

USING BATCH STUDIES TO OPTIMIZE ON-SITE WASTEWATER TREATMENT  
SYSTEMS FOR REMOVING EMERGING CONTAMINANTS

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## **ABSTRACT**

Charles Ryan McCall: Using Batch Studies to Optimize On-Site Wastewater Treatment Systems  
for Removing Emerging Contaminants  
(Under the direction of Howard Weinberg)

Decentralized or “on-site” wastewater treatment systems offer a number of advantages over conventional centralized systems, including reduced capital costs, adaptability, and ease of implementation for water reuse. While the ability of these systems to remove traditional wastewater constituents is well known, their ability to address contaminants of emerging environmental and public health concern, such as pharmaceuticals and personal care products, is less established. This report explores the effects of various design and operating parameters on emerging contaminant removal, with a focus on the use of batch studies to identify which removal mechanisms are most relevant. The results of an initial batch study, using wastewater from the Jordan Lake Business Park (Apex, NC), suggest that mixed media consisting of gravel, Stalite, and sand were more effective than sand alone at removing five selected contaminants from the bulk solution.

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## **LIST OF ABBREVIATIONS**

BOD	Biochemical Oxygen Demand
COD	Chemical Oxygen Demand
DEET	N,N-diethyl-meta-toluamide
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
E2	17 $\beta$ -Estradiol
EC	Emerging Contaminant
EPA	Environmental Protection Agency
GC-MS	Gas Chromatography Mass Spectrometry
HLR	Hydraulic Loading Rate
HRT	Hydraulic Residence Time
JLBC	Jordan Lake Business Center
LGW	Lab Grade Water
MGD	Million Gallons per Day
MLD	Million Liters per Day
NOM	Natural Organic Matter
OWASA	Orange Water and Sewer Authority
OWTS	On-Site Wastewater Treatment System
PPCP	Pharmaceuticals and Personal Care Products
SPE	Solid Phase Extraction
SSF	Subsurface Flow
STP	Sewage Treatment Plant

TSS	Total Suspended Solids
VF	Vertical Flow
VSF	Vegetated Sand Filtration
WWTP	Wastewater Treatment Plant

# **CHAPTER 1: INTRODUCTION TO ON-SITE WASTEWATER TREATMENT SYSTEMS INCLUDING CONSTRUCTED WETLANDS AND EMERGING CONTAMINANTS OF CONCERN**

## **1.1 Executive Summary**

Decentralized or “on-site” wastewater treatment systems offer a number of advantages over conventional centralized systems, including reduced capital and operating costs, adaptability, and ease of implementation for water reuse. These systems have been installed throughout the U.S. and internationally in a wide range of settings, including schools, business centers, and small residential communities. One of the most popular methods of onsite wastewater treatment involves the use of biologically active sand filtration to reduce wastewater contaminants. While the ability of these systems to remove traditional wastewater constituents, such as biochemical oxygen demand and nutrients, are well documented, their ability to remove a number of emerging chemical contaminants is relatively unknown. Such contaminants include pharmaceuticals, personal care products, and various agricultural and industrial chemicals.

Through the use of bench-scale experiments, it is possible to assess contaminant removal efficiencies by various processes that occur within an onsite wastewater treatment system. Batch studies provide a simple and effective way to isolate processes and examine the effects of various parameters on contaminant removal. Five chemicals representing different groups of emerging contaminants (ECs) are evaluated in this study, namely carbamazepine, triclosan, caffeine, atrazine, and N,N-diethyl-meta-toluamide (DEET). These chemicals are found in domestic and small-scale commercial waste streams, and were selected because of their

persistence in the environment. The Jordan Lake Business Center (JLBC), located in Apex, NC, which is host to an on-site wastewater treatment system that includes vegetated sand filtration, provided a case study for this technical report.

## **1.2 Introduction to On-Site Wastewater Treatment Systems (OWTSs)**

Conventional wastewater treatment processes have focused historically on removing biochemical oxygen demand (BOD) and nutrients, such as nitrogen and phosphorus (U.S. EPA, 2004), using a secondary biological treatment following primary clarification. During biological treatment, naturally occurring microorganisms break down organic matter under varied environmental conditions to achieve the desired effluent concentrations. In centralized wastewater treatment plants (WWTPs), these processes are typically energy-intensive, as aeration and mixing of large water volumes are required to maintain consistent substrate and bacterial concentrations throughout the reactor. Centralized WWTPs also require large capital investments in terms of the up-front construction costs, the distribution networks needed to convey the waste, and in the operation and maintenance of such systems.

As population growth and drought conditions have strained water resources, the use of decentralized or “on-site” wastewater treatment methods has increased substantially. Although the majority of systems are septic tanks, it is estimated that over 4 billion gallons of wastewater are treated every day in the U.S. by on-site systems at over 26 million sites, including homes, businesses, and recreational facilities (U.S. EPA, 2004). They have become recognized not as temporary solutions to wastewater treatment, but as long-lasting systems that, if designed and maintained properly, can bring significant benefits over conventional, centralized treatment processes. Although these systems treat smaller flows than centralized plants, they are significantly less expensive to construct, and require less energy to operate. Figure 1 shows the





health effects as well. The U.S. Environmental Protection Agency (EPA) estimates that only a third of land area in the U.S. has soils suitable for treatment of septic effluent. Furthermore, many systems are located near groundwater sources that can quickly become contaminated.

Over the past century, OWTs have evolved to include a number of options, or “alternative technologies,” for post-septic tank treatment. These technologies provide environments suitable for removal of nutrients and pathogens at higher rates than conventional subsurface soil treatment. However, the proper design, implementation, and maintenance of these systems are paramount to their success in achieving improved treatment goals. A continuing investigation of the effects of key design parameters on system performance has resulted in vast improvements over conventional on-site treatment systems.

One of the most popular additional processes in on-site treatment uses sand or media filters. In these systems, septic tank effluent passes through compacted layers of rock, sand, gravel, or other materials, undergoing a variety of transformation processes (U.S. EPA, 2002). Sand filters are, essentially, fixed-film bioreactors where microorganisms affix to and accumulate as a film on the various types of filter media, breaking down organic matter in the process. The majority of biological treatment in a sand filter system takes place within 6 inches of the filter surface, due in part to this region’s proximity to the air above (U.S. EPA, 2002). The physical process of sedimentation also takes place as water percolates through the media. Finally, in some cases constituents such as phosphorus are chemically adsorbed to the media surface. However, adsorption capacity is limited, as open sites are quickly saturated and unavailable. The capacity of a system to retain contaminants via adsorption is also dependent upon the media composition, the target chemicals, and the pH of the bulk liquid.

In terms of biological treatment, a number of key design parameters must be considered. Retention time has a significant effect on overall performance, as the microbial population must have sufficient time to acclimatize to and consume wastewater constituents. Media composition and dissolved oxygen (DO) concentration are two important parameters that are interrelated. Despite having porosities similar to sand, larger media types such as rock and gravel create larger effective pore sizes, which allow for greater interaction between the open air and wastewater near the filter surface. As such, larger media sizes will aerate the wastewater more effectively. At the same time, however, the use of larger media decreases the overall surface area available for biofilm growth. **Table 1** provides some basic parameters for gravel and sand media. Dosing volume and dosing frequency also affect the overall treatment capacity of media filter systems. If influent doses are too large or too frequent, microbial communities are at risk of experiencing a “shock”, where they become overwhelmed and ineffective at affecting the wastewater constituents.

**Table 1. Properties of gravel and sand media (Fetter, 1994)**

	Gravel	Fine Sand
Grain Size (mm)	>2.0	0.10 - 0.25
Specific surface area (cm <sup>2</sup> /g)	11.5	445
Porosity <sup>1</sup> (%)	25 - 50	25 - 50
Permeability <sup>2</sup> (cm/s)	10 <sup>-2</sup> to 1	10 <sup>-3</sup> to 10 <sup>-1</sup>

<sup>1</sup> Defined as the percentage of total volume that is void of material.

<sup>2</sup> Defined as a measure of the medium's ability to transmit a fluid (i.e., water).

Due to the relative stability of fixed-film processes, sand filters are generally easy to operate and maintain. Sand filters are typically operated with either intermittent or recirculating flows. With intermittent filters, also called “single-pass” filters, effluent is discharged after a single pass through the filter. Septic tank effluent is intermittently pumped from a dosing tank to the filter, where it is distributed along the surface of the media bed. Single-pass filters typically

use finer media types, as they allow for longer retention times and greater interaction between the wastewater and the biofilms on the media surfaces. Recirculating filters collect and recycle the treated water, passing it back through the media a number of times before it is discharged as effluent. Typically, the only difference mechanically between a recirculating system and an intermittent system is that the filter discharge line is equipped with a flow-splitting device, which returns a portion of the filter discharge to the dosing tank. This returned water is mixed with septic tank effluent before being reintroduced to the media filter.

A number of media types are used in sand filters, including various types of sand, gravel, and rock. Nonconventional media has also been used, including crushed glass, plastics, synthetic fibers, and other man-made materials. Sand media filters in particular have been used in a broad range of applications, including single households, small domestic communities, and commercial buildings. They have been used as both the primary means of treatment prior to discharge onto land or surface waters and as a pretreatment step prior to discharge into sewer systems.

Increasingly, effluent from these systems is being reused for non-potable applications, including irrigation and toilet flushing. Producing reuse quality water on site means there is no need for costly infrastructure to move it from a centralized location to a point of use. As mentioned previously, a number of treatment processes take place within these sand filter systems which are affected by conditions within the reactor, such as temperature, pH, retention time, media type, and the concentrations of the various contaminants. Section 1.3 will discuss methods by which these parameters are controlled, allowing for optimizing treatment conditions for removal of chemical contaminants.

### **1.3 Performance, Design, and Operation of OWTSSs**

In domestic applications, single-pass or “intermittent” sand filters are typically able to produce effluent with less than 10 mg/L BOD and 10 mg/L total suspended solids (TSS) (U.S. EPA, 2002). They are somewhat less effective in treating total nitrogen, with removal rates average around 25% (Pell and Nyberg, 1989). For both intermittent and recirculating filters, a 2- to 4-log reduction in fecal coliform is common. While recirculating filters are slightly better at treating BOD and TSS, they are much better equipped to remove total nitrogen, especially if an anoxic tank is placed before the recirculating tank. Addition of an anoxic treatment step allows nitrate removal to take place, as nitrified wastewater is recycled back to the front of the system.

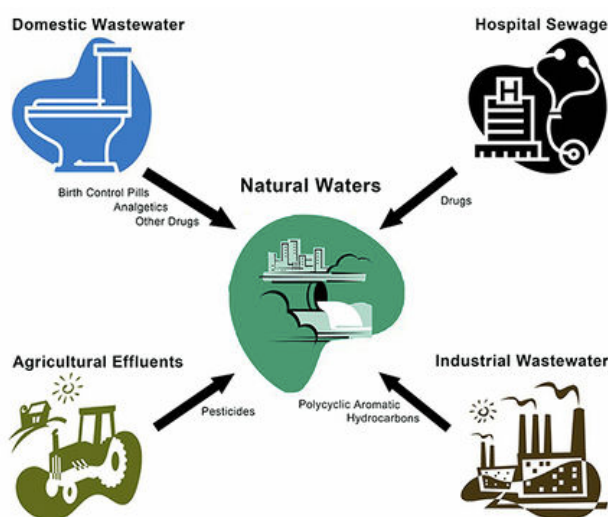
Modes of operation and mechanisms for aeration are two other design considerations for OWTSSs, which may be operated as batch or continuous flow systems. Batch systems will release a specified amount of septic tank effluent to the treatment train over a period of time, whereas continuous flow systems will seek to equalize flows continuously. Batch flow systems are preferable in settings where there is high variability in incoming flow, such as schools or business parks. These systems often meet oxygen demand via diffused aeration, in which manifolds beneath the sand filter supply diffused air from a blower or compressor. Surface mixers may also be used to introduce air to the wastewater matrix. A third way oxygen may be introduced is through vegetation. Plants placed above the sand filters will grow roots near the media surface, supplying oxygen to the microbes below. This type of setup is known as vegetated sand filtration (VSF). Finally, sand filters may simply be exposed to the open air, relying on the partial pressure of atmospheric oxygen to maintain sufficient DO levels in the bulk liquid. Although this method requires the least amount of energy, it is also limited in its ability to provide oxygen at depths beyond six inches in the media filter (U.S. EPA, 2002).

One of the ways that design parameters are chosen prior to field implementation is through laboratory-scale studies. Batch studies in particular are an approach to help modify and measure the relative effects of design variables at a smaller scale. They have been used in a variety of water treatment applications to determine how specific constituents can be remediated more effectively. For example, the American Water Works Association Research Foundation has published studies involving the removal of natural organic matter (NOM) in biofilters using batch processes (Bouwer et al., 1995). The main advantage of using batch studies to assess design parameters is that due to their small scale, they can be easily managed, adapted, and replicated. In addition, they can be used alongside continuous-flow studies that incorporate multiple process conditions to determine the relative effectiveness of each isolated process. However, the fact that batch studies are typically performed at scales much smaller than their real-world application means there are also some disadvantages. The results of batch testing are not always transferable to their full-scale counterparts, as real-world conditions are not always as ideal or as controllable as in a laboratory setting. These pros and cons must be considered and accounted for in any laboratory-scale testing.

#### **1.4 Emerging Contaminants and their Significance**

In recent years, public concern has grown regarding the release of pharmaceuticals and personal care products (PPCPs) from treated wastewater into surface and groundwaters used as drinking water sources. PPCPs refer to any chemical that is used in a domestic, commercial, or agricultural setting for health or cosmetic purposes (U.S. EPA, 2010). While some PPCPs are known to be readily biodegradable, others are released from these settings without undergoing any physical or chemical transformations, eventually finding their way to the natural

environment. As the number and use of anthropogenic chemicals has increased over time, they have become potential threats both from an environmental and public health perspective. Such chemicals include hormones, surfactants, and anti-microbial agents. Figure 2 illustrates various pathways such chemicals may take to arrive in natural waters.



**Figure 2. Sources of micro-contaminants in natural waters (iGEM, 2012)**

Despite increasing concern over the fate of these and other contaminants, little is known about how these micro-pollutants are affected by onsite wastewater treatment processes, or the mechanisms by which they can be remediated. This information is especially important in water reuse settings, where treated effluent may be used for irrigation purposes. Advances in detection technology for chemicals at this scale now allow for more precise measurements of their concentrations. As such, small differences in removal rates through wastewater treatment can be measured as a result of varying experimental conditions, such as media type, DO concentration, and retention time.

When deciding which chemicals to study in regard to their removal in wastewater treatment systems, it is important to choose a subset that represents a wide range of uses, chemical structures, and environmental and health effects. The five chemicals chosen for this

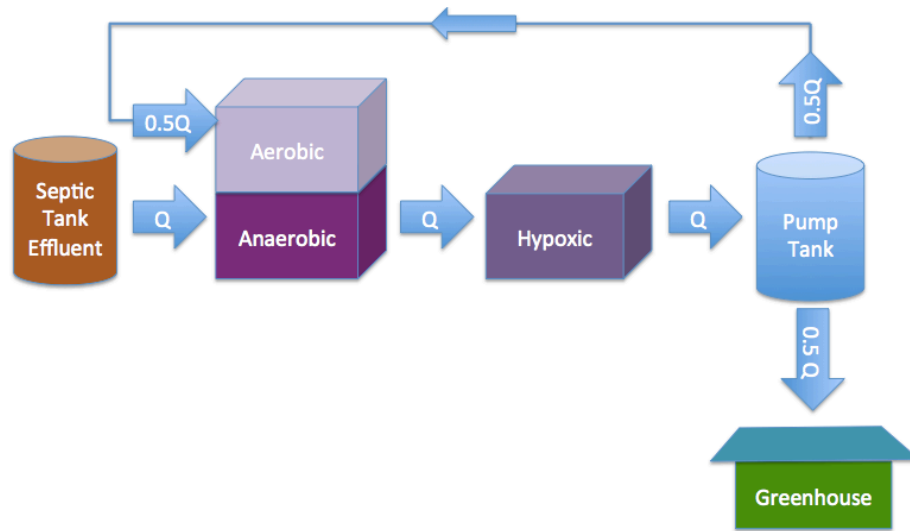
report are carbamazepine, triclosan, caffeine, atrazine, and DEET. Carbamazepine is a prescription drug used to treat seizures. Triclosan is an antibacterial and antifungal agent found in many household soaps. Caffeine is a widely used stimulant that is consistently found in septic tanks (Matamoros et al., 2009). Atrazine is an herbicide used to prevent weeds in the farming of corn and sugarcane. It is used to a lesser extent on golf courses and residential lawns, particularly in the Southeast compared to elsewhere in the U.S. Finally, DEET is the active ingredient in many insect repellants. Collectively, these chemicals represent those that can be found in domestic and small-scale commercial waste streams, and are of particular environmental and public health concern. In addition, the differences in chemical structures among these five contaminants means that their susceptibility to different removal mechanisms, such as biodegradation, adsorption, or photodegradation, could be different.

## **1.5 Overview of the Jordan Lake Business Center Wastewater Treatment System**

The Jordan Lake Business Center (JLBC), located in Apex, NC, is host to an on-site wastewater treatment system that includes sand filtration, and is the case study for this report. The wastewater treated by this system, which consists of vegetated sand filters known as “constructed wetlands,” is reused on-site for both irrigation and toilet flushing. The system, designed by Integrated Water Strategies, was the first water reclamation and reuse project in North Carolina (Integrated Water Strategies, 2013). Due to temporal variability in sewage flows, it utilizes a batch loading process. The system consists of three process zones: anaerobic ( $DO < 1$  mg/L), hypoxic ( $DO = 1-2$  mg/L), and aerobic ( $DO > 2$  mg/L). The main process tank contains both the aerobic and anaerobic zones, while a secondary tank houses the hypoxic zone. Septic effluent enters at the anaerobic stage and flows from there to the hypoxic stage. Water then flows



to a pump tank, where 50% of the forward flow is recycled back into the aerobic portion of the main process tank. A flow scheme of the JLBC process is given in Figure 3, where  $Q$  is the flow rate into the constructed wetlands system originating from the septic tank.



**Figure 3. Schematic of JLBC on-site wastewater treatment system**

While this system achieves consistent removal of BOD and total nitrogen, little is known about its ability to remove the types of contaminants discussed in Section 1.4. The next chapter will explore approaches for the removal of anthropogenic chemical contaminants from domestic wastewater using a constructed wetland.

## **CHAPTER 2: APPROACHING EMERGING CONTAMINANT REMOVAL BY CONSTRUCTED WETLANDS: IMPACT OF VARIOUS DESIGN AND OPERATING FACTORS**

### **2.1 Introduction to the Current Research**

Several recent studies have shown that both surface flow and subsurface flow constructed wetlands can be effective at removing ECs such as PPCPs from wastewater (Hijosa-Valsero et al., 2010; Matamoros and Bayona, 2006) while meeting conventional wastewater treatment objectives. However, less is known about the mechanisms responsible for the removal of specific contaminants, or how these mechanisms may be enhanced by altering specific wetland design parameters, including but not limited to filter media type, water depth, and the relative presence of aerobic versus anoxic conditions. This chapter will examine some of the current research regarding the effect of design and operating parameters on the removal of various emerging wastewater contaminants. Such research typically requires the use of smaller scale experimental setups to hold certain parameters constant while others are changed, providing valuable insight into the processes contributing to the net reaction rates. A number of researchers have begun to incorporate full-scale wetlands into their studies, allowing a determination of their difference in terms of treatment or behavior from laboratory- or pilot-scale studies. This review will focus primarily on the influence of design parameters on the removal or attenuation of emerging contaminants that are most abundant in municipal or residential wastewaters. The findings of this review will be considered in developing a set of batch-scale experiments to assess the impact of specific design and operating parameters on the removal of selected contaminants.

## 2.2 Effect of Hydraulic Loading Rate and Design Configuration on Emerging Contaminant Removal Mechanisms

Westerhoff et al. (2014) describe a number of key parameters of constructed wetland design that affect contaminant removal. Hydraulic residence time (HRT) and hydraulic loading rate (HLR) are related parameters that are considered to be two of the most influential. HRT, sometimes expressed as  $\tau$ , is an estimate of the average amount of time water spends within a treatment system:

$$\text{HRT } (\tau) = V/Q \quad (1)$$

where  $V$  is the wetland volume and  $Q$  is the average flow rate. HLR, sometimes expressed as  $q$ , is the flow per unit of wetland area and can be calculated in two ways:

$$\text{HLR } (q) = Q/A = h/\tau \quad (2)$$

where  $A$  is the wetland surface area from the top, and  $h$  is the water depth. For subsurface constructed flow wetlands, hydraulic loading rates are typically between 2 and 20 cm/day (U.S. EPA, 2000). Typically, the treatment efficiency of constructed wetlands can be improved by decreasing the hydraulic loading rate and thus increasing the HRT. This provides greater contact time between wastewater constituents and filter media. However, since increasing the HRT of a system typically involves increasing its size, it must be considered alongside the related costs of construction and operation.

Water depth is another physical aspect of wetlands systems that affects treatment efficiency. Relative to their overall size, beds with a shallower depth are expected to remove greater proportions of BOD, chemical oxygen demand (COD), and nutrients than deeper beds (Matamoros et al., 2008; Huang et al., 2004). This is likely due to greater diffusion of oxygen from the water surface compared to deeper beds with smaller surface area relative to bed volume.

However, deeper beds are also more susceptible to clogging and, thus, can be more costly to maintain.

Filter bed media type is another parameter that may affect the transformation or removal of certain contaminants. For example, removal efficiencies for contaminants that are susceptible to biodegradation may be improved through the use of filter bed materials that promote greater biofilm growth. Similarly, materials with greater surface area and, thus, more available adsorption sites, may be able to adsorb greater amounts of contaminants to the media surface. It has been suggested that filter bed materials do not have a significant effect on removal efficiency for conventional wastewater parameters (Huang et al., 2004, 2005; Brisson and Chazarnec, 2009). Yet, there is an acknowledgement that further research is necessary to determine conclusively whether filter bed materials have a measurable effect on the removal of emerging contaminants in constructed wetlands.

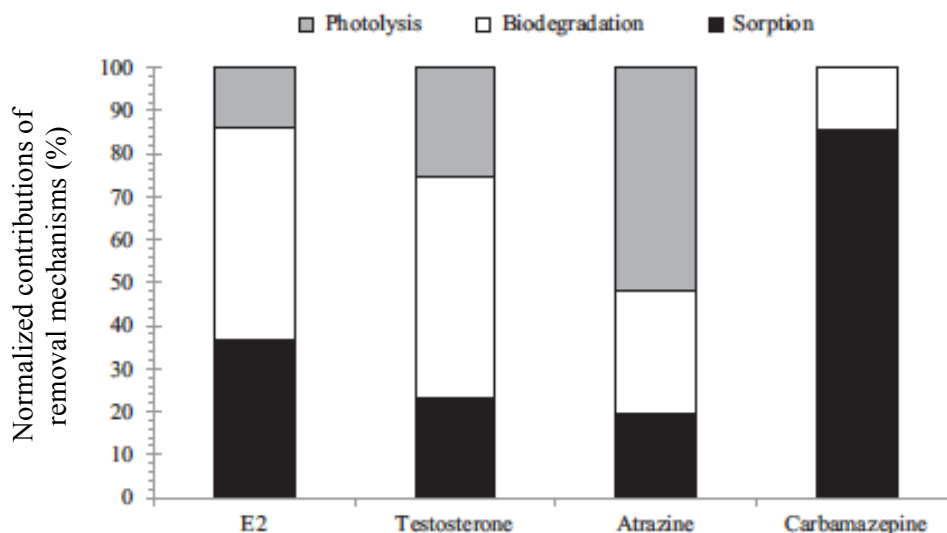
In order to determine how the modification of such parameters affects the ability of constructed wetlands to remove emerging contaminants, one must consider the various processes these contaminants may be engaged in while flowing through a wetland system. In determining the net reaction rate,  $r_T$ , for a particular contaminant, Westerhoff et al. (2014) considered five processes as indicated in Equation 3:

$$r_T = r_P + r_S + r_V + r_H + r_B \quad (3)$$

where  $r_P$ ,  $r_S$ ,  $r_V$ ,  $r_H$ , and  $r_B$  are the rates of photolysis, sorption, volatilization, hydrolysis, and biodegradation, respectively. The rate of photolysis of a compound such as an emerging contaminant is dependent upon a number of factors including light intensity, surface water depth and quality, and the physico-chemical properties of the contaminant itself. Analgesic drugs and antimicrobials (such as triclosan) are some of the PPCPs that are most susceptible to

photodegradation (Westerhoff et al., 2014). Sorption rates are dependent upon properties of the filter media as well as properties of the sorbate, including its water solubility. Since most emerging contaminants have low Henry's Law constants, their rates of volatilization are typically much smaller than those of other processes, and are thus ignored in determining the net reaction rate. Hydrolysis of contaminants is largely dependent upon the temperature and pH of the wastewater. Finally, biodegradation occurs when microbes break down chemicals for metabolism under either aerobic or anaerobic conditions.

Each emerging contaminant used in the Westerhoff et al. (2014) study was chosen from a different class, representing a group of chemicals that have the potential to be hazardous to the environment or public health. The seven chemicals chosen were atrazine, 17 $\beta$ -estradiol (E2), testosterone, caffeine, carbamazepine, primidone, and sucralose. The authors developed batch-scale experiments to quantify the amounts of these contaminants removed by each of the four main processes (photolysis, sorption, hydrolysis, and biodegradation). These experiments were used along with literature values to assign rate constants for each contaminant under each process. Rate constants were then input into a predictive model combining each reaction element to calculate the net reaction rate for each contaminant. As an example, **Figure 4** provides the predicted contributions from three of the main removal processes for four organic ECs.



**Figure 4. Contributions of different removal mechanisms for organic ECs from wastewater (Westerhoff et al., 2014)**

Based on the results of the batch study and the modeling of full-scale wetlands, Westerhoff et al. (2014) conclude that both operating parameters and design configurations influence which removal mechanisms contribute to EC removal, and thus affect overall treatment capacity of constructed wetlands for ECs. Atrazine, testosterone, and E2 were shown to be capable of photolysis in wetland water whereas carbamazepine, primidone, and caffeine were unaffected. Higher levels of dissolved organic carbon (DOC) from leached plant material negatively impacted photolysis rates. In modeling the removal of organic contaminants, the authors found that HLR was more influential than carbon loading rate. After normalizing for wetland area, the removal rates of all organic ECs except carbamazepine increased significantly with increased HLR. At the same time, however, BOD removal efficiency is likely to decrease with increased HLR, as the microbial community has less time to degrade organic material. Increasing the carbon loading rate, on the other hand, can be beneficial in removing contaminants that are most susceptible to sorption and biodegradation. For example, in cases where land is limited and wetlands must be operated at HLRs greater than 5 cm/day, increasing

the carbon loading rates can improve the removal rates for contaminants such as testosterone and estradiol. In terms of design configurations, wetlands with areas of open water will have greater impacts from photolysis, while sorption will dominate for designs with more available surface area (as plant material or as filter media). In addition, it is suggested that combining an open water wetland in series with a completely vegetated wetland will achieve greater overall EC removal than these two wetland types when operating alone (Westerhoff et al. 2014). These conclusions are valuable considerations when designing a set of batch experiments to determine the extent and mechanisms for removal of individual or mixtures of ECs.

### **2.3 Effects of Filter Media Size, Loading Frequency and Aeration**

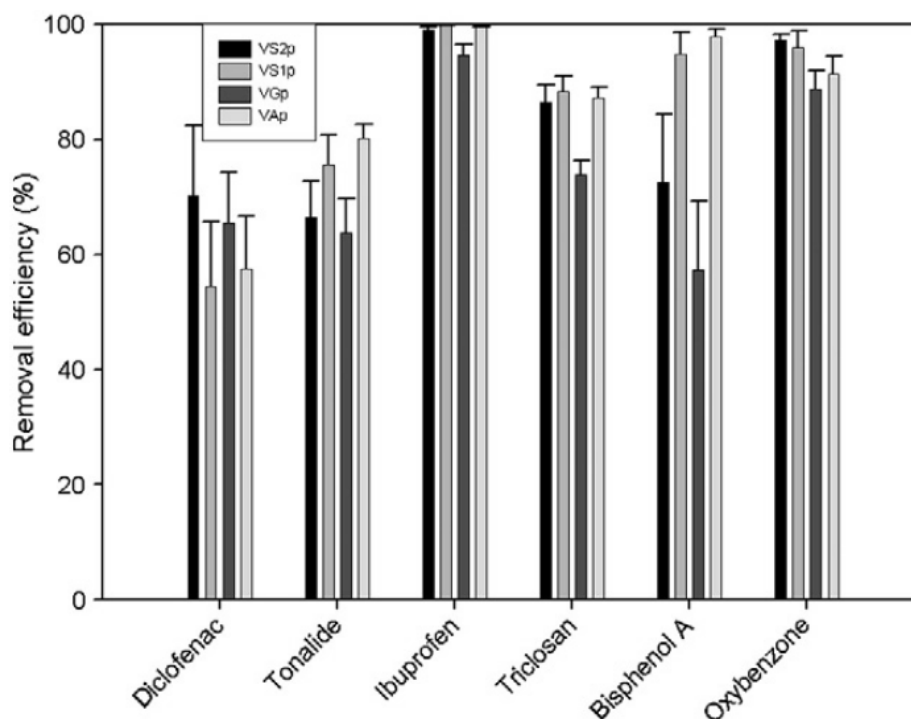
Avila et al. (2014) used four pilot-scale vertical flow (VF) constructed wetlands to evaluate the removal of eight widely used emerging contaminants from municipal wastewater. The selected contaminants, which include ibuprofen, acetaminophen, diclofenac, tonalide, oxybenzone, triclosan, ethinylestradiol, and bisphenol A, were chosen based on their widespread use and representation of a wide range of physico-chemical characteristics. Two of the wetland systems contained a gravel substrate, while the other two contained a sand substrate. One of the gravel systems included an active aeration system that supplied diffused air across the bottom of the gravel bed. All four systems had a surface area of 6.2 m<sup>2</sup> and received a hydraulic loading rate of 95 mm/day. The systems were pulse-loaded on an hourly basis except for one of the sand systems which was loaded every two hours. With this set of systems, the authors were able to evaluate the effects of media size, loading frequency, and the use of active aeration versus passive ventilation on emerging contaminant removal in subsurface flow constructed wetlands.

In terms of loading frequency, Avila et al. (2014) found that among two sand systems, the one with the higher pulse volume may have become more saturated with water, reducing air movement through pores and potentially decreasing aerobic activity in the wetland. Although the two sand systems with different loading frequencies performed equally well in terms of conventional wastewater parameters, it is suspected that the greater media saturation in the more frequently loaded system reduced air movement and thus oxygen transfer into the media bed. This negatively affected the removal of contaminants susceptible to aerobic biodegradation, such as diclofenac. On the other hand, tonalide and bisphenol A were removed more efficiently in the more frequently loaded system. However, contaminants primarily removed by sorption including tonalide may not have been removed as efficiently at increased hydraulic loading rate due to decreased contact time with filter bed materials. In addition, the reduced oxygen transfer in the more frequently loaded systems may have led to the formation of both aerobic and anaerobic zones within the media bed. The combination of different removal pathways through both aerobic and anaerobic reactions may increase the removal efficiency for certain biodegradable contaminants such as diclofenac (Hijosa-Valsero et al., 2010). In terms of overall EC removal efficiency, the hourly-dosed wetland performed slightly better than the one which was bihourly-dosed.

As for media size, the gravel-based wetland performed significantly worse than the sand-based system for all contaminants except for diclofenac. There are a number of potential explanations for this difference in treatment. First, the authors found that redox and DO concentrations were lower in the gravel-based vertical flow wetlands compared to the sand-based system. This could be a result of lower HRT in gravel media systems due to their larger material size, which allows water to pass through more quickly. The lower HRT means there is a higher



likelihood of contaminants passing through the system without much contact time with the biofilm, resulting in lower biodegradation rates. Further, gravel media has a lower filtering capacity than sand, which has smaller pore spaces and a higher surface area relative to its volume (Avila et al., 2014). With gravel, a higher proportion of solids are likely to pass through, some of which may have contaminants adsorbed to their surfaces. The positive influence of increasing HRT on EC removal is also in accordance with results presented in Westerhoff et al. (2014). Removal efficiencies reported by Avila et al. for the various wetland systems are provided in **Figure 5**.



**Figure 5. Contaminant removal efficiencies for various wetland systems (Avila et al., 2014).** Efficiencies are for a sand bed loaded every two hours (VS2p), sand bed loaded hourly (VS1p), gravel bed loaded hourly, and a gravel bed loaded hourly with active aeration (VAp). Lines above the bars indicate standard deviations (n = 10).

In terms of an aeration mechanism, the gravel-based bed with active aeration performed similarly to the sand bed without active aeration, with almost identical effluent quality for all ECs and conventional water quality parameters. This finding suggests that oxygen transfer is

higher in sand-based systems compared to gravel-based systems due to longer HRTs, which result in more contact time between wastewater and air. Assuming that a greater HRT is not an issue and the potential for clogging is not significantly increased, these results suggest that gravel-based systems struggling to meet performance requirements could be improved by either replacing a portion of gravel media with sand, or by installing an active aeration system below the media bed. Although Avila et al. (2014) provide some valuable comparisons between different types of constructed wetlands, they acknowledge that these systems were only tested in the months immediately following startup. As such, further studies are needed to assess their long-term performance.

#### **2.4 Kinetic Rate Determinations for Treatment of PPCPs in Subsurface Flow Constructed Wetlands**

While Avila et al. (2014) examined the ability of subsurface flow (SSF) wetlands to remove emerging contaminants over a few summer months, Matamaros and Bayona (2006) investigated the ability of horizontal flow constructed wetlands to remove contaminants over a two-year period. Specifically, two systems of different water depths were compared: a shallow bed with a 0.27 m water depth and a deeper bed with a 0.5 m water depth. Both beds had a surface area between 54 and 56 m<sup>2</sup>. Both wetland cells received the same urban wastewater, which was generated by a housing development with approximately 200 inhabitants. The eleven contaminants studied were salicylic acid, ibuprofen, OH-ibuprofen, CA-ibuprofen, naproxen, diclofenac, ketoprofen, caffeine, methyl dihydrojasmonate, galaxolide, and tonalide. These chemicals were chosen based on their presence in the actual wastewater and their ability to be accurately measured using gas chromatography-mass spectrometry (GC-MS).

**Table 2** provides the contaminant removal percentages for both beds in three sampling campaigns between May 2004 and July 2005. The results showed that the shallower wetland (0.27m depth) provided more efficient removal than the deeper one for PPCPs classified as efficiently removed, moderately removed, and recalcitrant. The authors note that similar findings have been reported elsewhere for BOD (Garcia et al., 2005) and other selected pharmaceuticals (Matamoros et al., 2005). However, the removal of the polycyclic musks (methyl dihydrojasmonate, galaxolide, and tonalide) was not affected by water depth. This was attributed to these chemicals' high hydrophobicity and stronger interaction with the gravel bed. While the wetlands generally provided less removal than conventional wastewater treatment (represented as STP — sewage treatment plant — in Table 2), they performed comparably to activated sludge plants for a number of the contaminants studied.

**Table 2: Contaminant removal percentages<sup>1</sup> and comparison to conventional sewage treatment plants (Matamoros and Bayona, 2006).**

	May 2004		May 2005		July 2005	STP
	C2	D2	C2	D2	D2	
	0.5 m	0.27 m	0.5 m	0.27 m	0.27 m	
<b>Pharmaceuticals</b>						
Salicylic acid	97 ± 1	98 ± 1	77 ± 5	92 ± 2	97 ± 1	99
Ibuprofen	52 ± 1	80 ± 2	17 ± 11	71 ± 8	62 ± 2	60 - 70
OH-Ibuprofen	33 ± 7	69 ± 9	20 ± 10	67 ± 11	50 ± 4	95
CA-Ibuprofen	73 ± 8	92 ± 2	25 ± 9	94 ± 3	75 ± 6	95
Naproxen	0 ± 10	90 ± 3	47 ± 22	85 ± 4	80 ± 9	40 - 55
Diclofenac	11 ± 42	45 ± 17	0 ± 12	0 ± 5	0 ± 10	9 - 75
Ketoprofen	0 ± 14	69 ± 5	0 ± 29	0 ± 8	45 ± 5	48-69
Caffeine	94 ± 1	98 ± 1	85 ± 2	94 ± 1	99 ± 1	99
<b>Fragrances</b>						
Methyl dihydrojasmonate	94 ± 2	99 ± 1	61 ± 8	99 ± 1	99 ± 1	98
Galaxolide	31 ± 11	45 ± 1	44 ± 6	50 ± 7	61 ± 10	70-85
Tonalide	32 ± 10	44 ± 9	53 ± 6	65 ± 3	64 ± 4	75-90

<sup>1</sup>C2 and D2 columns are wetland cells with deep and shallow bed depths, respectively. STP presents typical removal percentages in conventional activated sludge treatment plants.

Some clogging was observed in the deeper wetland, which caused a decrease in hydraulic retention time. This likely resulted in lower biodegradation rates. The results presented in Table 2 show a statistically significant difference between the two wetlands in the removal of biodegradable compounds. This observation represents an interesting design consideration for constructed wetlands: although deeper systems and those with sand-based media are more likely to perform favorably immediately following start-up, they also have a higher likelihood of clogging later in operation. This can often have the opposite of the desired effect for using sand, in which HRT is actually decreased rather than increased. Zero- and first-order kinetic areal rate constants were calculated for each contaminant. In most cases, first-order kinetic rates provided the better fit, as shown for the shallow wetland in **Table 3**. These values are in accordance with BOD removal rates for subsurface flow wetlands from Rousseau et al. (2004) suggesting that the kinetics of emerging contaminant removal are similar to that of other biodegradable organics.

**Table 3: Areal rate constants<sup>1</sup> for various emerging contaminants in a shallow wetland (Matamoros and Bayona, 2006).**

Contaminant	Zero-order kinetic			First-order kinetic	
	$K_{0A}$ ( $\text{mg m}^2 \text{ day}^{-1}$ )	$K_{0A}/C_0$ ( $\text{m}^{-1}$ )	$r^2$	$K_A$ ( $\text{m day}^{-1}$ )	$r^2$
Ibuprofen	0.523	0.028	0.888	0.051	0.951
OH-Ibuprofen	0.514	0.026	0.930	0.044	0.973
CA-Ibuprofen	0.973	0.035	0.671	0.130	0.505
Naproxen	0.057	0.036	0.984	0.076	0.919
Galaxolide	0.204	0.015	0.504	0.050	0.497
Tonalide	0.133	0.022	0.540	0.060	0.589
Caffeine	0.270	0.038	0.796	0.137	0.863
Methyl dihydrojasmonate	0.632	0.034	0.603	0.208	0.907

<sup>1</sup>  $K_{0A}$  is the zero-order kinetic rate constant and  $K_{0A}/C_0$  is the zero-order rate constant normalized for the chemical loading rate ( $C_0$ ) which is in units  $\text{mg} \cdot \text{m}^3/\text{day}$ .  $r^2$  is coefficient of determination which is a statistical measure of how well the presented rate constants the real data points.

## 2.5 Biotransformation, Sorption, and Photolysis in Open-Water Wetlands

The main objective of Jasper et al. (2014) was to assess the impact of biotransformation, sorption, and photolysis on the removal of six organic contaminants (atenolol, metoprolol, propranolol, trimethoprim, sulfamethoxazole, and carbamazepine) in a shallow open-water, pilot-scale constructed wetland. The bottom of the wetland cell consisted of a dense biomat of detritus that was colonized by a diverse microbial community, which was later characterized using scanning electron microscopy and DNA extraction. The experimental wetland received nitrified, non-disinfected effluent on a continuous basis from a nearby oxidation ditch wastewater treatment plant. It is important to note that this wetland had been operating for three years before the experiment began, meaning that microbial communities had been established prior to sampling. Hydraulic residence time in the experimental wetland cell ranged from 1 to 3 days. These relatively short HRTs were implemented to prevent the growth of algae or other floating vegetation that may have affected the efficacy of photolysis as a removal mechanism. Further, baffles were installed within the cell to prevent short-circuiting and ensure plug flow conditions.

Water samples were collected over a three-year period, with overall contaminant attenuation rates calculated using pseudo-first order kinetics, as shown in Equation 4:

$$C = C_o e^{-k_{pilot} V/Q} \quad (4)$$

where  $C$  is the contaminant concentration,  $C_o$  is the influent concentration,  $k_{pilot}$  is the pseudo-first order kinetic rate constant (per day),  $V$  is the wetland cell liquid volume (L), and  $Q$  is the flow rate, measured at the influent (in million liters per day (MLD)). In the case of this experiment, the volume of the cell was  $8 \times 10^4$  L and the flow rate was between  $2.6 \times 10^{-2}$  and  $7.6 \times 10^{-2}$  MLD. In addition to the pilot-scale wetland, microcosm experiments were conducted

to determine the rates of sorption and biotransformation. In addition to the wastewater used in the wetland cell study, the microcosms were spiked with a mixture of six contaminants at a concentration of 5 µg/L each. Both illuminated and dark microcosms were used to simulate treatment during daytime and nighttime, respectively, so that the determined rates of sorption and biotransformation would be closer to the true, in-field values.

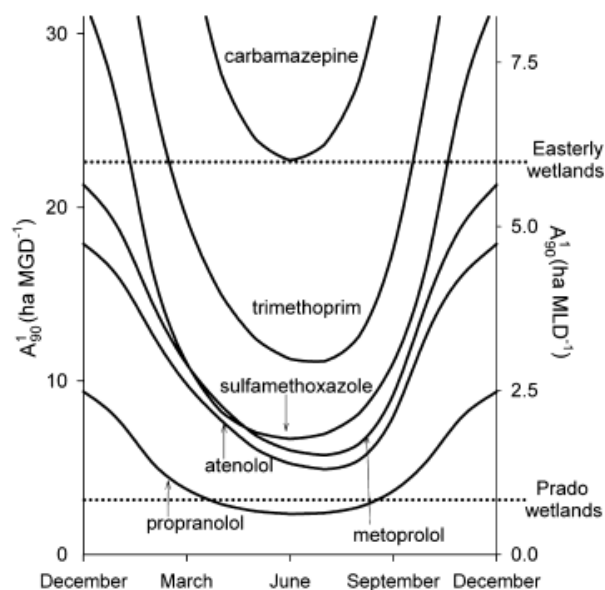
Microcosm test results suggested that highly hydrophobic compounds were most likely to adsorb to the biomat, while moderately hydrophobic compounds (carbamazepine and metoprolol) adsorbed to a lesser extent. On the other hand, for hydrophilic compounds such as atenolol, trimethoprim, and sulfamethoxazole, sorption accounted for less than 5% of observed removal. The microcosm experiments allowed the authors to categorize test compounds as follows: those undergoing rapid biotransformation (the beta-blockers atenolol, metoprolol, and propranolol ( $t_{1/2} < 1$  day)); those moderately amenable to biotransformation (trimethoprim and sulfamethoxazole,  $t_{1/2}$  of 2-20 days); or recalcitrant (carbamazepine ( $t_{1/2} > 40$  days)). These classifications agreed well with the rates measured in microcosms inoculated with activated sludge, although they were slower than the microcosms. A summary of first-order transformation rates for the compounds studied in various treatment systems is provided in **Table 4**.

**Table 4. Test compound first-order transformation rates (per day) (Jasper et al., 2014)**

Compound	Dark microcosms	Illuminated microcosms	Open-water wetland, summer	Activated sludge microcosms	Surface flow treatment wetlands
Atenolol	0.80 ± 0.03	0.59 ± 0.01	1.34 ± 0.60	6	0.05 - 0.10
Metoprolol	0.22 ± 0.05	0.82 ± 0.07	1.10 ± 0.30	1.2 - 2.6	0.03
Propranolol	0.11 ± 0.02	0.86 ± 0.02	1.15 ± 0.53	1.7	0.09
Trimethoprim	0.29 ± 0.05	0.056 ± 0.004	0.36 ± 0.23	0.2 - 0.4	0.04 - 0.20
Sulfamethoxazole	0.08 ± 0.01	0.034 ± 0.001	0.45 ± 0.49	0.09 - 0.26	0 - 0.02
Carbamazepine	0.016 ± 0.004	0.016 ± 0.003	0.02 ± 0.05	< 0.4	0 - 0.04

The results of this study can be applied to both full-scale wetland design and bench-scale studies in several ways. First, the fact that test compound attenuation rates decreased by more than 50% for all test contaminants during the winter months suggests that water surface temperatures can significantly affect microbial activity. Laboratory and bench-scales studies can be designed to vary temperature while other variables are held constant to measure a single variable's effect on contaminant removal. Further, Jasper et al. (2014) found that contaminants susceptible to biotransformation could be effectively removed using wetlands that were sized for denitrification of wastewater effluent. Denitrification activity itself may also have contributed to EC biotransformation in these cases. This suggests, however, that the size of a bench-scale system for emerging contaminant removal should be based on previous studies involving denitrification of wastewater effluent, especially if the majority of the contaminants of interest are susceptible to biodegradation.

**Figure 6** shows the wetland area necessary to achieve a 90% reduction via biotransformation and photolysis for all compounds listed in Table 4 at 1 million gallons per day (MGD) effluent flow at different times of the year. The combination of photolysis and biotransformation mechanisms in open-water wetlands allowed for the effective removal of a broader range of emerging contaminants. Water depth and thickness of the biomat were found to be primary factors to consider for the operation and maintenance of full-scale, open water constructed wetlands. While it is unlikely that either biomat thickness or photolysis will be a significant factor in bench-scale studies, it should be noted that contaminant removal rates in full-scale wetlands with larger areas of open water are likely to be higher than those in smaller experimental setups.



**Figure 6. Seasonal effects on removal of emerging contaminants as a function of open-water wetland area (Jasper et al., 2014).  $A_{90}$  represents areas predicted to provide 90% removal of emerging contaminants susceptible to biodegradation.**

## **2.6 Implications for the Use of Bench-Scale, Batch Experiments in Assessing the Removal of Emerging Wastewater Contaminants**

Overall, the current research on the use of constructed wetlands to remove emerging contaminants from wastewater suggests that these types of systems are a promising low-cost treatment method in both secondary treatment and secondary effluent polishing applications. Still, aspects of the research that have significant knowledge gaps include the determination of well-established removal efficiencies for pharmaceuticals and personal care products (Li et al., 2014). Further, the influence of traditionally monitored wastewater parameters on contaminant removal, such as pH, DO concentration, ammonia, and nitrate concentrations is relatively unknown. Finally, there are differing results in the literature regarding the influence of media type on contaminant removal. A set of controlled bench-scale batch experiments lends itself well to addressing these knowledge gaps, as they provide the ability to quickly and easily compare the effects of different parameters on the removal efficiencies of the contaminants of interest.



## **CHAPTER 3: INVESTIGATING CONTAMINANT REMOVAL MECHANISMS USING BATCH STUDIES: EXPERIMENT, RESULTS, AND DESIGN IMPLICATIONS**

### **3.1 Introduction to Batch Experiments (Explanation of Experimental Matrix)**

Using the experimental methods and analysis from the existing research presented in Chapter 2 as a guide, this chapter will present the methods and findings of bench-scale experiments conducted to assess the removal of emerging contaminants in batch studies. While column studies employ the plug flow that is typically seen in full-scale wetland systems, batch studies allow the assessment of various removal mechanisms individually. The primary objectives of this chapter are to demonstrate the ability of constructed wetlands media to remove selected organic contaminants of emerging concern and to assess their removal efficiencies using batch systems that varied in terms of physical media type and level of aeration. This was accomplished by using the contents of Chapter 2 to aid in the design and implementation of a series of batch experiments that replicated certain design and operating features of constructed wetlands. The results of these batch studies can then be used in conjunction with column studies to evaluate whether the removal efficiencies of the selected contaminants in these laboratory-scale experiments can aid in the design and operation of a full-scale constructed wetlands wastewater treatment system.

There are a number of inherent weaknesses to batch studies that this study sought to overcome. First, traditional batch studies involving wastewater typically involve introduction of a known wastewater matrix to a physical substrate, or media, over a predetermined time period. During this period, contaminant concentrations are measured to generate a decay rate which

represents the removal per unit time for the system. In these cases, the decay rate is assumed to follow a specific order uniformly over the experimental period. Typically, the objective of a batch study is to calculate a decay rate, similar to a thermodynamic decay constant, that quantifies the removal of the contaminant as a function of time. However, wastewater streams can be highly variable in terms of both wastewater constituents and flow. As such, the use of the same wastewater source for a number of batch experiments spread out over time is likely to produce different results in terms of decay rates, with large margins of error between different experimental runs. So, while this study seeks to obtain contaminant removal rates for the contaminants tested in a somewhat constant loading small system, they will not be used as a conclusive means of determining actual removal rates in all constructed wetlands systems.

Another aspect of batch studies involving treatment with physical media that this study attempts to overcome is the common practice of using media that has not been introduced to wastewater prior to testing. Although the use of virgin media aids in measuring the full sorptive capacity of the media, it does not accurately reflect real-world conditions in which wastewater treatment systems operate under a dynamic equilibrium. As such, the media used in the batch experiments described here were introduced to water from University Lake (Carrboro, NC) prior to testing for contaminant removal. This limited the sorptive capacity of the media and established a biomass within the reactor, both of which more closely align with expected conditions in an operating constructed wetlands system.

### **3.2 Methods**

As described in Chapter 1, the source water for this set of experiments was the Jordan Lake Business Center (JLBC), which operates a constructed wetlands system that provides non-

potable reclaimed water for both toilet flushing and irrigation of an on-site greenhouse.

Wastewater was collected in February 2014 from the distribution box (**Figure 7**) located between the first and second wetland cells. Water was collected using a siphoning hand pump and a 20 L polyethylene container. Upon returning to the UNC laboratories, this container was stored in a cold room until initial water quality analysis was performed.



**Figure 7. Distribution box containing source water**

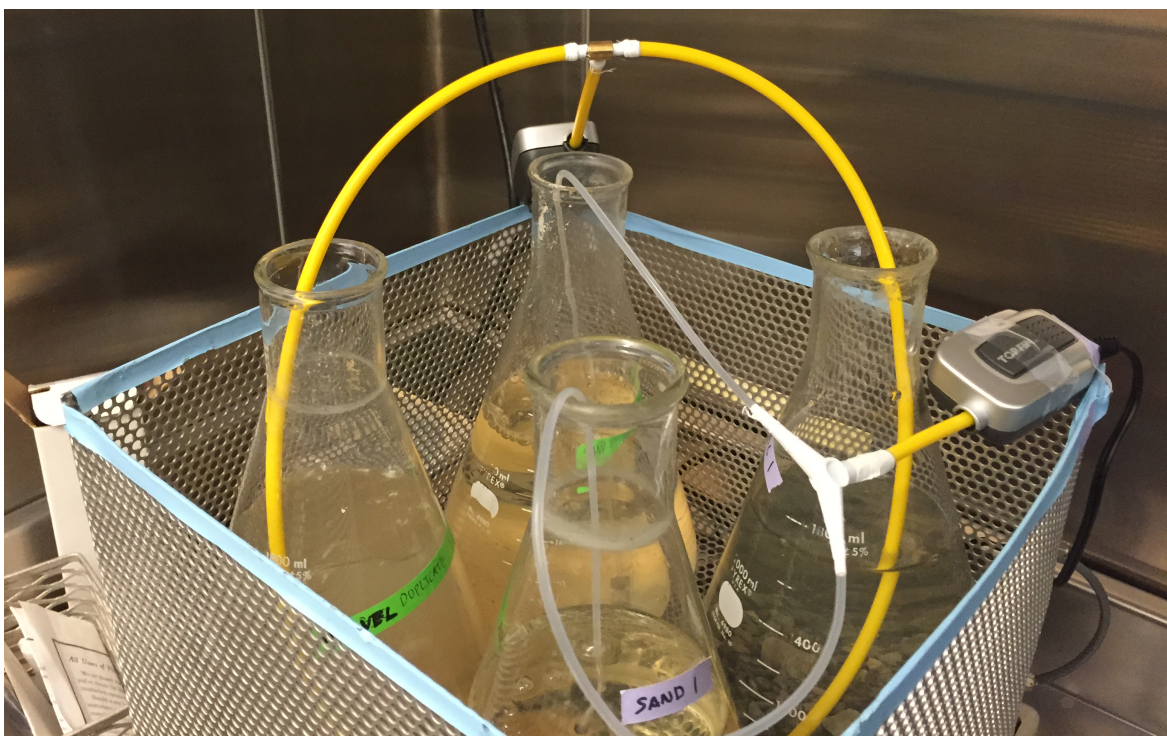
Two weeks prior to wastewater collection, four batch reactors were prepared in 2 L Erlenmeyer flasks using similar media to that used in the JLBC system. Two reactors consisted of sand only while the other two were a combination of sand, gravel, and Stalite, a locally sourced lightweight aggregate used in concrete, masonry, and asphalt products. The amount of sand added to the sand-only flasks was well in excess of the amount that was needed for a single round of batch experiments, to ensure that there was enough media to establish a biomass: 1,200 g of sand was placed in each of the two sand-only reactors, whereas the mixed-media reactors consisted of 500 g of sand, 150 g of gravel, and 250 g of Stalite. On the same week, 10 L of water sourced from University Lake was collected at the Orange Water and Sewer Authority (OWASA) Water Treatment Plant in Carrboro, NC. This water was used instead of JLBC wastewater to establish a

biomass on the media, ensuring that the contaminants of interest were not present in any detectable quantity while the media was being acclimatized. The University Lake water was added to each of the four reactors until one inch of freestanding water was visible above the top surface of the media. This method of biomass establishment ensured that the contaminant levels detected in subsequent testing resulted solely from spiked wastewater and not from re-dissolution of adsorbed contaminants from the media.

During the two-week period of biomass establishment with University Lake water, stock solutions for the five contaminants of interest (atrazine, caffeine, carbamazepine, DEET, and triclosan) were prepared in laboratory grade water (LGW). Each primary stock created was 50 mL, and was stored in amber bottles in the laboratory refrigerator. The concentrations of these five original stock solutions ranged from 44 mg/L (atrazine) to 212 mg/L (DEET). Intermediate stock solutions were then created in LGW as 3X dilutions of the five original stock solutions. To prepare the spiked wastewater, 24.2 mL of each intermediate stock solution was added to 10 L of JLBC wastewater to produce a spiked concentration in the range of 35.4 to 170.6 µg/L for each of the added ECs. The 20 L polypropylene container holding the wastewater was shaken by hand to ensure that the solution was well mixed. 500 mL of this solution was immediately removed from this 10 L and stored for solid phase extraction and gas chromatography/mass spectrometry (SPE/GC-MS) analysis of contaminant initial concentrations ( $t_{\text{initial}}$ ).

24 hours prior to the introduction of this spiked wastewater to the flasks, the flasks were drained of the University Lake water. The mouth of each flask was covered with a mesh cloth secured at the top before inverting over a plastic lab dish container. The flasks were drained for 24 hours. Excess sand was then removed from the two sand-only flasks such that only 90 g of sand remained in each flask. The original 900 g of sand, gravel, and Stalite was kept in each of

the two mixed media flasks. The spiked wastewater solution was then added to all four 2 L flasks. Wastewater was added according to the ratio of media to wastewater described by Cucarella & Renman (2009), who evaluated the sorption capacities of sand, gravel, and other media types observed in OWTS studies to more easily compare their sorptive ability. For the mixed-media flasks, which consisted primarily of gravel and Stalite, the ratio of media to wastewater (in g/mL) of 0.5 (900 g in 1800 mL of spiked wastewater). For the all-sand flask, this ratio was 0.05 (90 g in 1800 mL of spiked wastewater). After being filled, the flasks were placed on a shaker inside a temperature-controlled room and shaken continuously at 75 RPM. In addition to keeping the flasks open to air, each flask was also fitted with plastic tubing that reached the bottom of the flasks, connected to one of two small air pumps to ensure that conditions were aerobic within each flask. **Figure 8** shows the experimental setup. Measurements for  $UV_{254}$ , pH, ammonia, and nitrate for time  $t=0$  were performed on the same day that spiked wastewater was introduced to the media. The rest of the sample was held for SPE/GC-MS analysis at a later date. Samples awaiting analysis were stored in the laboratory refrigerator at 4 °C.



**Figure 8. Batch study experimental setup**

Immediately following the spiked wastewater introduction to the flasks, 55 mL of this wastewater was collected from each flask and stored for later analysis. The results of this analysis represent the time  $t=0$  results. The tests performed on this sample and the amount of sample used for each were as follows:

- Ammonia-Nitrogen (1 mL diluted with 9 mL of LGW)
- Nitrate-Nitrogen (1 mL diluted with 9 mL of LGW)
- $UV_{254}$  (1 mL)
- pH (10 mL)

At  $t = 4, 8, 24, 48, 72, 96, 120, 144$  and 192 hours after introduction of the spiked wastewater, 55 mL samples were collected in amber vials from each of the four flasks. The shaker was temporarily turned off, and the samples were collected using a graduated transfer pipette. They were labeled with the appropriate time stamp and stored in the fridge. The samples were used to measure the same parameters in the list above. These batch-treated samples

required filtration prior to spectrophotometric analysis. A 45-micron, non-acetate filter was used to remove suspended solids prior to analysis.

At  $t = 96$  and  $192$  hours, a  $555$  mL sample was collected from each of the four  $2$  L flasks. The additional  $500$  mL was used for SPE/GC-MS analysis. At timestamps  $t=0, 4, 8, 24, 48, 72, 96, 120, 148,$  and  $196$  hours, the DO concentration in each of the flasks was also measured using a DO benchtop analyzer. This did not require a sample to be taken from the flasks. Rather, the shaker was temporarily turned off while the DO probe was placed into the flask. The DO probe and meter were cleaned and prepared for use before turning off the shaker to keep the shaker downtime to a minimum and to ensure accurate readings each time the analyzer was used.

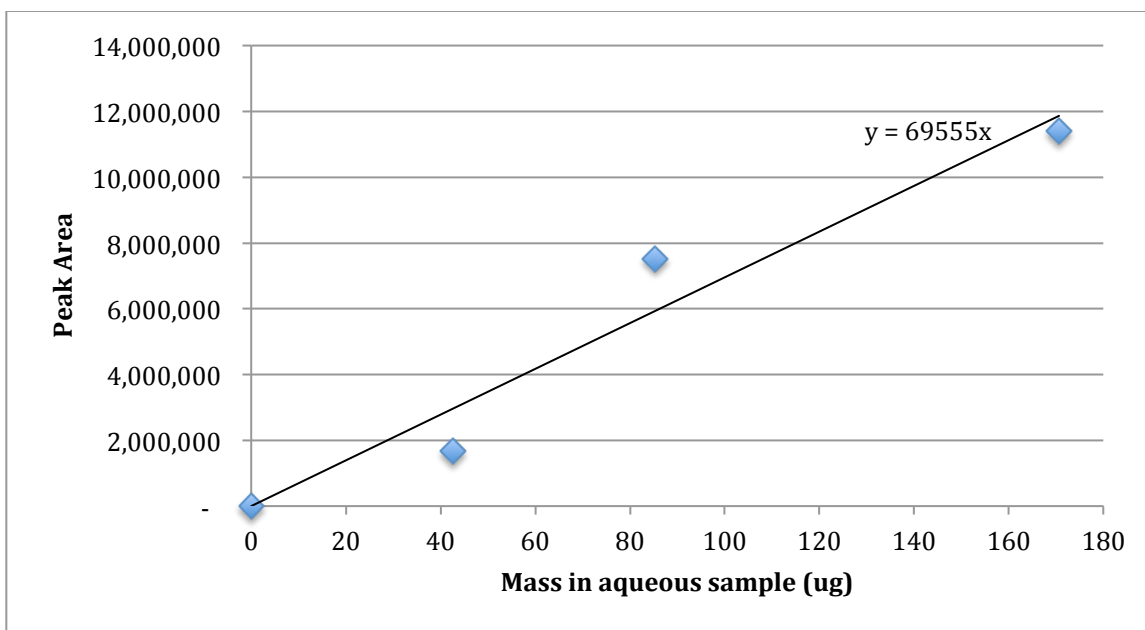
### **3.3 Analysis of Emerging Contaminants of Interest**

The procedure for detection and analysis of the seven emerging contaminants involved SPE/GC-MS as described by Stanford and Weinberg (2007). The SPE process concentrates the analytes of interest while eliminating other interfering components of the wastewater that would affect contaminant recovery and the detector signal. Prior to extraction,  $500$  mL samples were filtered using  $2.7, 1.5,$  and  $0.45\ \mu\text{m}$  filters, in sequence. Filtrate was then drawn through a series of two sorbent SPE cartridges that are located on top of a vacuum manifold. The first cartridge was used as a pre-filtering step to remove co-contaminants before the sample runs through the second cartridge, which retains the contaminant of interest. The cartridges are preconditioned using a series of solvents to allow the absorbent material in the cartridge to react selectively with the analytes of interest. Following the extraction process, the retained solids in the second cartridge were eluted into  $25$  mL vials using a mixture of methyl tert-butyl ether and methanol. The extracted material in the vials was then blown down to dryness using a low flow of nitrogen

gas and a heating block. Bis(trimethylsilyl)-trifluoroacetamide + 1% trimethyl-chlorosilane and pyridine, a catalyst, were added to the extracts. After a 30-minute reaction, 100  $\mu$ L of hexane was added to the vials which were vortexed to ensure transfer of the analyte into the hexane solvent. The latter was then drawn off using a Pasteur pipette and stored in an autosampler vial for subsequent GC-MS analysis.

The concentrations of each contaminant were quantified by measuring the areas of chromatographic peaks. Peaks representing each derivatized analyte were found as having the same retention time as that observed for a pure derivatized standard. The concentrations at each time step were calculated by first creating a calibration curve for each contaminant using peak area measurements for at least three samples of known concentrations. An example calibration curve for DEET is shown in **Figure 9**. These curves were used to correlate peak areas to mass amounts (in  $\mu$ g) so that contaminant concentrations could be calculated at each of the three time points where sampling for GC-MS analysis took place. For DEET, each  $\mu$ g in the wastewater sample corresponded to approximately 70,000 in peak area.

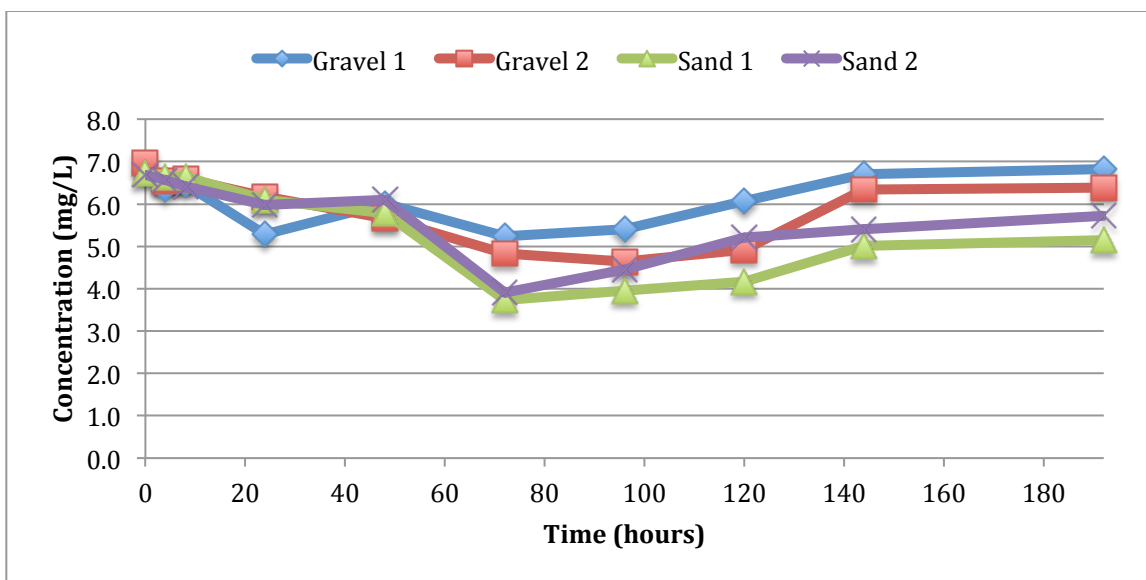




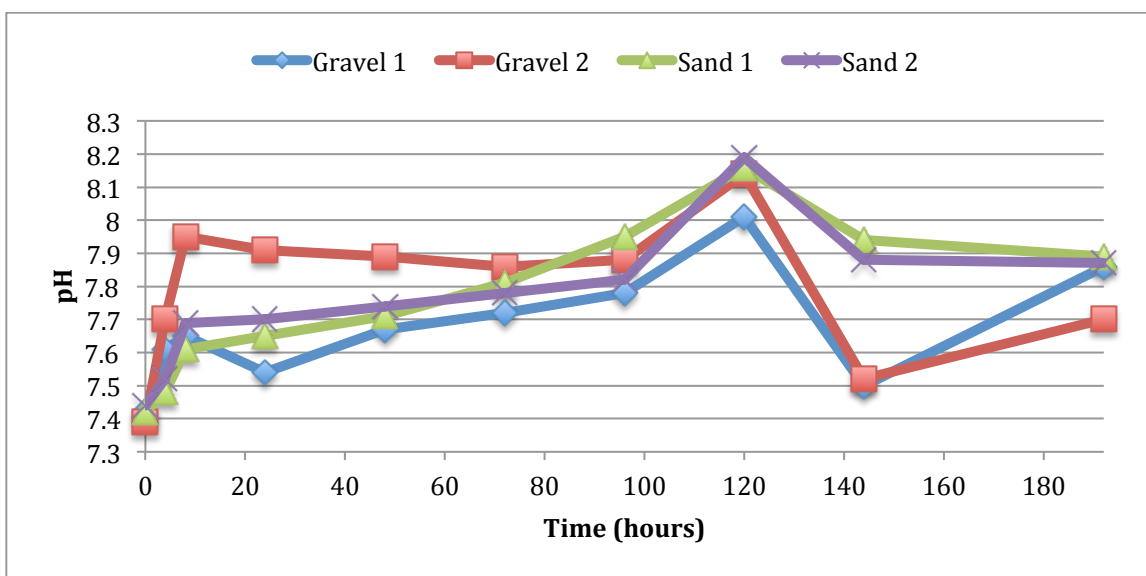
**Figure 9. GC-MS calibration curve for DEET.**

### 3.4 Experimental Results

**Figures 10 and 11** show the DO concentration and pH, respectively, in each of the four flasks throughout the 192-hour experiment. In these and the subsequent two figures, “Gravel 1” and “Gravel 2” are the two identical experimental setups with media consisting of gravel, Stalite, and sand. “Sand 1” and “Sand 2” are the two identical experimental setups with media consisting of sand only. All initial DO values were near 7 mg/L, which equates to about 75% oxygen saturation for water at 20° C. No flask fell below 3.7 mg/L DO at any point in the experiment, which suggests that conditions in the bulk solution remained aerobic at all times. By the end of the experiment, DO concentrations in all flasks had risen to at least 5.0 mg/L and held steady. Similarly, pH values remained relatively steady throughout, with nearly all values in the range 7.4 to 8.0. This suggests that biological activity was not hindered by acidic or basic conditions at any point during this period.



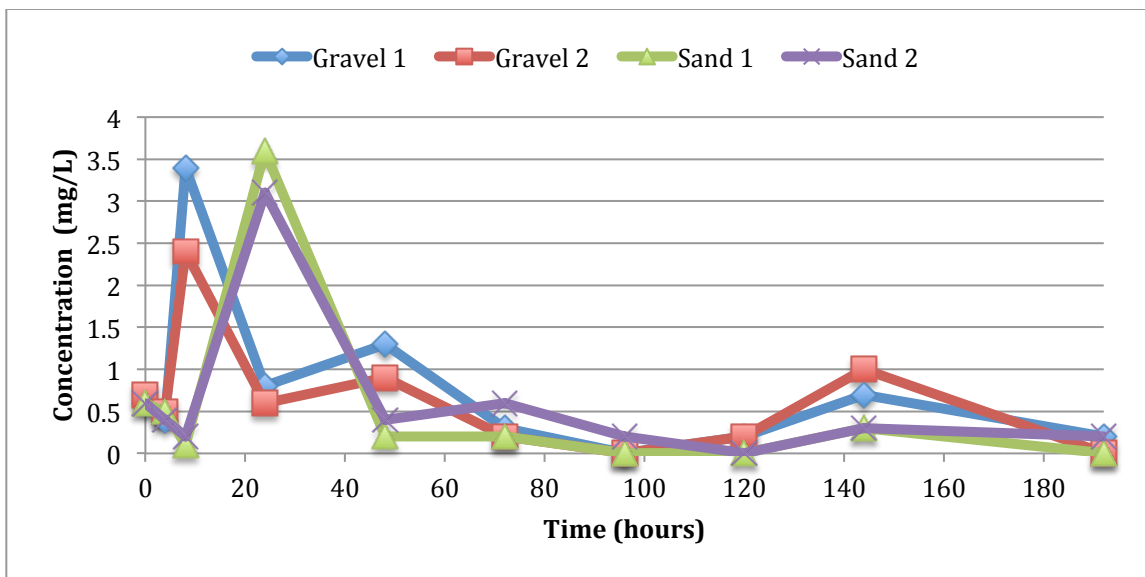
**Figure 10. Dissolved oxygen concentrations in the batch flasks**



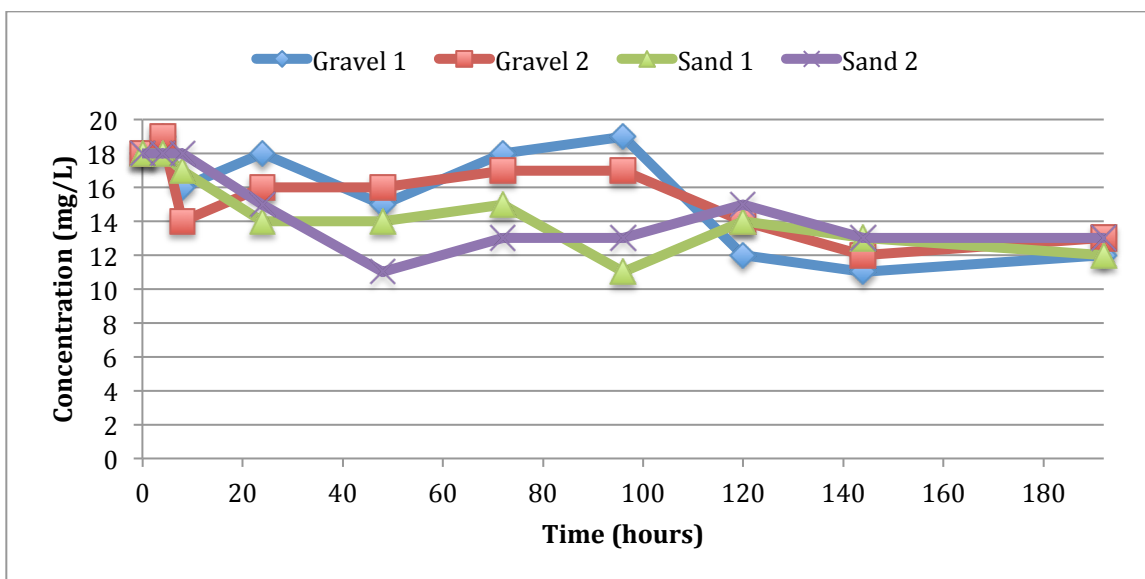
**Figure 11. pH in the batch flasks**

Concentrations of ammonia and nitrate in each flask throughout the experimental period are presented in **Figure 12** and **Figure 13**, respectively. Ammonia concentrations were relatively low for all readings, with small increases occurring at 8 hours in the gravel flasks, and at 24 hours in the sand flasks. The increase in ammonia corresponded with decreases in nitrate, which suggests that some ammonification took place, a process in which ammonium is released from

decaying organic matter. After these increases, ammonia levels fell back to near the initial levels and remained below 1 mg/L for the remainder of the experiment. There were no significant differences in ammonia or nitrate levels between the sand and the mixed-media flasks after the 100-hour time point. However, the correlations between increases in ammonia and decreases in nitrate, and vice-versa, suggest that active microbial populations were present.



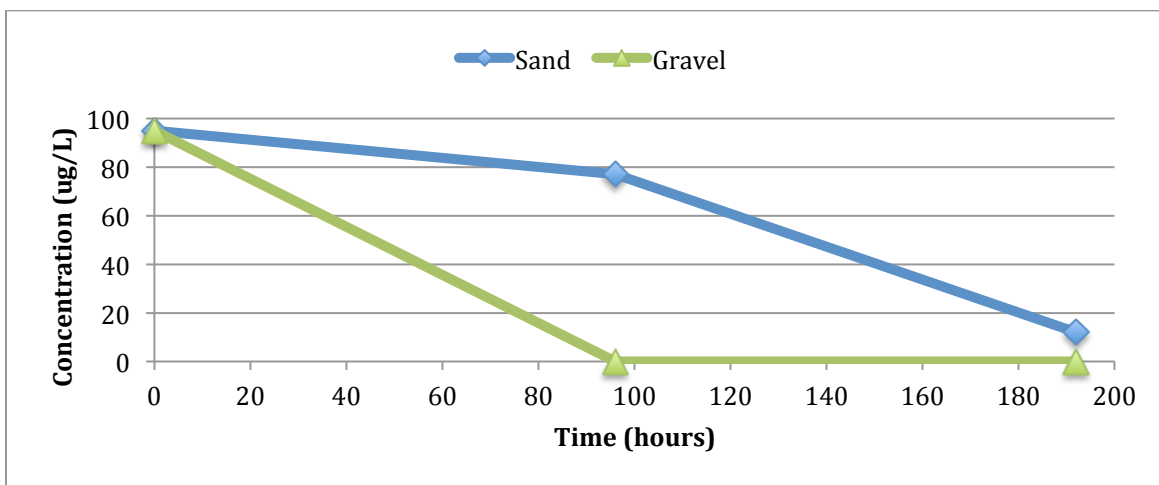
**Figure 12. Ammonia concentrations in the batch flasks (as  $\text{NH}_3\text{-N}$ )**



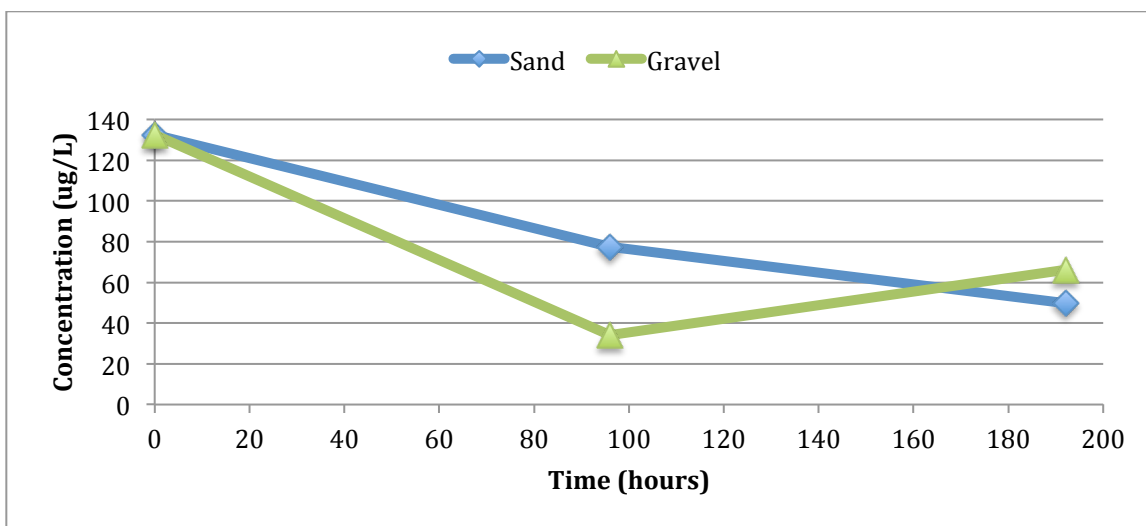
**Figure 13. Nitrate concentrations in the batch flasks (as  $\text{NO}_3\text{-N}$ )**

The concentrations over time for the five selected emerging contaminants are shown for both sand and mixed (gravel) media in **Figures 14 through 18**. The duplicate sample concentrations at each time point for both gravel (mixed) and sand only flasks were averaged to create a single representative plot for each media type. Overall, the gravel flasks outperformed the sand only flasks in terms of removing the selected contaminants from the bulk solution over the experimental period. In the cases of caffeine and DEET, concentrations fell to non-detectable levels in the gravel flasks by the 96-hour time point, which suggests that complete removal may have taken place at an earlier time. As such, more frequent sampling towards the beginning of the experimental period may have provided a more accurate representation of the removal rates of these contaminants. Sand and gravel performed similarly for atrazine in the first 96 hours, but while the gravel flasks were able to remove atrazine even further in the remainder of the experiment, concentrations in the sand flasks remained relatively steady. In the case of triclosan, only the concentrations for the gravel flasks are shown. Triclosan was measured for the sand flasks, but the analysis produced much higher concentrations at  $t = 96\text{h}$  than would be expected given the initial concentration in the spiked wastewater. Of the contaminants analyzed, carbamazepine experienced the lowest relative amount of removal, with approximately 50% of the initial concentration remaining in the gravel flasks and 38% remaining in the sand flasks, after 192 hours. Similar results were reported by Matamoros et al. (2009) where removal of carbamazepine was less than 50%. Carbamazepine was also the only contaminant in the current study for which the concentration in the gravel flask was greater at  $t = 192\text{h}$  than it was at  $t = 96\text{h}$ . Since none of the other analytes experienced this increase in concentration, it seems unlikely that it was a result of evaporation in the flasks. Rather, this suggests that some carbamazepine, which is neutral in solution, may have been adsorbed and subsequently dissolved

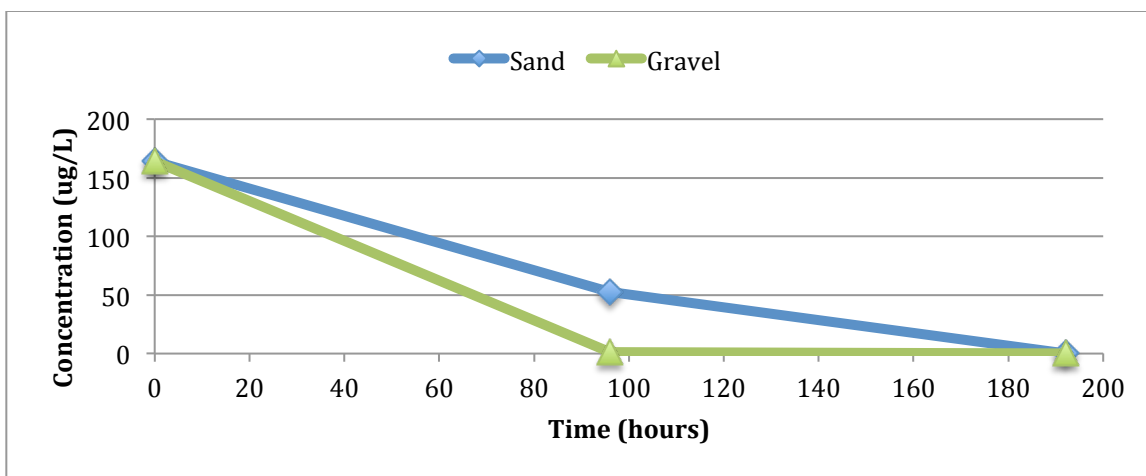
between 96 and 192 hours when pH became slightly more basic. Carbamazepine has a relatively high octanol-water partition coefficient ( $\log K_{OW} = 2.45$ ) (Yu et al., 2008), which suggests poor water solubility and a greater tendency to adsorb to media. A summary of removal percentages for each contaminant at each time point is provided in **Table 5**.



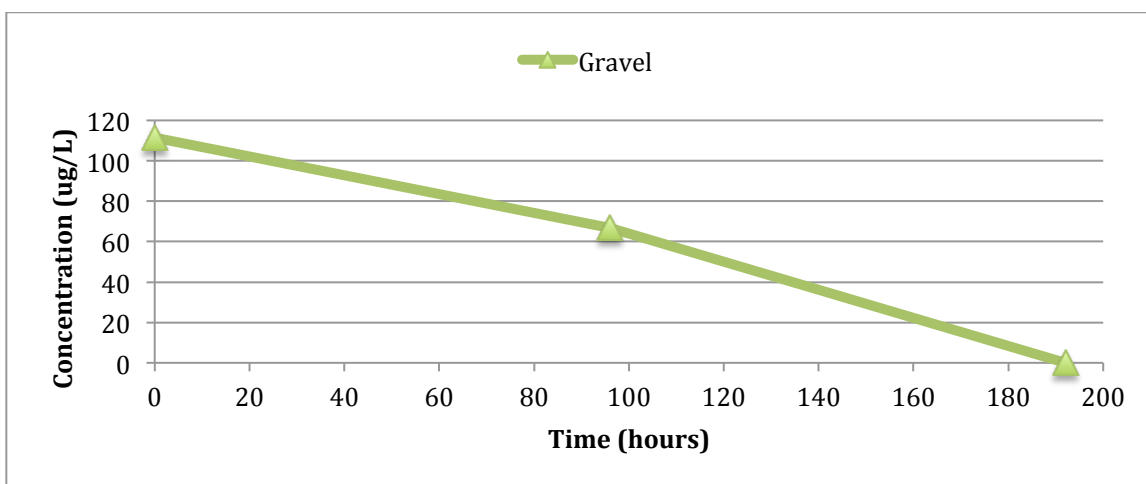
**Figure 14. Aqueous caffeine concentrations in sand and gravel batch flasks**



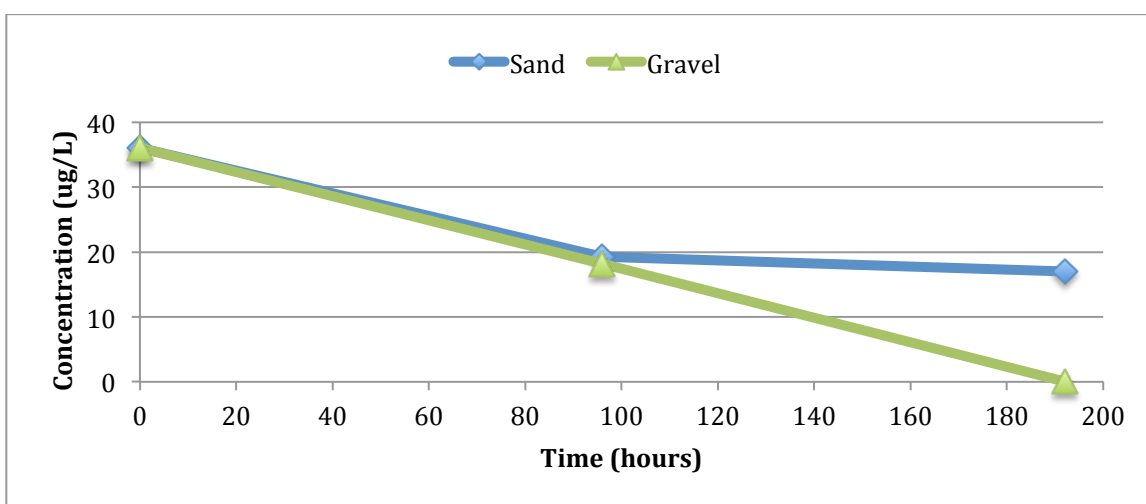
**Figure 15. Aqueous carbamazepine concentrations in sand and gravel batch flasks**



**Figure 16. Aqueous DEET concentrations in sand and gravel batch flasks**



**Figure 17. Aqueous triclosan concentrations in gravel batch flasks**



**Figure 18. Aqueous atrazine concentrations in sand and gravel batch flasks**

**Table 5. Contaminant removal summary in the batch experiments**

Contaminant	Initial Concentration (µg/L)	% Removal at 96 hours		% Removal at 192 hours	
		Sand	Gravel	Sand	Gravel
Caffeine	95.0	19	>98 <sup>2</sup>	87	>98 <sup>2</sup>
Carbamazepine	132.6	42	74	62	50
DEET	170.6	68	>99 <sup>2</sup>	>99 <sup>2</sup>	>99 <sup>2</sup>
Triclosan <sup>1</sup>	119.1	-	40	-	>99 <sup>2</sup>
Atrazine	35.4	46	50	53	>97 <sup>2</sup>

<sup>1</sup>Sand flasks were tested, but concentrations were significantly higher than initial levels and believed to be erroneous. <sup>2</sup> Indicates that the concentration at this time point was below the method detection limit (1 µg/L).

### 3.5 Interpretation of Results and Design Implications for Constructed Wetlands

The results presented above suggest that the gravel media (consisting of gravel, Stalite, and sand) were more effective than sand alone at removing the selected contaminants from the spiked wastewater. Although a certain background decay rate for the selected compounds in wastewater is expected, the majority of contaminant removal in this case is likely a result of both adsorption and biodegradation. It is believed that the mixed media allowed the wastewater to permeate and move through the flask more freely, which led to greater interaction with adsorption sites. Although biomass amounts were not quantified, it seemed that the gravel flasks were able to produce a greater amount of biomass than the sand flasks, which likely resulted in both enhanced adsorption and biodegradation. However, because the contaminants reached non-detectable levels in many of the samples taken, including a number of the t=96h samples, it would be difficult to determine accurate rate constants for these removal mechanisms from these particular results. A media-free control flask also would have helped to more accurately determine the impact of adsorption and biodegradation on overall removal. If more samples were taken in the first half of the experiment, first-order rate constants could be determined using the adsorption kinetic model, which states that the rate of adsorption is proportional to the concentration of the reactant and the concentration of available reaction sites as shown in Equation 5:

$$-r_A = k C_A C_S \quad (5)$$

where  $r_A$  is the rate of reaction of contaminant A,  $k$  is the adsorption rate constant,  $C_A$  is the concentration of contaminant “A”, and  $C_S$  is the concentration of available reaction sites (Fogler, 2011). Since the concentrations of reactants are relatively low in this case (on the order of  $\mu\text{g/L}$ ) it can safely be assumed that  $C_A$  is much less than  $C_S$ . As such, the above rate law reduces to a pseudo-1<sup>st</sup> order rate law shown in Equation 6:

$$-r_A = k' C_A \quad (6)$$

The flasks can be modeled as a constant volume batch reactor, and a mole balance on reactant “A” yields the combined rate law in Equation 7:

$$-dC_A/dt = k' C_A \quad (7)$$

Since this is a first order reaction, plotting  $\ln(C_{A0}/C_A)$  against time, where  $C_{A0}$  is the initial concentration, can be used to determine  $k'$ , the pseudo-1<sup>st</sup> order rate constant.

The results of this batch study also suggest that the OWTS at the Jordan Lake Business Center, which consists of vegetated filters with gravel, Stalite, and sand media and typically operates in a batch mode, may already be well-suited to attenuate organic emerging contaminants such as those studied here. While the system does not have a form of active aeration, the intermittent flow of batch loading allows air to permeate the upper portion of the media filter during periods of no flow. This ensures that both aerobic and anaerobic processes can take place, increasing the potential for biodegradation. In addition, uptake by the roots in the planter boxes may also contribute to emerging contaminant attenuation.

While the design considerations for constructed wetlands are varied, and are often specific to the site and the contaminants being targeted for removal, an experimental methodology similar to the one described in this report can be used to determine the effect of



other design parameters on specific removal mechanisms. For example, while keeping all other variables constant, the effect of aeration type (i.e., mechanical, diffused, open air) on the removal of contaminants that are known to be biodegradable could be determined. In addition, the impact of photolysis could be evaluated by comparing the results of batches at varying levels of light exposure. Lastly, an experiment involving re-suspension of the biomass into the bulk solution in the final stages of testing would help distinguish between the mechanisms of biodegradation, in which the target compounds are transformed, and enhanced adsorption due to biomass accumulation. While these studies focus on the effects of specific removal mechanisms, their results can form a basis of design for larger scale treatment systems, such as constructed wetlands, in which all of these mechanisms are contributing to contaminant removal. Overall, batch studies can be an extremely useful tool that allows engineers to focus on enhancing the most effective removal mechanisms, while reducing the need for costly and time-consuming redesigns of full-scale treatment systems.

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