFOA, $^{13}C_8$-PFOS, and d_6-venlafaxine as surrogate standards.

During instrumental analysis, standards and solvent blanks were injected every 10 samples as a means of validating instrument stability. A curve of seven standards (10, 25, 50, 100, 250, 500, and 1000 ng/mL; linearity correlations $r^2 \geq 0.99$) was used for UHPLC-MS/MS calibration Method detection limits (MDLs), determined using US Environmental Protection Agency protocols [98], limits of quantification (LOQs), defined as two times the MDL, and compound recoveries are provided in Table SI-F2 of the Supplemental Information. GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA) was utilized for statistical analysis.

8.4 Results and Discussion

8.4.1 Pharmaceuticals and Personal Care Products (PPCPs)

Of the fifteen PPCP compounds analyzed, ten were detected above the LOQ in at least one sample. The beta blockers betaxolol and bisprolol, carbamazepine, an anticonvulsant and mood stabilizer, resperidone, an antipsychotic, and venlafaxine, an antidepressant, were not detected in samples collected from any of the six sampled WWTPs. Concentrations of detected PPCPs during each individual stage of treatment from the six WWTPs, as well as the percent change between each treatment step, are provided in Table SI-F3 of the Supplemental Information. Overall changes in detected PPCP compound concentrations (changes between treatment influent and final solids) are provided in Table 8-2.

The antibiotic ciprofloxacin was detected in all six WWTPs. Concentrations are displayed in Figure 8-1a. Overall, the compound was poorly removed during both anaerobic and aerobic wastewater treatment processes. Concentrations of ciprofloxacin were only significantly changed between treatment influent and final solids in WWTP #4, where an 82.6% decrease (Tukey's multiple comparisons test, $P = 0.0005$) was observed.

The assorted treatment systems had a varied effect on n,n-diethyl-3-methylbenzamide (DEET), an insect repellent, as demonstrated in Figure 8-1b. WWTP #1-3, and #6 did not cause significant changes in concentrations between influent and final solids samples. Anaerobic treatment at WWTP #4, however, resulted in a 28.5% increase in DEET overall (Tukey's multiple comparisons test, $P = 0.0126$) while the aerobic sequencing batch reactor employed by WWTP #5 caused concentrations to decrease by 77.6% (Tukey's multiple comparisons test, $P = 0.0003$). While concentrations of DEET did not change overall at WWTP #3, a

Table 8-2: Overall Changes in Detected PPCP Compound Concentrations

		WWTP #1 (Anaerobic)	WWTP #2 (Anaerobic)	WWTP #3 (Anaerobic)	WWTP #4 (Anaerobic)	WWTP #5 (Aerobic)	WWTP #6 (Aerobic)
Aspartame	**Overall Change**	- 76.9%	- 100%	NS	- 77.6%	NS	- 100%
	P-value [a]	0.0162	< 0.0001	> 0.9999	0.0002	> 0.9999	0.0013
Chlorpyrifos	**Overall Change**	- 53.4%	NS	- 71.8%	- 81.0%	- 100%	NS
	P-value [a]	0.0075	0.0787	0.0036	< 0.0001	0.0004	> 0.9999
Ciprofloxacin	**Overall Change**	NS	NS	NS	- 82.6%	NS	NS
	P-value [a]	0.6494	0.2612	0.6969	0.0005	0.5327	0.0744
DEET	**Overall Change**	NS	NS	NS	+ 28.5%	- 77.6%	NS
	P-value [a]	0.9690	0.0557	0.9613	0.0126	0.0003	0.9882
Diltiazem	**Overall Change**	- 65.4%	- 57.4%	- 100%	- 60.3%	- 26.7%	NS
	P-value [a]	0.0018	0.0003	0.0084	< 0.0001	0.0121	0.0798
Diphenhydramine	**Overall Change**	NS	+ 473%	NS	NS	NS	+ 1496%
	P-value [a]	0.6909	0.0276	0.7819	0.4418	0.4680	0.0063
Fluconazole	**Overall Change**	NS	NS	NS	NS	- 88.2%	+ 83.1%
	P-value [a]	0.2271	0.5852	0.6995	0.3360	0.0010	0.0036
Irbesartan	**Overall Change**	NS	- 97.8%	+ 95.2%	NS	- 32.3%	NS
	P-value [a]	0.2728	0.0098	0.0234	0.0644	0.0459	0.0710
Norethindrone	**Overall Change**	- 89.0%	NS	- 95.4%	- 95.4%	- 95.7%	- 67.4%
	P-value [a]	0.0004	0.7108	0.0005	< 0.0001	0.0002	0.0080
Oxybenzone	**Overall Change**	NS	- 93.9%	- 97.2%	- 49.3%	- 96.1%	- 67.4%
	P-value [a]	0.0552	0.0020	0.0005	0.0139	< 0.0001	0.0110
Pendimethaline	**Overall Change**	- 27.1%	NS	- 56.4%	- 45.0%	NS	NS
	P-value [a]	0.0118	0.1035	< 0.0001	0.0004	0.3914	0.9078
PFOS	**Overall Change**	NS	NS	+ 187%	NS	+ 135%	+ 156%
	P-value [a]	0.9639	0.6046	0.0011	0.1196	0.0033	0.0010
Prednisone	**Overall Change**	- 73.8%	NS	- 92.2%	- 79.7%	+ 13.4%	NS
	P-value [a]	0.0251	0.8700	0.0118	0.0007	0.0110	0.7764
Testosterone	**Overall Change**	- 100%	NS	- 100%	NS	- 100%	NS
	P-value [a]	0.0002	> 0.9999	0.0012	0.3519	0.0008	> 0.9999
Triphenyl phosphate	**Overall Change**	NS	NS	NS	- 40.4%	- 53.4%	- 65.2%
	P-value [a]	0.5735	0.0503	0.5730	0.0004	0.0185	0.0102
Tris(2-butoxyethyl) phosphate	**Overall Change**	NS	NS	+ 128%	+ 376%	- 72.2%	- 61.5%
	P-value [a]	0.4309	0.1536	0.0068	0.0012	< 0.0001	0.0298

NS = change in concentration not significant and, thus, not calculated; [a] Tukey's multiple comparisons test

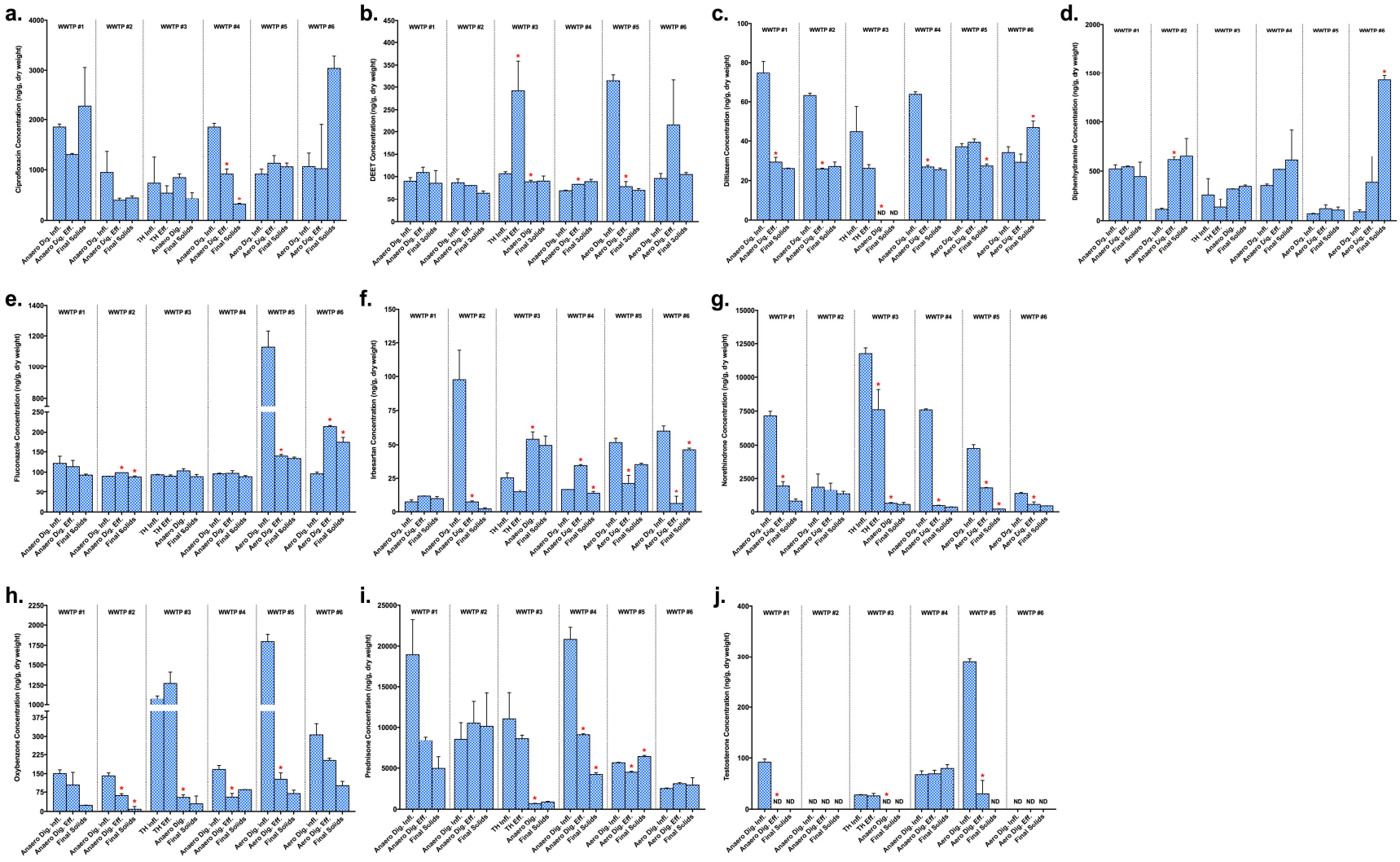


Figure 8-1: Concentrations of Detected PPCPs in Each WWTP Treatment Stage. * indicates that concentration at that stage of treatment is significantly different than the previous stage. (n = 2)

significant increase of 176% (Tukey's multiple comparisons test, P = 0.0189; Table SI-3) during thermal hydrolysis was observed, followed by a significant decrease in concentrations during anaerobic digestion (Tukey's multiple comparisons test, P = 0.0137; Table SI-F3) by 88.9%.Diltiazem, used to treat hypertension, was consistently degraded during anaerobic digestion (Figure 8-1c). The anaerobic treatment systems employed by WWTPs #1 – 4 all resulted in significant decreases in concentrations, with overall removal rates ranging from 57.4% to 100%.

Diltiazem concentrations decreased by 26.7% at WWTP #5 (Tukey's multiple comparisons test, P = 0.0121), a reduction that was due to the dewatering process, not the aerobic SBR. No significant overall change occurred during treatment at WWTP #6.

Concentrations of diphenhydramine (Figure 8-1d), an antihistamine, did not change significantly during treatment at WWTPs #1 and #3 - 5. However, anaerobic digestion treatment at WWTP #2 resulted in a 473% increase in concentrations (Tukey's multiple comparisons test, P = 0.0276) and the aerobic membrane bioreactor (MBR) treatment at WWTP #6 increased diphenhydramine concentrations by 1,496% (Tukey's multiple comparisons test, P = 0.0063).

Concentrations of fluconazole (Figure 8-1e), an antifungal agent, were not impacted overall by the anaerobic processes at WWTPs #1 – 4. WWTP #5, the facility with starting concentrations approximately an order of magnitude higher than the other WWTPs, was able to significantly decrease fluconazole during aerobic treatment with and overall decrease of 88.2% during treatment (Tukey's multiple comparisons test, P = 0.0010). Conversely, aerobic MBR treatment at WWTP #6 resulted in an increase of 83.1% in fluconazole (Tukey's multiple comparisons test, P = 0.0036).

The influence of treatment processes on concentrations of irbesartan, prescribed for the treatment of hypertension, widely differed amongst the six WWTPs (Figure 8-1f). Overall, concentrations did not change at WWTP #1 while as the anaerobic system at WWTP #2 significantly reduced irbesartan levels between influent and final solids by 97.8% (Tukey's multiple comparisons test, P = 0.0098). Conversely, WWTPs #3 and 4, the facilities with more advanced anaerobic systems, saw concentrations increase during anaerobic treatment. Irbesartan levels significantly increased between influent and final solids by 95.2% in WWTP #3. While there was no significant change in overall concentrations in WWTP #4, irbesartan did increase significantly during the anaerobic digestion stage of treatment (Tukey's multiple comparisons test, P = 0.0006; Table SI-F3) before decreasing during the dewatering process (Tukey's multiple comparisons test, P = 0.0004; Table SI-F3).

The treatment systems employed at the study WWTPs were generally quite effective at degrading norethindrone, a steroid hormone (Figure 8-1g). Anaerobic digestion treatments at WWTPs #1, 3, and 4 significantly reduced overall norethindrone levels by 89.0%, 95.4%, and 95.4%, respectively. Furthermore, the thermal hydrolysis stage of treatment at WWTP #3 reduced concentrations by 35.4 % (Tukey's multiple comparisons test, P = 0.0205; Table SI-F3). Aerobic treatments at WWTPs #5 and 6 reduced overall norethindrone levels by 95.7% and 67.4%, respectively.

Oxybenzone, a UV stabilizer and absorber, was effectively degraded under anaerobic and aerobic conditions (Figure 8-1h). Anaerobically, oxybenzone was removed by 93.9%, 97.2%, and 49.3% at WWTPs #2 – 4, respectively. Additionally,

the compound was readily degraded under aerobic conditions as well, with 96.1% and 67.4% removal at WWTPs #5 and 6, respectively.

Generally, the corticosteroid prednisone was effectively removed under anaerobic conditions while aerobic treatments appeared unable to degrade the compound (Figure 8-1i). Treatment at WWTP #1 was able to significantly degrade prednisone by 73.8% Tukey's multiple comparisons test, P = 0.0251) while WWTPs #3 and 4 reduced levels by 92.2% and 79.7% (Tukey's multiple comparisons test, P = 0.0118 and 0.0007), respectively. Treatment at WWTP #2 could not significantly change concentrations of the compound. While the aerobic SBR at WWTP #5 reduced prednisone levels by 20.2% (Tukey's multiple comparisons test, P = 0.0033; Table SI-F3), overall concentrations increased by 13.4% (Tukey's multiple comparisons test, P = 0.0110) between treatment influent and final solids. Concentrations did not change significantly during treatment at WWTP #6.

Testosterone (Figure 8-1j) was degraded to concentrations below LOQ during anaerobic treatment in WWTP #1 and 3. The compound was not significantly removed by treatment at WWTP #4. WWTP #5's aerobic SBR reduced testosterone levels by 89.9% (Tukey's multiple comparisons test, P = 0.0011; Table SI-F3) with overall concentrations being reduced to below the compound LOQ. The compound was not detected at or above the LOQ from WWTPs #2 or 6.

8.4.2 Pesticides

Of the four pesticides analyzed, two (emamectin benzoate and flubendiamide) were not detected at or above the LOQ from the six WWTPs. Concentrations of chlorpyrifos and pendimethalin, the detected pesticides, during each individual WWTP treatment stages and the percent change between each treatment step, are provided in Table SI-F3 of the Supplemental Information. Overall

changes in concentrations between treatment influent and final solids are provided in Table 8-2.

Both anaerobic and aerobic treatments were generally able to degrade the insecticide chlorpyrifos in the six study WWTPs (Figure 8-2a). In WWTP #1, chlorpyrifos was not significantly reduced during anaerobic digestion but, rather,

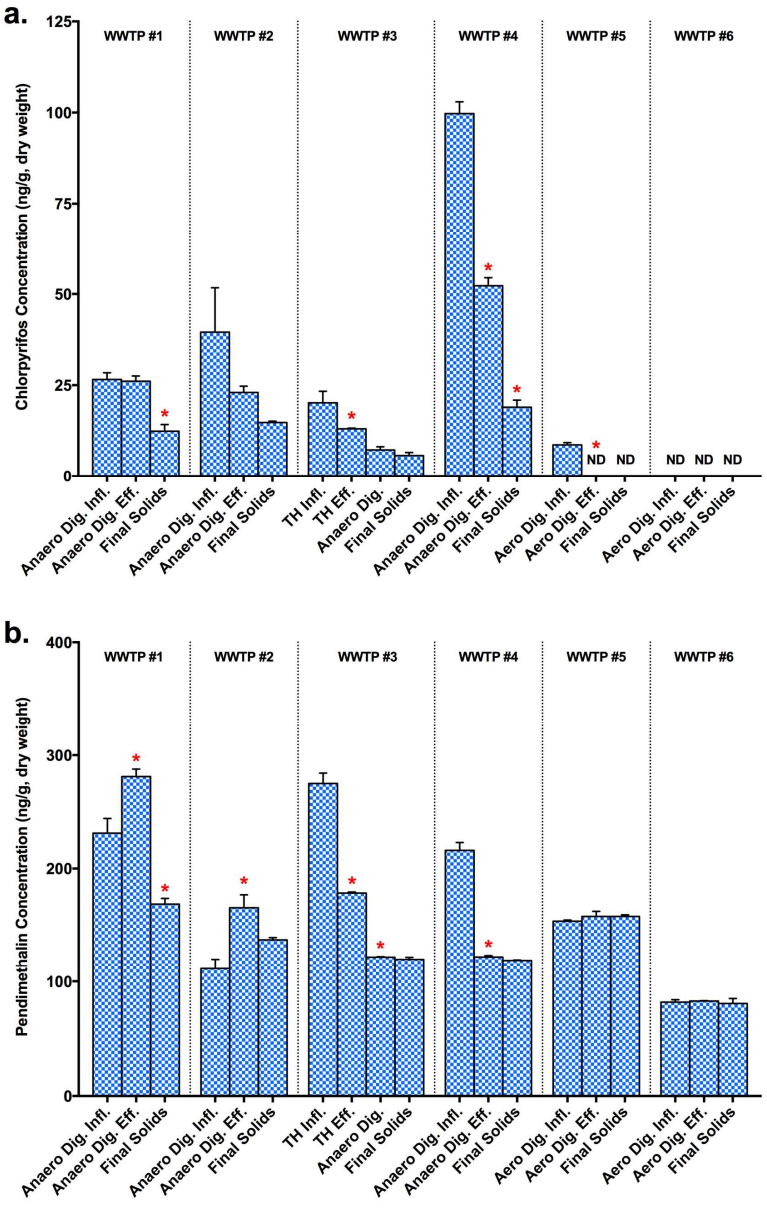


Figure 8-2: Concentrations of Detected Pesticides in Each WWTP Treatment Stage. * indicates that concentration at that stage of treatment is significantly different than the previous

during the dewatering process (52.5%, Tukey's multiple comparisons test, P = 0.0083; Table SI-F3). Concentrations were not significantly changed during treatment at WWTP #2. Chlorpyrifos levels were reduced by 71.8% (Tukey's multiple comparisons test, P = 0.0036) during the treatment process at WWTP #3, with a 35.5% reduction occurring due to thermal hydrolysis (Tukey's multiple comparisons test, P = 0.0453; Table SI-F3). The treatment system at WWTP #4 was also able to degrade the insecticide (81.0%, Tukey's multiple comparisons test, P = < 0.0001). Aerobic treatment at WWTP #5 reduced chlorpyrifos to levels below the LOQ (Tukey's multiple comparisons test, P = 0.0004) while the insecticide was not detected at or above the LOQ in any samples collected from WWTP #6.

Influences of the different treatment systems on concentrations of the herbicide pendimethalin were varied (Figure 8-2b). In WWTPs #1 and 2, concentrations increased by 21.6% and 49.5% [Tukey's multiple comparisons test, P = 0.0224 (WWTP #1), P = 0.0149 (WWTP #2); Table SI-F3], respectively, during the anaerobic stage of treatment. The treatment process employed at WWTP #3 reduced concentrations by 56.4% (Tukey's multiple comparisons test, P = < 0.0001) with significant decreases occurring during both thermal hydrolysis treatment and anaerobic digestion treatment. Anaerobic digestion treatment also significantly degraded pendimethalin at WWTP #4 (Tukey's multiple comparisons test, P = 0.0004; Table SI-F3). However, the aerobic processes employed by WWTPs #5 and 6 were not able to significantly change concentrations during treatment.

8.4.3 Fire Retardants

The flame retardants triphenyl phosphate and tris(2-butoxyethyl) phosphate were detected at all six WWTPs whereas tris(1-chloro-2-propyl) phosphate was not detected at or above the LOQ in any samples. Overall changes in concentrations of

triphenyl phosphate and tris(2-butoxyethyl) phosphate between treatment influent and final solids are provided in Table 8-2, while concentrations and percent change associated with individual treatment steps are provided in Table SI-F3 of the Supplemental Information.

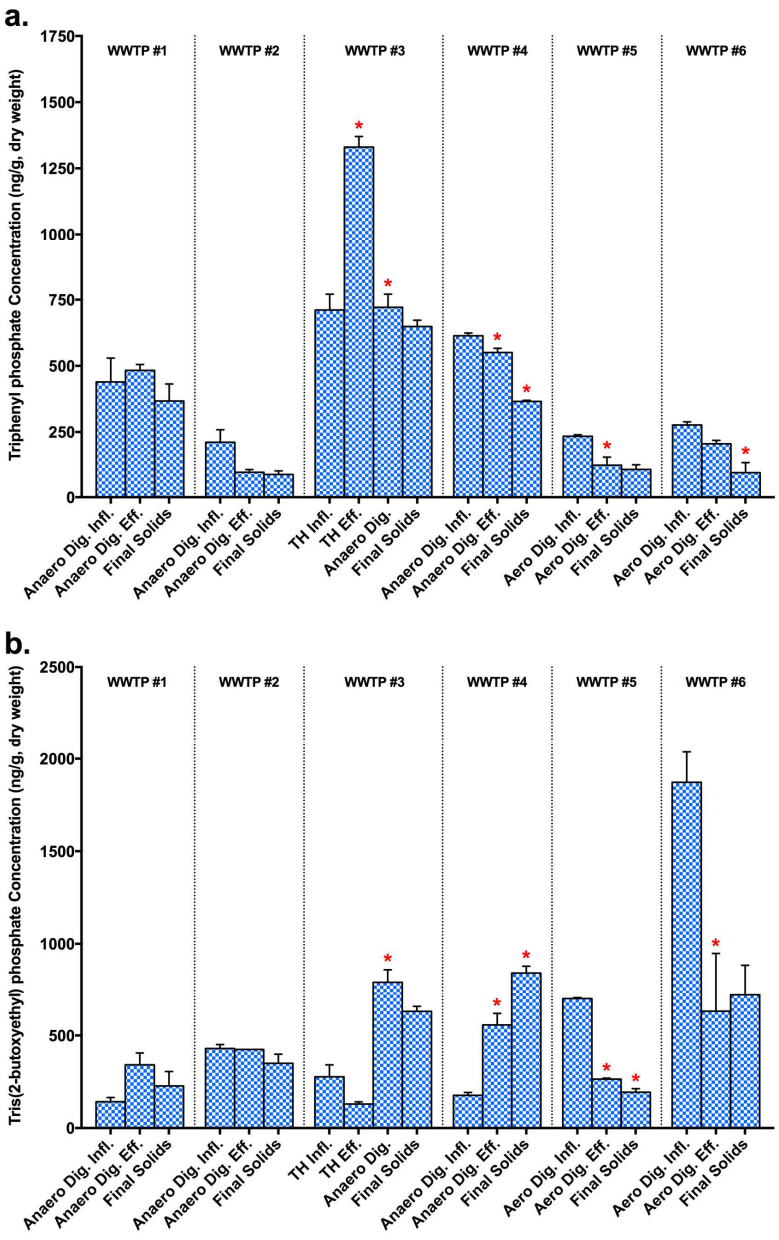


Figure 8-3: Concentrations of Detected Flame Retardants in Each WWTP Treatment Stage. * indicates that concentration at that stage of treatment is significantly different than the previous stage. (n = 2)

Triphenyl phosphate levels (Figure 8-3a) were not significantly changed during treatment at WWTPs #1 and 2. At WWTP #3, concentrations were not significantly different between treatment influent and final solids. However, the thermal hydrolysis stage of treatment resulted in a significant increase in triphenyl phosphate by 87.1% (Tukey's multiple comparisons test, P = 0.0006) while anaerobic digestion caused a 45.8% decrease (Tukey's multiple comparisons test, P = 0.0006). Treatment at WWTP #4 was able to decrease overall concentrations by 40.4% (Tukey's multiple comparisons test, P = 0.0004). The aerobic treatments utilized by WWTPs #5 and 6 were also able to significantly reduce triphenyl phosphate concentrations by 53.4% and 65.2%, respectively.

As with triphenyl phosphate, concentrations of tris(2-butoxyethyl) phosphate (Figure 8-3b) were not significantly changed during the treatment processes at WWTPs #1 and 2. The anaerobic digestion stage of treatment at WWTPs #3 and 4 resulted in large and significant increases in tris(2-butoxyethyl) phosphate: 509% and 215%, respectively [Tukey's multiple comparisons test, P = 0.0006 (WWTP #3), P = 0.0062 (WWTP #4); Table SI-F3]. Aerobic processes, however, were able to effectively degrade the fire retardant. The treatment processes at WWTP #5 was able to remove tris(2-butoxyethyl) phosphate by 72.2% overall (Tukey's multiple comparisons test, P = < 0.0001) while those at WWTP #6 degraded the compound by 61.5% (Tukey's multiple comparisons test, P = 0.0298).

8.4.4 Food Additives

The artificial sweetener aspartame was readily removed by anaerobic and aerobic treatment processes (Figure 8-4a). Concentrations were significantly reduced due to aerobic processes by 76.9% at WWTP #1 (Tukey's multiple

comparisons test, P = 0.0162), below levels of LOQ at WWPT #2 (Tukey's multiple comparisons test, P = < 0.0001), and by 77.6% at WWTP #4 (Tukey's multiple comparisons test, P = 0.0002). Aerobic MBR treatment at WWTP #6 also reduced aspartame concentrations to below the LOQ (Tukey's multiple comparisons test, P = 0.0013). The artificial sweetener was not detected at or above the LOQ at WWTPs # 3 and 5.

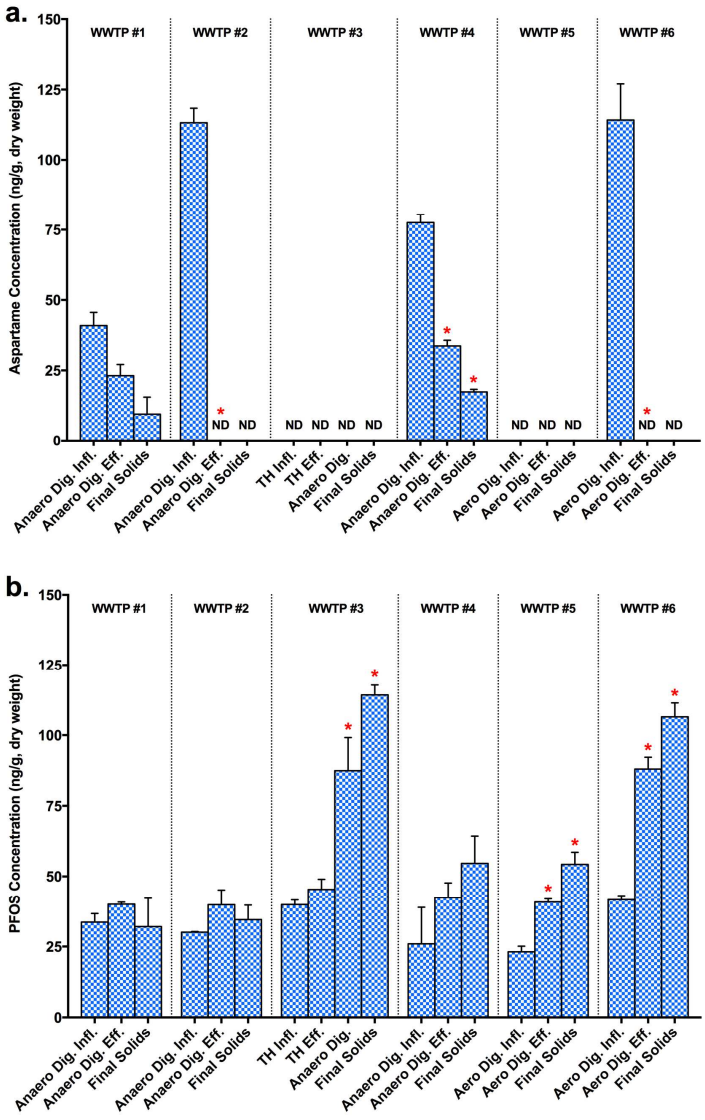


Figure 8-4: Concentrations of Detected a) Food Additives and b) Surfactants in Each WWTP Treatment Stage. * indicates that concentration at that stage of treatment is significantly different than the previous stage. (n = 2)

8.4.5 Surfactants

Perfluorohexanoic acid (PFHxA), perfluorononanoic acid (PFNA), and perfluorooctanoic acid (PFOA) were not detected at or above the LOQ in all samples. The treatment processes employed by the study WWTPs were unable to remove PFOS and, in some cases, resulted increases concentrations of the compound (Figure 8-4b). The anaerobic digestion stage of treatment at WWTP #3 resulted in a 92.5% increase in PFOS (Tukey's multiple comparisons test, P = 0.0098; Table SI-F3) while concentrations increased an additional 30.4% due to the dewatering process (Tukey's multiple comparisons test, P = 0.0467; Table SI-F3). The aerobic treatment processes employed by WWTPs #5 and 6 resulted in a 135% and 156% increase, respectively, [Tukey's multiple comparisons test, P = 0.0033 (WWTP #5), P = 0.0010 (WWTP #6)] in PFOS concentrations.

8.4.6 Predicted Environmental Concentrations in Soil

In order to fully assess the ecological implications of the detected ECs, predicted environmental concentrations (PECs) of the compounds in soil after a single biosolids application from each WWTP were calculated using Equation 1:

$$PEC = C_{soil} + \frac{C_{biosolids} \times AR}{D \times SD} \qquad (1)$$

The initial compound concentration in soil (C_{soil}) was assumed to be zero, indicating no prior biosolids application. The concentrations of ECs in final solids samples (provided in Table SI-F3) were used as the assumed concentrations in biosolids ($C_{biosolids}$). The application rate (AR) was assumed to be 6,950 dry kg/ha for Class A biosolids (WWTP #3) and 9,420 dry kg/ha for Class B biosolids (WWTPs #1, 2, and 4) and landfilled final solids (WWTPs #5 and 6) [55]. The soil density (D) was assumed to be 1.35 g/cm^3 [55] and the depth of the soil (SD) was set at 7.6 cm as a

means of simulating no till biosolids application [34]. The calculated PECs are provided in Table 8-3. Ciprofloxacin, diphenhydramine, norethindrone, prednisone, and tris(2-butoxyethyl) phosphate had the highest PECs in soil at 27.9 ng/g (WWTP #6), 13.1 ng/g (WWTP #6), 12.1 ng/g (WWTP #2), 93.1 ng/g (WWTP #2), and 7.74 ng/g (WWTP #4), respectively.

Table 8-3: PECs in Soil for Detected ECs

	Predicted Environmental Concentration in Soils (ng/g)					
	WWTP #1	**WWTP #2**	**WWTP #3**	**WWTP #4**	**WWTP #5**	**WWTP #6**
Aspartame	0.0869	NC	NC	0.160	NC	NC
Chlorpyrifos	0.114	0.136	0.0385	0.174	NC	NC
Ciprofloxacin	20.9	4.21	2.98	2.96	9.71	27.9
DEET	0.788	0.589	0.612	0.820	0.645	0.964
Diltiazem	0.238	0.247	NC	0.232	0.250	0.429
Diphenhydramine	4.10	6.02	2.38	5.59	0.982	13.1
Fluconazole	0.852	0.810	0.603	0.815	1.23	1.62
Irbesartan	0.0883	0.0200	0.336	0.125	0.321	0.421
Norethindrone	7.20	12.1	3.68	3.23	1.87	4.03
Oxybenzone	0.217	0.0786	0.201	0.776	0.636	0.917
Pendimethalin	1.55	1.26	0.813	1.09	1.45	0.740
PFOS	0.295	0.317	0.772	0.501	0.499	0.982
Prednisone	45.6	93.1	5.80	38.9	58.8	26.3
Testosterone	NC	NC	NC	0.731	NC	NC
Triphenyl phosphate	3.37	0.825	4.40	3.36	1.00	0.884
Tris(2-butoxyethyl) phosphate	2.09	3.21	4.27	7.74	1.78	6.61

NC = compound not detected at or above the LOQ in final solids samples, thus PEC not calculated

The presence of ECs in soils after biosolids applications can have varying effects on the local environment, such as microbial and biotic toxicity, plant uptake, mobilization into other environmental compartments, etc. In a study focusing on the impact of PPCPs on mosquito development, it was found that a PPCP mix that included norethindrone and ciprofloxacin delayed pupation time of *Culex*

quinquefasciatus. Additionally, when the mosquito larva were exposed to both *Bacillus thuringiensis* subspecies *israelensis* (Bti), a bacterial insecticide used for mosquito population control, and the PPCP mixture or antibiotic-only mixture (comprised of lincomycin, oxytetracycline, and ciprofloxacin), a significantly higher mortality rate was observed when compared to those exposed only to Bti treatments. Finally, exposure to the PPCP mixture, the antibiotic-only mixture, or a hormone mixture (consisting of norethindrone, 17β estradiol, and 17α ethynylestradiol) resulted in an alteration of the mosquito microbiome [203]. Furthermore, the presence of ciprofloxacin in soil has been demonstrated to not only negatively impact the activity of natural microbial populations but to persist within soils with retention of biologically active properties [204]. Pot studies performed by Eggen et al. (2011) indicated the ability of plants to uptake a fraction of ciprofloxacin into their roots, with root accumulation factors estimated to be 0.26 and 0.059 for barley and carrot, respectively. Moreover, the antibiotic negatively impacted the growth and development of carrots at concentrations of 6 – 10 mg/kg in the soil [205].

Column experiments conducted on a silty clay loam and a sandy loam soils resulted in half-lives of 20.1 days and 13.3 days, respectively, for norethindrone [206]. Laboratory studies of three agricultural soil types (loam, sandy loam, and clay loam) showed the ability of diphenhydramine to persist with and without liquid municipal biosolids (LMB) supplementation. The average 50% time of dissipation of the antihistamine during the experiments ranged from 88 days (clay loam, no LMB supplementation) to 335 days (loam, no LMB supplementation) [207]. Furthermore, a study of contaminants in rainfall-runoff from biosolids amended fields performed by Gray et al. (2017) demonstrated that rain events have the ability to mobilize numerous compounds and spread them from agricultural plots to other ecological.

One such compound was the flame retardant tris(2-butoxyethyl) phosphate, which demonstrated a high frequency of detection and an elevated concentration in runoff when compared to other analyzed compounds [208].

8.5 Conclusions

This paper outlines the impact that solids treatment systems at 6 WWTPs had on various ECs. Specifically, anaerobic digestion (both conventional and advanced) and aerobic digestion treatment systems were studied. Of the 27 compounds analyzed, 16 were detected at or above the LOQ in at least one sample. Impact of anaerobic and aerobic treatments on compound concentrations varied widely and was compound-specific. Additionally, PECs were calculated to estimate the impact that land application of biosolids from the study WWTPs would have on soil concentrations of the detected ECs. The ECs with the highest PECs in soil were ciprofloxacin, diphenhydramine, norethindrone, prednisone, and tris(2-butoxyethyl) phosphate. Based on the results of this study, further research should focus on the transformation products of compounds that increased and/or decreased during the treatment processes to better understand the dynamics of the compound groups and the pathways of how individual treatment processes specifically influence a parent compound and its metabolites.

Chapter 9: Conclusions and Future Work

9.1 Conclusions

The study of the fate of various anthropogenic pollutants of concern during wastewater treatment both on-site and in laboratory simulations has allowed for a better understanding regarding the presence of these compounds and their transformation products in wastewater, how new treatment technologies influence compound concentrations and transformation product levels, and how altering already established treatment technologies can help to improve upon compound degradation. Overall, the research presented in Chapters 2 through 8 can be summarized as:

1) Increases in temperature, hydraulic retention time (HRT), and sludge retention time (SRT) during activated sludge treatment improved TCS and TCC degradation while substantial formation of methyl triclosan (MeTCS), a TCS metabolite, occurred during most treatment conditions. While removal of TCS is desirable, formaiton of MeTCS is not due to its persistence and endocrine disrupting properties.
2) Degradation of TCS during nitrification treatment was more efficient at pH range of 8.5 - 9.5 than at a range of 6.5 - 7.5. MeTCS was formed in both pH ranges, but formation occurred more rapidly during the higher range. Again, while improvement of TCS is desireable during treatment, the formation of MeTCS is not. Concentrations of TCC and 2,4-dichlorophenol (2,4-DCP), a TCS degradation product, did not significantly change during any nitrification treatment conditions.

3) sludge treatment via CambiTHP and anaerobic digestion increased concentrations of TCS, MeTCS, and 2,4-DCP in biosolids, when compared to lime treatment of sludge. This was due to concentration via solids reduction during digestion. TCC concentrations decreased by over 95% during CambiTHP pretreatment.

4) Levels of four phthalate plasticizers in increased due to CambiTHP/anaerobic digestion treatment, when compared to treatment of sludge via liming. Increases were not solely due to solids reduction during digestion. It is theorized that transformation of metabolites during treatment added to increases in concentrations.

5) Comparison of solids treatment at six different WWTPs showed that the plasticizer di(2-ethylhexyl) phthalate (DEHP) was readily degraded during aerobic treatments while anaerobic digestion resulted in either no significant change in concentrations or an increase in concentration, in the case of more advanced anaerobic processes. Impacts of the various treatment systems on concentrations of the remaining three plasticizers were more varied – anaerobic digestion led to significant decreases, increases, or no significant change for these compounds, depending on the treatment facility.

6) Comparison of anaerobic treatment with and without CambiTHP showed that TCS and MeTCS increased during anaerobic digestion with no significant impact when pretreated via CambiTHP while concentrations of TCC increased more rapidly when sludge was not pretreated and 2,4-dichlorophenol levels increased at a faster rate with CambiTHP pretreatment. Anaerobic digestion and CambiTHP pretreatment had a

more varying effect on plasticizers with DEHP decreasing, diisononyl phthalate levels remaining steady, and benzyl butyl phthalate concentrations increasing with pretreatment and decreasing without. Plasticizer metabolites generally increased during the course of the experiments; and

7) a method utilizing sonication/solid phase extraction and analysis via ultra high performance liquid chromatography/mass spectrometry was developed for 27 compounds. Sixteen of the 27 compounds were detected in solids samples from 6 WWTPs, a majority of which were PPCPs. Concentrations of compounds increased/decreased/remained unchanged depending on the individual treatment system and compound chemistry.

9.2 Future Work

Based on the results of this research project, the following questions have arisen and should be addressed with future research:

1. What are additional triclosan and triclocarban transformation products and what is their fate/formation rates under various treatment processes?
2. What are additional PAE transformation products and what is their fate/formation rates under various treatment processes?
3. How can an effective and efficient analytical method for TCS, TCC, and PAE transformation products be developed in complex matrices such as sludge using currently available instrumentation?
4. What additional PAE conjugates/metabolites are present in wastewater and what are their fate during anaerobic and aerobic treatment? Do these compounds explain the patterns of PAE formation observed in this thesis?

5. What are the transformation products/metabolites formed from compounds degraded in the Chapter 8 research?

Chapter 10: Supplemental Information

10.1 Supplemental A: Degradation of Triclosan and Triclocarban and Formation of Transformation Products in Activated Sludge Using Benchtop Bioreactors

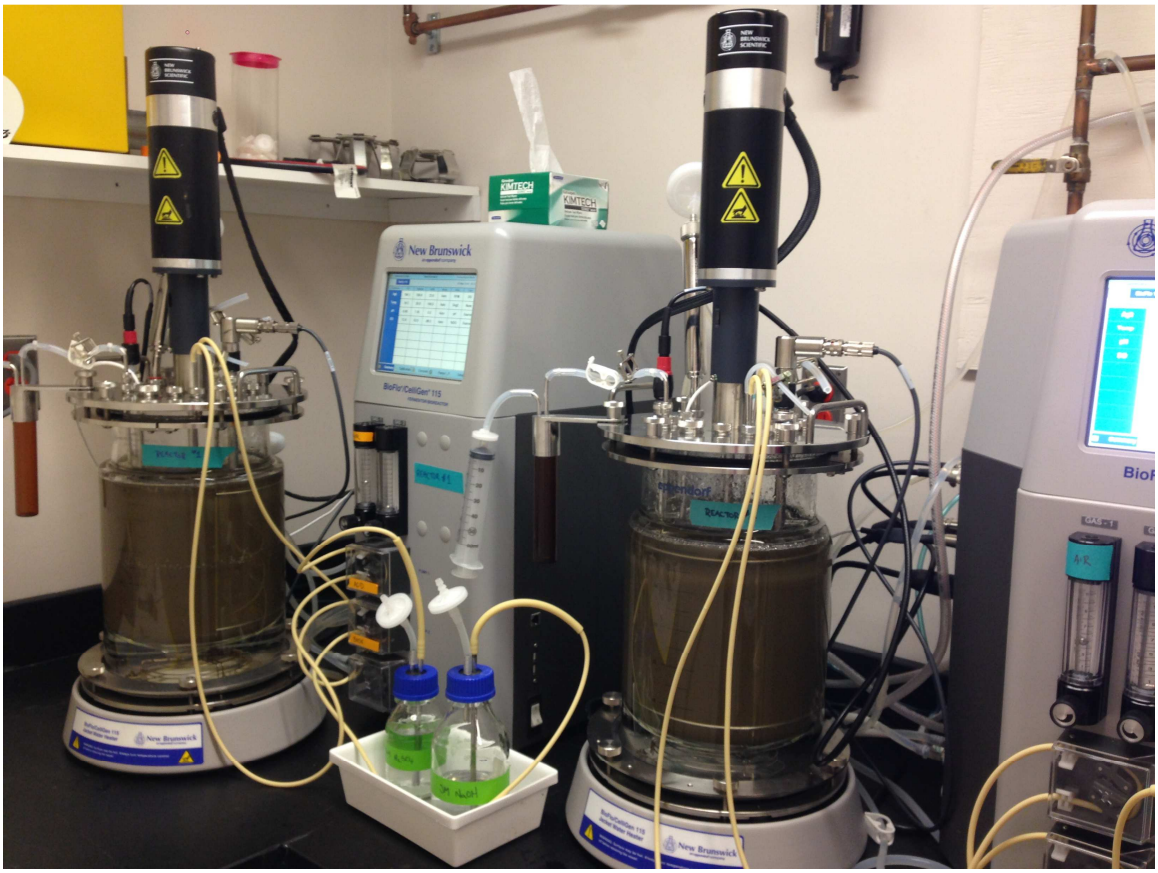


Figure SI-A1: BioFlo® 115 Benchtop Bioreactors Set-up Prior to Covering in Aluminum Foil

10.1.1 Instrumental Analysis

10.1.1.1 TCC and TCS HPLC-MS/MS conditions

The LC column temperature was maintained at 50 °C. The initial solvent management was 55% solvent A (70-1% formic acid:30 methanol) and 45% solvent B (methanol) and these conditions were changed by linear gradient to 50:50 (A:B) in 15 minutes whereupon the instrument is returned to initial settings in one minute and maintained there for 4 min for equilibration to the initial conditions. Total run time was 20 min. The flow rate was maintained at 0.3 ml min^{-1}. The injection volume was

10 µl. Source parameters were as follow: capillary voltage is set at 2.93 kV; cone voltage at 22 V; extractor voltage is set at 1 V; rf lens at 0.1 V; source and desolvation temperatures are 140 and 400 °C, respectively. A nitrogen generator is used to supply the nebulizer and desolvation gas (flow rates were approximately 60 and 600 L h^{-1}, respectively). Both quadrupoles were set at a resolution of 12.0. Analytes concentrations were determined by isotope dilution methods using $^{13}C_{12}$-TCC and $^{13}C_{12}$-TCS as an internal standard to quantitate the unlabeled triclosan and triclocarban. Peak integration and quantification was performed automatically using the MassLynx v4.0 software (Micromass Ltd., Machester, UK).

10.1.1.2 Transformation Products UHPLC-MS/MS conditions

A Shimadzu Nexera X2 UHPLC coupled with a Shimadzu 8040 triple quadrupole MS was used to qualitatively scan for 2,4-DCP, DCC, MCC, NCC, 4-chlorocatechol, and 4-CA. A mobile phase of 10 mM ammonium acetate in MeOH:acetonitrile:H_2O was run through a Supelco Ascentis® Express C18 column (2.7 µm, 50 x 2.1 cm) at a rate of 0.5 mL/min. The column over was maintained at 40°C. MS conditions were:

Compound	Source	Precursor m/z	Product m/z	Q1 (V)	CE (eV)	Q3 (V)
4-CA	ESI+	128.4	---	10	18	---
4-Chlorocatechol	ESI+	145.6	---	12	18	---
2,4-DCP	ESI-	161.1	125.05	10	18	24
DCC	ESI-	278.9	125.95	25	15	13
MCC	ESI-	245.1	126.05	16	11	24
NCC	ESI-	211.1	92.1	13	13	18

10.1.1.3 MeTCS GC-MS conditions

GC operating parameters were as follows: helium carrier gas flowing at 1 ml/min through column, injection inlet temperature was 250 °C, 2 µl of sample was injected at 14 kPa in splitless mode. The column temperature was programmed as

follow: initial setting was 70 °C (held for 5 min), then ramped at different rates as follows: first 20°C/min to 100 °C then 10 °C/min to 160 °C, next 1 °C/min to 169 °C and finally 18 °C/min to 325 °C. The interface to the detector was 280 °C. The MS detector was operated in electron impact ionization mode (EI, 70 eV) with the ion source temperature at 230 °C. The acquisition mode was single ion monitoring (SIM) and for quantification, the mass fragment 302.0 and 306.0 was used for MeTCS and 314.0 and 316.0 for $^{13}C_{12}$-MeTCS. The analyte concentrations were determined by isotope dilution methods using $^{13}C_{12}$-MeTCS as an internal standard to quantitate the unlabeled MeTCS. Peak integration and quantification was performed automatically using the MSD ChemStation software (Agilent Technologies).

10.1.2 Secondary Analysis Results

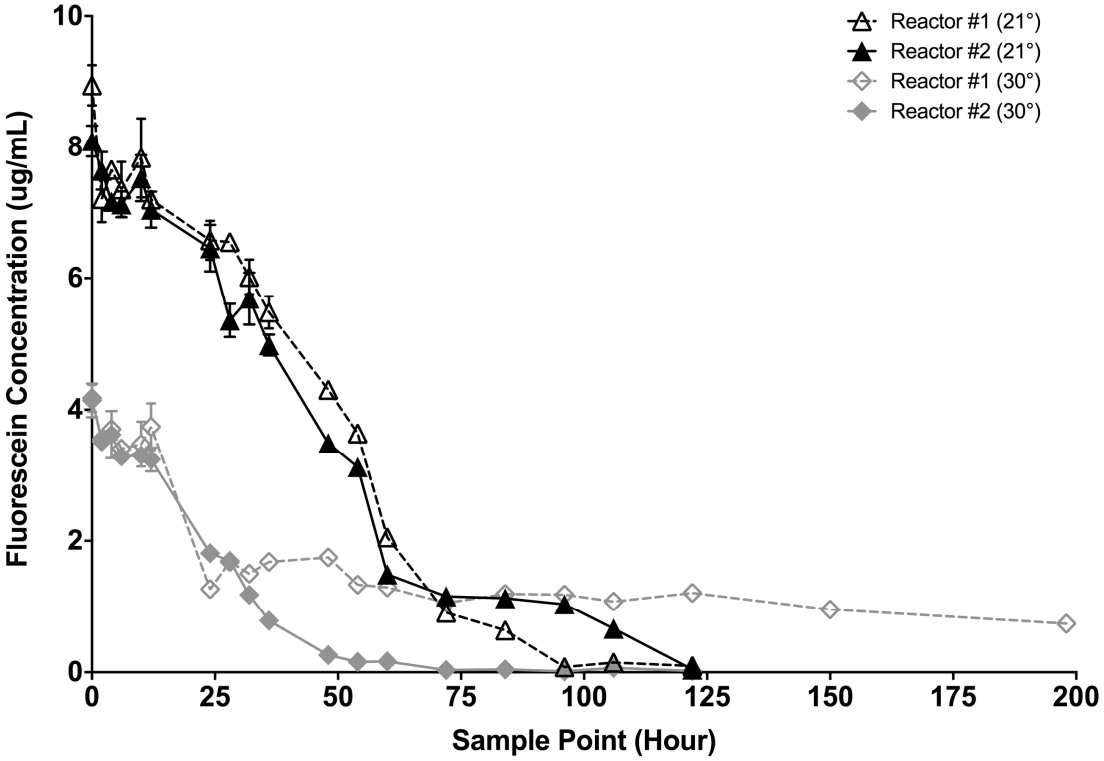


Figure SI-A2: Microbial Activity Results for Each Reactor Run at 21°C and 30°C

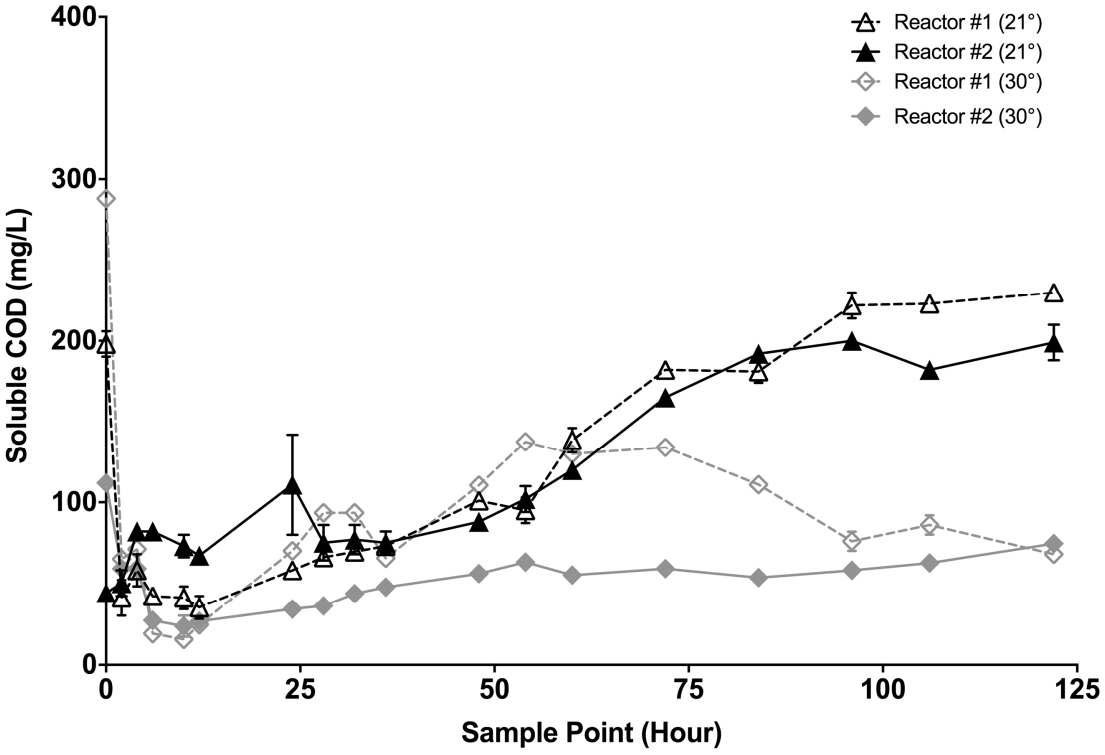


Figure SI-A3: Soluble COD Concentrations for Each Reactor Run at 21°C and 30°C

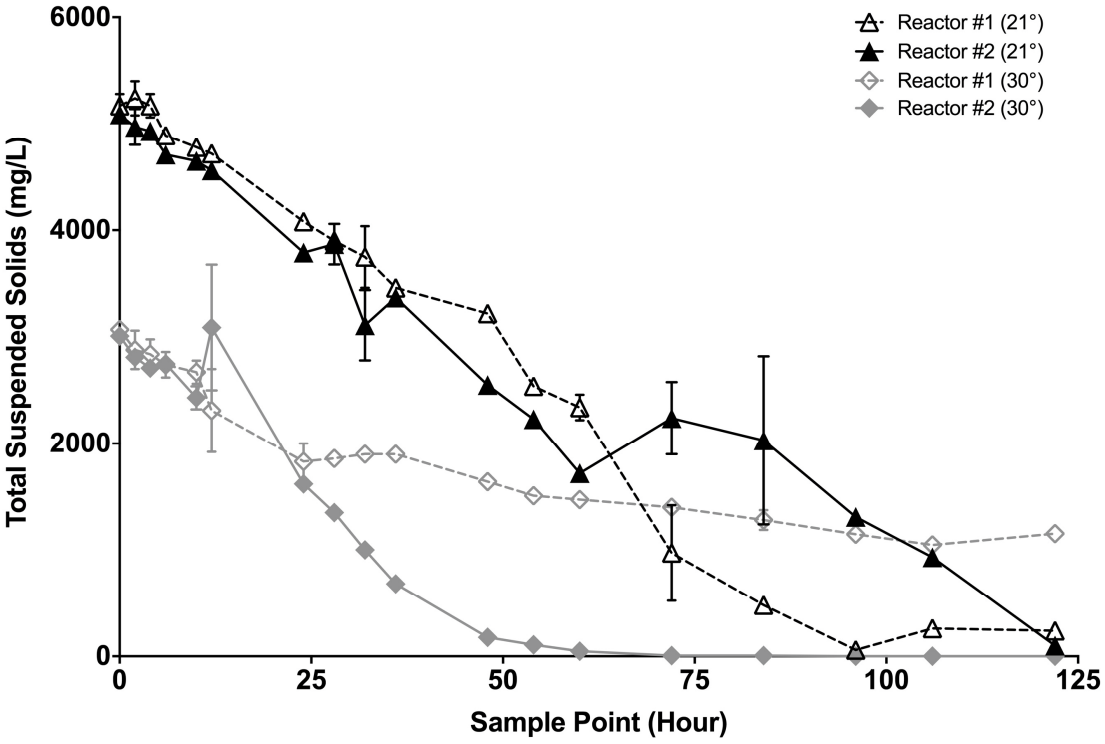


Figure SI-A4: Total Suspended Solids Concentrations for Each Reactor Run at 21°C and 30°C

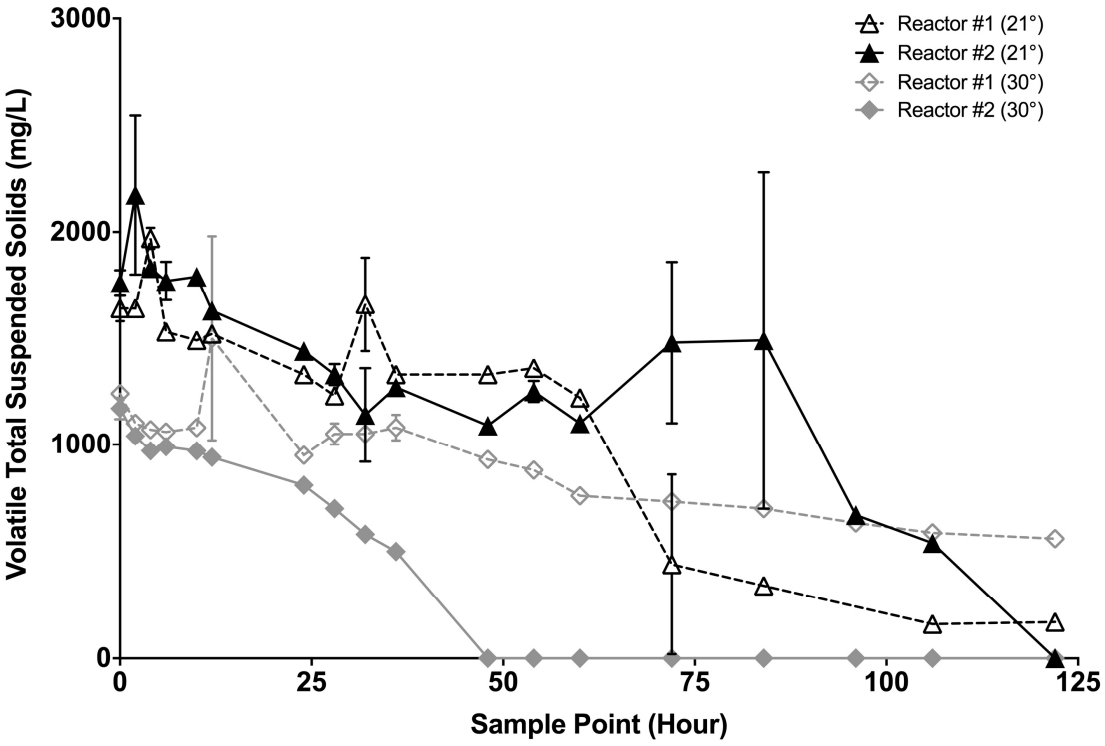


Figure SI-A5: Volatile Total Suspended Solids Concentrations for Each Reactor Run at 21°C and 30°C

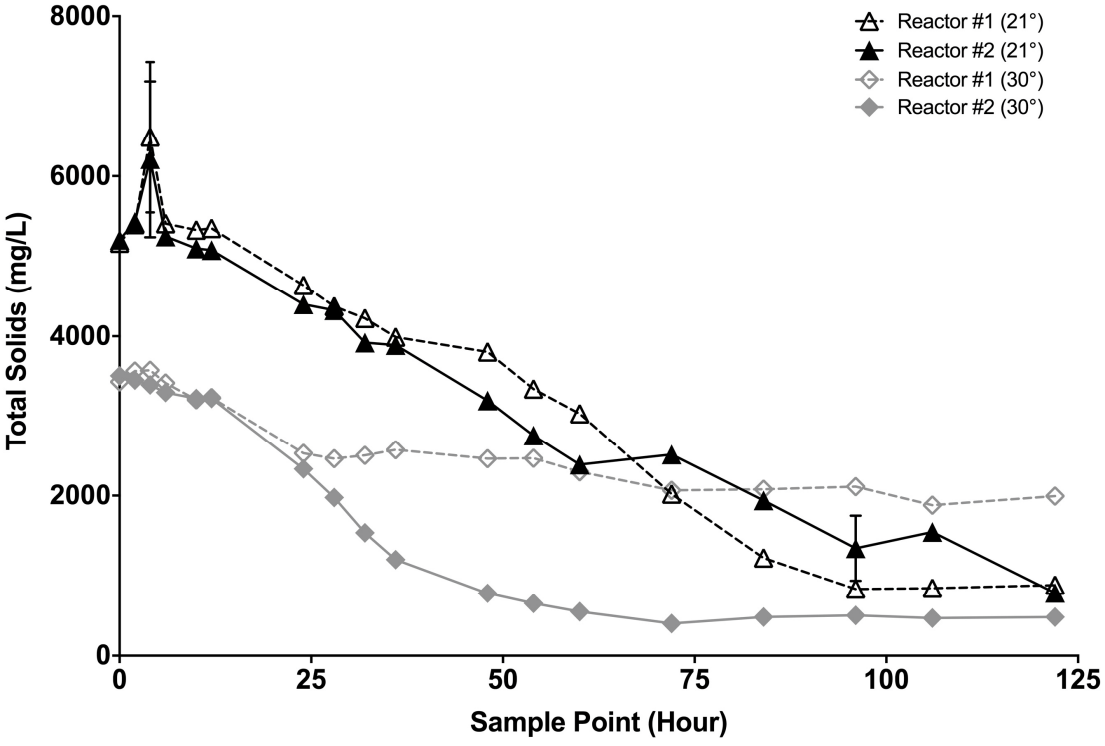


Figure SI-A6: Total Solids Concentrations for Each Reactor Run at 21°C and 30°C

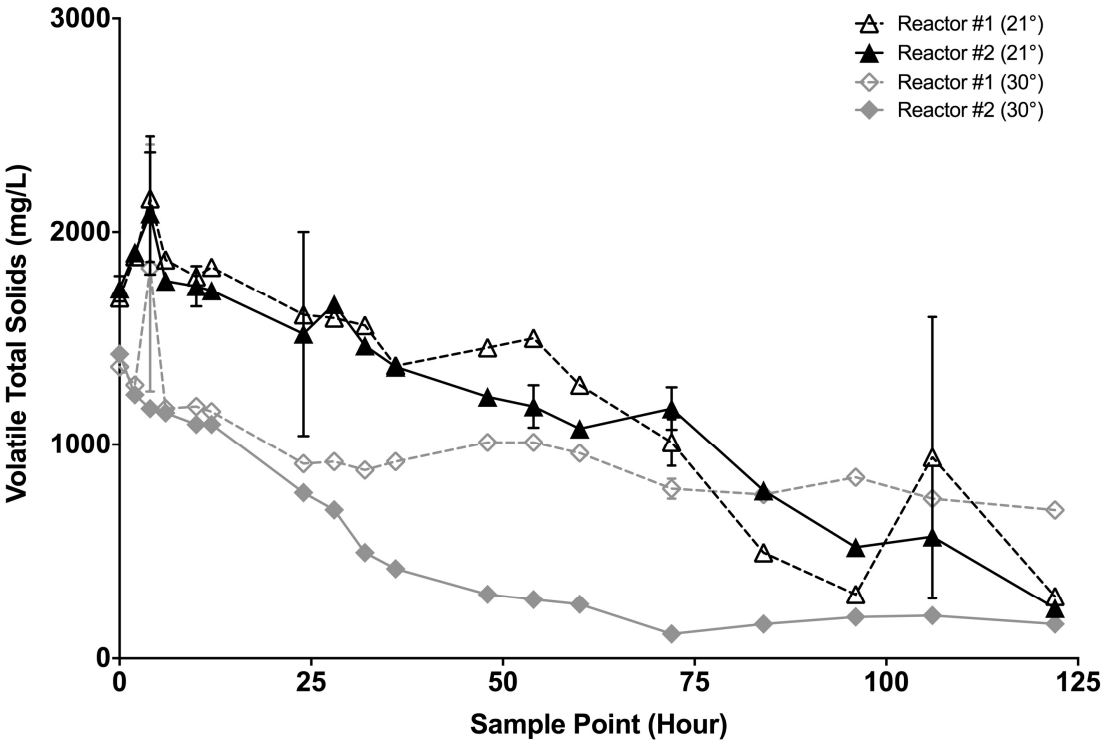


Figure SI-A7: Volatile Total Solids Concentrations for Each Reactor Run at 21°C and 30°C

10.2 Supplemental B: Fate of Triclosan, Triclocarban, and their Transformation Products in Wastewater Under Nitrifying Conditions

Table SI-B1: Standard Suppliers and Purities

Compound	Purity	Supplier
TCS	> 97%	Wellington Labs
TCC	> 97%	Wellington Labs
MeTCS	> 97%	Wellington Labs
$^{13}C_{12}$-TCS	≥ 99%	Wellington Labs
$^{13}C_{13}$-TCC	≥ 99%	Wellington Labs
$^{13}C_{12}$-MeTCS	≥ 99%	Wellington Labs
DCC	N/A	Oakwood Chemicals
MCC	N/A	Sigma Aldrich
NCC	98%	Sigma Aldrich
2,4-DCP	≥ 97%	Sigma Aldrich
d_3-2,4-DCP	98%	Cambridge Isotope
TCS-o-sulf	N/A	Toronto Research Chemicals

Table SI-2: Compound MDLs, LOQs, and Recoveries

	MDL	LOQ	Recovery (%)	MDL	LOQ	Recovery (%)	MDL	LOQ	Recovery (%)	MDL	LOQ	Recovery (%)
	TCS			MeTCS			2,4-DCP			TCS-O-Sulf		
Water (ng/L)	7.9	15.8	82.3 ± 11.8	8.5	17.0	68.1 ± 7.6	49.3	98.6	68.0 ± 11.2	53.9	107.8	64.9 ± 12.5
Sludge (ng/g)	14.4	28.8	87.5 ± 4.1	16.7	33.4	70.1 ± 5.2	14.5	29.0	77.4 ± 10.7	29.2	58.4	65.3 ± 16.1
	TCC			DCC			MCC			NCC		
Water (ng/L)	2.7	5.4	70.8 ± 3.5	58.9	117.8	73.4 ± 6.9	53.3	106.6	69.5 ± 6.4	60.7	121.4	72.7 ± 7.8
Sludge (ng/g)	8.2	16.4	90.8 ± 7.3	35.6	71.2	77.5 ± 9.2	45.8	91.6	87.3 ± 7.8	43.9	87.8	85.5 ± 4.6

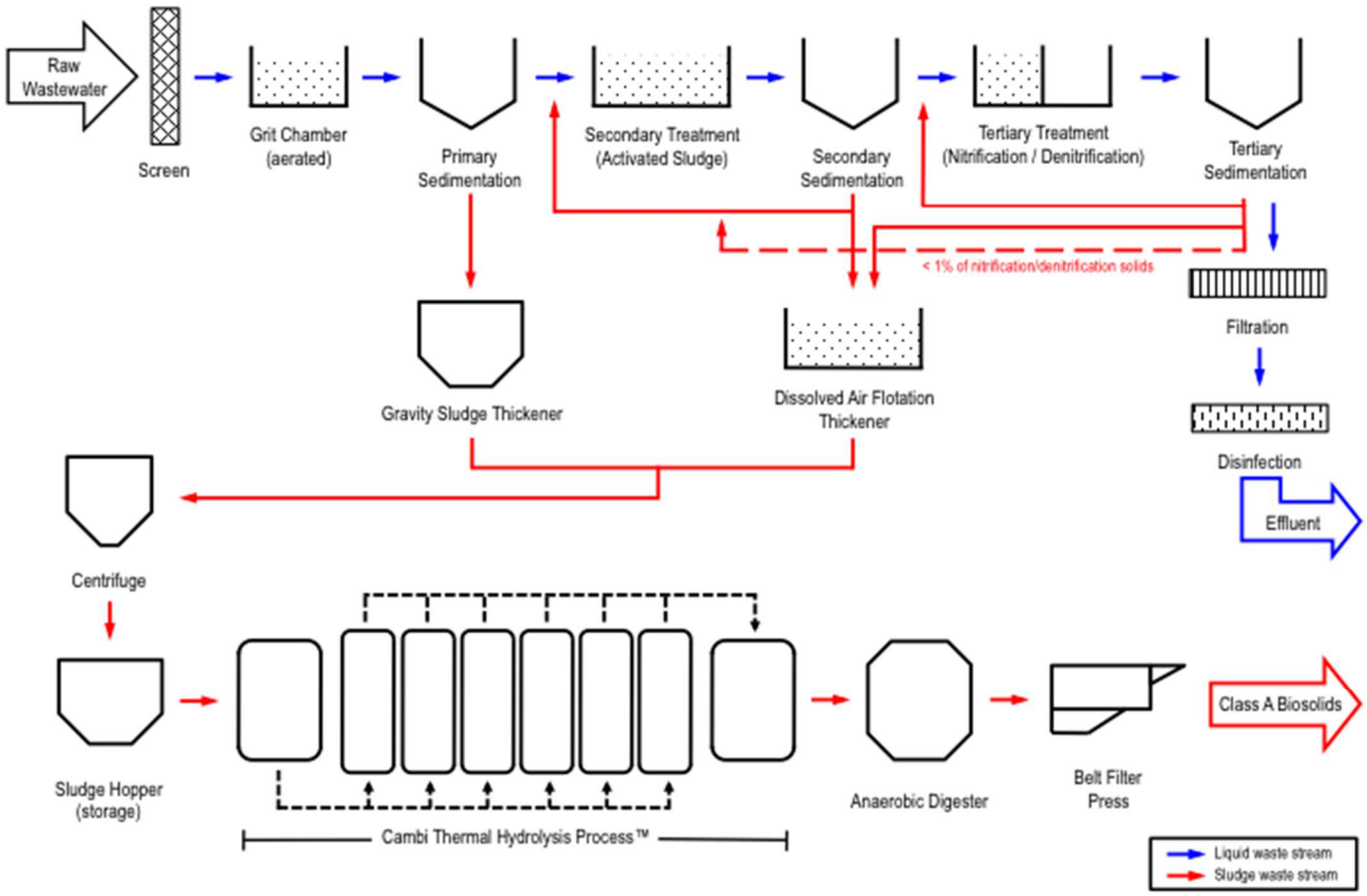


Figure SI-B1: Process Flow Diagram of Study WWTP

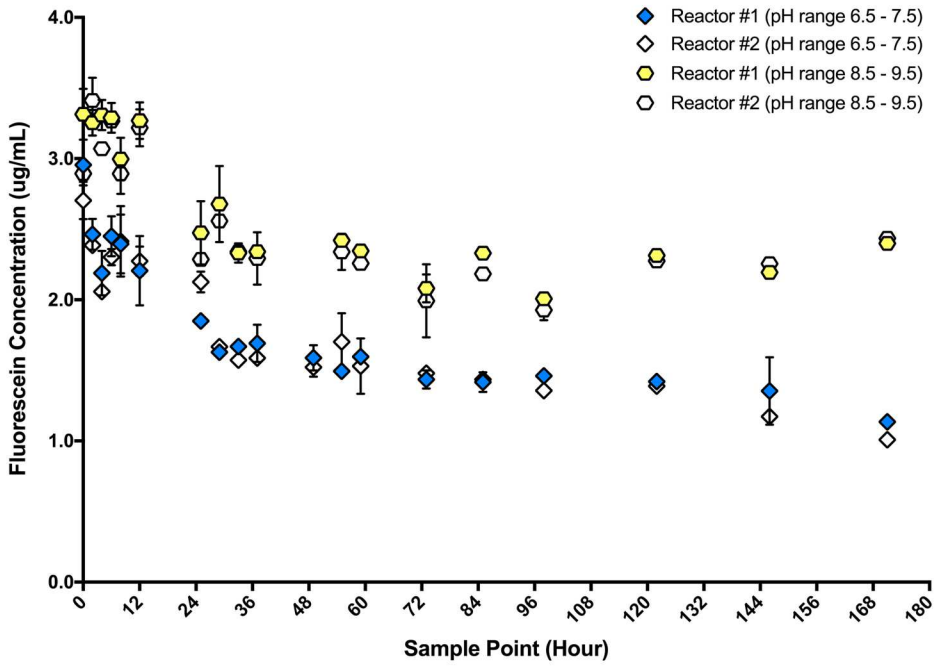


Figure SI-B2: Microbial Activity During Nitrification Treatment

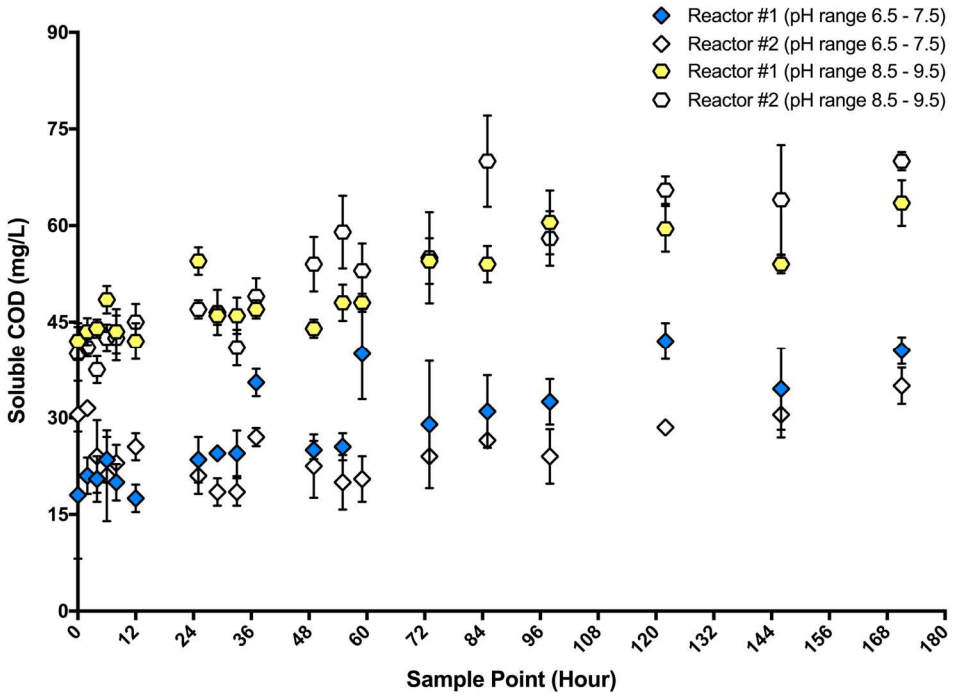


Figure SI-B3: sCOD During Nitrification Treatment

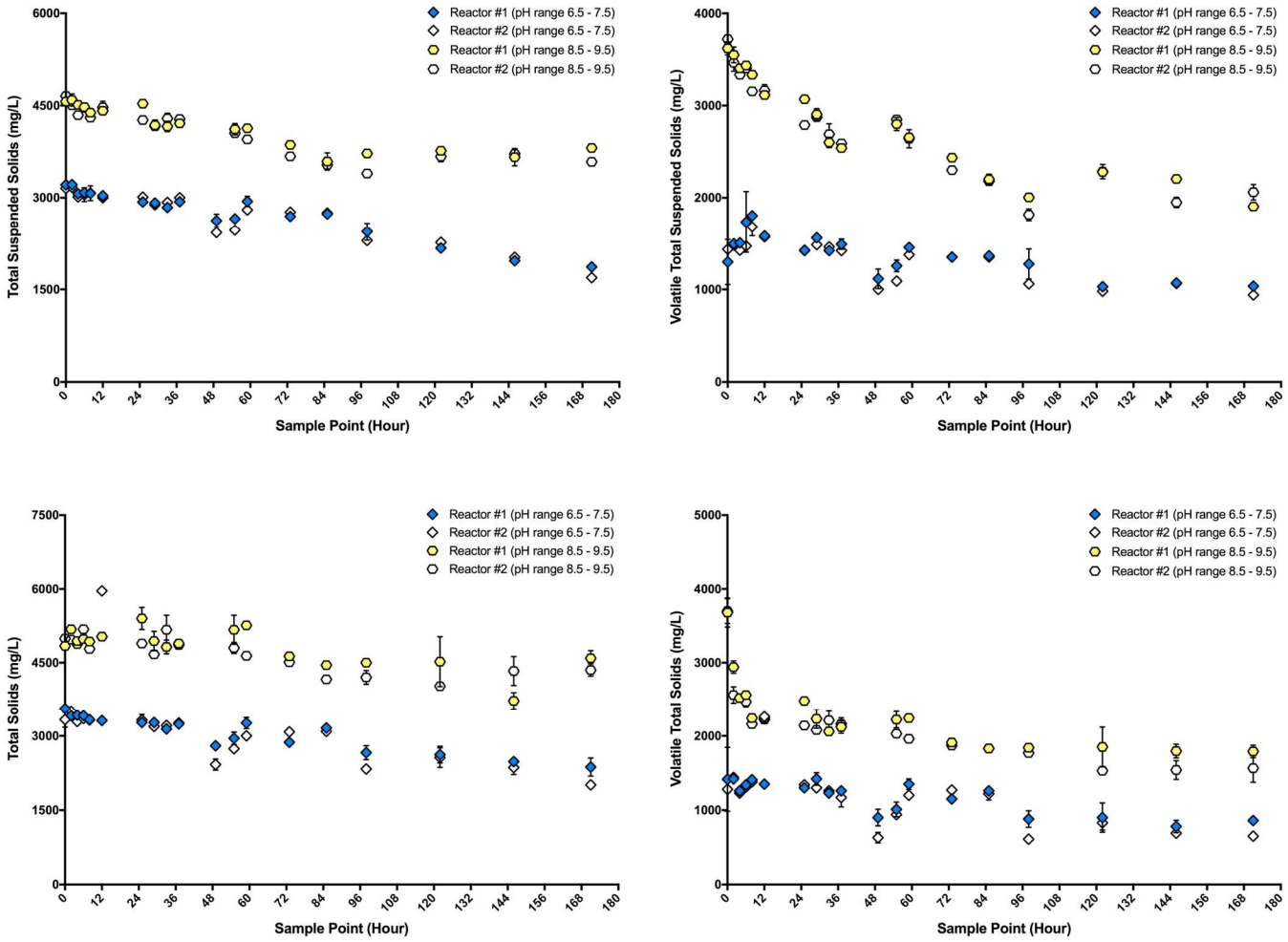


Figure SI-B4: Solids Concentrations During Nitrification Treatment

10.3 Supplemental C: Influence of Thermal Hydrolysis-Anaerobic Digestion Treatment of Wastewater Solids on Concentrations of Triclosan, Triclocarban, and their Transformation Products in Biosolids

10.3.1 Sample Extraction

10.3.1.1 TCC, TCS, MeTCS, TCS-O-Sulf, DCC, MCC, NCC, & 2,4-DCP

Samples were extracted using a Dionex Accelerated Solvent Extraction (ASE) #300 system (Dionex Corporation, Sunnyvale, CA, USA) with a 20:80 (v/v) blend of water:isopropyl alcohol (IPA). The ASE was run at a pressure of 2001 psi and a temperature of 120°C. The heat cycle was set for 6 minutes followed by a static cycle of 10 minutes and a purging of 200 seconds. Three extraction cycles in total were performed for each sample.

Sample cleanup was performed using Oasis® HLB solid phase extraction (SPE) cartridges (6 mL, 200 mg, 30 um) (Waters Corporation, Milford, MA, USA). Cartridges were conditioned with 25 mL dichloromethane (DCM):diethyl ether (DEE) (80:20 v/v). ASE extracts were mixed with 100 mL of phosphate buffer (pH ~7) and loaded on to the cartridges under vacuum. After sample loading, Oasis® HLB cartridges were washed with 10 mL of deionized water and left under vacuum for 20 minutes until dryness. A cartridge with 4 g of Na_2SO_4 on top of 1 g Florisil® was placed under the Oasis® HLB cartridges. Target analytes were then eluted with 3 x 10 mL DCM:DEE (80:20 v/v). Eluates were evaporated with a rotary evaporator and reconstituted in 1.5 mL methanol (MeOH) for instrumental analysis.

10.3.2. Instrumental Analysis

10.3.2.1 TCC, TCS, DCC, MCC, NCC, & 2,4-DCP

Instrument: Shimadzu Nexera X2 UHPLC w/ Shimadzu 8040 triple quadrupole MS

Mobile Phase: 10 mM Ammonium acetate in MeOH:ACN:H_2O (60:15:25 v/v) (0.5 mL/min)

Column: Supelco Ascentis® Express C18 reverse phase column (2.7 µm, 50 x 2.1 mm)

Column oven: 40°C

Compound	Source	Precursor m/z	Product m/z	Q1 (V)	CE (eV)	Q3 (V)
TCC	ESI-	313.00	160.00	19.0	13.0	30.0
$^{13}C_{13}$-TCC	ESI-	326.00	165.95	20.0	15.0	30.0
TCS	ESI-	287.00	34.95	17.0	9.0	13.0
$^{13}C_{12}$-TCS	ESI-	299.10	34.95	17.0	9.0	13.0
2,4-DCP	ESI-	161.10	125.05	10.0	18.0	24.0
d_3-2,4-DCP	ESI-	164.00	126.95	15.0	18.0	23.0
DCC	ESI-	278.90	125.95	25.0	15.0	13.0
MCC	ESI-	245.10	126.05	16.0	11.0	24.0
NCC	ESI-	211.10	92.10	13.0	13.0	18.0

10.3.2.2 TCS-O-Sulf

Instrument: Shimadzu Nexera X2 UHPLC w/ Shimadzu 8040 triple quadrupole MS

Mobile Phase: 0.2% Formic acid in MeOH (0.55 mL/min)

Column: Supelco Ascentis® Express C18 reverse phase column (2.7 µm, 50 x 2.1 mm)

Column oven: 40°C

Compound	Source	Precursor m/z	Product m/z	Q1 (V)	CE (eV)	Q3 (V)
TCS-O-Sulf	ESI-	366.90	286.90	15.0	15.0	30.0
$^{13}C_{12}$-TCS	ESI-	299.00	35.05	27.0	10.0	12.0

10.3.2.3 MeTCS

Instrument: Agilent 7890B gas chromatograph (GC) w/ Agilent 5977A mass selective detector (MSD)

Carrier gas: Helium (1 mL/min)

Column: DB-5-MS (length: 15 m; diameter: 0.25 mm; and film thickness: 0.1 µm)

Mode: Splitless

Injection temperature: 250°C

Chromatographic conditions: The initial setting was 70 °C (held for 5 min), ramped 20°C/min until 100 °C, then 10 °C/min to 160 °C, next 1 °C/min to 169 °C and finally 18 °C/min to 325 °C. The interface to the detector was 280 °C.

MS Conditions: The MS detector was run in electron impact ionization mode (EI, 70 eV) with an ion source temperature of 230 °C. The acquisition mode was single ion monitoring (SIM) and for quantification, the mass fragment 302.0 and 306.0 was used for MeTCS and 314.0 and 316.0 for $^{13}C_{12}$-MeTCS.

10.3.2.4 4-CA & 3,4-DCA

Instrument: Agilent 7890B gas chromatograph (GC) w/ Agilent 5977A mass selective detector (MSD)

Carrier gas: Helium (1 mL/min)

Column: DB-5-MS (length: 15 m; diameter: 0.25 mm; and film thickness: 0.1 µm)

Mode: Splitless

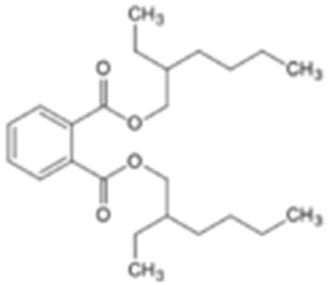
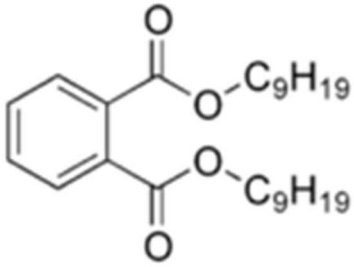
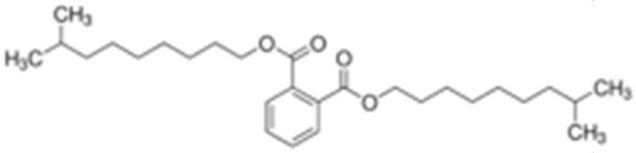
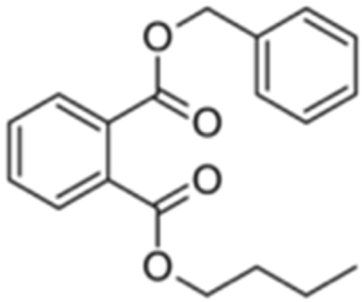
Injection temperature: 250°C

Chromatographic conditions: The initial setting was 40 °C (held for 5 min) and ramped 22°C/min until 290 °C (held for 10 min). The interface to the detector was 325 °C.

MS Conditions: The MS detector was run in electron impact ionization mode (EI, 70 eV). The acquisition mode was single ion monitoring (SIM) and for quantification, the mass fragment 127.0 was used for 4-CA, 161.0 for 3,4-DCA, and 163 for d_2-3,4-DCA.

10.4 Supplemental D: Effect of Cambi Thermal Hydrolysis Process-Anaerobic Digestion Treatment on Concentrations of Phthalate Plasticizers in Wastewater Sludge

Table SI-D1: Structures and log K_{ow} Values of Compounds Studied

Compound Name	Structure	log K_{ow}
Bis(2-ethylhexyl) phthalate (DEHP)		8.39 [18]
Diisononyl phthalate (DiNP)		9.37 [18]
Diisodecyl phthalate (DiDP)		10.36 [a]
Benzyl butyl phthalate (BBP)		4.84 [18]

[a] log K_{ow} value estimated using US EPA EPI Suite™ KOWWIN v 1.67

UHPLC-MS/MS Analytical Settings

Compound	Precursor (m/z)	Product (m/z)	Dwell time (msec)	Q1 (V)	CE	Q3 (V)
DEHP	*391.2*	*148.95*	*100*	*-27*	*-22*	*-25*
	391.2	113.15	100	-27	-10	-21
d4-DEHP	*395.1*	*153.05*	*100*	*-10*	*-21*	*-29*
	395.1	170.95	100	-10	-14	-30
DiNP	*419.3*	*71.1*	*100*	*-27*	*-22*	*-26*
	419.3	85.25	100	-27	-18	-15
DiDP	*447.3*	*85.2*	*100*	*-29*	*-21*	*-15*
	447.3	71.1	100	-29	-21	-27
BBP	*313.25*	*91.1*	*100*	*-19*	*-21*	*-15*
	313.25	148.95	100	-19	-14	-14
d4-BBP	*317.1*	*91.1*	*100*	*-14*	*-22*	*-16*
	317.1	153	100	-14	-15	-27

** Settings in *italics* indicate those used for quantitation

Nebulizing gas flow: 3 L/min
Drying gas flow: 15 L/min
DL temperature: 150°C
Heat block temperature: 350°C

Column oven temperature: 50°C

Table SI-D2: Method Detection Limits and Recoveries for Phthalate Plasticizers

	DEHP	DiNP	DiDP	BBP
MDL (ng/L)	22.3	27.2	20.9	14.6
Recovery (%)	87.1 ± 5.7	100.8 ± 10.7	89.2 ± 9.7	93.0 ± 4.9

10.5 Supplemental E: Effect of Cambi Thermal Hydrolysis Process-Anaerobic Digestion Treatment on Concentrations of Phthalate Plasticizers in Wastewater Sludge

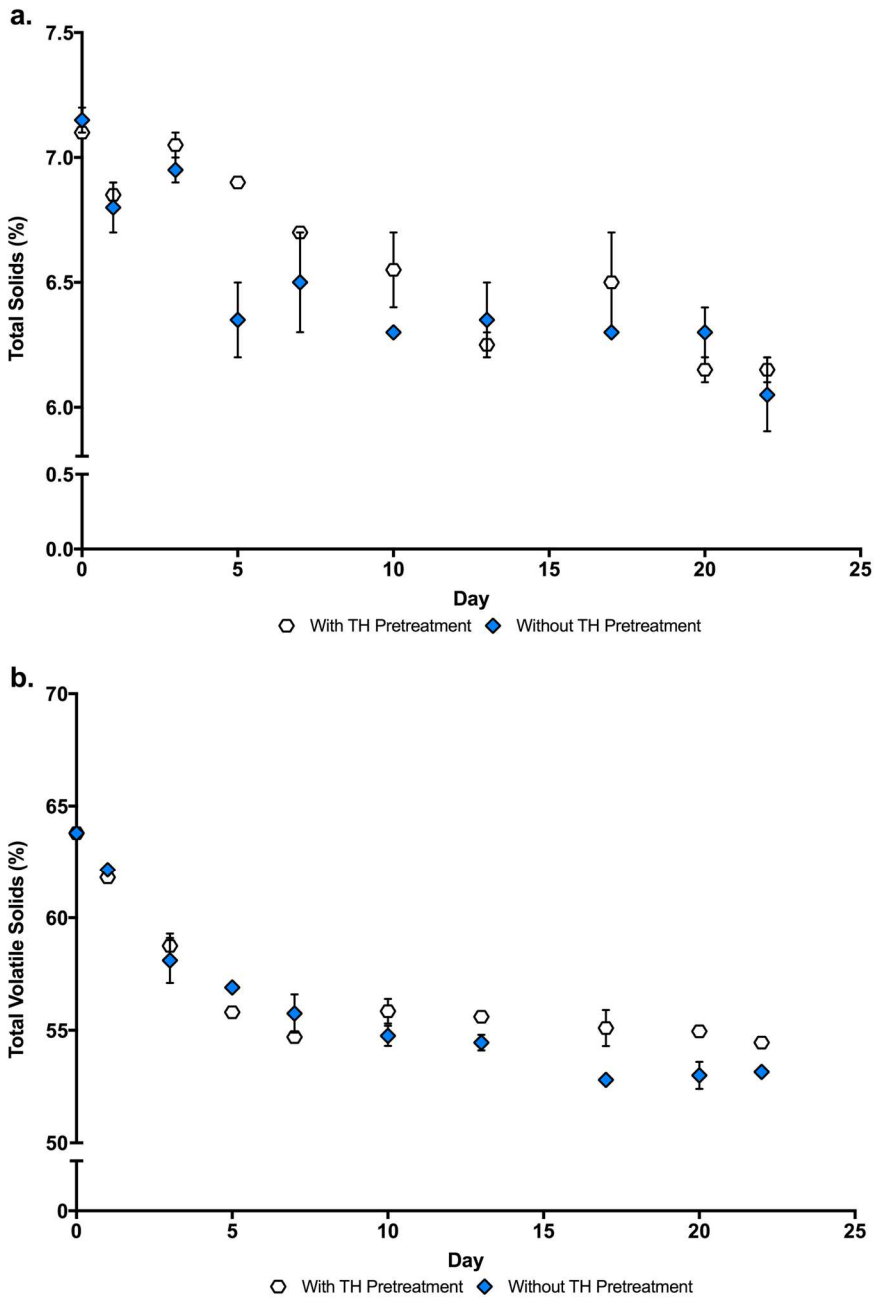


Table SI-E1: Solids Data During Anaerobic Digestion

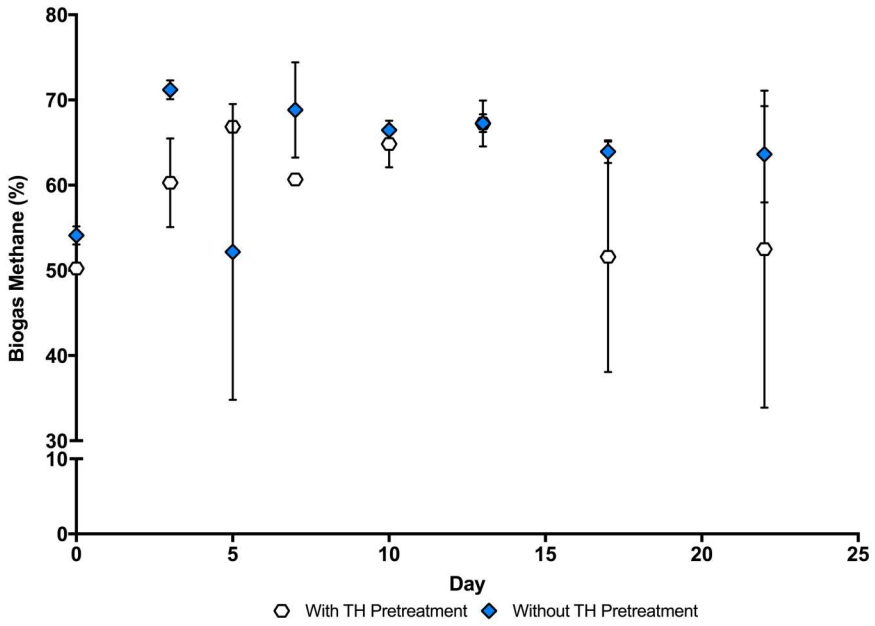


Figure SI-E2: Biogas (Methane) Makeup

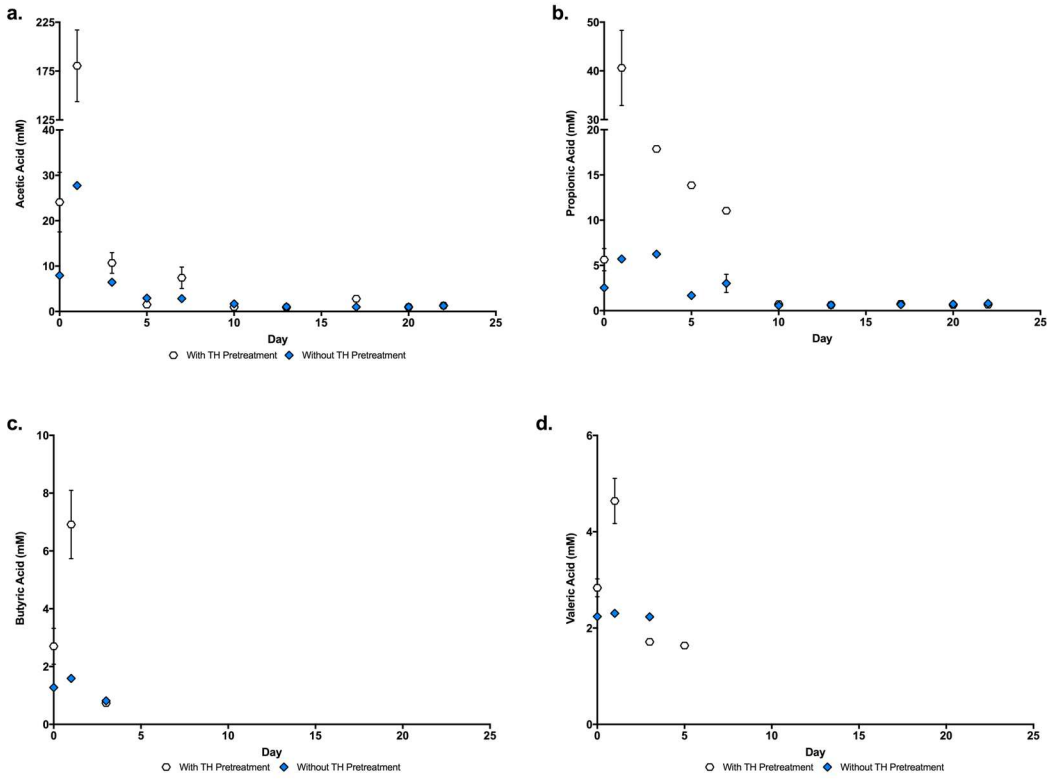


Figure SI-E3: Solids Concentrations During Anaerobic Digestion

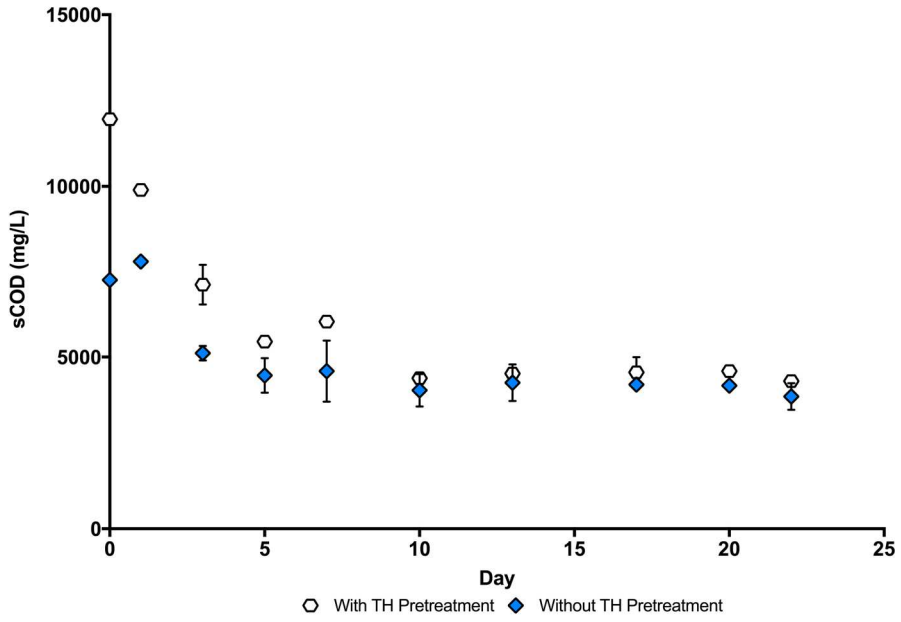


Figure SI-E4: sCOD Concentrations During Anaerobic Digestion

10.6 Supplemental F: Impact of Various Wastewater Treatment Conditions on Concentrations of 27 Emerging Contaminants and Their Estimated Concentrations in Soils

Table SI-F1: Target Compound Standard Information and UHPLC-MS/MS Conditions

Compound	Standard Supplier	Standard Purity	Retention Time (min)	ESI (+/-)	Precursor (m/z)	Product (m/z)	Pause Time (msec)	Dwell Time (msec)	Q1 (V)	CE (eV)	Q3 (V)
Aspartame	Supelco	99%	0.83	+	295.10	120.00	1.5	25	- 19	- 27	-20
				+	295.10	235.00	1.5	25	- 19	- 14	- 23
Betaxolol	Sigma Aldrich	> 98%	1.14	+	308.20	116.10	1.5	25	- 20	- 21	- 11
				+	308.20	72.15	1.5	25	- 20	- 24	- 28
Bisoprolol	Sigma Aldrich	≥ 98%	1.01	+	326.20	116.00	1.5	25	- 21	- 19	- 21
				+	326.20	74.05	1.5	25	- 21	- 26	- 29
Carbamazipine	Sigma Aldrich	≥ 99%	1.24	+	237.10	193.95	3.0	50	- 15	- 20	- 19
$^{13}C_6$-Carbamazipine	Cerilliant	99.5%	1.24	+	243.10	199.95	3.0	50	- 16	- 20	- 19
Chlorpyrifos	Sigma Aldrich	99.6%	3.15	+	349.90	97.00	1.5	25	- 21	- 35	- 17
				+	349.90	197.90	1.5	25	- 21	- 20	- 19
d_6-Methyl chlorpyrifos	Cambridge Isotope	98%	2.63	+	329.90	130.95	3.0	50	- 20	- 22	- 21
Ciprofloxacin	Sigma Aldrich	≥ 98%	0.98	+	332.10	313.85	1.5	25	- 21	- 21	- 30
				+	332.10	230.90	1.5	25	-21	- 38	- 22
$^{13}C_4$-Ciprofloxacin	Cambridge Isotope	99%	0.98	+	336.10	318.00	1.5	25	- 22	- 21	- 21
				+	336.10	290.90	1.5	25	- 22	- 18	- 30
DEET	Sigma Aldrich	97%	1.37	+	192.00	90.95	1.5	25	- 30	- 29	- 15
				+	192.00	118.95	1.5	25	- 30	- 16	- 20
d_6-DEET	Cambridge Isotope	98%	1.37	+	198.10	118.95	1.5	25	- 13	- 18	- 20
				+	198.10	90.95	1.5	25	- 13	- 31	- 16
Diltiazem	Cerilliant	99.9%	1.45	+	415.20	177.90	1.5	25	- 27	- 26	- 17
				+	415.20	149.90	1.5	25	- 27	- 44	- 27
Diphenhydramine	Sigma Aldrich	≥ 98%	1.18	+	256.10	167.05	1.5	25	- 17	- 12	- 16
				+	256.10	151.90	1.5	25	- 17	- 37	- 26
Emamectin benzoate	Sigma Aldrich	99.4%	3.45	+	886.50	158.00	1.5	25	- 40	- 41	- 29
				+	886.50	82.20	1.5	25	- 40	- 55	- 30
Flubendiamide	Sigma Aldrich	98.7%	2.00	-	681.10	253.90	1.5	25	+ 22	+ 29	+ 26
				-	681.10	273.95	1.5	25	+22	+ 17	+ 29
Fluconazole	Sigma Aldrich	≥ 98%	0.82	+	307.10	237.90	1.5	25	- 20	- 17	- 23
				+	307.10	219.95	1.5	25	- 20	- 18	- 21

Triphenyl phosphate	Supelco	≥ 96%	2.44	+	327.00	77.10	1.5	25	- 21	- 47	- 30
				+	327.00	151.90	1.5	25	- 21	- 40	- 27
Tris(2-butoxyethyl) phosphate	Sigma Aldrich	94%	2.61	+	399.20	45.00	1.5	25	- 26	- 25	- 17
				+	399.20	199.00	1.5	25	- 26	- 17	- 19
Venlafaxine	Sigma Aldrich	≥ 98%	1.04	+	278.20	58.10	1.5	25	- 17	- 22	- 21
				+	278.20	260.15	1.5	25	- 17	- 13	- 26
d_6-Venlafaxine	Cerilliant	98.6%	1.04	+	284.20	64.05	1.5	25	- 18	- 22	- 24
				+	284.20	266.10	1.5	25	- 18	- 13	- 27

** MS conditions highlighted in **RED** indicate those used for quantitation

Table SI-F2: Target Compound MDLs, LOQs, and Average Recoveries

Compound	MDL (ng/g)	LOQ (ng/g)	Recovery (%)
Aspartame	1.15	2.30	91.1 ± 6.33
Betaxolol	2.05	4.10	69.7 ± 4.39
Bisoprolol	24.7	49.4	76.3 ± 8.04
Carbamazipine	10.3	20.6	71.0 ± 3.96
Chlorpyrifos	1.04	2.08	68.3 ± 6.17
Ciprofloxacin	8.98	18.0	67.2 ± 12.1
DEET	10.4	20.8	73.4 ± 9.88
Diltiazem	7.83	15.7	82.5 ± 7.19
Diphenhydramine	13.2	26.4	60.6 ± 3.42
Emamectin Benzoate	6.17	12.3	74.8 ± 5.55
Flubendiamide	18.7	37.4	93.4 ± 3.02
Fluconazole	2.26	4.52	86.9 ± 4.11
Irbesartan	0.524	1.05	71.2 ± 7.20
Norethindrone	23.4	46.8	94.0 ± 5.06
Oxybenzone	1.69	3.38	59.6 ± 9.82
Pendimethalin	7.99	16.0	88.1 ± 3.57
PFHxA	24.8	49.6	75.3 ± 10.5
PFNA	13.8	27.6	69.4 ± 6.29
PFOA	20.0	40.0	80.2 ± 4.90
PFOS	6.87	13.7	106 ± 13.4
Prednisone	16.6	33.2	93.5 ± 13.6
Resperidone	43.1	86.2	57.3 ± 7.99
TCPP	14.2	28.4	62.8 ± 3.91
Testosterone	7.90	15.8	80.2 ± 5.31
Triphenyl phosphate	5.71	11.4	90.1 ± 4.28
Tris(2-butoxyethyl) phosphate	14.6	29.2	87.7 ± 8.23
Venlafaxine	25.5	51.0	77.9 ± 3.48

Table SI-3 Concentrations of Detected Target Compounds During Individual Treatment Stages at the Study WWTPs

		WWTP #1			WWTP #2		
		Anaerobic Digestion Influent	Anaerobic Digestion Effluent	Final Solids	Anaerobic Digestion Influent	Anaerobic Digestion Effluent	Final Solids
Aspartame	**Conc. ± SD (ng/g)**	40.9 ± 4.66	23.1 ± 3.97	9.47 ± 6.04	113 ± 5.18	ND	ND
	% Change*	---	NS	NS	---	- 100%	NS
	P-value	---	0.0739	0.1370	---	< 0.0001	> 0.9999
Chlorpyrifos	**Conc. ± SD (ng/g)**	26.6 ± 1.83	26.1 ± 1.42	12.4 ± 1.84	39.5 ± 12.2	23.0 ± 1.73	14.8 ± 0.376
	% Change*	---	NS	- 52.5%	---	NS	NS
	P-value	---	0.9578	0.0083	---	0.1946	0.5467
Ciprofloxacin	**Conc. ± SD (ng/g)**	1852 ± 58.8	1313 ± 21.2	2280 ± 775	950 ± 424	399 ± 53.3	458 ± 37.7
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.5291	0.2258	---	0.2120	0.9697
DEET	**Conc. ± SD (ng/g)**	90.0 ± 7.90	109 ± 11.4	85.8 ± 27.3	86.6 ± 8.39	80.9 ± 0.0422	64.1 ± 4.89
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.5881	0.4785	---	0.6158	0.1139
Diltiazem	**Conc. ± SD (ng/g)**	74.8 ± 5.82	29.1 ± 2.35	25.9 ± 0.207	63.2 ± 1.14	25.7 ± 0.411	26.9 ± 2.20
	% Change*	---	- 61.1%	NS	---	- 59.3%	NS
	P-value	---	0.0022	0.6929	---	0.0002	0.7159
Diphenhydramine	**Conc. ± SD (ng/g)**	521 ± 40.9	543 ± 8.37	447 ± 141	114 ± 11.2	614 ± 30.5	656 ± 177
	% Change*	---	NS	NS	---	+ 439%	NS
	P-value	---	0.9664	0.5653	---	0.0343	0.9146
Fluconazole	**Conc. ± SD (ng/g)**	122 ± 17.4	114 ± 15.3	92.8 ± 2.41	89.8 ± 0.409	98.8 ± 0.0310	88.2 ± 2.52
	% Change*	---	NS	NS	---	+ 10.0%	- 10.7%
	P-value	---	0.8246	0.3929	---	0.0179	0.0113
Irbesartan	**Conc. ± SD (ng/g)**	7.24 ± 1.47	11.5 ± 0.136	9.62 ± 1.54	97.7 ± 21.9	7.24 ± 0.731	2.18 ± 0.799
	% Change*	---	NS	NS	---	- 92.6%	NS
	P-value	---	0.0818	0.4078	---	0.0114	0.9178

Norethindrone	**Conc. ± SD (ng/g)**	7153 ± 336	1959 ± 315	784 ± 163	1864 ± 968	1618 ± 543	1322 ± 181
	% Change*	---	- 72.6%	NS	---	NS	NS
	P-value	---	0.0008	0.0505	---	0.9256	0.8958
Oxybenzone	**Conc. ± SD (ng/g)**	151 ± 14.7	103 ± 52.6	23.6 ± 1.09	141 ± 11.8	61.4 ± 7.35	8.56 ± 10.8
	% Change*	---	NS	NS	---	- 56.5%	- 86.1%
	P-value	---	0.4001	0.1676	---	0.0088	0.0279
Pendimethalin	**Conc. ± SD (ng/g)**	231 ± 12.9	281 ± 6.55	169 ± 5.03	111 ± 8.58	166 ± 11.4	137 ± 2.08
	% Change*	---	+ 21.6%	- 39.9%	---	+ 49.5%	NS
	P-value	---	0.0224	0.0022	---	0.0149	0.0828
PFOS	**Conc. ± SD (ng/g)**	33.6 ± 3.06	40.0 ± 0.734	32.1 ± 10.1	30.1 ± 0.194	39.8 ± 5.30	34.5 ± 5.15
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.6054	0.4850	---	0.2017	0.5106
Prednisone	**Conc. ± SD (ng/g)**	18958 ± 4227	8385 ± 437	4969 ± 1412	8568 ± 2012	10517 ± 2629	10140 ± 4128
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.0529	0.4765	---	0.8114	0.9917
Testosterone	**Conc. ± SD (ng/g)**	91.8 ± 6.16	ND	ND	ND	ND	ND
	% Change*	---	- 100%	NS	---	NS	NS
	P-value	---	0.0002	> 0.9999	---	> 0.9999	> 0.9999
Triphenyl phosphate	**Conc. ± SD (ng/g)**	439 ± 90.0	483 ± 21.9	367 ± 64.7	211 ± 47.4	97.8 ± 10.5	89.9 ± 13.4
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.7930	0.3148	---	0.0597	0.9607
Tris(2-butoxyethyl) phosphate	**Conc. ± SD (ng/g)**	142 ± 22.8	341 ± 63.9	228 ± 78.0	430 ± 21.0	425 ± 0.200	350 ± 49.0
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.0878	0.2825	---	0.9839	0.1772

SD = standard deviation; NS = change in concentration not significant (Tukey's multiple comparisons test) and, thus, not calculated; ND = compound not detected at or above the LOQ; * calculated change from previous treatment step

Table SI-3 (cont.) Concentrations of Detected Target Compounds During Individual Treatment Stages at the Study WWTPs

		WWTP #3				WWTP #4		
		Thermal Hydrolysis Influent	Thermal Hydrolysis Effluent	Anaerobic Digestion	Final Solids	Anaerobic Digestion Influent	Anaerobic Digestion Effluent	Final Solids
Aspartame	**Conc. ± SD (ng/g)**	ND	ND	ND	ND	77.5 ± 2.98	33.7 ± 2.02	17.4 ± 0.899
	% Change*	---	NS	NS	NS	---	- 56.5%	- 48.4%
	P-value	---	> 0.9999	> 0.9999	> 0.9999	---	0.0006	0.0096
Chlorpyrifos	**Conc. ± SD (ng/g)**	20.2 ± 3.16	13.0 ± 0.190	7.23 ± 0.899	5.69 ± 0.847	99.7 ± 3.23	52.3 ± 2.23	19.0 ± 1.93
	% Change*	---	- 35.6%	NS	NS	---	- 47.5%	- 63.7%
	P-value	---	0.0453	0.0853	0.8039	---	0.0007	0.0020
Ciprofloxacin	**Conc. ± SD (ng/g)**	743 ± 503	549 ± 143	850 ± 71.0	440 ± 117	1851 ± 73.7	918 ± 97.9	322 ± 15.5
	% Change*	---	NS	NS	NS	---	- 50.4%	- 64.9%
	P-value	---	0.8856	0.7020	0.5047	---	0.0020	0.0074
DEET	**Conc. ± SD (ng/g)**	106 ± 4.23	292 ± 67.1	88.9 ± 3.37	90.3 ± 11.0	69.5 ± 1.17	83.3 ± 0.234	89.3 ± 4.81
	% Change*	---	+ 176%	- 69.6%	NS	---	+ 19.9%	NS
	P-value	---	0.0189	0.0137	> 0.9999	---	0.0344	0.2366
Diltiazem	**Conc. ± SD (ng/g)**	44.7 ± 13.0	26.0 ± 1.84	ND	ND	63.8 ± 1.26	26.7 ± 0.773	25.3 ± 0.826
	% Change*	---	NS	- 100%	NS	---	- 58.2%	NS
	P-value	---	0.1436	0.0496	> 0.9999	---	< 0.0001	0.4446
Diphenhydramine	**Conc. ± SD (ng/g)**	265 ± 161	137 ± 81.4	324 ± 2.67	351 ± 12.5	359 ± 14.6	517 ± 1.79	609 ± 309
	% Change*	---	NS	NS	NS	---	NS	NS
	P-value	---	0.5555	0.3024	0.9896	---	0.6823	0.8692
Fluconazole	**Conc. ± SD (ng/g)**	93.6 ± 1.02	90.1 ± 3.11	103 ± 5.27	89.0 ± 5.44	95.9 ± 1.67	97.2 ± 6.47	88.8 ± 2.74
	% Change*	---	NS	NS	NS	---	NS	NS
	P-value	---	0.8364	0.1046	0.0817	---	0.9519	0.2563
Irbesartan	**Conc. ± SD (ng/g)**	25.4 ± 3.50	14.7 ± 1.44	54.1 ± 5.37	49.6 ± 6.82	16.9 ± 0.0591	34.3 ± 0.803	13.6 ± 1.28
	% Change*	---	NS	+ 268%	NS	---	+ 103%	- 60.3%
	P-value	---	0.2476	0.0040	0.7877	---	0.0006	0.0004

Norethindrone	**Conc. ± SD (ng/g)**	11775 ± 418	7604 ± 1508	627 ± 57.9	543 ± 147	7591 ± 75.3	462 ± 22.5	352 ± 14.6
	% Change*	---	- 35.4%	- 91.8%	NS	---	- 93.9%	NS
	P-value	---	0.0205	0.0031	0.9995	---	< 0.0001	0.1864
Oxybenzone	**Conc. ± SD (ng/g)**	1076 ± 41.3	1272 ± 139	54.4 ± 10.3	29.7 ± 30.0	167 ± 15.8	55.4 ± 14.3	84.5 ± 0.379
	% Change*	---	NS	- 95.7%	NS	---	- 66.8%	NS
	P-value	---	0.1737	0.0003	0.9856	---	0.0058	0.1882
Pendimethalin	**Conc. ± SD (ng/g)**	275 ± 9.11	178 ± 0.951	122 ± 0.440	120 ± 1.85	216 ± 6.96	122 ± 1.30	119 ± 0.550
	% Change*	---	- 35.5%	- 31.5%	NS	---	- 43.5%	NS
	P-value	---	0.0001	0.0009	0.9695	---	0.0004	0.7579
PFOS	**Conc. ± SD (ng/g)**	39.9 ± 1.66	45.4 ± 3.65	87.4 ± 11.8	114 ± 3.50	26.0 ± 12.9	42.3 ± 5.36	54.6 ± 9.65
	% Change*	---	NS	+ 92.5%	+ 30.4%	---	NS	NS
	P-value	---	0.8292	0.0098	0.0467	---	0.3488	0.5049
Prednisone	**Conc. ± SD (ng/g)**	11033 ± 3255	8643 ± 428	666 ± 37.3	856 ± 82.5	20816 ± 1449	9121 ± 127	4236 ± 215
	% Change*	---	NS	- 92.3%	NS	---	- 56.2%	- 53.6%
	P-value	---	0.5334	0.0278	0.9993	---	0.0017	0.0212
Testosterone	**Conc. ± SD (ng/g)**	27.2 ± 0.764	25.4 ± 4.75	ND	ND	67.6 ± 7.29	69.2 ± 6.92	79.6 ±7.55
	% Change*	---	NS	- 100%	NS	---	NS	NS
	P-value	---	0.8682	0.0016	> 0.9999	---	0.9725	0.4315
Triphenyl phosphate	**Conc. ± SD (ng/g)**	711 ± 59.7	1330 ± 40.1	721 ± 49.4	649 ± 23.6	614 ± 10.1	551 ± 16.1	366 ± 3.99
	% Change*	---	+ 87.1%	- 45.8%	NS	---	- 10.3%	- 33.6%
	P-value	---	0.0006	0.0006	0.4697	---	0.0223	0.0010
Tris(2-butoxyethyl) phosphate	**Conc. ± SD (ng/g)**	277 ± 65.0	130 ± 11.6	792 ± 67.7	631 ± 27.1	177 ± 15.4	557 ± 62.6	843 ± 36.5
	% Change*	---	NS	+ 509%	NS	---	+ 215%	+ 51.3%
	P-value	---	0.1264	0.0006	0.0967	---	0.0062	0.0140

SD = standard deviation; NS = change in concentration not significant (Tukey's multiple comparisons test) and, thus, not calculated; ND = compound not detected at or above the LOQ; * calculated change from previous treatment step

Table SI-3 (cont.) Concentrations of Detected Target Compounds During Individual Treatment Stages at the Study WWTPs

		WWTP #5			WWTP #6		
		Aerobic Digestion Influent	Aerobic Digestion Effluent	Final Solids	Aerobic Digestion Influent	Aerobic Digestion Effluent	Final Solids
Aspartame	**Conc. ± SD (ng/g)**	ND	ND	ND	114 ± 12.8	ND	ND
	% Change*	---	NS	NS	---	- 100%	NS
	P-value	---	> 0.9999	> 0.9999	---	0.0013	> 0.9999
Chlorpyrifos	**Conc. ± SD (ng/g)**	8.61 ± 0.653	ND	ND	ND	ND	ND
	% Change*	---	- 100%	NS	---	NS	NS
	P-value	---	0.0004	> 0.9999	---	> 0.9999	> 0.9999
Ciprofloxacin	**Conc. ± SD (ng/g)**	917 ± 96.7	1127 ± 165	1058 ± 73.8	1063 ± 277	1021 ± 884	3037 ± 241
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.3151	0.8363	---	0.9968	0.0706
DEET	**Conc. ± SD (ng/g)**	314 ± 13.2	78.5 ± 10.8	70.3 ± 3.75	96.0 ± 10.6	215 ± 101	105 ± 4.15
	% Change*	---	- 75.0%	NS	---	NS	NS
	P-value	---	0.0004	0.7225	---	0.2537	0.2893
Diltiazem	**Conc. ± SD (ng/g)**	37.1 ± 1.57	39.4 ± 1.62	27.2 ± 0.928	34.2 ± 2.96	29.0 ± 4.43	46.7 ± 3.27
	% Change*	---	NS	- 31.0%	---	NS	+ 61.0%
	P-value	---	0.3683	0.0067	---	0.4329	0.0328
Diphenhydramine	**Conc. ± SD (ng/g)**	68.5 ± 5.15	120 ± 38.0	107 ± 30.9	89.7 ± 20.7	391 ± 259	1431 ± 43.3
	% Change*	---	NS	NS	---	NS	+ 266%
	P-value	---	0.3038	0.8855	---	0.2632	0.130
Fluconazole	**Conc. ± SD (ng/g)**	1128 ± 103	140 ± 4.18	134 ± 3.52	95.9 ± 4.41	214 ± 2.86	176 ± 11.8
	% Change*	---	- 87.6%	NS	---	+ 123%	- 17.8%
	P-value	---	0.0010	0.9943	---	0.0012	0.0288
Irbesartan	**Conc. ± SD (ng/g)**	51.7 ± 3.08	21.3 ± 5.85	35.0 ± 1.02	60.0 ± 3.82	6.09 ± 5.26	45.8 ± 1.81
	% Change*	---	- 58.8%	NS	---	- 89.9%	+ 652%
	P-value	---	0.0087	0.0753	---	0.0017	0.0041

Norethindrone	**Conc. ± SD (ng/g)**	4756 ± 282	1816 ± 31.0	204 ± 10.5	1347 ± 73.1	544 ± 180	439 ± 4.60
	% Change*	---	- 61.8%	- 88.8%	---	- 59.6%	NS
	P-value	---	0.0008	0.0046	---	0.0114	0.6566
Oxybenzone	**Conc. ± SD (ng/g)**	1797 ± 89.3	128 ± 25.3	69.3 ± 13.2	306 ± 44.2	202 ± 8.44	99.9 ± 19.9
	% Change*	---	- 92.9%	NS	---	NS	NS
	P-value	---	< 0.0001	0.5838	---	0.0704	0.0725
Pendimethalin	**Conc. ± SD (ng/g)**	154 ± 1.16	158 ± 4.53	158 ± 1.41	81.8 ± 2.17	82.8 ± 0.364	80.7 ± 4.43
	% Change*	---	NS	NS	---	NS	NS
	P-value	---	0.4052	0.9992	---	0.9401	0.7473
PFOS	**Conc. ± SD (ng/g)**	23.1 ± 2.00	40.8 ± 1.18	54.3 ± 4.30	41.6 ± 1.50	88.0 ± 4.23	107 ± 4.96
	% Change*	---	+ 76.6%	+ 33.1%	---	+ 112%	+ 21.6%
	P-value	---	0.0168	0.0349	---	0.0026	0.0350
Prednisone	**Conc. ± SD (ng/g)**	5644 ± 58.6	4503 ± 108	6400 ± 131	2457 ± 64.7	3027 ± 158	2862 ± 976
	% Change*	---	- 20.2%	+ 42.1%	---	NS	NS
	P-value	---	0.0033	0.0008	---	0.6276	0.9556
Testosterone	**Conc. ± SD (ng/g)**	289 ± 5.58	29.3 ± 27.1	ND	ND	ND	ND
	% Change*	---	- 89.9%	NS	---	NS	NS
	P-value	---	0.0011	0.2999	---	> 0.9999	> 0.9999
Triphenyl phosphate	**Conc. ± SD (ng/g)**	234 ± 5.56	125 ± 30.8	109 ± 17.3	277 ± 12.2	206 ± 12.6	96.3 ± 38.1
	% Change*	---	- 46.6%	NS	---	NS	- 53.3%
	P-value	---	0.0266	0.7529	---	0.1194	0.0406
Tris(2-butoxyethyl) phosphate	**Conc. ± SD (ng/g)**	699 ± 5.21	264 ± 5.80	194 ± 18.6	1873 ± 165	631 ± 317	720 ± 164
	% Change*	---	- 62.2%	- 26.5%	---	- 66.3%	NS
	P-value	---	< 0.0001	0.0188	---	0.0243	0.9207

SD = standard deviation; NS = change in concentration not significant (Tukey's multiple comparisons test) and, thus, not calculated; ND = compound not detected at or above the LOQ; * calculated change from previous treatment step

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