Biotic and Abiotic Remediation of Acetaminophen with Woodchip and Biochar-amended Woodchip Adsorbents

James Patrick Wade

Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

Master of Science in
Biological Systems Engineering

Zachary M. Easton, Chair

Gene Yagow

Naraine Persaud

September 11, 2015

Blacksburg, Virginia

Keywords: acetaminophen, biochar, woodchips, adsorption, adsorbent, adsorbate

Biotic and Abiotic Remediation of Acetaminophen with Woodchip and Biochar-amended Woodchip Adsorbents

James Patrick Wade

Abstract

Pharmaceuticals and personal care products found in the environment pose a significant hazard to human and ecosystem health. While there has been significant work on the fate and remediation of pharmaceuticals and personal care products in wastewater treatment, relatively little work has explored the fate, transport and remediation of these compounds in non-point source input. This is concerning given the increasing use of pharmaceuticals in livestock production and wastewater treatment derived biosolids frequently applied to land. These experiments aimed to quantify the abiotic adsorption and biotic transformation and uptake potential of woodchips and biochar-amended woodchips as a potential sorbent strategy for diffuse acetaminophen (ACT) pollution.

Batch reactions were created in triplicate, supplied with 5 mM ACT, and analyzed over an eight hr period using ultraviolet spectrophotometry (298 nm). Ultraviolet absorbance readings for each time step then were compared to standard curves and solution ACT concentration was determined. Decreases in ACT from initial concentrations were the result of either abiotic and/or biotic. Overall, the woodchips and biochar-amended woodchips showed similar removal efficiency (16-21% of initial concentration). Whole model ANOVA analysis showed biologic activity having no significant effect on ACT solution concentration. However, within group ANOVA comparison showed significant differences between abiotic and biotic WC and abiotic and biotic WC treatments (controlling for media).

Thus, the media effect could have masked the effect of biology on ACT removal. Species capable of degrading ACT exist and further study into their ability to grow and survive on these sorbents requires further work.

Dedication

This thesis is dedicated to Timothy Rion Wade II.

Acknowledgements

I wish to thank the Biological Systems Engineering staff and faculty for their commitment, advice and help in this project. Specifically, these include Zach Easton[1], Martin Davis[1], Justin Barone[1] and Gene Yagow[1]. Additionally, I wish to thank Dr. Xia[2] and Dr. Persaud[2] for their advice and support on the project.

This work was funded by a grant from the USDA-NRCS-CIG program.

[1] Biological Systems Engineering, Virginia Tech, Blacksburg, VA 24060

[2] Crop and Soil Environmental Science, Virginia Tech, Blacksburg, VA 24060

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ACT = acetaminophen

PPCP = personal care products and pharmaceuticals

WC = woodchips

BC = biochar

ppm = part per million

ppb = part per billion

kg = kilogram

mg = milligram

µg = microgram

ng = nanogram

L = liter

Introduction

The detection, evaluation and discussion of emerging contaminants in the global environment have increased in recent years (Deo and Halden, 2013; Lapworth et al., 2012; Pal et al., 2010; Tijani et al., 2013). Due to advances in detection and analytical methods, contaminant concentrations approaching µg/g levels in soil and ng/L levels in water may now be detected (Gros et al., 2006; Hilton, 2003; Loffler, 2005; Loffler, 2003). Nevertheless, population growth and subsequent increases in human contaminant production, use and disposal will lead to greater environmental contaminant input. The consequence of emerging contaminants, on both ecosystems and individual organisms, is an area of expanding research (Gillis et al., 2014; Lawrence et al., 2012; Ortiz de Garcia et al., 2014). The regulation of these contaminants has led to mandating Environmental Risk Assessments in Europe (Meisel et al., 2009) and creating Candidate Contaminant Lists in the United States (Richardson and Ternes, 2014).

This literature review outlines the current source, transport, and fate of acetaminophen (ACT). Relevant sources are point and non-point sources while transport can be followed through water and soil matrices. The fate is dependent on various abiotic and biotic transformations and interactions. Finally, the review outlines knowledge gaps and potential solutions to remediate ACT in the environment.

a. Environmental Relevance

Organic pollutant loads have increased dramatically in soils and water bodies worldwide. One subset of organic pollutants of concern in water and soil include Personal Care Products and Pharmaceuticals (PPCPs). One pharmaceutical is acetaminophen (paracetamol), an analgesic and

antipyretic. ACT (Figure 1) is a COX-2 inhibitor – meaning it possesses biological activity by disrupting prostaglandin production – and is sold worldwide as a pain and inflammation reliever in doses ranging from 200-1000 mg. ACT is also compounded with a multitude of other prescription medication like codeine. ACT is incompletely metabolized within the liver and is excreted in urine where it can enter the environment. Fecal matter is then processed with wastewater treatment and the treated water enters waterways or activated sludge biosolids are land applied. Other ACT sources include agriculture, industry and hospitals. As a result the concentration of ACT in receiving waters and soils has increased to quantifiable levels (ppb, ppm). Important ACT properties are given in Figure 2 and Table 1 (NCBI, 2015).

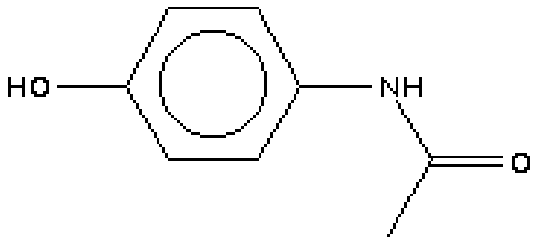

Figure 1. Acetaminophen structure.

Table 1. Acetaminophen chemical properties.

Chemical formula	$C_8H_9NO_2$
Molecular weight, g/mol	151.1626
Density, g/cc	1.263
Solubility, mg/mL (20^{o}C)	12.78
pKa	9.38
Vapor pressure, mmHg	$6.29x10^{-5}$
Henry's constant, (atm-m^3)/mol	$6.4x10^{-13}$
Melting temperature, ^{o}C	169

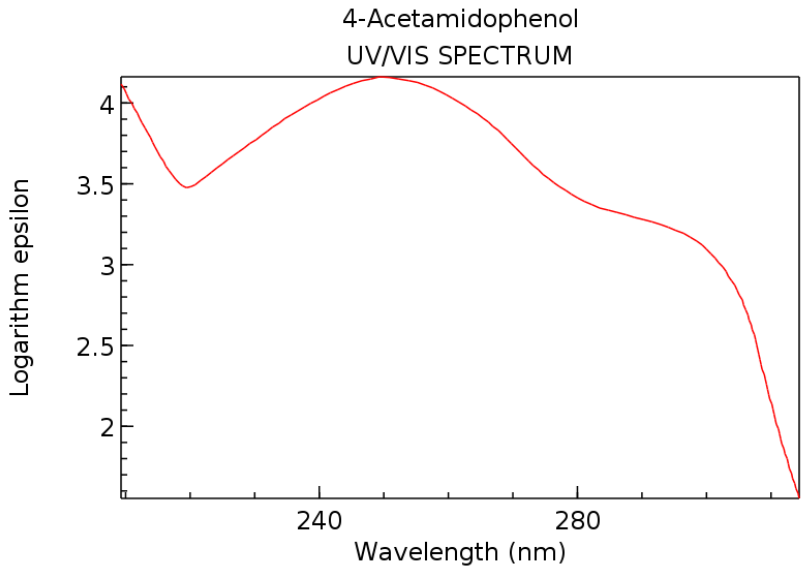

Figure 2. Ultraviolet-visible spectrum for ACT.

Emerging contaminants, particularly PPCPs, have gained attention because their ubiquity in the environment has recently been highlighted, and their numerous metabolites have unknown effects on human and ecosystem health. As PPCPs are designed for biological activity, their presence in soil and water systems, even at low ng/L and ng/kg concentrations, can be troublesome. ACT specifically (paracetamol, 4-acetamidophenol) ranks high in human over-the-counter drug usage. In 2002, the United States produced and sold 3.6×10^9 g of ACT, and many other countries also use significant quantities of ACT (Yamamoto et al., 2009). Furthermore, usage and waste from hospitals (Verlicchi et al., 2012), agriculture (Baron et al., 2014; Bernot et al., 2013) and production waste from industry (Lin and Tsai, 2009) all contribute ACT to the environment (Figure 3). The transport and fate of the parent compound varies depending on how ACT enters the environment. ACT can enter the environment as a biosolid soil application (Lapen et al., 2008; Topp et al., 2008; Xia, 2005), wastewater treatment plant discharge (Behera et al., 2011; Kasprzyk-Hordern et al., 2009; Li et al., 2012; Pedrouzo et al., 2010), reclaimed

water use for irrigation (Calderon-Preciado et al., 2011a; Calderon-Preciado et al., 2011b; Kinney, 2006) aquifer recharge (Fram and Belitz, 2011), non-point diffuse pollution such grazing livestock excretion (Kinney, 2006) and septic systems (Katz et al., 2010). Fate also depends on physiochemical properties of ACT, such as solubility, stability, and hydrophobicity, as well as its sorption, diffusion, transformation and accumulation in the environment (Tijani et al., 2013). Two physicochemical properties of ACT, Henry's constant ($6.29x10^{-5}$ mmHg) and the octanol-water partition coefficient (0.46), indicate negligible volatility and high mobility in the soil (NCBI, 2015) thus there is the potential for significant environmental diffusion and accumulation. Despite apparent environmental ubiquity the lifespan and effects of ACT in liquid and solid environments, under variable drivers like pH and temperature, is not well understood and should continue to be studied in order to develop mitigation technologies. One promising area of research could explore the physiochemical and microbial (biological) remediation strategies. For instance, delaying and trapping ACT on active surfaces could provide time for microbial degradation to remove ACT or convert it to non-toxic products (Li et al., 2014; Wu et al., 2012).

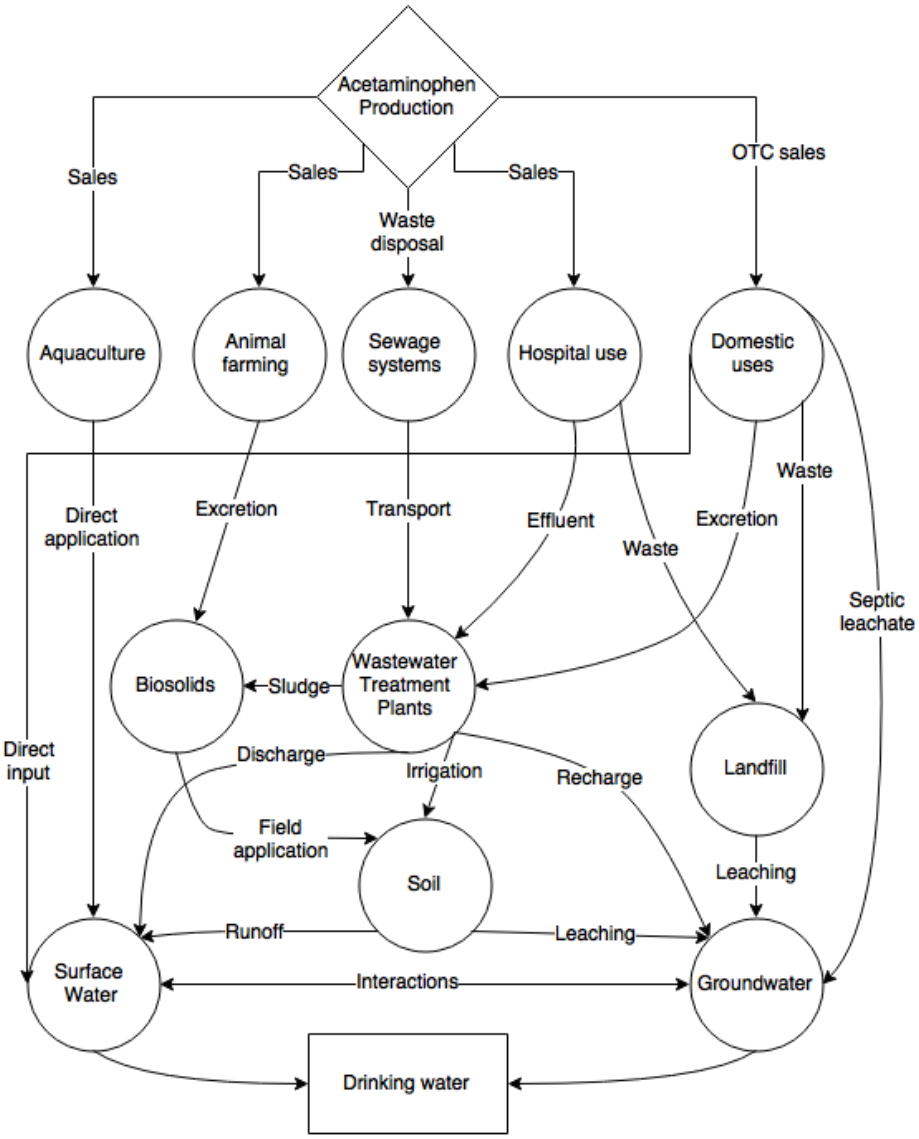

Figure 3. Sources, routes and fate of ACT. Adapted from Deo, R., and R. Halden. 2013. Pharmaceuticals in the Built and Natural Water Environment of the United States. Water 5(3):1346-1365. Used with author's permission.

b. <u>Frequency of Detection and Concentrations in the Environment</u>

ACT has been detected in many environmental matrices. Surface water, groundwater, ocean, soil, and sediment have been assessed with a range of ACT concentrations. Researchers (Bernot et al., 2013) detected ACT in surface water 56% of the time with mean concentration of 0.0172

µg/L, while United States scientists (Kolpin et al., 2002) detected ACT in surface water in 24% of samples with mean concentration of 0.110 µg/L. Groundwater concentrations detected ACT in 0.32% of samples with maximum concentration of 1.89 µg/L (Fram and Belitz, 2011). Seawater ACT levels were measured as high as 297 ng/L in one study (Afonso-Olivares et al., 2013). Soil irrigated with reclaimed wastewater showed ACT accumulating over 1000% relative to the applied load resulting in maximum 1,640 ng in 30 cm soil cores (Kinney, 2006). Biosolid application to agricultural fields showed rapid ACT disappearance (3-5 hours post-application) depending on application method (Lapen et al., 2008). This suggests a rapid dispersion into the environment following biosolids application, though the ultimate fate of the biosolids-derived ACT has yet to be assessed. Transport of ACT was shown to be rapid through large soil pores that directly access tile drains (Lapen et al., 2008). Furthermore, the fluidity of the applied biosolids was an assumed driver of this transport. However, the accumulation of ACT in soil was observed despite its high water solubility (Lapen et al., 2008). ACT fate in biosolid field application can be attributed to a few variables, such as application method, pore networks, fluidity of biosolids, rainfall, and drainage characteristics.

c. Common Sources of Acetaminophen in the Environment

As with other emerging contaminants, an important focus of work concerning ACT has been the search for environmental sources of the compound. Much focus has been paid to incomplete wastewater treatment processes and land-applied biosolids as primary sources of emerging contaminants (Mompelat et al., 2009). Interaction with groundwater and surface water implies non-point sourcing of ACT to waterways by discharge and incomplete degradation. In a comprehensive study of United States waterways, the maximum ACT concentration observed in

surface water was 10 µg/L (Kolpin et al., 2002). Other studies have observed ACT in global waterways: 42 ng/L mean detected Ebro river basin (Gros et al., 2006), 0.11 µg/L maximum detected in Indiana streams (Bunch and Bernot, 2011), 85 ng/L ACT detected in Australian rivers (Scott et al., 2014), and 297 ng/L maximum ACT concentration detected in seawater (Afonso-Olivares et al., 2013). Acetaminophen inputs range from residential, industrial, and agricultural sources.

i. Point Sources

Most well characterized sources of environmental ACT are point sources. Improper household disposal of drugs through toilets and refuse discharge into waterways are obvious origin points (Bound and Voulvoulis, 2005). Septic tanks have also been identified as ACT sources among a list of emerging contaminants found in household discharge (Dougherty et al., 2010; Katz et al., 2010). As urbanization and population increase, the contaminant burden of septic systems will grow in proportion to their prevalence. Another important source of environmental ACT is hospital wastewater effluent (Mompelat et al., 2009; Sim et al., 2011). In one case study, 4.1 µg/L ACT was observed in hospital effluent (Verlicchi et al., 2012). Other reports have observed hospital ACT effluents with median concentrations of 62.25 µg/L (Lin and Tsai, 2009) and 36.95 µg/L (Lin et al., 2008) in Taiwan. Finally, a mean concentration of 16 µg/L was observed in a Spanish private-health care center sewer (Gomez et al., 2006). Thus, hospital wastewaters, in places where there is leakage in the wastewater infrastructure or direct discharge to receiving waters, can provide a significant source of ACT to the environment. A final point source that must be accounted for in the search for the origins of environmental ACT is industrial

wastewater. ACT was identified in the wastewater effluent of pharmaceutical production facilities with a maximum concentration of 417 µg/L (Lin and Tsai, 2009).

ii. Non-Point Sources

In addition to the point sources listed above, non-point sources of environmental acetaminophen exist in agriculture. Both land-applied biosolids (Gottschall et al., 2012; Topp et al., 2008) and animal excretion (Bernot et al., 2013; Veach and Bernot, 2011) have been identified as indirect routes of entry of ACT into the environment. A rural Indiana stream, receiving confined animal feeding operation discharge, was screened for a suite of contaminants. ACT had mean concentration of 17.2 ng/L and was detected in 56% of samples collected. ACT loading was higher downstream where there was increased discharge up to 171 µg/m/d (Bernot et al., 2013). While land-application of biosolids has been identified as an entry route for environmental ACT, different application practices have been correlated with differing environmental burdens. One study compared surface runoff of fields that had been treated with injected biosolids versus broadcast application (Topp et al., 2008). Initially, runoff from the broadcast applied biosolids field had ACT concentrations below detection limits, but by day 36, this had increased to 146 ng/L. The authors hypothesized this delayed increase in concentration was due to release of chemically conjugated or physically sequestered ACT species. Injected biosolid ACT concentrations were below limits of detection and quantification, 10.6 and 35.3 ng/L, respectively, denoting measurable or reliably measurable quantification, respectively. The results indicate that biosolid injection, as opposed to broadcast application, reduced ACT runoff. Thus, a potential best management practice to reduce ACT burden is injecting biosolid directly into the subsoil. Injecting ACT subsurface could increase the potential for leaching to groundwater, but

the authors found that the ACT was quickly bound to soil residue, (88% immobilized after 2 days incubation), which indicates reduced potential for groundwater leaching (Li et al., 2014). Other studies have corroborated these findings, for instance, groundwater ACT concentrations down gradient of landfill leachate, showed ACT below detection limits (0.009 µg/L) (Buszka et al., 2009). Agricultural tile drainage systems have also been monitored for ACT. Liquid municipal biosolids were applied either to soil surface and then incorporated within 24 hours or with a subsurface deposition slurry applicator. ACT was not present in biosolid and was spiked to 4,475 ng/L (Lapen et al., 2008). The soil surface applied biosolid resulted in a maximum observed ACT mass load of 42,107 ng/15 min; however, tile drainage ACT concentrations collected 3-5 hours post-application were below the limit of quantification (35.3 ng/L) (Lapen et al., 2008). Overall, the authors observed a higher loading from surface applied biosolid versus subsurface applied biosolid with an predicted export ratio of 0.14 subsurface to surface application (Lapen et al., 2008).

Interaction between sediment and water matrices also has been studied. Kinney, (2006) found that ACT, possessing a low octanol-water partition coefficient ($\log K_{ow}$ of 0.46) can rapidly form bound residues with sediment leading to low persistence (< 20 days). Another environmental ACT source is reclaimed wastewater, commonly used to meet increasing water demands for irrigation and groundwater around the globe. However, this water is still designated non-potable and is thus only used for crop irrigation and aquifer recharge. ACT has been observed in reclaimed water at concentrations between 8.3 and 65.3 ng/L (Kinney, 2006). Increases in total ACT mass in soil irrigated with reclaimed water denotes potential for long-term increases in ACT soil concentration (Kinney, 2006). Furthermore, biotic transformations were the dominant removal mechanism for ACT from water/sediment systems, again leading to low persistence in

water (Loffler, 2005). Non-point ACT sources can be mitigated with different strategies – biosolid injection, pre-treating reclaimed water with sludge, biofilter sorption and microbial metabolism – to reduce groundwater and surface water pollution.

d. Fate and Transport of Environmental ACT

i. Sorption Kinetics

Adsorption is the physiochemical process through which molecules attach to solid surfaces by physical and chemical interactions. Characteristics involved in adsorption include surface area, pore network, hydrophobicity and quantity and quality of surface energy. Adsorption may also involve capturing and retaining the three-dimensional structure through pore networks present in the adsorbent (Cabrita et al., 2010). Adsorption, easily evaluated through the octanol-water partition coefficient, K_{ow}, increases as the K_{ow} increases. A low K_{ow} value predicts low soil sorption and high K_{ow} value predicts high soil sorption. As ACT has a low K_{ow} (0.46) relative to other over-the-counter drugs like ibuprofen (K_{ow} of 3.97), it has low hydrophobicity and is unlikely to quickly adsorb to soil (Lin et al., 2010). However, a better predictor for soil sorption is the specific soil characteristics. For instance, surface charge, directly and indirectly controlling the physical and chemical soil properties, acts as a filter for pollutants. Dispersion, flocculation, coagulation, electrophoretic mobility, solubility, and adsorption and movement are all affected by the surface charge characteristics of soils (Bolan et al., 1999). Specific solutes, such as ACT, can therefore behave variably depending on the specific soil characteristics. Two types of surface charges exist, permanent which is independent of solution composition, and variable which changes with solution concentration changes. These charges are predicated on the functional groups inherent to the soil composition. Both positive and negative charges exist due

to three processes: isomorphous substitution (permanent charge), protonation and deprotonation (variable charge), and specific adsorption of cations and anions (variable charge) (Bolan et al., 1999; Meuser and Van de Graaff, 2011). Thus, the amount of positive and negative charge present in the soil can affect the transport or adsorption of ACT and alterations in soil surface charge can release adsorbed ACT or increase ACT adsorption.

Other substances, either in isolation or in various matrices have the potential to increase adsorption of ACT. When utilizing activated carbons for ACT adsorption, both Langmuir and Freundlich adsorption models may be applied depending on whether monolayer (Cabrita et al., 2010; Chang et al., 2015; Ribeiro et al., 2011) or multilayer (Jung et al., 2015) adsorption is assumed. The adsorption kinetics are dependent on the adsorbent surface area and pore quantity and quality (Ruiz et al., 2010). Pseudo-second order kinetics best describe acetaminophen adsorption with activated carbons (Basha et al., 2015; Cabrita et al., 2010; Chang et al., 2015; Ruiz et al., 2010). Uptake is initially rapid, followed by a slower phase as equilibrium conditions are reached. The greatest uptake rate (52.7 mg/g-min) was observed for commercially prepared wood activated carbon, while the lowest uptake rate (2.5 mg/g-min) was observed for polyethylene sourced activated carbon. Half-lives were 3.3 and 46 min, respectively. Furthermore, commercially activated coal carbon produced an observed uptake rate of $1.30*10^3$ mg/g-min and half-life of 59 min and performed better than its oxidized form ($0.50*10^3$ mg/g-min; 214 min). A well-developed, interconnected pore network, which allows aqueous solution to enter inner pores (i.e. wettability of carbon structure), is predicted to best adsorb ACT. The mesopores allow movement into internal surfaces (also called transport pores). Then micropores can be utilized for adsorption given a correct micropore diameter (0.7 to 1.2 nm) (Cabrita et al., 2010; Ruiz et al., 2010). These micropores were screened and measured ranging from 0.7 nm to

1.2 nm depending on the carbon source used (Cabrita et al., 2010). The 1.2 nm pore size reflected the greatest adsorptive capacity (commercially activated wood) while the 0.7 nm pore size, reflecting peach stone and cork powder biomass residues, had less adsorptive capacity. The plastic carbon source, with mean pore size of 1.0 nm, resulted in least adsorption (Cabrita et al., 2010).

ii. Partitioning in Different Phases

The partitioning of ACT into solid or liquid phases is vital to understanding its environmental fate and transport. ACT volatility to gas is negligible, owing to its Henry's law constant of ($6.4*10^{-13}$ (atm-m^3)/mol). Yamamoto et al. (2009), measuring temporal ACT photolysis potential, revealed half-lives ranging from 35 to 56 hr and was a function of temperature while Basha et al. (2015) measured negligible photolysis removal. Thus, the sorption of aqueous ACT to soil and sediment is essential to predict transport and fate. River sediment adsorption of ACT was observed with a K_d (sorption coefficient) ranging from 1.0 to 2.8 L/kg. Additionally, organic carbon-based sorption coefficient (K_{oc}) ranged from $1.7*10^2$ to $1.3*10^4$ L/kg C. These results indicated that sorption to soil is moderately dependent on the organic content of soil. Furthermore, the pK_a value of ACT (9.38) denotes a positive charge at neutral pH and capacity for cation exchange with soil (Yamamoto et al., 2009). Activated sludge, a biosolid often used in field application, showed moderate ACT adsorption potential when compared to sterilized sludge (28.1% removed), which was due to sorption only. The neutral and hydrophilic character of ACT cannot efficiently adsorb to the hydrophobic character intrinsic to sludge. The authors noted a positive correlation between the K_{ow} value and adsorption only removal efficiency. However, when using natural sludge (microorganisms present), ACT showed 50.7% removal in the first

hour, steady aqueous concentrations for the next two hours, and an overall 85.2% removal by the sixth hour. This is the combination of biodegradation and sludge adsorption. Aerobic biodegradation is promoted by the electron donating hydroxyl group present in ACT and the results are consistent with this hypothesis (Fan et al., 2014). Thus, ACT physiochemical adsorption is a baseline which biodegradation can increase given correct environmental parameters and organisms.

iii. Mobility

Spatial and temporal variation of environmental ACT presence creates unique challenges for developing mitigation strategies. A temporal variation study of Indiana agriculture and suburban watersheds concluded that ACT concentrations were similar between the two land uses. ACT was detected in 84% of the samples ranging from 1.6 to 460 ng/L. The suburban area had highest ACT concentration in July, attributed to low flow and thus higher concentration, while the agriculture concentrations were highest in the winter, attributed to lower irradiance (e.g., sunlight photolysis) , temperature and higher loading. Overall, ACT concentrations were positively correlated only to water column dissolved oxygen. Other factors, such as discharge, turbidity, total rainfall, pH and temperature, showed weak relationships. The agriculture site total ACT concentration was influenced by pH, chlorophyll-A concentration and total rainfall in previous 10 days (Veach and Bernot, 2011). Veterinary medicine concentrations were increased significantly adjacent to a swine confined animal feeding operation. However, these concentrations declined rapidly downstream. ACT, in contrast, showed no spatial variation nor temporal variation across or within the study sites (mean 0.017 µg/L) (Bernot et al., 2013). The upper White River watershed was screened at ten headwater streams for ACT (and other PPCPs)

during baseflow conditions in July 2008. Two agricultural sub-watershed samples (>90% land use) showed acetaminophen ranging from 0.088 to 0.318 µg/L. The other, urbanized samples showed negative correlations to pharmaceutical concentrations. Thus, ACT entering waterways from urban sources is buffered through sewage treatment (degradation, dilution), while agriculturally dominated lands are not buffered (Bunch and Bernot, 2011). Overall, non-point acetaminophen sources (agriculture, individual sewage treatment systems) are significant ACT contamination contributors. An Indiana stream study, receiving only confined animal feeding operations effluent, used a conservative tracer study to determine travel distance for four PPCPs. Results showed ACT was transported 30.5 km downstream with a loss rate of 0.002 mg/km^2/min and loss velocity (loss relative to abundance) 0.04 km/min (Bernot et al., 2013). This transport distance was an order of magnitude greater than nitrate and triclosan. Additionally, Bernot et. al (2013) observed ACT concentration decline over 20 km relative to a conservative tracer, though the not significantly temporally variable (below LOD to 0.14 ppm). Urban sewage treatment dilutes endogenous concentration from any non-point upstream sources. However, another study showed hydrolysis and biodegradation are negligible over 30 days in microcosm experiments, which led authors to conclude that indirect photolysis reactions degrade ACT (Lam et al., 2004).

Li et al. (2014) quantified ACT fate in soil and measured eight intermediates produced under a 120-day aerobic incubation. ^{14}C labelled ACT parents were mineralized to 17% $^{14}CO_2$ in soils. Furthermore, sterilization of the soil or amendment with biosolids inhibited ACT mineralization. A high proportion of bound and non-extractable residues were observed in all soils after 2 days and the fraction of ACT parent compound in the soil was negligible after 30 days. Thus, microbial activity affects mineralization, non-extractable and bound residues (Li et al., 2014). A water/sediment compartment study showed ACT forming rapid and extensive bound residues

with low persistence in water column (Loffler, 2005). Upon sediment contact, the authors state that ACT was bound rapidly; moreover, the transformation products (TP) were assumed to aid in strong sediment binding. These TPs then can be incorporated into active biomass (Loffler, 2005).

Municipal biosolid application (ACT at 4,475 ng/L) to tile drained agricultural fields was below detection limits 3-5 hours after application. Lapen et al. (2008) cite microbial degradation, lability, or adsorption to solids in the soil as mechanisms for removal (Lapen et al., 2008). Another biosolid application study measured ACT below detection limits for subsurface injected biosolids but when biosolids were surface applied ACT was detected concentrations in runoff ranged from 47 ng/L to 146 ng/L, which increased over time. Authors hypothesized this was the result of chemically bound sediments being mobilized (Topp et al., 2008). Thus, the ability of acetaminophen to move in soil depends on microbial communities, bound residue formation, and potential for water solvation. Once suspended in water, acetaminophen has the ability to travel many kilometers. The key to reducing waterway ACT concentrations is to foster the interaction of ACT with sediment or other chemically active surfaces through best management practices (injection, pre-treatment), which can then maximize physiochemical adsorption and microbial metabolism.

e. Mitigation and Remediation Strategies

i. Physiochemical Remediation Approaches

While there are many studies in the literature detailing wastewater treatment plant technology to remediate water-based ACT (Tijani et al., 2013), there are comparatively few studies that explore non-point source ACT mitigation technologies. WWTP technology, such as activated carbon can

be applied to non-point source pollution mitigation. Several different materials, which have been used as adsorbents in industry, could potentially be repurposed for field-level ACT mitigation. These include various carbon media, titanium dioxide (TiO_2) coated pellets, and cork/plastic adsorbents.

Activated carbons were used to determine ACT removal kinetics and adsorption capability and authors observed pseudo-second order kinetics with intraparticle diffusion (Ruiz et al., 2010). The surface heterogeneity of the activated carbon with oxidation was correlated to adsorption capability and removal rate. Furthermore, carbon wettability enhanced ACT transfer to pores and thus increased removal. In contrast, oxidized carbon surfaces reduced adsorption rate and removal efficiency due to water competition. Mestre et al. (2009) studied cork and plastic carbon adsorbents and found that they outperformed commercial activated carbons for ACT removal because of retention in mesopores and accessibility to inner pores – mesopores and micropores must be large enough to fit the molecule internally (Mestre et al., 2009). Another study (Mestre et al., 2007) used cork activated carbons and cork powdered activated carbons for ACT removal and observed that powdered activated carbon, possessing more developed micropores, resulted in greater than 90% removal. The study assumed pseudo-second order kinetics and Langmuir adsorption. Activating a carbon base for photocatalytic degradation, Basha et al. (2015) showed a 10% by weight TiO_2 coating of adsorbents provided 28.4 mg/g adsorption that followed Langmuir kinetics.

A study of two bioadsorbents (sugar cane bagasse and vegetable sponge) on ACT removal under varying influent concentrations (1 to 100 µM) at pH 7 quantified ACT removal rates of 120.5 and 37.5 µg/g for sugar cane and vegetable sponge, respectively (Ribeiro et al., 2011). Another

study used three activated carbons created from urban (plastic; physical activation) and industrial residues (cork and peach stone; chemical activation) and compared them to two commercial (wood and coal; physical activation) carbons for ACT removal. Overall, highest adsorption was observed for wood commercial carbon, followed by cork powder, peach stone, coal and plastic activated carbon. Porosity experiments showed wood carbon possessing well-developed mesopores and high volumes of small micropores. The chemically activated carbon (cork and peach stone) also showed a large network of micropores. However, the authors noted desorption from this micropore network is easier than from the activated carbon. Reaction kinetics showed similar results, pseudo-second order fitting, where wood carbon showed fastest adsorption, followed by cork, peach stone, coal and plastic (Cabrita et al., 2010). Eucalyptus lignin-based chemically activated carbons was used for ACT adsorption study (Cotoruelo et al., 2011). Three different carbons were produced with increasing micropore amounts. ACT was most adsorbed with highest total surface area, micropore and mesopore volume, and external surface area. However, authors noted as temperature decreased, ACT adsorption also decreases, which denotes an endothermic adsorption mechanism (Cotoruelo et al., 2011). This is opposite to observations using benzoic and salicylic acid adsorbates. Kinetics showed a multi-step adsorption mechanism. First, a sharp decrease in adsorption rate is observed as surface area is covered and easily accessed pores are utilized. After half an hour an intermediate decreasing adsorption rate was observed. Finally, equilibrium was reached after several days. The more developed structures showed higher adsorption rates due to better macropore and mesopore volume. Overall, van der Waals interactions controlled adsorption, modelled with Langmuir. The controlling step for ACT adsorption involved mass transfer to internal pores (diffusion coefficients 10^{-12} to 10^{-11} cm^2/s) (Cotoruelo et al., 2011).

Biochar adsorbents are another class of carbon media. Field-scale application of biochars has produced promising results for agriculture: nutrient use, crop enhancement, carbon sequestration and contaminant sequestration (Huang et al., 2014; Ippolito et al., 2012; Laird et al., 2010; Park et al., 2013). Furthermore, biochar has similar properties (surface area, pore area, pore volume, pore diameter, cation exchange capacity) to commercially activated carbon used in WWTPs (Azargohar and Dalai, 2006; Park et al., 2013).

Abiotic adsorption is dependent on the surface and pore properties of the adsorbent and the physiochemical characteristics of the adsorbate. Activated carbons are observed to have high adsorption rates and affinity when chemically produced resulting in hydrophobic surfaces with well-developed micropores (Cabrita et al., 2010). In addition, oxidation of carbon structures favored ACT transfer to carbon pores. However, overall rate and removal of ACT in oxidized carbon is reduced due to the competition from water molecules (Ruiz et al., 2010).

Physicochemical remediation strategies are based on the quality of the adsorbent utilized. The adsorbent surface area, pore diameter, pore area and pore volume all contribute to the monolayer adsorption of ACT. Since ACT adsorption is endothermic and spontaneous (Cotoruelo et al., 2011), the capacity for adsorption is limited by the quantity and quality of interactive sites and pores available and the quantity of activated sites. Thus, the best adsorbent will have well-developed pore networks, large surface areas, and a high area of activated surface sites (Cabrita et al., 2010; Ruiz et al., 2010).

ii. Biological Remediation

Biological metabolism may also aid in ACT removal. In a batch study of biotic and abiotic pharmaceutical removal with variable organic matter concentrations (Maeng et al., 2011), noted ACT removal greater than 88% under biotic conditions, but only 11% under abiotic conditions. They concluded that biodegradation is a significant fate of pharmaceutically active compounds.

To further investigate this, microorganisms capable of degrading ACT have been studied. A *Penicillium* species had been isolated from a mold in ACT solution and found to metabolize ACT as a sole carbon source, degrading the compound to 4-aminophenol and acetate with specialized enzyme systems and pathways (Hart and Orr, 1975). In addition, aerobic *Pseudomonas* and *Stenotrophomonas* species, isolated from a membrane bioreactor, are capable of complete ACT degradation for sole energy, carbon and nitrogen sources. A mixed culture was observed to synergistically interact to degrade ACT through 4-aminophenol and hydroquinone up to 4,000 mg/L ACT (Zhang et al., 2013). For each of these organisms, the degradation pathway is debated; however, most cite ACT converting to hydroquinone via 4-aminophenol (Wu et al., 2012). Hydroquinone is eventually mineralized to ammonia, nitrate, water and carbon dioxide. Other transformation pathways also have been proposed: pyrocatechol, 4-aminophenol, *ortho* and *meta*-cleavage (Hughes et al., 2002; Zhao et al., 2000), and photocatalytic degradation (Yang et al., 2009). Pathway dependent intermediates thus have the potential to appear in the environment (Wu et al., 2012). The authors note that the laboratory strains have unknown survival potential in the field and thus require additional study. In another study, a *Pseudomonas* strain ST1 was isolated and found to utilize ACT as the sole carbon and energy source (Ahmed et al., 2001). At 72 hours in mineral salt agar media, this strain degraded 76.8% of an initial 1000

ppm ACT solution. This strain could be used for remediation of phenol and aminophenol contaminated sites and wastewater treatment of phenolic compounds (Ahmed et al., 2001). These aerobic heterotrophic microorganisms can utilize ACT as a carbon, nitrogen and energy sources effectively and rapidly.

A study on ACT bio-sorption and biodegradation showed strong sorption, weak desorption and strong biodegradability on immobilized cells. Sodium azide was used to inhibit microbial degradation and determine sorption potential; hydrolysis and volatilization were negligible in the control batch. ACT showed 100% sorption within 8 days and greater than 25% degradation in 2 days. From the bio-sorption/degradation rate constant, ACT is readily bio-sorbed and degraded (1.06/day). It also had sorption constant of 36 L/kg and desorption constant of 206 L/kg denoting strong sorption and weak desorption (Yu et al., 2011). Under anoxic conditions microorganisms have less impact ACT removal. Anaerobic conditions for methanogenic bacteria were predicted to correlate with little ACT biodegradability under anaerobic conditions (Musson et al. 2010). At 56 days of incubation, ACT showed an 11% reduction in high initial concentration tests. At environmentally relevant concentrations ACT showed reduction through abiotic adsorption but eventually desorbed once the organic adsorbent substrate was depleted (Musson et al., 2010).

Nitrifying sludge may also react with ACT parent compound in wastewater treatment plants to create reactive nitrogen species (Chiron et al., 2010). In the WWTP, 90-95% of ACT was removed; however, 7-15% of ACT was transformed to 3-chloro-ACT. Batch reactions using an enriched nitrifying community revealed a half-life of ACT ranging from 1.77 to 2.43 days. Furthermore, nitrification reactions involving peroxidase, produced 3-nitro-ACT and 3,5-dinitro-ACT. Creation of nitrophenols, like nitro-ACT, may negatively impact aquatic life (Chiron et al.,

2010). Thus, microorganisms are capable of metabolizing ACT to non-toxic products and eventual mineralization. The degradation time required depends on environment (anoxic or oxic) and substrate available (ACT alone or in mixed solution). Engineered environments, such as biofilters, may be constructed to redirect microorganisms to degrade the priority pollutant.

iii. Combined Mitigation Strategies

Lab-scale aqueous environments, utilizing soil and water batches, observed significant ACT removal (Lin et al., 2010). The impact of microbial metabolism was much greater than adsorption. ACT had a half-life of 2.1 days with weak (9%) desorption (Lin et al., 2010). Furthermore, respiking the batch led to faster degradation denoting microorganism adaptation to ACT. Authors noted that given a chance to adsorb to particles, ACT could then be quickly degraded with adapted microorganisms (Lin et al., 2010).

Membranes have also been studied for contaminant fate. ACT was used as the sole carbon source in membrane flow reactions (De Gusseme et al., 2011). Results showed greater than 99.9% of the 100 µg/L ACT influent removed after 16 days with flow through hydraulic residence time of 5 days (ACT loading rate of 20 µg/L-day). Further batch experiments denoted no adsorption to biomass, no influence of wastewater treatment plant effluent matrix and the presence of heterotrophic microbes (*Delftia tsuruhatensis* and *Pseudomonas aeruginosa*) able to remove environmentally relevant ACT concentrations (8.3 µg/L). Membrane removal of ACT, *p*-aminophenol, aspirin and salicylic acid was studied (Khamis et al., 2011). Overall, supplied wastewater resulted in a degradation rate tenfold faster than pure water solution degradation. Further, activated carbon and clay-micelle complex adsorbed contaminants most effectively.

A synthetic wastewater solution was supplied to activated sludge (sterilized and unsterilized) to determine sludge biodegradation and adsorption. 5 µg/L ACT supplied to an aerated submerged membrane reactor revealed adsorption only conditions removing 28.1% while adsorption and biodegradation together removed 92.2% of initial ACT. The hydrophilic and uncharged nature of ACT (Table 1) denotes a tendency for microbial metabolism and low sorption affinity. The authors noted a positive correlation between octanol-water partition coefficient and adsorption removal efficiency (Fan et al., 2014). As ACT has a low K_{ow} the ability to adsorb to soil or matrix is also assumed low. However, this assumption can be too simple; the surface charge characteristics of the soil or adsorbent must also be evaluated. Retaining ACT for a period of time may allow for biotic and abiotic transformations to occur. Specifically, adsorbing ACT to specific adsorbent may allow time for photolysis, microbial degradation, and mineralization.

The objective of this study is to develop a passive, non-point source mitigation technology to remove ACT from ground or surface water. The study utilizes two carbon sources, woodchips and biochar, to facilitate both physiochemical and biological ACT removal. The removal efficiency between sterilized and active matrices, composed of woodchips or biochar-amended woodchips (10% v/v), is compared. This comparison will lead to better understanding for specific adsorbent use in mitigating ACT pollution.

Experimental Methods

Woodchips from mixed hardwood (poplar and oak) and high temperature biochar from Biochar Solutions Inc. (Berthoud, CO) were used to develop water-based ACT treatment matrices. The biochar was created from a pine feedstock and produced using a two-stage process where it is first carbonized under low O_2 conditions at 500-700 ˚C for less than one minute, and then held for up to 14 minutes at 300-550 ˚C in an anoxic hot gas environment. After milling, the final product consists of two size fractions: 80% of the material is approximately 1.5 cm by 1 cm by 0.5 cm (0.75 cm^3) and 20% is a fine dust fraction 10-100 µm in length. The woodchips were comprised of mixed hardwood ranging approximately from 2.5 by 2.5 by 0.3 cm to 6 by 6 by 0.6 cm.

The study consisted of a factorial design with two primary treatments, a woodchip only (WC) and a woodchip + 10% (w/w) addition of biochar (WC+BC) created in triplicate. Secondary treatments consisted of abiotic conditions (e.g., microbial activity was suppressed) versus biotic conditions. 5.3100 g of woodchips were weighed out for the WC treatment. For the WC+BC treatment 5.3100 g of woodchips were combined with 0.5963 g of biochar. These masses were measured as the 10% v/v ratio of BC to WC. These treatments were then placed in 50 mL Falcon tubes for incubation. A stock 5 mM ACT solution was prepared, with and without 20 mM sodium azide (NaN_3). The purpose of adding NaN_3 is to remove biological activity through destruction of biological organisms (Yu et al., 2013). After washing the adsorbent (sorbent) in the Falcon tubes for 2 hours at 120 rpm and 21^{o}C, centrifuging at 8000 rpm for 8 minutes, three times, the storage solution was decanted and 40 mL of 5 mM ACT solution was added to the tubes in triplicate. The wetted sorbent was massed and taken to increase above initial mass as

follows: WC equaled 10.9675 g and WC+BC equaled 12.1211 g. The wetted sorbent masses then can account for dilution of the initial 5 mM ACT solution. An immediate sample (time zero) was taken, filtered, and absorbance response recorded. The batches were shaken for 0.5, 1, 2, 4, and 8 hours at 120 rpm and 21^{o}C. At each time step, liquid samples were filtered and analyzed for the absorbance response.

UV-Visual spectrophotometry (190-325 nm) was used to measure ACT in samples. Spectrum scan analysis of pure solution shows consistent presence and signal of ACT at 298nm (Behera, 2012; Murtaza et al., 2011). Matrix effects of WC and WC+BC are assumed and plots of spiked ACT concentration versus absorbance response will produce matrix-matched standard curves (Table 2). These standard curves can then be used to analyze unknown ACT solution concentrations over time for biotic and abiotic WC and WC+BC matrices.

a. Preparation of Standard Curves

Due to confounding compounds in WC and BC material the matrix effect on solution absorbance must be standardized. Matrix effects testing requires spiking a known matrix solution volume with known analyte mass and determining the consequent response. This approach was developed to matrix-match the sorbent and ACT solution.

Initially, standard curves were created for determining dose-response relationships. The abiotic setup required a 20 mM sodium azide (NaN_3) solution in deionized (DI) water while the biotic setup used only DI water. A 1:10 volume ratio sorbent was created using WC and BC; the control sorbent was woodchips only. The WC and WC+BC were placed in 50 mL Falcon tubes. To these tubes either 40 mL of DI water or 40 mL of 20 mM NaN_3 solution was added and

saturated for 2 hours on a shaker table at 60 rpm and 21^{o}C. Following saturation, the batches were centrifuged at 8000 rpm for 8 minutes. The supernatant was poured out carefully to maintain the sorbent. Once decanted another 40 mL of appropriate solution was added and the tubes were inverted to re-suspend the BC and WC particulates. The wash, centrifuge and resuspension steps were repeated twice more. After three washes, a final 40 mL solution was added and shaken for 2 hours at 60 rpm and 21^{o}C. These three washes remove dissolved organic carbon (DOC), which was shown to interfere with the UV signal used to analyze ACT solution concentration.

Once the tubes were washed with the respective solutions, 10 mL volumes of the biotic sample solution from washed batches was aliquoted into three separate beakers. A known mass (Table 2) of ACT was added to each aliquot and mixed completely. The spectrophotometer (Shimadzu Visible Spectrophotometer UVmini-1240V) was autozeroed with DI water. 3 mL of ACT spiked solution was filtered with a 0.45 micron filter into a polystyrene cuvette and scanned for absorbance from 220-300 nm. A peak absorbance region for ACT over the background signal was noted (Table 3). Peak absorbance was recorded for multiple wavelengths for each spiked solution. Finally, the spiked sorbent solution response was plotted against the ACT concentration to create the DI water standard curve.

The abiotic treatment matrix-matched standard curve was similarly created. First, 10 mL of treated 20 mM sodium azide solution was aliquoted into three separate beakers, equal ACT masses added and mixed completely. The spectrophotometer with was auto-zeroed with the 20 mM NaN_3 solution. 3 mL of ACT spiked solution was filtered with a 0.45 micron filter into a polystyrene cuvette and scanned for absorbance response from 220-300 nm. A peak absorbance

region relative to matrix signal was selected and noted for individual wavelengths. The absorbance response was recorded for the spiked solution at each wavelength noted. Finally, absorbance response was plotted against ACT concentration to create an abiotic calibration curve. Table 3 shows the wavelength chosen for analysis for each treatment.

Table 2. ACT masses used for matrix-matched standard curve creation.

Mass number	ACT mass, g	Volume, L	g/L	mM
0	0.0000	0.01	0.00	0.00
1	0.0054	0.01	0.54	3.57
2	0.0083	0.01	0.83	5.49
3	0.0130	0.01	1.30	8.60

Table 3. Wavelengths used for treatment analysis.

Sorbent Type	Wavelength, nm
Biotic WC	298
Abiotic WC	298
Biotic WC+BC	298
Abiotic WC+BC	298

b. Testing Removal Efficacy in Batch Reactions

The purpose of the abiotic adsorption experiments was to determine the amount of ACT capable of adsorbing to a fixed mass of sorbent. The secondary objective was to determine any differences between sorption to woodchips and biochar-amended woodchips. This will aid in determining what substrate is more efficient for capturing ACT to allow time for degradation by microorganisms or by chemical and physical means (photolysis, free radicals).

ACT removal efficiency (RE) was calculated according to equation 1.

$$RE(\%) = \left[\frac{C_0 - C_t}{C_0}\right] * 100 \quad (1)$$

Where C_0 is the initial ACT concentration (mg/L) and C_t is the ACT concentration at time t (mg/L).

c. Kinetics

Kinetic model was assumed to be pseudo-second order based on previous work (Cabrita et al., 2010; Ruiz et al., 2010). The amount adsorbed at time t (mg/g), q_t, was calculated using equation 2.

$$q_t = \frac{(V_0 C_0 - V_t C_t)}{W} \quad (2)$$

Where C_0 is the initial ACT concentration (mg/L), C_t is the ACT concentration at time t (mg/L), W is the mass (g) of dry sorbent, V_t is the volume of adsorbate solution at each time step (L). The equilibrium-adsorbed amount, q_e, is given in equation 3.

$$q_e = \frac{(V_0 C_0 - V_t C_t)}{W} V_t \quad (3)$$

Where C_e is the concentration at equilibrium (mg/L).

Pseudo-second order kinetics is modeled and linearized via equation 4. The half-life for this model is given in equation 5. The pseudo-second order model has been used in previous studies of ACT adsorption to activated carbon.

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \quad (4)$$

$$t_{1/2} = \frac{1}{k_2 q_e} \quad (5)$$

Where q_e is the equilibrium adsorption density (mg/g), q_t is the adsorption density at time t (mg/g), and k_2 is the adsorption rate constant (g/mg-min). Plotting t/q_t versus t results in a linear relationship. Then k_2 was calculated from the slope of the line. This value reflects the amount of sorbent needed for attaching unit adsorbate over unit time. The higher the value the more sorbent required per unit adsorbate and time.

d. Statistical Analysis

To differentiate between the treatments', linear mixed-effects models or *lme4* (Bates et. al, 2012) were created in R (R Core Team, 2015). The null hypothesis states there is no difference between biotic and abiotic media types and ACT adsorption. Alternatively, there is a difference between biotic and abiotic media types and ACT adsorption. Normalizing the sample to starting concentration for each batch allows statistical comparison between treatments. The null and alternate models assumed are given below:

$$[ACT] = Media * Time + (1 + Bio|id) + (1|id) \quad (6)$$

$$[ACT] = Media * Time * Bio + (1 + Bio|id) + (1|id) \quad (7)$$

Where Media, Time, Bio and id reflect the treatment media, time step, abiotic or biotic treatment, and the replicate identifier. The last two factors in equation 6 and 7 denote the random effects for each sample ("id") and differing baseline levels of ACT and differing responses to the main factor in question ("1+Bio|id"). These factors incorporate random intercepts and random slopes to describe ACT concentration.

Within each biotic or abiotic group (WC and WC+BC) the model may be altered as below to compare media types with either biotic or abiotic treatment. The alternate (equation 8) and null (equation 9) models reflect the influence of media treatment on ACT concentration.

$$[ACT] = Media * Time + (1|id) \quad (8)$$

$$[ACT] = Time + (1|id) \quad (9)$$

The other comparison is whether biological activity within the same treatment sorbent causes significant change in ACT concentration. The alternate (equation 10) and null (equation 11) models reflect the influence of biology on ACT concentration.

$$[ACT] = Bio * Time + (1|id) \quad (10)$$

$$[ACT] = Time + (1|id) \quad (11)$$

Assumptions of the test include normality of the data and equal residual variance. Figures 4, 5 and 6 show the boxplot, histogram and Q-Q plots, respectively. The normality of the data is confirmed by inspection of the Q-Q plot. Normality is required before analysis with linear mixed effects models. By definition, linear models must obey linearity and normality before subsequent analysis. Thus, Figure 6 sample values should follow the theoretical line given. Excluding the initial, normalized value of 1, the Q-Q plot appears normal. Final data input and coding of equations 6, 7, 8, 9, 10 and 11 with *lme4* (Bates et. al, 2012) and output using R is given in Appendix A (R Core Team, 2015).

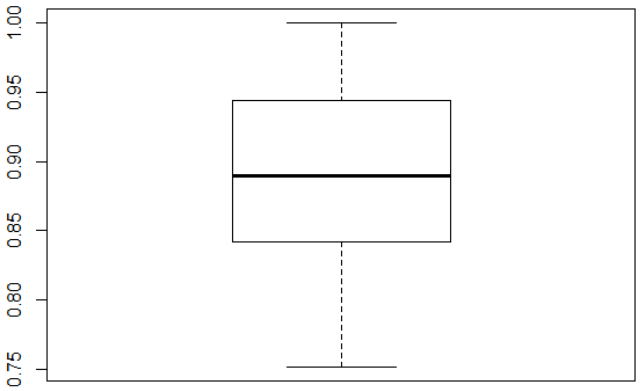

Figure 4. Boxplot of all ACT normalized concentration.

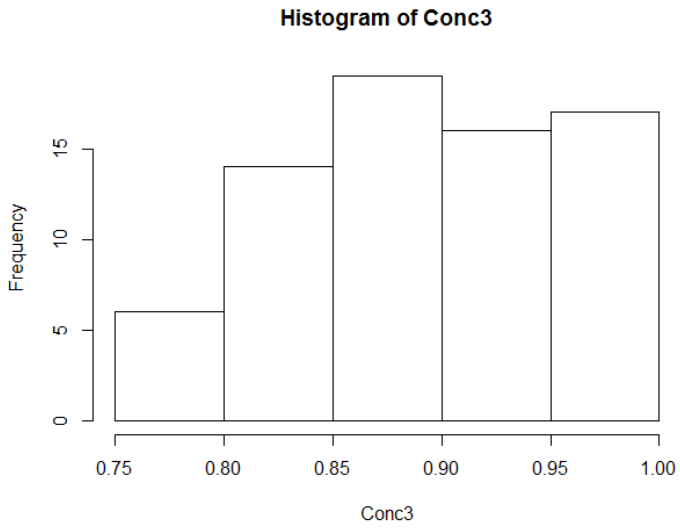

Figure 5. Histogram of ACT normalized concentration.

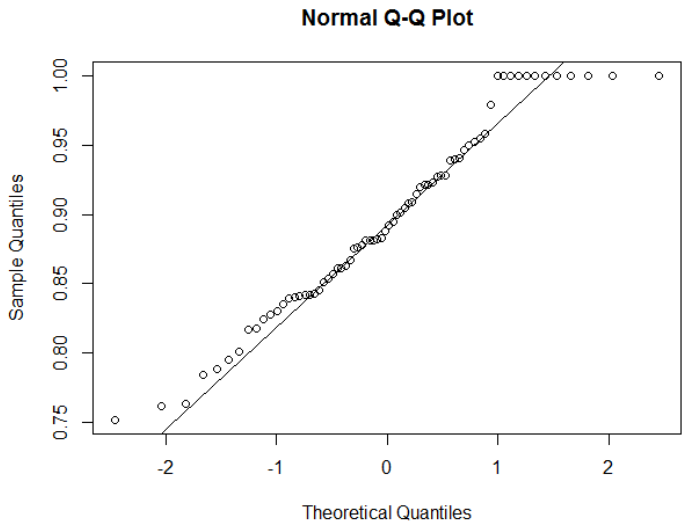

Figure 6. Q-Q plot of normalized ACT concentration.

Results

a. Standard curves and matrix-matched standard curves

Due to the matrix effects observed in standard curve creation, a matrix-matched standard curve was used for determining unknown ACT concentration in analyte solution through time. The standard curve was reproduces below for the biotic (Figure 7) and abiotic (Figure 8) batches. The matrix-matched curves were given in Figure 9 and 10. The spiked solution standards covered an ACT concentration range from 3.5 mM to 8.6 mM. No intercept was seen to pass through zero with zero ACT based on the three-point calibration. This implied that a baseline absorbance for the sorbent exists. Furthermore, any absorbance at or below this baseline cannot be used to measure ACT concentration. From Figure 9 and 10, the enhancement of the signal was greatest for WC+BC. Enhancement was also observed over the spike concentrations for woodchips treatment. Enhancement reflected the increase in signal due to the aqueous solvation of sorbent. As such, the signal response for WC+BC was expected higher than WC alone; both signals were higher than pure solution dose responses. Appearing somewhat curvilinear, the curves should be carefully used. Utilizing any concentrations outside this regression is not advised.

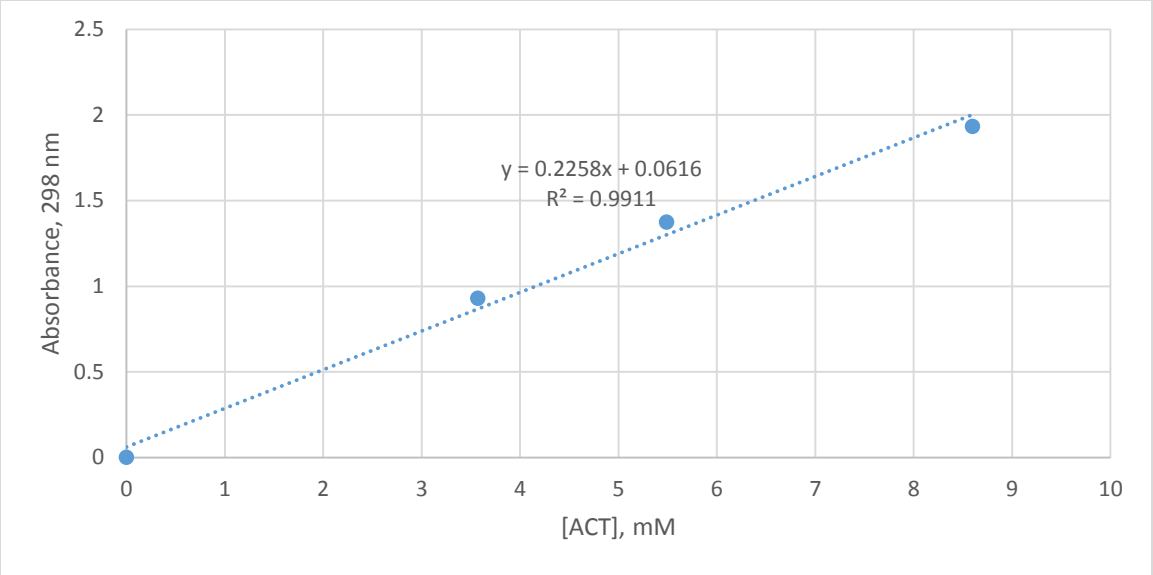

Figure 7. DI water standard curve at 298 nm.

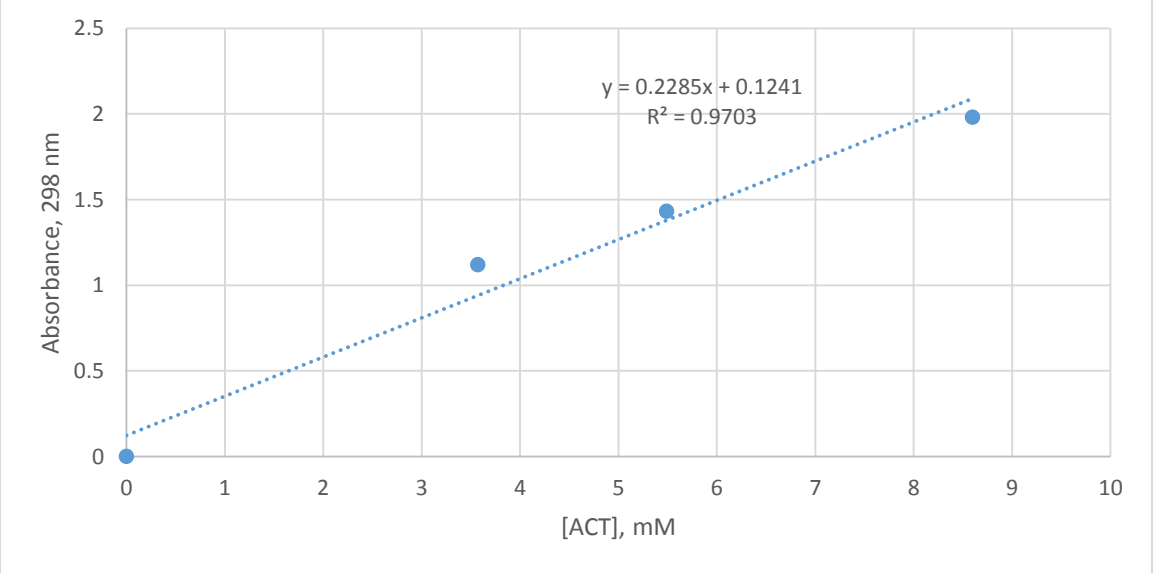

Figure 8. 20 mM NaN_3 DI water standard curve at 298 nm.

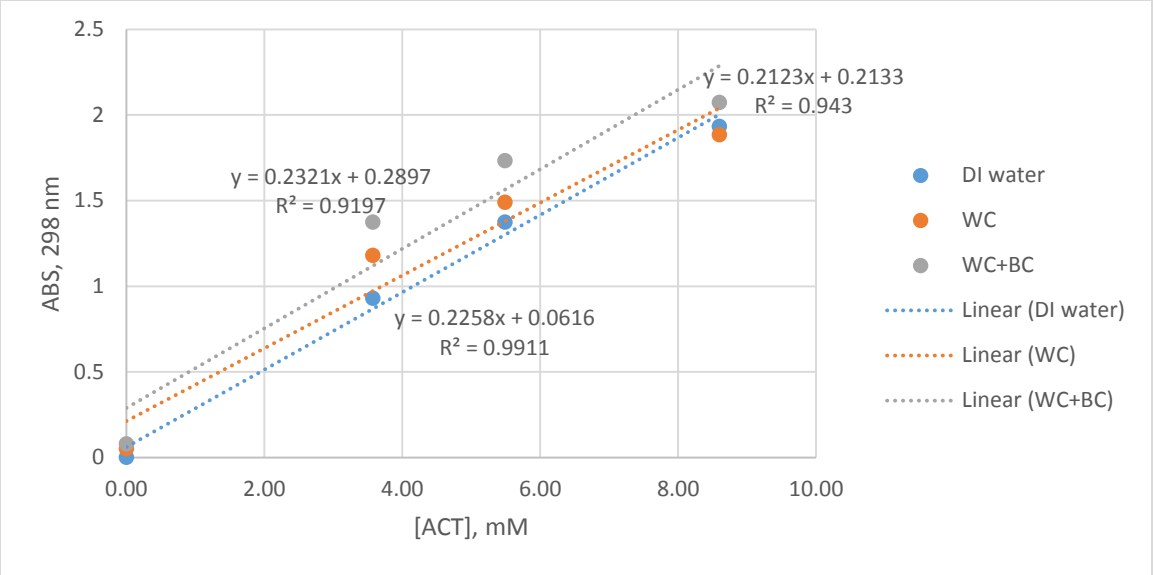

Figure 9. Biotic standard overlay at 298 nm.

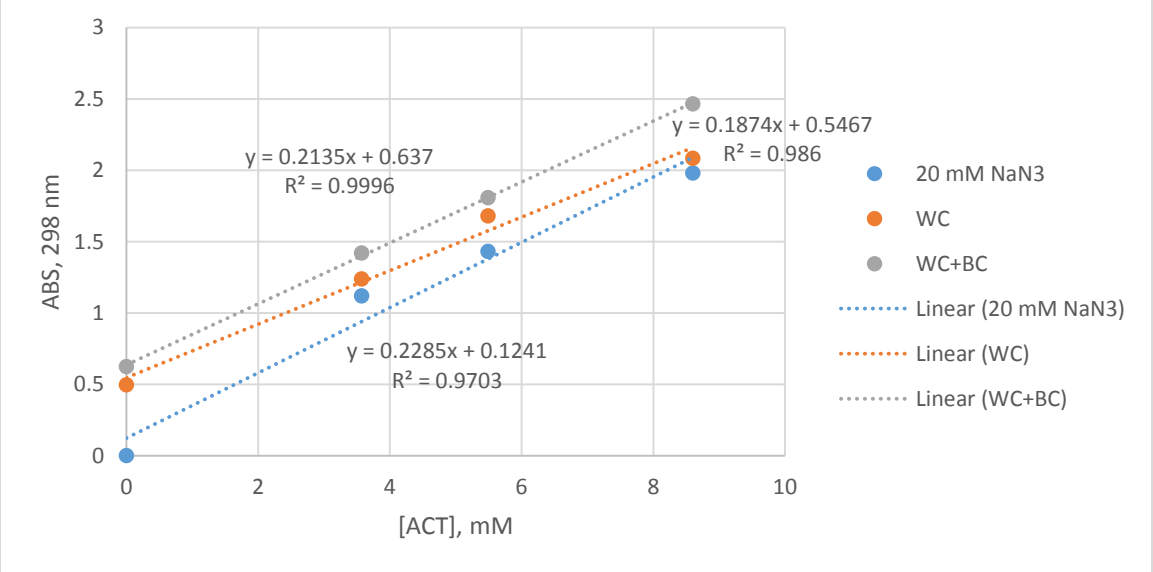

Figure 10. Abiotic standard curve overlay at 298 nm.

b. *<u>Abiotic Adsorption</u>*

Abiotic results were given in Table 4 with normalized results shown in Table 5. Table 6 showed the control batch (no sorbent) and normalization. Figure 11 showed the normalized concentration

over time for WC, WC+BC and control batches. From Table 6, the control batch showed negligible change over time denoting insignificant photolysis or adsorption to tube surface. From Tables 4 and 5, over 8 hours the total ACT removed was 19% for WC and 21% for WC+BC. The ANOVA test, using equations 6 and 7, resulted in a chi-squared value of $X^2(2) = 7.589$, $p=0.1078$ (Table 10). This p-value reflected no significant difference between WC and WC+BC. Rapid removal was observed in the first hour, with 11% and 13% of total removal for WC and WC+BC. By hour 8, the adsorption was negligible and equilibrium was assumed. The plot in Figure 11 depicted the trend toward equilibrium at hour eight. Thus, within this abiotic group, WC sorbent was not significantly affected by BC amendment.

Table 4. 5 mM ACT time-series, 298 nm absorbance: concentrations for triplicate abiotic run.

Time, hr	Sample #	WC, unitless	[ACT], mM	Sample #	WC + BC, unitless	[ACT], mM
0	1	1.378	4.436	13	1.395	3.550
0	2	1.393	4.516	14	1.408	3.611
0	3	1.410	4.607	15	1.388	3.518
	Average	1.394	4.520	Average	1.397	3.560
0.5	1	1.314	4.094	13	1.340	3.293
0.5	2	1.348	4.276	14	1.325	3.222
0.5	3	1.348	4.276	15	1.329	3.241
	Average	1.337	4.215	Average	1.331	3.252
1	1	1.281	3.918	13	1.305	3.129
1	2	1.316	4.105	14	1.298	3.096
1	3	1.305	4.046	15	1.284	3.030
	Average	1.301	4.023	Average	1.296	3.085
2	1	1.254	3.774	13	1.275	2.988
2	2	1.293	3.982	14	1.281	3.016
2	3	1.292	3.977	15	1.278	3.002
	Average	1.280	3.911	Average	1.278	3.002
4	1	1.246	3.732	13	1.256	2.899
4	2	1.308	4.062	14	1.286	3.040
4	3	1.272	3.870	15	1.259	2.913
	Average	1.275	3.888	Average	1.267	2.951
8	1	1.202	3.497	13	1.207	2.670
8	2	1.260	3.806	14	1.277	2.998
8	3	1.238	3.689	15	1.226	2.759
	Average	1.233	3.664	Average	1.237	2.809

Table 5. 5 mM ACT normalized concentration results.

Time, hr	WC, C(t)/C(0)	WC+BC, C(t)/C(0)
0	1.000	1.000
0	1.000	1.000
0	1.000	1.000
Average	1.000	1.000
0.5	0.923	0.927
0.5	0.947	0.892
0.5	0.928	0.921
Average	0.933	0.914
1	0.883	0.881
1	0.909	0.857
1	0.878	0.862
Average	0.890	0.867
2	0.851	0.842
2	0.882	0.835
2	0.863	0.854
Average	0.865	0.843
4	0.841	0.817
4	0.900	0.842
4	0.840	0.828
Average	0.860	0.829
8	0.788	0.752
8	0.843	0.830
8	0.801	0.784
Average	0.811	0.789

Table 6. 5 mM abiotic control solution results and normalization.

Time, hr	ABS, 298 nm	[ACT], mM	C(t)/C(0)
0	1.417	5.658	1.000
0.5	1.430	5.715	1.010
1	1.422	5.680	1.004
2	1.417	5.658	1.000
4	1.417	5.658	1.000
8	1.365	5.431	0.960

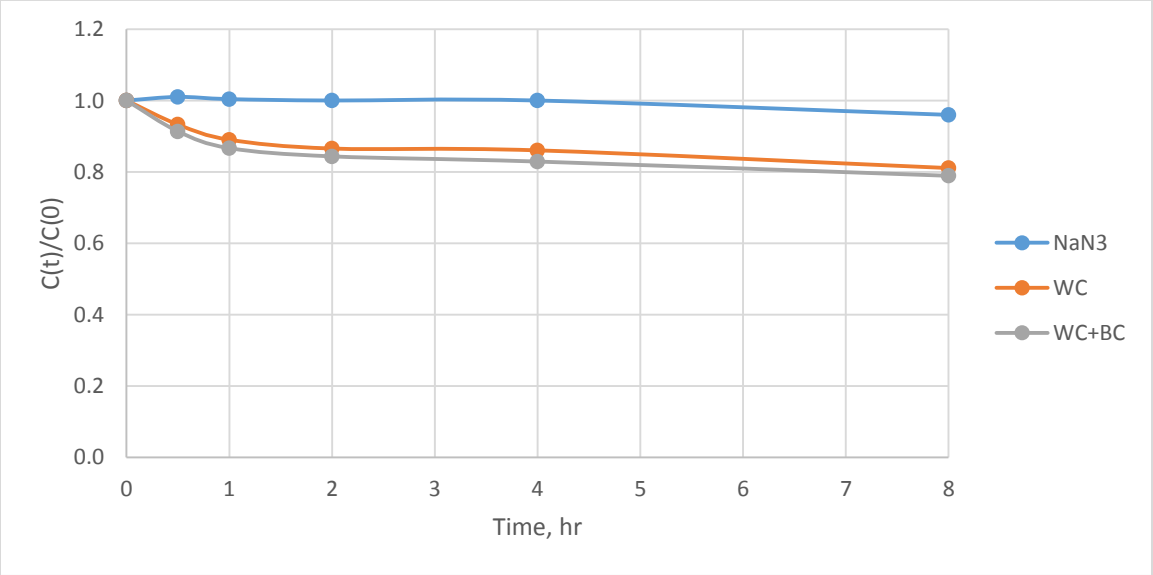

Figure 11. Time-series plot of ACT concentration for abiotic control, WC, and WC+BC sorbent.

c. Microbial Remediation

For biotic batches, 5 mM initial ACT concentrations were reduced over time (Table 7, Figure 12). Normalized batch treatment results (Table 8) and control (Table 9) were reproduced. The control solution remained constant denoting negligible photolytic degradation loss and negligible adsorption to tube surface or photolysis (Figure 12). Microbial presence, assumed due to lack of sodium azide biological inhibition, showed insignificant removal. Again, the between group chi-squared value of $X^2(2) = 7.589$, p=0.1078, reflected no significant difference between equation 6

and 7. By hour 8, woodchips showed 20% aqueous ACT reduction. The assumed biologically active WC+BC showed 16% removal at hour 8.

Table 7. 5 mM ACT time-series 298 nm absorbance and concentrations for triplicate biotic run.

Time, hr	Sample #	WC, unitless	[ACT], mM	Sample #	WC + BC, unitless	[ACT], mM
0	4	1.280	5.024	16	1.240	4.094
0	5	1.261	4.935	17	1.260	4.181
0	6	1.264	4.949	18	1.249	4.133
	Average	1.268	4.970	Average	1.250	4.136
0.5	4	1.227	4.775	16	1.200	3.922
0.5	5	1.239	4.831	17	1.216	3.991
0.5	6	1.200	4.648	18	1.203	3.935
	Average	1.222	4.751	Average	1.206	3.949
1	4	1.182	4.563	16	1.165	3.771
1	5	1.198	4.638	17	1.190	3.879
1	6	1.146	4.393	18	1.192	3.888
	Average	1.175	4.531	Average	1.182	3.846
2	4	1.153	4.426	16	1.108	3.526
2	5	1.177	4.539	17	1.177	3.823
2	6	1.133	4.332	18	1.158	3.741
	Average	1.154	4.433	Average	1.148	3.697
4	4	1.115	4.247	16	1.045	3.254
4	5	1.122	4.280	17	1.164	3.767
4	6	1.095	4.153	18	1.148	3.698
	Average	1.111	4.227	Average	1.119	3.573
8	4	1.028	3.837	16	1.014	3.121
8	5	1.070	4.035	17	1.140	3.664
8	6	1.080	4.082	18	1.135	3.642
	Average	1.059	3.985	Average	1.096	3.475

Table 8. 5 mM ACT biotic normalized concentration results.

Time, hr	WC, C(t)/C(0)	WC+BC, C(t)/C(0)
0	1.000	1.000
0	1.000	1.000
0	1.000	1.000
Average	1.000	1.000
0.5	0.950	0.958
0.5	0.979	0.955
0.5	0.939	0.952
Average	0.956	0.955
1	0.908	0.921
1	0.940	0.928
1	0.888	0.941
Average	0.912	0.930
2	0.881	0.861
2	0.920	0.914
2	0.875	0.905
Average	0.892	0.894
4	0.845	0.795
4	0.852	0.901
4	0.839	0.895
Average	0.845	0.864
8	0.764	0.762
8	0.818	0.876
8	0.825	0.881
Average	0.802	0.840

Table 9. ACT control results and normalization.

Time, hr	ABS, 298 nm	[ACT], mM	C(t)/C(0)
0	1.316	17.70	1.00
0.5	1.328	17.89	1.01
1	1.331	17.94	1.01
2	1.334	17.99	1.02
4	1.343	18.14	1.02
8	1.322	17.80	1.01
12	1.327	17.88	1.01

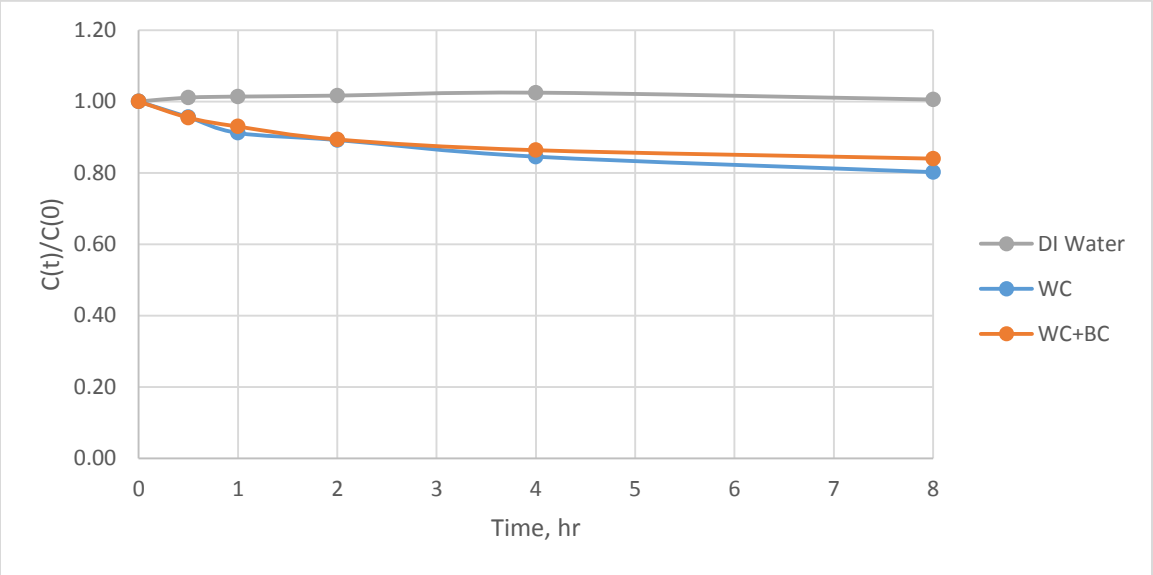

Figure 12. Time-series plot of ACT concentration for biotic control, WC, and WC+BC sorbent.

Table 10 shows the overall removal efficiency for each treatment. The greatest removal achieved was due to abiotic WC+BC (21.1%), biotic WC (19.8%), abiotic WC (18.9%), and finally biotic WC+BC (16.0%). Between treatment ANOVA used equation 6 (null) and 7 (alternate) for comparison. No significance ($p > 0.05$) was observed between treatments with $p = 0.1078$ (Table 10). Thus, the between group ANOVA result cannot reject the null hypothesis and there was no significant statistical difference between equations 6 and 7. The interaction among time, media and biology did not significantly affect the ACT concentration. However, simplification of the model with equations 8, 9, 10 and 11 reduced the interaction to only two terms; time and media or time and biology. The null hypothesis then required only time to explain differences in ACT concentration while alternate hypotheses included media or biology interacting with time.

Table 11 and 12 show the within group ANOVA results. Essentially, the model from equations 6 and 7 was simplified to hold biology or media type constant. Overall, no significance was

observed between variable media types under controlled abiotic (p = 0.1620) or biotic (p = 0.4366) treatment (Table 11). However, a statistically significant result was found within each media treatment for varying biological activity (Table 12). Assumed biotic treatments led to statistically significant ACT concentrations versus abiotic treatments. Thus, the biotic WC resulted in significantly different ACT concentrations versus abiotic WC (p = 0.003038) and biotic WC+BC resulted in significantly (p = 1.369e-05) different ACT concentrations versus abiotic WC+BC.

Table 10. Removal efficiency and between treatment ANOVA chi-squared, p, and deviance values for all treatments at eight hours.

Treatment	Removal Efficiency, %	Deviance
Abiotic WC	18.9	-258.84
Abiotic WC+BC	21.1	
Biotic WC	19.8	-266.43
Biotic WC+BC	16.0	
Between group ANOVA	$X^2(4) = 7.589$, p=0.1078	-7.59

Table 11. Chi-squared, p, and deviance values for within biotic/abiotic treatments ANOVA test.

Treatment	X^2(df) value	p-value	Deviance
Abiotic WC	(2) = 3.6405	0.1620	-123.66
Abiotic WC+BC			-127.30
Biotic WC	(2) = 1.6576	0.4366	-136.21
Biotic WC+BC			-137.86

Table 12. Chi-squared, p, and deviance values for within media treatment ANOVA test.

Treatment	X^2(df) value	p-value	Deviance
Abiotic WC	(2) = 11.593	0.003038	-129.81
Biotic WC			-141.40
Abiotic WC+BC	(2) = 22.397	1.369e-05	-104.62
Biotic WC+BC			-127.02

Deviance defines how far the actual model is from the predicted model. Because the concentration used in the model was normalized these units were then mM(t=t)/mM(t=0) (mg/L(t=t) per mg/L(t=0)). For the WC media type, the difference between abiotic and biotic was 11.59 units. For the WC+BC media, the difference was 22.4 units. So with biotic WC overall 0.076 mM (11.59 mg/L) lower ACT concentration was expected at time t versus abiotic WC. Additionally, biotic WC+BC expected 0.148 mM (22.4 mg/L) lower ACT concentration at time t versus abiotic WC+BC.

There was no test for biological activity or presence. Thus, the assumption of sodium azide inhibiting biology could also be made for the treatments using DI water only; i.e. the DI water treatments also did not possess biological activity. This may explain why the difference between groups was insignificant. Yet, comparing within treatments (Table 12), the significance of biology was observed. Interestingly, the original three variable interaction model may be masking the biotic effect due to the media effect.

Finally, speaking to the ACT structure available to adsorb in the model, an analysis of each setup pH was conducted. Table 13 shows that each pH was below the pKa for ACT, and thus ACT was available in molecular form.

Table 13. pH measurements for all media types

Media Type	pH
Abiotic WC	6.260
Abiotic WC+BC	6.499
Biotic WC	5.343
Biotic WC+BC	5.840

Conclusions

The purpose of these experiments was to quantify the ACT removal potential of WC versus WC+BC. For the 5 mM ACT solutions, both treatment matrices reduced the ACT concentration over the 8 hr sampling interval.

Overall removal efficiency between treatments (interaction of time, media and biology) was statistically similar. Thus, the null hypothesis cannot be rejected and there is no difference between treatments. However, within the treatments (controlling either media or biology) the biotic versus abiotic media showed significant differences. Thus the null hypothesis within treatments could be rejected when controlling for media type. The null hypothesis cannot be rejected when controlling for biology within media types. Additionally, the results of abiotic and biotic kinetic fitting could not be used to draw any relevant conclusions as a zero concentration was not observed. Because DOC was removed from the experiment to reduce signal interference, heterotrophic microorganisms were assumed to be limited in carbon and energy sources. Heterotrophs must then look for other carbon and energy sources. Species capable of utilizing ACT as their carbon and energy source exist but the specialized enzymes and metabolic pathways could require time or different environmental conditions for activation. Results indicated that, controlling for media type, a significant difference was observed between biotic and abiotic ACT removal (Table 12). Thus, more ACT was removed under biotic treatment than abiotic treatment for WC or WC+BC treatments.

Differences between the biotic and abiotic treatment groups produced additional questions. The initial speculation was that the microbial community present in the biotic setup could aid in ACT removal but this was found to be significant only when controlling for media treatment (Table

12). The overall model interaction showed no significant difference between abiotic and biotic media. The abiotic WC and WC+BC removal efficiencies of 20-21% over 8 hrs are similar or slightly lower than values others have reported for abiotic removal (Ribeiro et al., 2011). The biotic WC (19.8%) and WC+BC (16%) removal was much lower than anticipated. This is counter to many studies showing activated carbon or sludge adsorbing 90% or more of ACT (Cabrita et al., 2010; Yu et al., 2011). This may be due to key material washout during the removal of DOC for signal interference reduction. Tests for presence of microorganisms in the treatment setups should be performed.

In conclusion, the ability of the two matrices studied to adsorb ACT was evident. The matrices used are capable of reducing aqueous ACT concentration, albeit not environmentally relevant, within hours. Since non-point sources contribute to ACT pollution, biofilters composed of WC+BC can act to diminish ACT effluent to waterways. Furthermore, other emerging contaminants observed in these non-point sources can be studied using these sorbents. A pure solution versus mixed solution (composed of ACT and other relevant contaminants like nitrate, pesticides or other pharmaceuticals) experiment can be performed to determine maximum concentrations capable of adsorption, adsorption sites competition, and possible influences on microbial communities. Field conditions are neither pure nor ideal and comparing solution mixtures to mimic different field conditions is important. Will the interaction between pollutants compete for adsorption sites? What threshold concentration of a mixed (or pure) solution can adsorb? ACT does adsorb to the treatments given here, however, desorption from these treatments should also be evaluated. Furthermore, the longevity of the sorbent for variable pH and temperature conditions should be analyzed to account for seasonal variation. As ACT has been shown to degrade in a reasonable amount of time (days), the biofilters ability to regenerate

adsorption sites over its lifespan is important. Intercepting ACT before hydrological input, and kilometer transport through surface water, could reduce the concentrations observed worldwide. Furthermore, the specific surface charges of both WC and BC should be studied; like soil, the specific sorbent setup may vary and alter the amount or type of ACT sorbed. Finally, isotherm development for ACT on biochar and woodchips should be conducted for comparison to commercially activated carbon models. Economics and sustainability of non-point source remediation treatments is vital for scale-up, applicability, and feasibility.

While the media type in the overall model masked the biotic treatment, significant difference could be observed when comparing within treatment results. Further work should seek to determine the requirements for bacterial incubation for greater ACT removal. Bacterial communities and species can be analyzed for various ACT concentrations and environmental parameters (pH, oxidative state). Plate counts with this experimental setup would physically show biological presence of some species (instead of assuming presence). Inoculation of known ACT degrading species may display maximum biodegradation rates for the WC and WC+BC treatments. Labelled ACT experiments could trace ACT from solution to surface adsorption, bacterial metabolism and integration, or mineralization.

Finally, the ACT concentration used in this study (5 mM; 755,800 ppm) was well above that found in natural systems (1 ppm). Thus, a more precise technique (High-Performance Liquid Chromatography-tandem Mass Spectrometry) could be applied to environmentally relevant ACT concentrations. Additionally, the experiment could be carried out over a longer time period to achieve the zero concentration needed for half-life and kinetic calculations. A more sensitive method could determine if the assumed pseudo-second order model is defensible with this

sorbent setup. Additionally, does this kinetic model hold with and without biological activity under environmentally relevant ACT concentrations? Perhaps abiotic setups are first-order while biotic setups are pseudo-second order. This analytical technique could also distinguish the various ACT transformation products (hydroquinone, *p*-aminophenol, nitrate, etc.) and fate within the sorbent (adsorption, mineralization, microbial integration). This experiment showed the basic ability of WC and WC+BC matrices to adsorb ACT over an eight hour time period. Determining overall ACT fate – degradation, transformation, mineralization, desorption - once adsorbed to biotic or abiotic treatments is a reasonable next step.

Appendix A. Data Analysis: R code and output

```
read.csv("data.csv")
my.data<-read.csv("data2.csv")
attach(my.data)
summary(my.data)

hist(Conc3)
boxplot(Conc3)
qqnorm(Conc3)
qqline(Conc3)

ACT.model1 <- lmer(Conc3 ~ Media*Time+(1|id), data=my.data)
summary(ACT.model1)
ACT.model2 <- lmer(Conc3 ~ Media*Bio+(1|id), data=my.data)
summary(ACT.model2)
ACT.model3 <- lmer(Conc3 ~ Media*Bio*Time + (1|id), data=my.data)
summary(ACT.model3)
ACT.model4 <- lmer(Conc3 ~ Media*Time + Media*Bio + Media*Bio*Time + (1|id), data=my.
data)
summary(ACT.model4)

Introduce biotic to determine if differences are due to time alone or biotic and time.
Use ANOVA between the two models for Chisquare test.

ACT.model <- lmer(Conc3 ~ Media*Time + Media*Time*Bio + (1+Bio|id) + (1|id), data=my.da
ta, REML=FALSE)
ACT.modelnull <- lmer(Conc3 ~ Media*Time + (1+Bio|id) + (1|id), data=my.data, REML=FAL
SE)
anova(ACT.model,ACT.modelnull)
coef(ACT.model)


ACT.modelnull: Conc3 ~ Media * Time + (1 + Bio | id) + (1 | id)
ACT.model: Conc3 ~ Media * Time + Media * Time * Bio + (1 + Bio | id) +
ACT.model: (1 | id)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
ACT.modelnull9 -240.84 -220.35 129.42-258.84
ACT.model 13 -240.43 -210.83 133.21-266.43 7.5894 0.1078
```

```
read.csv("wc.csv")
wc<-read.csv("wc.csv")
attach(wc)
```

Within group: WC bio vs abio

```
ACT.modelwc <- lmer(Conc3 ~ Time*Bio + (1|id), data=wc, REML=FALSE)
ACT.modelnullwc <- lmer(Conc3 ~ Time + (1|id), data=wc, REML=FALSE)
anova(ACT.modelwc,ACT.modelnullwc)
```

```
ACT.modelnullwc: Conc3 ~ Time + (1 | id)
ACT.modelwc: Conc3 ~ Time * Bio + (1 | id)
Df AIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
ACT.modelnullwc4 -121.81 -115.47 64.904-129.81
ACT.modelwc6 -129.40 -119.90 70.701-141.40 11.5932 0.003038**
```

Within group: WC/BC

```
read.csv("wcbc.csv")
wcbc <- read.csv("wcbc.csv")
attach(wcbc)
```

```
ACT.model.wcbc <- lmer(Conc3 ~ Bio*Time + (1|id), data=wcbc, REML=FALSE)
ACT.modelnull.wcbc <- lmer(Conc3 ~ Time + (1|id), data=wcbc, REML=FALSE)
anova(ACT.model.wcbc,ACT.modelnull.wcbc)
```

```
ACT.modelnull.wcbc: Conc3 ~ Time + (1 | id)
ACT.model.wcbc: Conc3 ~ Bio * Time + (1 | id)
 DfAIC BIC logLik deviance Chisq Chi Df Pr(>Chisq)
ACT.modelnull.wcbc4-96.624-90.29 52.312-104.62
ACT.model.wcbc6 -115.021 -105.52 63.511-127.02 22.397 21.369e-05***
```

```
read.csv("abio.csv")
abio<-read.csv("abio.csv")
attach(abio)
```

Within group: Abiotic

```
ACT.model.abio <- lmer(Conc3 ~ Media*Time + (1|id), data=abio, REML=FALSE)
ACT.modelnull.abio <- lmer(Conc3 ~ Time + (1|id), data=abio, REML=FALSE)
anova(ACT.model.abio,ACT.modelnull.abio)
```

```
ACT.modelnull.abio: Conc3 ~ Time + (1 | id)
ACT.model.abio: Conc3 ~ Media * Time + (1 | id)
 Df AICBIC logLik devianceChisq Chi Df Pr(>Chisq)
ACT.modelnull.abio4 -115.66 -109.32 61.829-123.66
ACT.model.abio6 -115.30 -105.80 63.649-127.30 3.64052 0.162
```

Within group: Biotic

```
read.csv("bio.csv")
bio <- read.csv("bio.csv")
attach(bio)
```

```
ACT.model.bio <- lmer(Conc3 ~ Media*Time + (1|id), data=bio, REML=FALSE)
ACT.modelnull.bio <- lmer(Conc3 ~ Time + (1|id), data=bio, REML=FALSE)
anova(ACT.model.bio,ACT.modelnull.bio)
```

```
ACT.modelnull.bio: Conc3 ~ Time + (1 | id)
ACT.model.bio: Conc3 ~ Media * Time + (1 | id)
Df AIC BIC logLik devianceChisq Chi Df Pr(>Chisq)
ACT.modelnull.bio4 -128.21 -121.87 68.103-136.21
ACT.model.bio6 -125.86 -116.36 68.932-137.86 1.65762 0.4366
```

References

Afonso-Olivares, C., M. Torres-Padrón, Z. Sosa-Ferrera, and J. Santana-Rodríguez. 2013. Assessment of the Presence of Pharmaceutical Compounds in Seawater Samples from Coastal Area of Gran Canaria Island (Spain). *Antibiotics* 2(2):274-287.

Ahmed, S., M. A. Javed, S. Tanvir, and A. Hameed. 2001. Isolation and characterization of a *Pseudomonas* strain that degrades 4-acetamidophenol and 4-aminophenol. *Biodegradation* 12:303-309.

Azargohar, R., and A. K. Dalai. 2006. Biochar as a precursor of activated carbon. *Applied Biochemistry and Biotechnology* 129-132:762-773.

Baron, P. A., D. C. Love, and K. E. Nachman. 2014. Pharmaceuticals and personal care products in chicken meat and other food animal products: a market-basket pilot study. *Sci Total Environ* 490:296-300.

Basha, S., D. Keane, K. Nolan, M. Oelgemoller, J. Lawler, J. M. Tobin, and A. Morrissey. 2015. UV-induced photocatalytic degradation of aqueous acetaminophen: the role of adsorption and reaction kinetics. *Environ Sci Pollut Res Int* 22(3):2219-2230.

Behera, S. 2012. UV-Visible Spectrophotometric Method Development and Validation of Assay of Paracetamol Tablet Formulation. *Journal of Analytical & Bioanalytical Techniques* 03(06).

Behera, S. K., H. W. Kim, J. E. Oh, and H. S. Park. 2011. Occurrence and removal of antibiotics, hormones and several other pharmaceuticals in wastewater treatment plants of the largest industrial city of Korea. *Sci Total Environ* 409(20):4351-4360.

Bernot, M. J., L. Smith, and J. Frey. 2013. Human and veterinary pharmaceutical abundance and transport in a rural central Indiana stream influenced by confined animal feeding operations (CAFOs). *Sci Total Environ* 445-446:219-230.

Bolan, N. S., R. Naidu, J. K. Syers, and R. W. Tillman. 1999. Surface Charge and Solute Interactions in Soils. 67:87-140.

Bound, J. P., and N. Voulvoulis. 2005. Household Disposal of Pharmaceuticals as a Pathway for Aquatic Contamination in the United Kingdom. *Environmental Health Perspectives* 113(12):1705-1711.

Bunch, A. R., and M. J. Bernot. 2011. Distribution of nonprescription pharmaceuticals in central Indiana streams and effects on sediment microbial activity. *Ecotoxicology* 20(1):97-109.

Buszka, P. M., D. J. Yeskis, D. W. Kolpin, E. T. Furlong, S. D. Zaugg, and M. T. Meyer. 2009. Waste-indicator and pharmaceutical compounds in landfill-leachate-affected ground water near Elkhart, Indiana, 2000-2002. *Bull Environ Contam Toxicol* 82(6):653-659.

Cabrita, I., B. Ruiz, A. S. Mestre, I. M. Fonseca, A. P. Carvalho, and C. O. Ania. 2010. Removal of an analgesic using activated carbons prepared from urban and industrial residues. *Chemical Engineering Journal* 163(3):249-255.

Calderon-Preciado, D., C. Jimenez-Cartagena, V. Matamoros, and J. M. Bayona. 2011a. Screening of 47 organic microcontaminants in agricultural irrigation waters and their soil loading. *Water Res* 45(1):221-231.

Calderon-Preciado, D., V. Matamoros, and J. M. Bayona. 2011b. Occurrence and potential crop uptake of emerging contaminants and related compounds in an agricultural irrigation network. *Sci Total Environ* 412-413:14-19.

Chang, E. E., J. C. Wan, H. Kim, C. H. Liang, Y. D. Dai, and P. C. Chiang. 2015. Adsorption of Selected Pharmaceutical Compounds onto Activated Carbon in Dilute Aqueous Solutions Exemplified by Acetaminophen, Diclofenac, and Sulfamethoxazole. *ScientificWorldJournal* 2015:186501.

Chiron, S., E. Gomez, and H. Fenet. 2010. Nitration processes of acetaminophen in nitrifying activated sludge. *Environ Sci Technol* 44:284-289.

Cotoruelo, L. M., M. D. Marqués, A. Leiva, J. Rodríguez-Mirasol, and T. Cordero. 2011. Adsorption of oxygen-containing aromatics used in petrochemical, pharmaceutical and food industries by means of lignin based active carbons. *Adsorption* 17(3):539-550.

De Gusseme, B., L. Vanhaecke, W. Verstraete, and N. Boon. 2011. Degradation of acetaminophen by Delftia tsuruhatensis and Pseudomonas aeruginosa in a membrane bioreactor. *Water Res* 45(4):1829-1837.

Deo, R., and R. Halden. 2013. Pharmaceuticals in the Built and Natural Water Environment of the United States. *Water* 5(3):1346-1365.

Dougherty, J. A., P. W. Swarzenski, R. S. Dinicola, and M. Reinhard. 2010. Occurrence of Herbicides and Pharmaceutical and Personal Care Products in Surface Water and Groundwater around Liberty Bay, Puget Sound, Washington. *Journal of Environment Quality* 39(4):1173.

Fan, H., J. Li, L. Zhang, and L. Feng. 2014. Contribution of sludge adsorption and biodegradation to the removal of five pharmaceuticals in a submerged membrane bioreactor. *Biochemical Engineering Journal* 88:101-107.

Fram, M. S., and K. Belitz. 2011. Occurrence and concentrations of pharmaceutical compounds in groundwater used for public drinking-water supply in California. *Sci Total Environ* 409(18):3409-3417.

Gillis, P. L., F. Gagne, R. McInnis, T. M. Hooey, E. S. Choy, C. Andre, M. E. Hoque, and C. D. Metcalfe. 2014. The impact of municipal wastewater effluent on field-deployed freshwater mussels in the Grand River (Ontario, Canada). *Environ Toxicol Chem* 33(1):134-143.

Gomez, M. J., M. Petrovic, A. R. Fernandez-Alba, and D. Barcelo. 2006. Determination of pharmaceuticals of various therapeutic classes by solid-phase extraction and liquid chromatography-tandem mass spectrometry analysis in hospital effluent wastewaters. *J Chromatogr A* 1114(2):224-233.

Gottschall, N., E. Topp, C. Metcalfe, M. Edwards, M. Payne, S. Kleywegt, P. Russell, and D. R. Lapen. 2012. Pharmaceutical and personal care products in groundwater, subsurface drainage, soil, and wheat grain, following a high single application of municipal biosolids to a field. *Chemosphere* 87(2):194-203.

Gros, M., M. Petrovic, and D. Barcelo. 2006. Development of a multi-residue analytical methodology based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for screening and trace level determination of pharmaceuticals in surface and wastewaters. *Talanta* 70(4):678-690.

Hart, A., and D. L. J. Orr. 1975. The degradation of paracetamol (4-hydroxyacetanilide) and other substituted acetanilides by a *Penicillium* species. *Antonie van Leeuwenhoek* 41:239-247.

Hilton, M. J. T., K. V. 2003. Determination of selected human pharmaceutical compounds in effluent and surface water samples by high-performance liquid chromatography-electrospray tandem mass spectrometry. *Journal of Chromatography A* 1015:129-141.

Huang, M., L. Yang, H. Qin, L. Jiang, and Y. Zou. 2014. Fertilizer nitrogen uptake by rice increased by biochar application. *Biology and Fertility of Soils* 50(6):997-1000.

Hughes, M. A., M. J. Baggs, J. a. al-Dulayymi, M. S. Baird, and P. A. Williams. 2002. Accumulation of 2-Aminophenoxazin-3-one-7-Carboxylate during Growth of Pseudomonas putida TW3 on 4-Nitro-Substituted Substrates Requires 4-Hydroxylaminobenzoate Lyase (PnbB). *Applied and Environmental Microbiology* 68(10):4965-4970.

Ippolito, J. A., D. A. Laird, and W. J. Busscher. 2012. Environmental benefits of biochar. *J Environ Qual* 41(4):967-972.

Jung, C., J. Oh, and Y. Yoon. 2015. Removal of acetaminophen and naproxen by combined coagulation and adsorption using biochar: influence of combined sewer overflow components. *Environ Sci Pollut Res Int*.

Kasprzyk-Hordern, B., R. M. Dinsdale, and A. J. Guwy. 2009. The removal of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs during wastewater treatment and its impact on the quality of receiving waters. *Water Res* 43(2):363-380.

Katz, B. G., D. W. Griffin, P. B. McMahon, H. S. Harden, E. Wade, R. W. Hicks, and J. P. Chanton. 2010. Fate of Effluent-Borne Contaminants beneath Septic Tank Drainfields Overlying a Karst Aquifer. *Journal of Environment Quality* 39(4):1181.

Khamis, M., R. Karaman, F. Ayyash, A. Qtait, O. Deeb, and A. Manssra. 2011. Efficiency of advanced membrane wastewater treatment plant towards removal of aspirin, salicyclic acid, paracetamol and p-aminophenol. *Journal of Environmental Science and Engineering* 5:121-137.

Kinney, C. A. F., E. T.; Werner, S. L.; Cahill, J. D. 2006. Presence and distribution of wastewater-derived pharmaceuticals in soil irrigated with reclaimed water. *Environ Toxicology and Chemistry* 25(2):317-326.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: A national reconnassaince. *Environ Sci Technol* 36(1202-1211).

Laird, D., P. Fleming, B. Wang, R. Horton, and D. Karlen. 2010. Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma* 158(3-4):436-442.

Lam, M. W., C. J. Young, R. A. Brain, D. J. Johnson, M. A. Hanson, C. J. Wilson, S. M. Richards, K. R. Solomon, and S. A. Mabury. 2004. Aquatic persistence of eight pharmaceuticals in a microcosm study. *Environ Toxicol Chem* 23(6):1431-1440.

Lapen, D. R., E. Topp, C. D. Metcalfe, H. Li, M. Edwards, N. Gottschall, P. Bolton, W. Curnoe, M. Payne, and A. Beck. 2008. Pharmaceutical and personal care products in tile drainage following land application of municipal biosolids. *Sci Total Environ* 399(1-3):50-65.

Lapworth, D. J., N. Baran, M. E. Stuart, and R. S. Ward. 2012. Emerging organic contaminants in groundwater: A review of sources, fate and occurrence. *Environ Pollut* 163:287-303.

Lawrence, J. R., B. Zhu, G. D. Swerhone, J. Roy, V. Tumber, M. J. Waiser, E. Topp, and D. R. Korber. 2012. Molecular and microscopic assessment of the effects of caffeine, acetaminophen, diclofenac, and their mixtures on river biofilm communities. *Environ Toxicol Chem* 31(3):508-517.

Li, J., Q. Ye, and J. Gan. 2014. Degradation and transformation products of acetaminophen in soil. *Water Res* 49:44-52.

Li, Y., R. Jindal, K. Choi, Y. L. Kho, and P. Garcia de Bullen. 2012. Pharmaceutical residues in wastewater treatment plants and surface waters in Bangkok. *Journal of Hazardous, Toxic, and Radioactive Waste* 16(88-91).

Lin, A. Y., C. A. Lin, H. H. Tung, and N. S. Chary. 2010. Potential for biodegradation and sorption of acetaminophen, caffeine, propranolol and acebutolol in lab-scale aqueous environments. *J Hazard Mater* 183(1-3):242-250.

Lin, A. Y., and Y. T. Tsai. 2009. Occurrence of pharmaceuticals in Taiwan's surface waters: impact of waste streams from hospitals and pharmaceutical production facilities. *Sci Total Environ* 407(12):3793-3802.

Lin, A. Y., T. H. Yu, and C. F. Lin. 2008. Pharmaceutical contamination in residential, industrial, and agricultural waste streams: risk to aqueous environments in Taiwan. *Chemosphere* 74(1):131-141.

Loffler, D. R., J.; Meller, M.; Ternes, T. A. 2005. Environmental fate of pharmaceuticals in water/sediment systems. *Environ Sci Technol* 39:5209-5218.

Loffler, D. T., T. A. 2003. Determination of acidic pharmaceuticals, antibiotics, and ivermectin in river sediment using liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* 1021:133-144.

Maeng, S. K., S. K. Sharma, C. D. Abel, A. Magic-Knezev, and G. L. Amy. 2011. Role of biodegradation in the removal of pharmaceutically active compounds with different bulk organic matter characteristics through managed aquifer recharge: batch and column studies. *Water Res* 45(16):4722-4736.

Meisel, M. L., M. do Ceu Costa, and A. Pena. 2009. Regulatory approach on environmental risk assessment. Risk management recommendations, reasonable and prudent alternatives. *Ecotoxicology* 18(8):1176-1181.

Mestre, A. S., J. Pires, J. M. Nogueira, J. B. Parra, A. P. Carvalho, and C. O. Ania. 2009. Waste-derived activated carbons for removal of ibuprofen from solution: role of surface chemistry and pore structure. *Bioresour Technol* 100(5):1720-1726.

Mestre, A. S., J. Pires, J. M. F. Nogueira, and A. P. Carvalho. 2007. Activated carbons for the adsorption of ibuprofen. *Carbon* 45(10):1979-1988.

Meuser, H., and R. H. M. Van de Graaff. 2011. Characteristics of Natural and Urban Soils.91-136.

Mompelat, S., B. Le Bot, and O. Thomas. 2009. Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water. *Environ Int* 35(5):803-814.

Murtaza, G., K. S. A., A. Shabbir, A. Mahmood, M. H. H. Bin Asad, K. Farzana, N. S. Malik, and I. Hussain. 2011. Development of a UV-spectrophotometric method for the simultaneous determination of aspirin and paracetamol in tablets. *Scientific Research and Essays* 6(2):417-421.

Musson, S. E., P. Campo, T. Tolaymat, M. Suidan, and T. G. Townsend. 2010. Assessment of the anaerobic degradation of six active pharmaceutical ingredients. *Sci Total Environ* 408(9):2068-2074.

Ortiz de Garcia, S. A., G. Pinto Pinto, P. A. Garcia-Encina, and R. Irusta-Mata. 2014. Ecotoxicity and environmental risk assessment of pharmaceuticals and personal care products in aquatic environments and wastewater treatment plants. *Ecotoxicology* 23(8):1517-1533.

Pal, A., K. Y. Gin, A. Y. Lin, and M. Reinhard. 2010. Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. *Sci Total Environ* 408(24):6062-6069.

Park, J., I. Hung, Z. Gan, O. J. Rojas, K. H. Lim, and S. Park. 2013. Activated carbon from biochar: influence of its physicochemical properties on the sorption characteristics of phenanthrene. *Bioresour Technol* 149:383-389.

Pedrouzo, M., F. Borrull, E. Pocurull, and R. M. Marcé. 2010. Presence of Pharmaceuticals and Hormones in Waters from Sewage Treatment Plants. *Water, Air, & Soil Pollution* 217(1-4):267-281.

Ribeiro, A. V. F. N., M. Belisario, R. M. Galazzi, D. C. Balthazar, M. G. Pereira, and J. N. Ribeiro. 2011. Evaluation of two bioadsorbents for removing paracetamol from aqueous media. *Electronic Journal of Biotechnology*.

Richardson, S. D., and T. A. Ternes. 2014. Water analysis: emerging contaminants and current issues. *Anal Chem* 86(6):2813-2848.

Ruiz, B., I. Cabrita, A. S. Mestre, J. B. Parra, J. Pires, A. P. Carvalho, and C. O. Ania. 2010. Surface heterogeneity effects of activated carbons on the kinetics of paracetamol removal from aqueous solution. *Applied Surface Science* 256(17):5171-5175.

Scott, P. D., M. Bartkow, S. J. Blockwell, H. M. Coleman, S. J. Khan, R. Lim, J. A. McDonald, H. Nice, D. Nugegoda, V. Pettigrove, L. A. Tremblay, M. S. Warne, and F. D. Leusch. 2014. A national survey of trace organic contaminants in Australian rivers. *J Environ Qual* 43(5):1702-1712.

Sim, W. J., J. W. Lee, E. S. Lee, S. K. Shin, S. R. Hwang, and J. E. Oh. 2011. Occurrence and distribution of pharmaceuticals in wastewater from households, livestock farms, hospitals and pharmaceutical manufactures. *Chemosphere* 82(2):179-186.

Tijani, J. O., O. O. Fatoba, and L. F. Petrik. 2013. A Review of Pharmaceuticals and Endocrine-Disrupting Compounds: Sources, Effects, Removal, and Detections. *Water, Air, & Soil Pollution* 224(11).

Topp, E., S. C. Monteiro, A. Beck, B. B. Coelho, A. B. Boxall, P. W. Duenk, S. Kleywegt, D. R. Lapen, M. Payne, L. Sabourin, H. Li, and C. D. Metcalfe. 2008. Runoff of pharmaceuticals and personal care products following application of biosolids to an agricultural field. *Sci Total Environ* 396(1):52-59.

Veach, A. M., and M. J. Bernot. 2011. Temporal variation of pharmaceuticals in an urban and agriculturally influenced stream. *Sci Total Environ* 409(21):4553-4563.

Verlicchi, P., M. Al Aukidy, A. Galletti, M. Petrovic, and D. Barcelo. 2012. Hospital effluent: investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment. *Sci Total Environ* 430:109-118.

Wu, S., L. Zhang, and J. Chen. 2012. Paracetamol in the environment and its degradation by microorganisms. *Appl Microbiol Biotechnol* 96(4):875-884.

Xia, K. B., A.; Das, K.; Pillar, G. 2005. Occurrence and fate of pharmaceuticals and personal care products (PPCPs) in biosolids. *Journal of Environment Quality* 34:91-104.

Yamamoto, H., Y. Nakamura, S. Moriguchi, Y. Nakamura, Y. Honda, I. Tamura, Y. Hirata, A. Hayashi, and J. Sekizawa. 2009. Persistence and partitioning of eight selected pharmaceuticals in

the aquatic environment: laboratory photolysis, biodegradation, and sorption experiments. *Water Res* 43(2):351-362.

Yang, L., L. E. Yu, and M. B. Ray. 2009. Photocatalytic oxidation of paracetamol: dominant reactions, intermediates, and reaction mechanisms. *Environ Sci Technol* 43:460-465.

Yu, T. H., A. Y. Lin, S. C. Panchangam, P. K. Hong, P. Y. Yang, and C. F. Lin. 2011. Biodegradation and bio-sorption of antibiotics and non-steroidal anti-inflammatory drugs using immobilized cell process. *Chemosphere* 84(9):1216-1222.

Yu, Y., Y. Liu, and L. Wu. 2013. Sorption and degradation of pharmaceuticals and personal care products (PPCPs) in soils. *Environ Sci Pollut Res Int* 20(6):4261-4267.

Zhang, L., J. Hu, R. Zhu, Q. Zhou, and J. Chen. 2013. Degradation of paracetamol by pure bacterial cultures and their microbial consortium. *Appl Microbiol Biotechnol* 97(8):3687-3698.

Zhao, J., A. Singh, H. Huang, and O. P. Ward. 2000. Biotransformation of Hydroxylaminobenzene and Aminophenol by Pseudomonas putida 2NP8 Cells Grown in the Presence of 3-Nitrophenol. *Applied and Environmental Microbiology* 66(6):2336-2342.