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Advanced Treatment Technologies for Mitigation of Nitrogen and Off-flavor Compounds in Onsite Wastewater Treatment and Recirculating Aquaculture Systems

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Advanced Treatment Technologies for Mitigation of Nitrogen and Off-flavor Compounds in Onsite Wastewater Treatment and Recirculating Aquaculture Systems

by

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DEDICATION

To my husband. For his love and support. I will forever be thankful.

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ABSTRACT

Non-point sources (NPS) of pollution are non-discernable, diffuse sources of pollution that are often difficult to localize and in turn mitigate. NPS can include stormwater runoff, agricultural/aquaculture wastes and wastes from small decentralized wastewater treatment systems, such as conventional septic systems. The mitigation of these NPS is imperative to reduce their potential detrimental effects on the water environment. This dissertation addresses novel treatment technologies for the mitigation of nutrients, particularly nitrogen, in Recirculating Aquaculture Systems (RAS) and onsite wastewater treatment systems (OWTS). The removal of trace organics limiting RAS production and water reuse were also investigated.

The first question this dissertation addressed is: *Can the application of a UV-TiO_2 reactor reduce the concentration of off-flavor compounds in RAS?* In the UV-TiO_2 reactor, spray-coated TiO_2 plates were placed in an aluminum reactor and exposed to UV light. The process was applied in both a full-scale sturgeon RAS and a bench-scale RAS for the degradation of Geosmin (GSM) and 2-methylisoborneol (MIB). Improved performance on the removal of GSM and MIB was observed when the UV-TiO_2 was applied as a batch reactor since it allowed for a longer treatment time without the effect of constant production of the compounds in the biological treatment processes. Treatment performance of UV-TiO_2 was affected by GSM and MIB concentrations and dissolved oxygen. No harmful effects were observed on other water quality parameters when the UV-TiO_2 reactor was operated as a batch or side stream process.

The second question this dissertation addressed is: *Does the application of Tire-Sulfur Hybrid Adsorption Denitrification (T-SHAD) in RAS improve nutrient and off-flavor compound removal when compared to conventional heterotrophic denitrification?* T-SHAD combines tire mulch as an adsorbent and sulfur oxidizing denitrification for the removal of NO_3^--N from the aquaculture waters. Adsorption studies showed the tire has significant adsorption capacity for the off-flavor compounds GSM and MIB but can be limited by contact time and, possibly, the presence of competing organic matter in RAS. The application of T-SHAD as an effluent polishing step in RAS with a high empty bed contact time (EBCT) of 720 min removed 96.6% of NO_3^--N and 69.6% of GSM. The application of T-SHAD within RAS as denitrification side treatment for NO_3^--N removal resulted in lower EBCT (185 min) that limited NO_3^--N removal to 21% and showed no significant removal of off-flavor compounds. The comparison between T-SHAD and a molasses fed heterotrophic upflow packed bed reactor (UPBR), showed no significant differences in N species concentrations as well as off-flavor compound removal. However, high production of SO_4^{2-} resulted from sulfur oxidizing denitrification (SOD) processes was noted.

Hybrid Adsorption and Biological Treatment Systems (HABiTS), is composed of two biofilters in series employing ion exchange (IX) and nitrification for removal of NH_4^+ and tire scrap coupled with sulfur chips and oyster shells for both adsorption and SOD of NO_3^-. The third question addressed in this dissertation is: *What IX/adsorption media best balances both ammonium removal and cost effectiveness for application in OWTS?* Adsorption isotherms performed with different media materials showed that the zeolite material, clinoptilolite, was the best medium for the nitrification stage of HABiTS due to its high IX capacity for $NH_4^+$and cost. An adsorption capacity of 11.69 mg g^{-1} NH_4^+-N when in competition with other cations present in septic tank effluents was determined by the IX model fit to the data.

The cost of clinoptilolite is significantly higher than the other media materials tested. However, the high adsorption capacity would allow for low dosages that can be combined with non-adsorptive material reducing overall costs.

The fourth question this dissertation addressed is: *How is the BNR process within HABiTS affected by IX*? Results from side-by-side biofilter studies with HABiTS and a conventional nitrification/denitrification biofilter showed that the combined IX and nitrification in HABiTS can allow for faster startup, sustain variable loading, and achieve over 80% removal of NH_4^+ at a hydraulic loading rate of 0.34 $m^3\ m^{-2}$-d^{-1} when compared to the conventional biofilter with 73% removal. Under lower loading rates the biological treatment was enhanced and dominated the NH_4^+ removal processes in both columns. The addition of a denitrification stage decreased Total Inorganic Nitrogen (TIN) by 53.54% and 40.97%, for the HABiTS treatment and the control treatment, respectively, under loading rates of 0.21 $m^3\ m^{-2}$-d^{-1}. Further decrease of NH_4^+-N loading rates results in high desorption of exchanged NH_4^+ in the clinoptilolite, resulting in lower TIN removal efficiencies (28.7%) when compared to the conventional control treatment (62%).

The final question addressed in this dissertation is: *Does the proposed hybrid system enhance the removal of TIN in OWTS under transient loading conditions?* Further studies with HABiTS and the conventional biofilter were performed to determine N removal performance on an hourly basis. It was found that the performance of HABiTS varies with daily and hourly loads, particularly when recovering from periods of very low loading to high loadings and vice versa. If recovering from low loading periods, IX is observed for HABiTS and the biofilter outperforms the conventional treatment in overall TIN removal. However, recovery from a high loading period results in release of NH_4^+-N stored in the clinoptilolite and increased production of NO_3^--N that could affect the performance of the denitrification stage.

CHAPTER 1: INTRODUCTION

1.1 Non-Point Sources of Pollution

Point sources of pollution have been the focus of the wastewater industry for decades, resulting in advances in the design, development, implementation and optimization of treatment technologies to mitigate them. More often than not these pollution sources (i.e. industrial and domestic wastewater) are mitigated with centralized wastewater treatment systems. These large-scale systems, most common in the developed world, collect the wastewater from industry and large residential areas where it is treated to achieve minimum standards enforced locally and/or nationally depending on the country. In the United States, for example, effluents from centralized wastewater treatment systems must comply with National Pollutant Discharge Elimination System (NPDES) permits, which often have stringent limits on solids, fecal coliforms, organics (i.e. biochemical oxygen demand) and nutrient (e.g. nitrogen (N) and phosphorus (P) species) concentrations (USEPA, 2002). Non-point sources (NPS) of pollution, on the other hand, are much more challenging to regulate and control. NPS are defined as non-discernable, diffuse sources of pollution (USEPA, 2002) that are often difficult to localize and in turn mitigate. NPS can include stormwater runoff, agricultural/aquaculture wastes and wastes from small decentralized wastewater treatment systems (DWTS), such as conventional septic systems. This dissertation focuses on two of the above mentioned NPS, aquaculture wastes and conventional septic systems, and addresses novel treatment technologies for the mitigation of nutrients and trace organics produced by these sources. Although stormwater runoff has also been recognized to have significant impact on aquatic environments, technologies for control of stormwater runoff are

outside of the scope of this dissertation. Further review on these NPS as well as nutrient and trace organic removal processes within the technologies that address NPS is addressed in Chapter 2, the literature review. Chapters 3, 4 and 5 address technologies for the management and advanced treatment of these NPS. Chapter 6 of this dissertation presents conclusions and recommendations on the studied treatment technologies.

1.2 Recirculating Aquaculture Systems

Aquaculture is a form of agriculture that involves the farming of aquatic species, be it for decoration (such as tropical fish), animal consumption (shrimp) or human consumption (catfish, salmon, sturgeon, caviar) (FDAC, 2013). Farm level aquaculture has become popular worldwide due to the high demand for fish protein (Christianson and Summerfelt, 2014) and declining wild fish stocks. Sales from farm-level aquaculture were reported to be approximately $1.1 billion in the US in 2005 (USDA, 2005). In the state of Florida, total aquaculture sales were reported of approximately $69 million in 2012, of which $24.1 million were products for human consumption (FDAC, 2013). Conventional aquaculture systems involve constant fresh water inputs and high wastewater production (Hamlin et al., 2008). Recirculating aquaculture systems (RAS) treat and recirculate wastewater back to fish tanks, reducing fresh water inputs and wastewater discharges, while providing more environmental control and higher aquaculture product production rates (Gonçalves and Gagnon, 2011). Treatment processes in RAS include media or drum filters for solids removal and biological nitrogen removal (BNR). Typical RAS treatment is focused on ammonium (NH_4^+) and subsequently nitrite (NO_2^-) removal due to the high toxicity of these species for aquatic organisms at concentrations above 1 mg L^{-1} and 0.3 mg L^{-1}, respectively (Timmons et al., 2002; Hamlin et al., 2008). Through BNR processes under aerobic conditions these compounds are oxidized to NO_3^-, which accumulates in the RAS and often climbs to high

concentrations in low water exchange systems. Depending on the type of fish and desired water recirculation rates and quality, additional treatment units can be utilized including denitrification and ultraviolet (UV) light and/or ozone for disinfection. Prior studies indicate detrimental effects of high NO_3^--N (>100 mg L^{-1}) on fish health (Hamlin, 2006; Davidson et al., 2014) highlighting the importance of the mitigation of all inorganic N species in RAS.

RAS are also challenged by their limited removal of trace organics, such as off-flavor compounds geosmin (GSM) and 2-methylisoborneol (MIB). These compounds are secondary metabolites of cyanobacteria and some actinomycetes (Guttman and van Rijn, 2008) that cause an earthy musty flavor that can be detected in water at extremely low concentrations (between 10 and 20 ng L^{-1}; Drikas et al., 2009). Additionally, GSM and MIB accumulate in the lipid-rich tissue of fish and can affect taste and quality of fish, particularly catfish, salmon and sturgeon (Howgate, 2004). The current removal approach for these compounds is to purge them from the fish prior to harvesting, which requires large amounts of highly treated water (Burr et al., 2012).

Multiple technologies have been studied for removal of GSM and MIB. Physical-chemical processes, such as activated carbon adsorption, have also been studied for the removal GSM and MIB. Activated carbon has been found effective at removal off-flavor compounds but require high dosing rates to achieve concentration levels below the detection threshold (Matsui et al., 2013) resulting in high cost of treatment (Bamuza-Pemu and Chirwa, 2012), and the need to regenerate or dispose of the spent material. The effect of other organic materials, such as humic acids, competing for the same adsorption space can also limit adsorption capacity and rate, further hindering the removal process (Matsui et al., 2013; Newcombe et al., 2002).

Advanced treatment technologies for GSM and MIB include oxidation processes such as ultraviolet radiation (UV), ozonation, and advanced oxidation processes (AOPs) using different

catalysts (Srinivasan & Sorial, 2011) or a combination of two or more oxidation processes. AOPs rely on highly reactive hydroxyl radicals, which are non-selective and able to oxidize electron-rich organic compounds (Howe et al., 2012). In aquaculture, UV or ozonation are commonly used for pathogen control but the dosing is often insufficient for GSM and MIB removal (Schrader et al., 2010). UV photocatalysis with titanium dioxide (TiO_2) is an AOP that has been used in multiple applications and found to oxidize up to 99% of GSM and MIB (Lawton et al., 2003). The catalyst is typically applied as a slurry, which requires post treatment for the removal of the particles. The application of this method in aquaculture is problematic since the filtration method typically used cannot remove fine particles and the effect of TiO_2 particles on fish health is unknown.

Chapter 3 of this dissertation discusses the application of a novel UV-TiO_2 photocatalysis reactor for the removal of GSM and MIB in RAS. In the UV-TiO_2 reactor, spray coated TiO_2 plates are placed in an aluminum reactor and submitted to UV light. The process was applied in both a full-scale sturgeon RAS and a bench-scale RAS for the degradation of GSM and MIB. The question this chapter aims to answer is: *Can the application of a UV-TiO_2 reactor reduce the concentrations off-flavor compounds in RAS?* The specific objectives of this chapter were as follows:

- Investigate the performance of the UV-TiO_2 treatment under batch and continuous flow reactor configurations for the removal of GSM and MIB in RAS.
- Evaluate and discuss the effect of the UV-TiO_2 treatment on water quality parameters and the possible impacts on biological wastewater treatment processes in RAS.

Chapter 4 of this dissertation discusses the application of a Tire Sulfur Hybrid Adsorption Denitrification (T-SHAD) reactor in RAS. T-SHAD combines tire mulch as an adsorbent and sulfur oxidizing denitrification for the removal of NO_3^- from the fish water. This chapter aims to

answer the following question: *Does the application of T-SHAD in RAS improve nutrient and off-flavor compound removal when compared to conventional heterotrophic denitrification?* The specific objectives of this study were to:

- Determine the adsorption capacity of tire mulch for GSM and MIB.
- Assess denitrification and off-flavor compound removal performance of T-SHAD in different reactor configurations in a bench-scale RAS.
- Compare T-SHAD to heterotrophic denitrification utilizing molasses as an organic electron donor and carbon source.

1.3 Onsite Wastewater Systems

Conventional septic tank systems, also known as onsite waste water treatment systems (OWTS), treat approximately one third of the wastewater in the US and in some states the use of these systems can exceed 40% (USEPA, 2002). In 2007, more than 26 million households employed a septic system for their wastewater treatment, with the majority located in rural and suburban areas (USEPA, 2008). Conventional OWTS consist of a septic tank for solids separation and biodegradation of organics and a soil infiltration system, or drainfield, to further remove solids, organics and pathogens. There are many advantages tied to OWTS, such as simplicity of operation, reliability, low cost when compared to centralized treatment in rural and suburban areas, and low maintenance requirements (USEPA, 1999). Major challenges of conventional OWTS include:

- Application limitations due to water table elevation and proximity to drinking water supplies and environmentally sensitive areas (USEPA, 1999; FDOH, 2013; Gorman and Halvorsen, 2006).

- Variable water usage within the household and long idle times (e.g. during vacations) result in highly variable loading rates, which in turn affect the biological treatment process (USEPA, 1999, 2002; Oakley, 2010).
- Little to no N removal (USEPA, 1999) causing contamination of groundwater and surface water (Howe et al, 2012; Liu et al, 2009).

Reported water quality data for domestic wastewater in the US are shown in Table 1. Most of the total nitrogen (TN) entering OWTS is in the form of organic N and NH_4^+ from urine and food wastes (Ahuja et al., 2014). Since anaerobic conditions persist in the septic tank, only pathogen inactivation and some organic matter degradation will occur. When the effluent is distributed over the surface of the drainfield, nitrification can occur as the wastewater percolates through the soil media surrounding the distribution system. The effluent of the drainfield, which is high in NO_3^-, can eventually reach the groundwater and other water bodies (Gill et al., 2009) and potentially affect the quality of drinking water if the OWTS is close to drinking water wells.

Table 1.1: Quality of domestic wastewater (modified from Siegrist et al., 2013).

Constituent	Units	Lowe et al. (2009)	USEPA (2002)	Crites & Tchobanoglous (1998)	Hirst et al. (2013)
		Range	Range	Range	Range
TSS_a	mg L^{-1}	22-1690	155-330	100-350	22-63
$cBOD5_b$	mg L^{-1}	112-1101	155-286	110-400	30-190
COD_c	mg L^{-1}	139-4584	500-600	250-1000	170-420
TOC_d	mg L^{-1}	35-738	Not reported	80-290	Not reported
TN_e	mg L^{-1}	9-240	26-75	20-85	71-97
TP_f	mg L^{-1}	0.2-32	6-12	4-15	6-11

[a] Total Suspended Solids (TSS)
[b] Carbonaceous Biological Oxygen Demand (cBOD5)
[c] Chemical Oxygen Demand (COD)
[d] Total Organic Carbon (TOC)
[e] Total Nitrogen
[f] Total Phosphorus

Passive OWTS are defined as systems that utilize only one pump, no artificial aeration for nitrification, and reactive media for denitrification (FDOH, 2013). A number of prior studies have investigated passive N-removing OWTS. The majority of these studies enhanced treatment within the drainfield to allow for BNR and remove N species (Chang et al., 2010; Xuan et al., 2012; Kong et al., 2014). However, removal of N species was very variable requiring additional research into the subject.

A combination of ion exchange (IX) and biological treatment has the potential to enhance passive nitrogen removal in OWTS. IX materials, such as the zeolite compounds chabazite and clinoptilolite, have the ability to adsorb positively charged ions, such as NH_4^+ (Jorgensen and Weatherley, 2003; Rozic et al., 2000; Wen et al., 2006). Bioregeneration can be carried out by nitrifying bacteria (Lahav and Green, 1999), allowing the reuse of the material. The combination of IX and biological nitrification has the potential to provide enhanced treatment of the variable NH_4^+ loadings observed in OWTS. During periods of high loading, NH_4^+ loads in excess of the capacity of the nitrifying bacteria are adsorbed by the IX media. During low loading periods, NH_4^+ is desorbed and is utilized by the nitrifying population. A study by the Florida Department of Health (FDOH) and Hazen and Sawyer (H&S) showed that application of clinoptilolite in the nitrification stage of a two-stage passive OWTS resulted in 94% removal of Total Kedhjal Nitrogen (TKN; NH_4^+-N + Organic N) entering the system (Hirst et al., 2013). The use of the combined IX/nitrification process coupled with sulfur oxidizing denitrification (SOD) resulted in average effluent TN below 3 mg L^{-1}. The residual TN was mostly in the form of organic nitrogen and NH_4^+ since NO_3^- and NO_2^- were reduced below 1 mg L^{-1} (Hirst et al., 2013). In a similar manner, scrap tire chips were recently shown in our laboratory to have a high IX capacity for NO_3^- (Krayzelova et al., 2014; Lisi et al., 2004). The tire chips can be bioregenerated by biological

denitrification. Krayzelova et al. (2014) combined IX on scrap tire chips and autotrophic SOD in bench-scale studies with synthetic OWTS wastewater. NO_3^- removal efficiencies of 90%, 89% and 94% were achieved under steady state, variable flow and variable concentrations, respectively.

Inspired by the studies of Hirst et al. (2013) and Krayzelova et al. (2014), Chapter 5 of this dissertation investigates Hybrid Adsorption and Biological Treatment Systems (HABiTS) for the removal of N in OWTS. HABiTS is composed of two biofilters in series employing IX and nitrification for removal of NH_4^+ and tire scrap coupled with sulfur chips and oyster shells for both adsorption and SOD of NO_3^-. This chapter aims to answer the following questions:

1. *What IX/adsorption media best balances both ammonium removal and cost effectiveness for application in OWTS?*
2. *How is the BNR process within HABiTS affected by IX?*
3. *Does the proposed hybrid system enhance the removal of TIN in OWTS under transient loading conditions?*

The specific objectives for this chapter are as follows:

- Determine NH_4^+ adsorption capacity, hydraulic properties, cost and availability of various IX media for application in HABiTS.
- Compare the performance of HABiTS enhanced OWTS with nitrification/denitrification biofilters without an adsorptive medium under transient loading conditions.
- Compare the hourly performance of HABiTS with nitrification/denitrification biofilters without an adsorptive medium under transient loading conditions.

CHAPTER 2: LITERATURE REVIEW

2.1 Non-Point Sources of Pollution

A non-point source (NPS) of pollution is defined by the Unites States Environmental Protection Agency (USEPA, 2008) as a source that does not meet the legal definition of a point source. These sources are diffuse and non-discernable sources and are more difficult to control than point sources, and include agricultural and urban runoff and on-site wastewater treatment systems (OWTS). This dissertation will address problems with the control of Nitrogen (N) from two important NPS, recirculating aquaculture systems (RAS) and OWTS. The following subsections expand the literature review presented in the introduction to provide a more in depth background of the NPS and the efforts at mitigating those. Additional review of relevant literature can be found in the introductions of Chapters 3, 4 and 5.

2.1.1 Recirculating Aquaculture Systems (RAS)

Land based farmed fish production has increased as a response to the high demand for fish protein (Christianson and Summerfelt, 2014) and the decline of wild fish stocks due to over fishing and habitat elimination. Traditional fish farming involves outdoor fish ponds and constant fresh water inputs to account for water losses due to evaporation and seepage (Verdegem et al., 2006) as well as to improve the water quality within the pond. Recirculating aquaculture systems (RAS) provide a more controlled and water efficient process for fish farming (Hamlin et al., 2008; Martins et al., 2010; Gonçalves and Gagnon, 2011). In RAS, water is treated onsite and recirculated back to the fish tanks for reuse. Water savings in RAS varies depending on water exchange rates, which can range anywhere from 90 to 99% (van Rijn, 2006; Badiola et al., 2012).

Treatment processes within RAS vary greatly. The most common treatment unit is solids removal to remove any fish waste and uneaten food and reduce ammonium (NH_4^+) production from decaying solids (Timmons et al., 2002). Fine particle removal is normally achieved using settling tanks, media filters, drum filters or foam fractionation (van Rijn, 2006; Martins et al. 2010, Timmons et al., 2002). Nitrification is another common treatment in RAS due to the toxicity of NH_4^+ and nitrite (NO_2^-) to aquatic species (Stickney et al., 2000; Timmons et al., 2002; Hamlin et al., 2008; Kuhn et al., 2010). Nitrification in RAS is often achieved using fluidized bed reactors, moving bed bioreactors (MBBR) or aerated media filters (Martins et al, 2010; van Rijn et al., Timmons et al., 2002) and requires high aeration rates to ensure complete nitrification.

Denitrification in RAS is less common. In many systems nitrate (NO_3^-) is managed through freshwater exchanges. In low water exchange systems, denitrification is achieved using reactors similar to those used for nitrification. Heterotrophic denitrification is most common in RAS and has been achieved in settling tanks due to anaerobic conditions in the sludge and utilizing the fish waste as the electron donor, fluidized media beds (Tsukuda et al., 2015, Kim et al., 2004) and upflow packed bed reactors (UPBRs) (Hamlin et al., 2008; Singer et al., 2008; Saliling et al., 2007).

Fluidized media beds and UPBRs have been found to provide high denitrification efficiency with a small footprint when using media materials as carriers for denitrifying biomass (Tsukuda et al., 2015). Prior RAS denitrification studies with fluidized media beds and UPBRs involve the addition of an external carbon source since biologically available organic carbon is significantly removed in the sedimentation basin (Tsukuda et al., 2015) as well as the aerobic nitrification stage in RAS. Limited carbon availability can result in incomplete denitrification and release of NO_2^- into the system causing toxicity. A variety of organic carbon sources and media materials have been studied. Saliling et al., (2007), for example, tested an UPBR with wood chips

and wheat straw and found NO_3^--N removal rates of 1340 mg $L^{-1}d^-$ with a flowrate of 15 mL min^{-1} of synthetic aquaculture water. In the study by Hamlin et al., (2008) methanol, acetate and molasses were used as organic carbon sources in an UPBR with plastic carriers. Removal rates were found to be 670 mg NO_3^--N L^{-1} d^{-1} for all three sources. Sulfur oxidizing denitrification (SOD) presents an alternative to heterotrophic denitrification by utilizing and inorganic solid phase electron donor, elemental sulfur (S^0), and eliminating the need for an external organic carbon source. There have been limited studies investigating SOD in RAS. One example is the study by Christianson et al. (2015), where fluidized granular sulfur biofilters treating wastewater from a full-scale RAS achieved removal rates as high as 800 mg NO_3^--N L^{-1}-d^{-1} with a short empty bed contact time (EBCT) of 4 minutes. However, NO_3^--N in the effluent remained high. Simard et al., (2015) on the other hand saw complete denitrification in sulfur granule columns connected in series with an EBCT of 640 minutes. Further research is needed for the application of SOD in RAS, with focus on optimization of EBCT and the effects of SOD as a denitrification unit within the RAS treatment loop.

RAS systems often also employ disinfection processes. These processes aim to inactivate bacteria that could possibly be pathogenic to fish species. Conventional RAS disinfection processes include UV disinfection and ozonation (Timmons et al., 2002; Robertson et al. 2005). Other compounds of concern in RAS that have been the most reported issues are off-flavors (Badiola et al., 2012). These off-flavors impart and earthy musty odor and flavor to water and fish and can be detected in concentrations as low as 10 ng L^{-1} (Drikas et al., 2009). Off-flavors in RAS are typically managed by depuration which is achieved by placing the fish in clean water without or with very low levels of the off-flavor compounds so they can be purged from the fish. Although common, this practice is costly due to high water, chemical and energy usage as well as the loss

of fish biomass caused by starvation and stress during the depuration process (Burr et al., 2012). Advanced treatment technologies for off-flavor compound removal are discussed in Section 2.3.

2.1.2 Conventional On-site Wastewater Treatment Systems (OWTS)

Conventional OWTS typically refer to a septic system coupled with a subsurface drain field (Xuan et al, 2012). These systems are used rural and suburban areas with low population density where it is not economically feasible to construct the infrastructure needed to convey wastewater to centralized systems (Luostarinen et al., 2005; Gorman and Halvorsen, 2006). OWTS have the advantages of being low cost, simple to operate and maintain and providing in-situ treatment for domestic wastewater (USEPA, 1999). Regardless of these benefits there are still major challenges for OWTS applications. Factors that limit the location for application of OWTS include the depth to the water table (FDOH, 2013), which limits the use of the drain field and might require the use of a mound. A mound system results in increased construction costs and land use (USEPA, 2002). Space availability is another limitation, since OWTS require a considerable amount of land area for each residence. Local regulations may also limit OWTS applications by specifying distances between OWTS and water sources, such as drinking water wells (FDOH, 2013). In Florida, for example, new OWTS cannot be installed within seventy five feet of a private potable well or a multi-family water well, one-hundred feet of a public drinking water well if such a well serves a facility with an estimated sewage flow of 2000 gallons or less per day and two-hundred feet of a public drinking water well if such a well serves a facility with an estimated sewage flow of more than 2000 gallons per day (FDOH, 2013).

The limited removal of nitrogen in OWTS is a critical challenge that needs to be addressed since it can limit applications of OWTS in sensitive ecosystems and cause overloading of N in both groundwater and surface water (Liu et al., 2009; Sahu et al., 2009). These issues are being

recognized and addressed in the state Florida, particularly for springsheds. For example, the Florida Department of Environmental Protection (FDEP) has determined the major contributors to nitrogen pollution in the Kings Bay springshed (Figure 2.1) and OWTS are recognized as the highest contributor to N pollution.

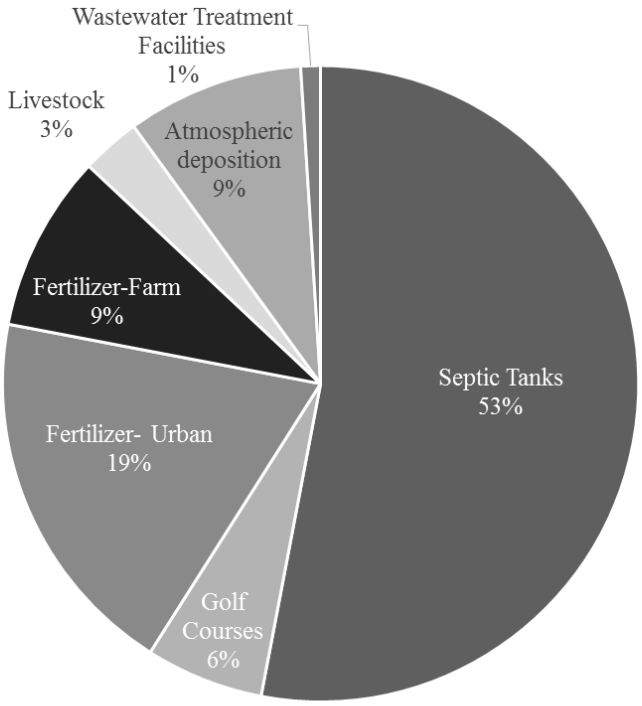


Figure 2.1: Nitrogen inputs into the Kings Bay springshed in the state of Florida (data from FDEP, 2015).

The treatment train in OWTS has two main components: the septic tank and the subsurface wastewater infiltration system or drain field (Figure 2.2). The primary purpose of the septic tank is for solids separation, anaerobic biodegradation of organics and ammonification of the organic N. The drain field acts as a media filter and intercepts solids while providing surface area for biofilm growth. Depending on the soil type, surface area and loading rate it may also promote other physical processes such as adsorption and/or ion exchange while also degrading organics and inactivating pathogens (USEPA, 2002). Distribution of the septic tank effluent to the drain field typically employs a perforated pipe, with either gravity or pumped distribution of the

wastewater. Nitrification can take place due to the presence of oxygen in the voids of the drain field soil media (USEPA, 2002). As mentioned previously, NO_3^- is highly mobile and can travel through soil and eventually reach the groundwater or surface water causing eutrophication and/or contamination of drinking water sources. Denitrification can occur within the soil if saturated conditions persist and oxygen is depleted (Gill et al., 2009).

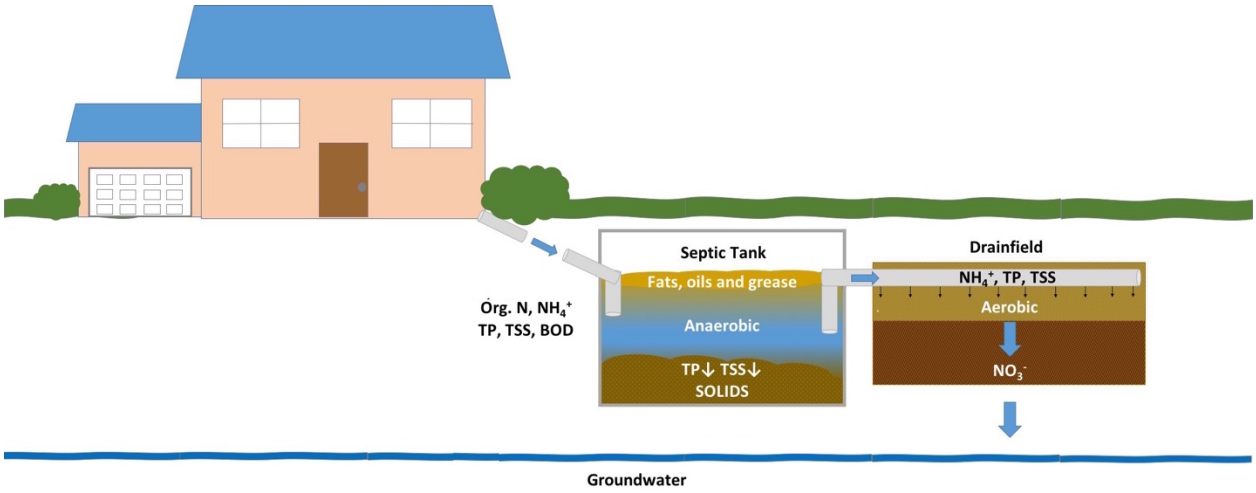


Figure 2.2: Conventional on-site wastewater treatment system.

A number of technologies have been developed to provide enhanced treatment in OWTS. These technologies vary from enhancement of the septic tank or the drainfield up to addition of new treatment units. An example of septic tank enhancement is the use of multi-chambered settling tanks that greatly improve solids removal. Another example was investigated by Moussavi et al. (2010) involving an upflow septic tank as opposed to the conventional horizontal flow system. Improved removal of total suspended solids (TSS) and chemical oxygen demand (COD) was observed at retention times as low as 24 hours. However, these modifications had little effect on N removal. Oh et al. (2014) studied the use of recycled rubber particles as filter media for the treatment of septic tank effluent and observed 93% removal of TSS and 90% removal of NH_4^+-N. Chang et al. (2010) studied a modification of the conventional drainfield design that included a

vertical flow area for nitrification and a horizontal flow area with a combination of sand, tire crumbs and sawdust for denitrification. Greater removal of total N (TN) was observed in the modified drainfield (70%) compared with a conventional drainfield (50%).

Passive N removal OWTS that incorporate biological nitrogen removal (BNR) in media filters after the septic tank have also been studied (Weiss et al., 2008; Smith, 2012; Tait et al., 2013; Hirst et al., 2013). Unsaturated nitrifying biofilters with sand media have been found to improve TSS and TKN removal (Anderson et al., 1998, USEPA, 2002). Expanded clay materials have also been used in a variety of studies resulting in high TKN removal (Smith, 2012; Hirst et al., 2013) due to passive aeration as the wastewater flows through the unsaturated layer. This passive aeration through the biofilter has been shown to provide sufficient dissolved oxygen (DO) for nitrification; however, transient loadings can result in variable N concentrations in the effluent (Petitjean et al., 2016). These transient loading rates are predominant in these systems due to temporal variations in water use within the household (USEPA, 2002; Henze, 2002). Peak flows occur in the morning and afternoon (NSF International, 2005) and are limited during the day and night time, resulting in inconsistent substrate for the microbial community in the biofilters. This is especially problematic for slow growing microorganisms such as nitrifyers. The quality of the wastewater also varies temporally and depending on the number of people living in the residence, eating and cleaning HABiTS and health conditions (USEPA, 1999). Cleaning HABiTS, such as use of bleach, can kill the bacteria necessary for biological processes in the systems (USEPA, 2002). Overcoming these challenges would require a robust treatment that could provide control of peak flows while promoting biological nitrogen removal in OWTS. More details on OWTS and biofilters is provided in Chapter 5.

2.2 Nutrient Mitigation

Nutrients, most specifically nitrogen (N) and phosphorus (P), are key elements needed for biological growth. However, when nutrients are discharged in excess to surface and groundwater they can have detrimental effects on the environment, including eutrophication (overgrowth of algae), which results in seagrass mortality, the release of toxins, death of aquatic species and hypoxia. Nitrate (NO_3^-), an oxidized form of N, can cause impairment of drinking water supplies due to health effects on humans such as methaemoglobinemia, which reduces the ability of the blood to carry oxygen. This disease is commonly referred to as "blue baby syndrome" since it mostly affects infants resulting in a bluish tint around the mouth, hands and feet (WHO, 1998). Other effects of NO_3^--N in drinking water include possible cancers and adverse effects on the reproductive system (Gill et al., 2009).

2.2.1 Biological Nitrogen Removal Processes

N is usually removed from wastewater by biological nitrogen removal (BNR) processes. In sewage and septic tank effluents the most prevalent species of N is NH_4^+, which is introduced in wastewater by the hydrolyzation of urea present in urine and feces. Nitrification is the aerobic oxidation of NH_4^+ to NO_2^- and NO_2^- to NO_3^- (Madigan et al., 2010). Nitrification occurs in a series of reactions requiring specific microorganisms, substrates and enzymes. The nitrification reaction as well as examples of the microoganisms and required substrates are described in Table 2.1. Recently, however, a bacterium from the Nitrospira genus was identified as a microorganism that can oxidize both NH_4^+ and NO_2^-, resulting in complete nitrification (Daims et al, 2015). This process, identified as comammox, results in a lower energy yield than the traditional two-step process described below. Although significant, this process was not investigated in this dissertation.

Table 2.1: Nitrification pathway reactions (Madigan et al., 2010).

Bacterium	*Substrate*	*Enzyme*
	NH_3^+	
Nitrosomas	⇓	Ammonia Monooxygenase
	NH_2OH	
	⇓	Hydroxylamine oxidoreductase
	NO_2^-	
Nitrospira/ Nitrobacter	⇓	Nitrite oxidoreductase
	NO_3^-	

In wastewater with pH values below 9.25 (pK_a), the NH_4^+ species is more prevalent than the ammonia (NH_3) species (Equation 1).

$$NH_4^+ \leftrightarrow NH_3 + H^+ \qquad \text{Equation 2.1}$$

The overall wo-step nitrification process is shown in Equation 2.2, assuming a yield coefficient of 0.15 g volatile suspended solids per gram of substrate (Ahuja, 2014).

$$NH_4^+ + 1.44O_2 + 0.316CO_2 + 0.081HCO_3^- \rightarrow 0.081C_5H_7O_2N$$
$$+0.919NO_3^- + 0.846H_2O + 1.83H^+ \qquad \text{Equation 2.2}$$

The nitrification process can be limited by many factors. First, because the process is aerobic, an external supply of oxygen is normally needed to fully nitrify NH_4^+ to NO_3^-. To satisfy the stoichiometric relation, 3.3 g of O_2 required for the complete oxidation of 1 g of NH_4^+-N. Lack of oxygen can result in incomplete nitrification and can result in accumulation of NO_2^-, which increases the toxicity of the wastewater further hindering the process by inhibition of NO_2^- oxidizing bacteria (NOB). Lack of alkalinity is another factor that can hinder nitrification. As described in Equation 2.2, alkalinity is consumed during nitrification at a ratio of 0.35 g HCO_3^- g $^{-1}$ NH_4^+-N, which may require addition of an external alkalinity source in areas with low alkalinity water. High organic content in the wastewater can also hinder the nitrification process since heterotrophic oxidizing bacteria can out compete nitrifiers for the available oxygen.

In wastewater treatment, nitrification is achieved using both suspended and attached growth processes (Metcalf and Eddy, 1979). Suspended growth processes are more typical in centralized wastewater treatment facilities and are designed similar to the activated sludge process. In suspended growth nitrification, however, aeration and mixing are used to maintain the microbes in a suspended state in the wastewater (Ahuja, 2014). For these systems, provision of an oxygen supply and mixing can be very energy intensive, expensive and complex. In attached growth processes, a high surface area medium is supplied to promote attachment and growth of nitrifying biofilm (Metcalf and Eddy, 1979) and forcer aeration may be supplied. Attached growth nitrification has been carried out in trickling filters (Metcalf and Eddy, 1979), similarly, by percolation of water through the drainfield soil in conventional OWTS (USEPA, 2002), in media bio-filters (Hirst et al., 2013), moving bed bioreactors (Luostarinen et al., 2006), constructed wetlands and bioretention cells (Ergas et al., 2010).

Denitrification occurs when lack of oxygen (<0.5 mg L^{-1}) causes facultative microorganisms to utilize NO_3^- as an alternate electron acceptor (Nazaroff and Cohen, 2001). Denitrification is a step-wise process that reduces NO_3^- to nitrogen gas (N_2). The denitrification pathway and an example of the microorganism, enzymes and substrates in this pathway are described in Table 2.2. Denitrifying bacteria also require an electron donor, which for heterotrophic denitrification is a source of organic carbon. Heterotrophic denitrification utilizing the organic carbon fraction available in wastewater and assuming a yield coefficient of 0.18 g volatile suspended solids per gram of substrate (Ahuja, 2014) is shown in Equation 2.4.

Table 2.2: Denitrification pathway

Bacterium	*Substrate*	*Enzyme*
	NO_3^-	
Escherichia Coli, Pseudomonas stutzeri, Paracoccus denitrificants,	⇓	Nitrate reductase
	NO_2^-	
Pseudomonas stutzeri/ Paracoccus denitrificants	⇓	Nitrite reductase
	NO	
Pseudomonas stutzeri/ Paracoccus denitrificants	⇓	Nitric oxide reductase
	N_2O	
Pseudomonas stutzeri/ Paracoccus denitrificants	⇓	Nitrous oxide reductase
	N_2	

$$NO_3^- + 0.127C_{10}H_{19}O_3N + H^+ \rightarrow 0.058C_5H_7O_2N + 0.468N_2 + 0.848CO_2$$
$$+0.127NH_4^+ + 1.84H_2O + 0.127HCO_3^- \qquad \text{Equation 2.3}$$

The process of heterotrophic denitrification generates alkalinity at a ratio of 0.12 g HCO_3^- g^{-1} NO_3^-, which can be useful when pre-anoxic zones are combined with nitrification processes. However, this process can be limited by the presence of oxygen or lack of electron donor, and result in incomplete denitrification and the production of some of the N compounds in the denitrification pathway. For example, incomplete denitrification can result in the increase of NO_2^- concentrations that have toxic effects to aquatic species (Timmons et al., 2002; Hamlin et al., 2008). Incomplete denitrification can also result in $N_2O_{(g)}$ emission, which is important since $N_2O_{(g)}$ is a potent greenhouse gas (Lashof & Ahuja, 1990). Furthermore, the organic carbon requirement can be a limiting factor since bioavailable organic carbon is typically removed in the aerobic stage of BNR processes. The addition of external sources of organic carbon, such as methanol or acetate, can be costly and complex (Park and Yoo, 2009; Hamlin et al, 2008) requiring close monitoring of C/N ratios to ensure complete denitrification and reduce carry-over of organic

carbon to the effluent, which result in permit violations or hinder other treatment processes (Metcalf and Eddy, 1979).

Autotrophic denitrification is an alternative metabolic process, where inorganic electron donors, such as elemental sulfur (S^0), H_2 or pyrite, are utilized (Sengupta et al.,2007; Ergas and Reuss, 2001; Tong et al., 2017). The use of S^0 is advantageous due its high NO_3^- removal rates and lower costs when compared to other electron donor sources (Sengupta et al., 2007). *Thiobacillus denitrificans* utilize NO_3^- as the electron acceptor and oxidize S^0 to SO_4^{2-} (Kelly, 1999). A stoichiometric equation S^0 oxidizing denitrification (SOD) is shown in Equation 2.4 (Sengupta et al., 2007). There are some disadvantages associated with SOD such as the low solubility of S^0 (Park and Yoo, 2009) that can potentially limit denitrification rates. In addition, the consumption of alkalinity (Equation 2.4) could require the addition of an external alkalinity source such as oyster shells or limestone (Oh et al, 2001). Furthermore, the production of high SO_4^{2-} concentrations could potentially limit applications, particularly for drinking water processes where a maximum SO_4^{2-} level of 250 mg L^{-1} is recommended under the National Secondary Drinking Water Regulations (USEPA, 2009)

$$NO_3^- + 1.1S^0 + 0.4CO_2 + 0.76H_2O + 0.08NH_4^+ \rightarrow 0.08C_5H_7O_2N$$
$$+1.1SO_4^{2-} + 0.5N_2 + 1.28H^+ \qquad \text{Equation 2.4}$$

Like nitrification, denitrification can be achieved using either suspended or attached growth treatment processes (Metcalfe and Eddy, 1979) only under anoxic conditions as described above. Suspended growth processes, like the Bardenpho process, are typically used in wastewater treatment plants (Metcalfe and Eddy, 1979). Attached growth processes for denitrification can utilize the same type of media utilized in nitrification but maintained under saturated conditions to ensure oxygen depletion and an anaerobic environment. Other media utilized for denitrification

includes wood chips (Ergas et al., 2010), sulfur powder (Christianson et al., 2015), sulfur pellets mixed with tire scraps (Krayzelova et al., 2014), pyrite (Tong et al., 2017). In all cases mentioned above, the media serves as both biofilm carrier and source of electron donor.

Other BNR processes have been studied. Simultaneous nitrification and denitrification (SND) can often occur in water/wastewater treatment in the same bioreactor without distinct aerobic or anoxic zone (Daigger and Littleton, 2000). This could potentially occur in aerobic media filters for example, where oxygen depletion along the length of the filter could result in anoxic conditions that are favorable for denitrification. On the other hand, SND can also occur within the layers of the biofilm in both suspended and attached processes. Thick biofilms would limit oxygen diffusion creating and anoxic zones within the biofilm even under aerobic conditions (Rittman and Langeland, 1985). This would result in SND within the biofilm and effectively reduce TN. Overall, the SND process reduces aeration requirements and the need for external organic carbon sources (Metcalf and Eddy, 1979; Rittman and Langeland, 1985).

Shortcut N removal processes have gained popularity in recent years. These processes, also known as nitrite shunt, are carried out by oxidizing NH_4^+ to NO_2^- and reducing it to N_2. This process results in energy savings, lower oxygen and organic carbon source demand (Schmidt et al., 2003). This process, however, requires carefully controlled DO conditions and short SRTs to limit the activity of NOBs and to prevent the production of NO_3^-. Anaerobic ammonium oxidation (Anammox) is another popular process where NH_4^+ is partially nitrified to NO_2^- and NO_2^- is utilized as an oxidant in the denitrification process to remove the remaining NH_4^+ (Van Loosdrecht, 2002). This process has no carbon source requirements but requires substantial oxygen and temperature control to allow for the partial nitrification required.

2.2.2 Ion Exchange and Adsorption

Ion exchange (IX) is the physical process where ions in the aqueous phase are exchanged with ions in the solid phase (Howe et al, 2012). The type of ion exchanged in the process is dependent on the media utilized and the pre-saturated ion. Zeolites are hydrated alumino-silicate minerals with a porous structure. They have an internal structure of channels and pores containing ions that can be exchanged with ions in solution (Wei et al., 2011). Zeolites are the most common media for cation exchange (Wang and Peng, 2010) and can also be used for anion exchange when chemically modified (Loganathan et al., 2013). The adsorption capacity of zeolites is dependent on the application, type of zeolite, the ion for exchange and presence of other competing ions. Wang and Peng (2010) determined that zeolites have a selected affinity for K^+ and NH_4^+ ions over Na^+ ions. Ames et al. (1960) reported that the cation selectivity of zeolites follows the order of: $Cs^+ > Rb^+ > K^+ > NH_4^+ > Ba^{2+} > Na^+ > Ca^{2+} > Fe^{3+} > Mg^{2+}$. This selectivity explains why typical modifications of zeolites include supersaturation of the IX sites with sodium (Na^+) using a highly-concentrated NaCl solution. This supersaturation or pretreatment can enhance adsorption of the desired cation but results in an effluent brine solution that will require further treatment or special disposal.

Other studies have shown the potential for biological regeneration of the NH_4^+ saturated zeolite by utilizing the medium as a carrier for nitrifying biofilms (Lahav and Green, 1997; Aponte-Morales et al, 2016). During nitrification, nitrifying bacteria utilize the NH_4^+ in solution, lowering the bulk liquid concentration, which in turn drives desorption of the NH_4^+ ion from the exchanger resulting in bioregeneration. An advantage of bioregeneration is that it provides reuse without requiring post treatment of brine solutions. A summary of prior studies of IX with zeolites, their applications, adsorption capacities and regeneration methods is provided in Table 2.4. Additional

media for ion exchange and nutrient adsorption include purolite for PO_4^{3-} removal (Kauspediene and Snukiskis, 2010), tire scraps for NO_3^- (Krayzelova et al., 2014; Chang et al., 2010; Lisi et al., 2004) and dried or pyrolized agricultural residues for NH_4^+ removal (Liu et al., 2010). Hybrid treatments combining IX and BNR are discussed further in Chapter 5.

Table 2.3: Applications of zeolite materials for ion exchange of nutrients

Zeolite Compound	Particle size (mm)	Reactor	Target ion	Surface modification	Adsorption capacity (mg g^{-1})	Regeneration	
Clinoptilolite	<0.25	Batch	NH_4^+	1 M Na^+	5.232	Nitrification in SBR (NH_4^+-N < 5 mg L^{-1})	Wei et al. (2011)
Modernite				None	7.586		
Clinoptilolite	0.5-1	Column	NH_4^+	1 M Na^+	10.4	None	Alkas et al. (2013)
Chabazite	2-4	Batch	NH_4^+	None	4.5	None	Cyrus and Reddy (2011)
Chabazite	1	Batch	NH_4^+	Groundwater	40.46	Nitrification in SBR (NH_4^+-N ~ 800 mg L^{-1})	Aponte-Morales et al., (2016)
Clinoptilolite	<0.09	Batch	Pb^{2+}	None	276	None	Inglezakis et al. (2010)
Bentonite					276		
Vermiculite					205		

2.3 Off-Flavor Compound Mitigation

Off-flavor compounds plague both drinking and wastewater treatment. Geosmin (GSM) and 2-methylisoborneol (MIB) are secondary metabolites of cyanobacteria and actinomycetes (Guttman and van Rijn, 2008). These metabolites are released when cells lyse together with other microtoxins, particularly during algae blooms. Although GSM and MIB are not toxic they are problematic due to their low odor threshold at concentrations as low as 10 ng L^{-1} (Drikas et al., 2009). Additionally, they accumulate in the lipid-rich tissue of fish and can affect taste and quality of the filet (Howgate, 2004), particularly in freshwater farm raised catfish, salmon and sturgeon. The current removal approach for these compounds is to purge them from the fish prior to

harvesting, which requires large amounts of fresh or highly treated water (Burr et al., 2012) and results in high cost.

Prior studies have identified the persistence of off-flavor compounds in highly aerobic and environments with high organic carbon content (Guttman and van Rijn, 2008; Schrader and Summerfelt, 2010), particularly in biofilters utilized in RAS (Schrader et al., 2005). Biological treatment has been used for removal of off-flavor compounds. Guttman and van Rijn (2009) indicated that anaerobic sludge from RAS has the potential to both absorb and biodegrade GSM and MIB. Hsieh et al. (2010), on the other hand, observed degradation of 93% and 63.7% of GSM and MIB, respectively, in aerobic slow sand filters with an empty bed contact time (EBCT) of 173 mins. The findings of Hamlin et al., (2008) supported Hsieh et al., (2010) where no significant difference of GSM and MIB was observed in the effluent of heterotrophic UPBRs with varying carbon sources.

A common process for GSM and MIB removal is the use of adsorbents, such as activated carbon, for the physical chemical removal of GSM and MIB. Activated carbon, both in powder or granular form, has been studied extensively and the capacity for GSM and MIB adsorption has been shown to be dependent on the base material of the carbon (Yu, et al., 2007; Cook et al., 2000) and interactions with natural organic matter (Matsui et al., 2013; Newcombe et al., 2002; Elhadi et al., 2006). Enhanced off-flavor removal has also been attributed to decreasing particle size as observed by Matsui et al., (2013). In batch test studies, Yu et al., 2007 investigated powdered activated carbons and found higher adsorption capacity of GSM and MIB in fruit shell-based carbon than wood and bituminous coal carbon. This high capacity was attributed to larger micropore volumes, rather than total pore volume or surface area. In batch studies by Lalezary et

al., (1986) a dose of 20 mg L^{-1} of powdered activated carbon was sufficient to remove over 90% of GSM and MIB with a contact time of 180 mins.

Combined activated carbon and biological degradation of GSM and MIB in biofilters has also been studied. Drikas et al., (2009) studied a variety of pre-treated granular activated carbons and found 20 mins EBCT in virgin carbon biofilters was sufficient to remove GSM and MIB in the presence of dissolved organic carbon varying from 2 to 4 mg L^{-1}. Combined biodegradation an adsorption processes were also observed by Drikas et al., (2009) resulting in reduced MIB removal in autoclaved granular activated carbon but no significant change in GSM removal. Preferential removal of GSM over MIB has been seen in studies with both powdered and granular activated carbon (Lalezary et al., 1986; Chen et al., 1997; Summers et al., 2013). Factors such as seasonal water temperatures, EBCT, hydraulic loading (HLR) and the transient nature of off-flavors can also affect removal of these compounds in granular activated carbon biofilters (Elhadi et al., 2006). The use of activated carbon, however, is limited by the potential high dose necessary for removal of GSM and MIB to below under threshold levels (Matsui et al., 2013), resulting in high capital and operational costs (Bamuza-Pemu and Chirwa, 2012). Alternative adsorbents for GSM and MIB were studied by Kelly et al. (2006) including polystyrene, polyethylene and natural rubber and found removals of 19.4 % and 30.1% of GSM and MIB, respectively with a dose of 1.43 g L^{-1} and 8 hr contact time. However, further studies into the application of alternative low cost adsorbents for the removal of GSM and MIB are needed.

Oxidation processes have also been used for GSM and MIB removal in RAS and drinking water. These processes rely on highly reactive hydroxyl radicals, which are non-selective and able to oxidize electron-rich organic compounds (Howe et al., 2012). Oxidation processes are more common for treatment of drinking water due to the importance of the public's perception of safe

drinking water and the need for a highly-treated product water. Oxidation processes include ultraviolet radiation (UV), ozonation and advanced oxidation processes (AOPs) with different catalysts (Srinivasan & Sorial, 2011) or a combination of two or more of the above. Photolysis by UV achieves limited removal of GSM and MIB but performance is enhanced when combined with a Vacuum UV process (Kutschera et al., 2009). Ozonation, which is conventionally used for disinfection purposes, can also oxidize off-flavor compounds (Gonçalves and Gagnon, 2011). Ozone, however, possess the threat of disinfection by-product formation requiring strict dosage control.

Photocatalysis is an AOP process that involves light activation of a catalyst for production of hydroxyl radicals. Titanium dioxide (TiO_2) is a common catalyst that has been studied for oxidation of trace organic compounds in both air and water applications (Obee and Brown, 1995; McCullagh et al. 2011). The slurry application of TiO_2 under UV light has been found extremely effective for GSM and MIB (Lawton et al., 2003). This application, however, is limited by the need for removal of the fine TiO_2 particles. Application of TiO_2 in pelletized form has shown high removal of GSM and MIB (Pestana et al., 2014) but results in very high catalyst dosage that could have detrimental effects of fish health. Prior studies have investigated immobilization of TiO_2 onto glass plates (Zhao et al., 2015) with significant removal of GSM and MIB under low catalyst dosage (Pettit et al., 2014). Immobilized UV-TiO_2 shows great promise for applications in RAS but further research is required to identify the operational parameters as well as the potential effects of the UV-TiO_2 reactor on the water quality conditions of RAS.

Due to the highly reactive and non-selective nature of the hydroxyl radicals, the removal of GSM and MIB in AOPs can be limited by competition of natural organic matter and carbonate scavengers (Bamuza-Pemu and Chirwa, 2012). AOPs with UV can also be limited in the treatment

of turbid waters due to lack of light penetration and reduced activation of the catalyst. These processes also have the potential to produce NO_2^- as a consequence of NO_3^- photolysis (Kutschera et al., 2009). Limitations in the full-scale application of AOP process are mostly due to the high cost, dominated by the energy requirements of the UV irradiation (Zoschke et al., 2012).

CHAPTER 3: OXIDATION OF OFF FLAVOR COMPOUNDS IN RECIRCULATING AQUACULTURE SYSTEMS USING UV-TiO_2 PHOTOCATALYSIS

3.1 Introduction

In most conventional aquaculture systems, fish are raised in tanks or ponds that require constant fresh water inputs and have high wastewater production (Hamlin et al., 2009). Recirculating aquaculture systems (RAS) are gaining popularity due to their water savings compared with conventional aquaculture. In RAS, the water is treated and recirculated, reducing fresh water inputs and wastewater discharges (Gonçalves and Gagnon, 2011). In addition, RAS provide environmental controls that can allow higher production rates (Gonçalves and Gagnon, 2011). A disadvantage of RAS is that conditions are created for the production of off-flavor compounds. These compounds, such as (-)-Geosmin (GSM) and 2-methylisoborneol (MIB), are secondary metabolites of cyanobacteria and some actinomycetes (Guttman and van Rijn, 2008) that cause an earthy musty flavor that can be detected in water at extremely low concentrations (between 10 and 20 ng L^{-1} in water; Drikas et al., 2009). Additionally, they accumulate in the lipid-rich tissue of fish and can affect taste and quality of the filet (Howgate, 2004), particularly in freshwater farm raised catfish, salmon and sturgeon. The current removal approach for these compounds is to purge them from the fish prior to harvesting, which requires large amounts of fresh or highly treated water (Burr et al., 2012).

Advanced treatment technologies are required for removal of GSM and MIB from RAS. Use of adsorbents, such as activated carbon, has been studied extensively and removal of GSM and MIB. Adsorbent capacity has been shown to be dependent on the base material of the carbon

(Yu, et al., 2007; Cook et al., 2000) and interactions with natural organic matter in the wastewater (Matsui et al., 2013; Newcombe et al., 2002). However, removal of GSM and MIB to below the detection threshold requires large dosages of activated carbon (Matsui et al., 2013), resulting in high capital and operational costs (Bamuza-Pemu and Chirwa, 2012). Other treatments for GSM and MIB include ultraviolet radiation (UV), ozonation and advanced oxidation processes (AOPs) using different catalysts (Srinivasan & Sorial, 2011) or a combination of two or more oxidation processes. AOPs rely on highly reactive hydroxyl radicals, which are non-selective and able to oxidize electron-rich organic compounds (Howe et al., 2012). These processes are more common for treatment of drinking water due to the importance of the public's perception of safe drinking water and the need for a highly-treated product water. In aquaculture, oxidation processes are commonly used for pathogen control but the dosing has been found insufficient for GSM and MIB removal (Schrader et al., 2010).

An AOP involving UV photocatalysis with titanium dioxide (UV-TiO_2) has been studied for oxidation of trace organic compounds in both air and water applications (Obee and Brown, 1995; McCullagh et al. 2011). When light waves of sufficient energy activate the catalyst, an electron is excited from the valence band to the conducting band as described by Equation 3.1.

$$TiO_2 + h_v \rightarrow e^- + h^+ \qquad \text{Equation 3.1}$$

The resulting hole in the valence band (h^+) has sufficient reduction potential to oxidize water molecules to hydroxyl radicals (Equation 3.2). The hydroxyl radicals in turn oxidize organics, eventually leading to carbon dioxide and mineral acid formation (Equation 3.3). Depending upon concentration levels, pH and surface charge of the catalyst (Pettit et al., 2014) some organics may be directly oxidized by the valence band holes (Equation 3.4).

$$h^+ + H_2O \rightarrow OH^{\cdot} + H^+ \qquad \text{Equation 3.2}$$

$$OH^{\cdot} + Organic \rightarrow CO_2 \quad \text{Equation 3.3}$$

$$h^{+} + Organic \rightarrow CO_2 \quad \text{Equation 3.4}$$

Previous studies have shown that UV-TiO_2 photocatalysis has the capacity to oxidize up to 99% of GSM and MIB (Lawton et al., 2003) when applied as a slurry. In these applications, after the reaction step the catalyst was removed by centrifugation or filtration. The application of this method; however, is problematic in aquaculture (Pestana et al., 2014) because filtration or centrifugation are unable to remove very fine particles and the effect of the TiO_2 particles on fish health is unknown. The TiO_2 catalyst has also been applied in pelletized form, resulting in high removal rates for GSM and MIB, but requiring a high catalyst load (Pestana et al., 2014). An alternative for application of the UV-TiO_2 treatment process that can be adapted to RAS is the immobilization of the catalyst onto a solid substrate. This application has been studied for degradation of trace organics, such as neonicotinoid insecticides, alachlor, methylene blue and pentachlorophenol (Zabar et al., 2012; Ryn et al., 2003; Nawi and Zain, 2012; Gunzalazuardi and Lindu, 2005). A number of catalyst immobilization methods have been used including sol-gel applications (Gunzalazuardi and Lindu, 2005), dip coating (Nawi and Zain, 2012) and spray coating (Pettit et al., 2014; Zhao et al., 2015). Likewise, there are a number of solid substrates for the immobilization, including glass (Nawi and Zain, 2012; Rubio et al., 2013), metals (Gunzalazuardi and Lindu, 2005) and carbon nano tubes (Yao et al., 2008).

In prior studies in our laboratory an AOP treatment was developed involving a UV reactor with TiO_2 immobilized on glass plates (Pettit et al., 2014; Zhao et al., 2015). High removal rates of GSM and MIB were observed with spiked de-ionized water and RAS wastewater in under 8-hours in batch studies. This study builds on the above-mentioned research by investigating the application of the UV-TiO_2 as a treatment unit for RAS. The specific objectives of this study were

to (1) investigate the performance of the UV-TiO_2 treatment under batch and continuous flow reactor configurations for the removal of GSM and MIB in RAS; and (2) evaluate and discuss the effect of the UV-TiO_2 treatment on water quality parameters and the possible impacts on biological wastewater treatment processes in RAS.

3.2 Materials and Methods

3.2.1 UV-TiO_2 AOP Reactor

A description of the bench-scale UV-TiO_2 reactor used in this study has been published elsewhere (Pettit et al., 2014). Briefly, the UV-TiO_2 reactor is an aluminum container with dimensions of 38.1 cm (l) x 5.08 cm (w) x 2.54 cm (h) that houses the TiO_2 coated glass plates and two T5 8-watt fluorescent GE Blacklight Blue bulbs (F8T5BLB), which provided irradiance in the UVA spectral range of 350 to 400 nm. The reactor was operated at a flow rate of approximately 200 mL min^{-1} using a Masterflex L/S peristaltic pump (Cole Palmer; Vernon Hills, IL). This flow rate results in a water height above the glass plates of approximately 2 cm.

3.2.2 Batch Reactor Application

Batch experiments were carried out at Healthy Earth Sarasota (HES) facility located within the MOTE Aquaculture Research Park where Siberian Sturgeon are raised in RAS. The sturgeon are fed at a rate of 70 kg d^{-1} and the dissolved oxygen (DO) concentrations are maintained at or near saturation to ensure fish health. Each RAS treats the wastewater from four 70 m^3 growout tanks. A common trough collects the wastewater, which flows through a series of treatments (Figure 3.1) and is later recirculated back to the tanks. The first treatment unit is a rotating 60 mm drum screen filter (PR Aqua Rotofilter Model 4872, Nanaimo, BC, Canada) for solids separation. The concentrate is routed to a settling pond while the filtered water flows to a Moving Bed Bioreactor (MBBR) for organic carbon removal and nitrification of ammonium (NH_4^+) to nitrite

(NO_2^-) and nitrate (NO_3^-). The MBBR is a 45 m^3 mechanically aerated tank with approximately 55% of the volume occupied by plastic carriers (AMB™ media, EEC North America LLC, Blue Bell, PA) that provide the superficial area for the growth of microorganisms. A portion of the wastewater (the side stream in Figure 3.1) is denitrified in two parallel 1.89 m^3 upflow packed bed reactors (UPBRs), with approximately 1 m^3 of the volume occupied by plastic carriers (AMB™ media, EEC North America LLC, Blue Bell, PA). Molasses ($C_{12}H_{22}O_{11}$), a locally available byproduct of sugar cane processing, is used as an electron donor and carbon source for the denitrification process at a carbon to NO_3^- ratio of 2:1 (Hamlin, 2008). The low NO_3^- effluent from the UPBR is pumped back to the main line prior to the drum filter. After the MBBR, the water enters a degassing basin where air is injected to remove CO_2 and restore the DO concentrations. The water is then subjected to UV disinfection and enhanced with pure oxygen before returning it to the fish tanks. Alkalinity is monitored in this same basin and controlled with the addition of sodium bicarbonate. This treatment scheme lowers nitrogen (N) concentrations and makes possible the reuse of approximately 88% of the treated water, reducing both the amount of freshwater inputs and wastewater production (the other 12% is replaced with groundwater). The UV-TiO_2 reactor was placed after the MBBR prior to the oxygenation basin and sodium bicarbonate addition for alkalinity control. Nitrified RAS effluent (2 L) was pumped to a glass retention tank and treated by recirculating through the reactor for six hours. The total treatment cycle (fill, react, decant) was about 8 hours and was repeated 3 times each day. A ChonTrol four-circuit programmable timer (Cole Palmer; Vernon Hills, IL) was used to control the cycles.

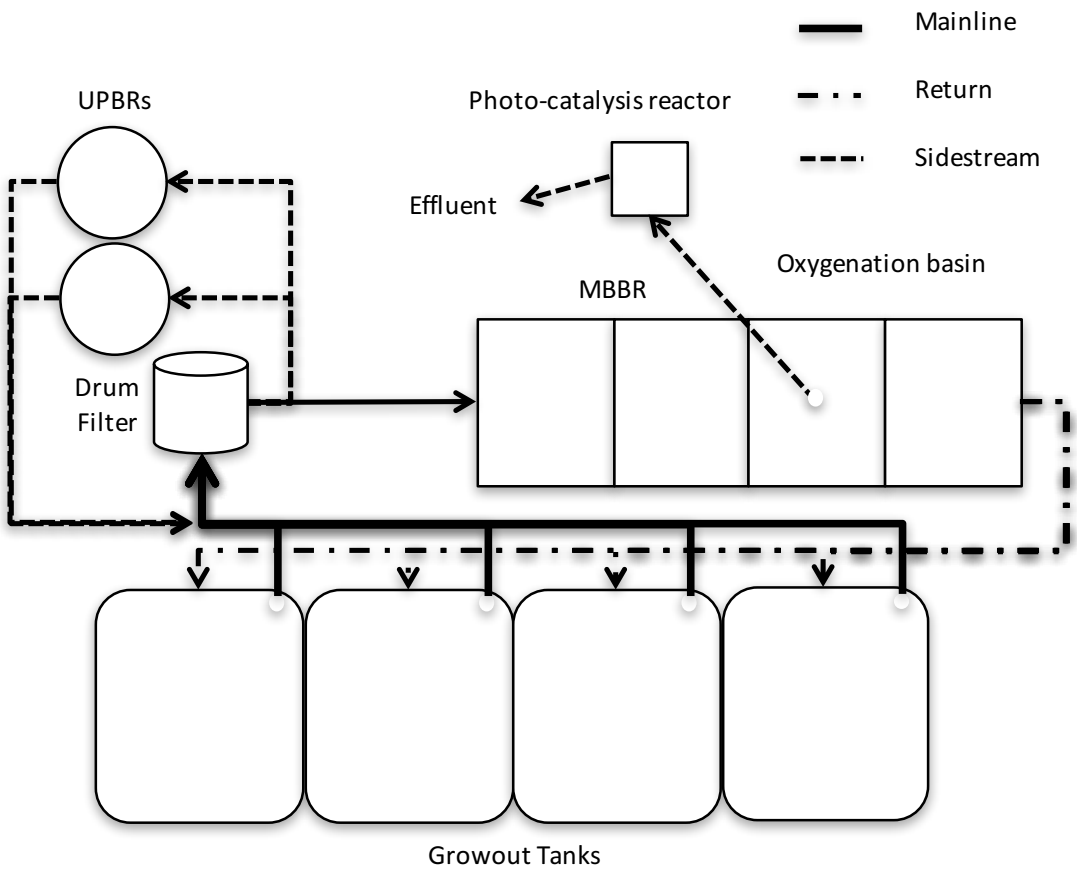


Figure 3.1: Treatment scheme at HES. Main line, return and side stream flow are shown using different line styles.

3.2.3 Continuous Flow Application

A laboratory scale RAS (Figure 3.2) was designed based on a 0.004% scale down of HES's RAS shown in Figure 3.1. Feeding rates were calculated based on a scale down of HES's feeding rates and equations for typical RAS Total Ammonia Nitrogen (TAN) loading rates from Timmons et al. (2002). Synthetic wastewater was pumped from a 2 L glass container into the system at a flow rate of 1.3 L d^{-1} using a Masterflex C/L Dual Channel Pump (Cole Palmer; Vernon Hills, IL). The synthetic wastewater consisted of a solution of 0.25 g L^{-1} NH_4Cl and 0.4 g L^{-1} $NaHCO_3$ and dissolved fish feed pellets (1.58 g L^{-1}) from the feeders at HES. Water was recirculated through the main line at a rate of 360 L d^{-1} using a Masterflex L/S peristaltic pump (Cole Palmer; Vernon Hills, IL). Two Cole-Parmer Valved Variable Area Acrylic flow meters were used for flow

measurement and control into the MBBR and UPBR. The MBBR consisted of a glass bottle with a working volume of 3 liters, with 55% filled with plastic carriers collected from the MBBR in MAP's RAS described in 2.1. A compressor and air diffuser (Tetra Whisper®, Blacksburg, VA) provided the DO needed for nitrification. A side stream was connected after the MBBR where 10.8 L d^{-1} were pumped to an UPBR consisting of an acrylic column (Koflo; Cary, IL) of approximately 0.75 L with 53% of its volume filled with plastic carriers from HES's UPBR described in 2.1. Plastic carriers were collected from HES since they provided biofilms acclimated to RAS conditions, which allowed for rapid start-up times for the bench-scale system. The bench-scale RAS was initially filled with aquaculture water from the growout tanks at HES. A single-syringe infusion pump (Cole Palmer; Vernon Hills, IL) was connected to the side stream to provide molasses (Hamlin, 2008) at a flow rate of 4 μL hr^{-1}. The effluent from the UPBR was pumped back to the main line and recirculated to the MBBR. A small glass reservoir (working volume less than 500 mL) was added to facilitate the installation of the photocatalytic reactor as a side treatment unit and to add an outlet stream for control of liquid build up.

Two different strategies were used to test the UV-TiO_2 reactor in the bench-scale RAS. A preliminary experiment was carried out where the UV-TiO_2 reactor was placed within the mainline of the RAS, just upstream of the MBBR (not shown). Due to problems with nitrification inhibition (described below), a second loop was added, and the UV-TiO_2 reactor was added as a side stream treatment (Figure 3.2). Samples were collected two to three times each week prior to installation of the UV-TiO_2 reactor (25 days), one hour after installation and two to three times each week after installation of UV-TiO_2.

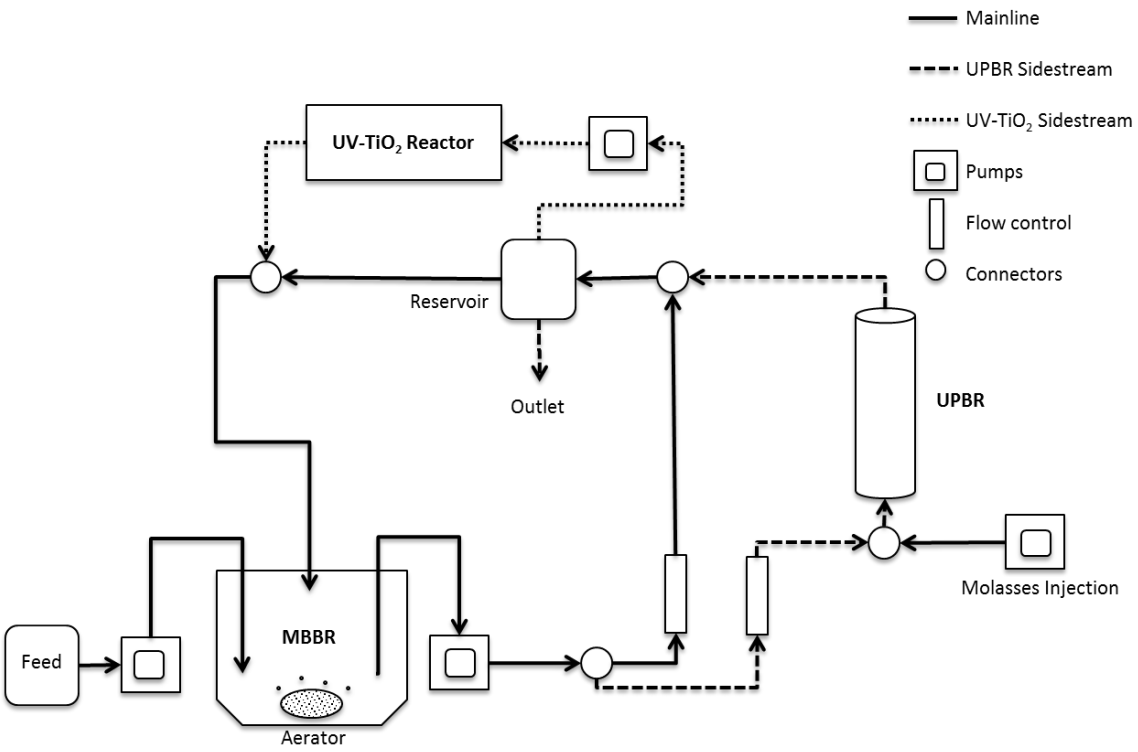


Figure 3.2: Bench scale RAS. Mainline and side stream flows for UPBR and UV-TiO_2 reactor are shown in different line styles (Not to scale).

3.2.4 Analytical Methods

Samples collected from the full and bench scale RAS were analyzed according to Standard Methods (APHA et al., 2012). pH and dissolved oxygen (DO) were measured using an Oakton Acorn Series meter (Orion 5 Star ThermoScientific) and calibrated electrodes. A portion of the samples was filtered through a 0.45 μm mixed Cellulose Esters filter (FisherScientific, Waltham, MA). Chemical oxygen demand (COD) was measured for unfiltered and filtered samples with the Vario Tube Test (Loveland Co) COD LR test kits ([5220]; MDL: 0-150 mg L^{-1}). During Phase I, ammonium was determined by the colorimetric method (Willis et al., 1996) and the resorcinol method was used for nitrate (Zhang and Fisher, 2006). NO_2^- was determined according to Standard Methods [4500]. The concentrations of all three N species were determined by absorbency in a HACH 4000 Spectrophotometer (Loveland, CO). During Phase II, concentrations of anions (NO_2^-

, NO_3, PO_4^{-3}, SO_4^{-2}) and cations (NH_4^+) were measured in the filtered samples using a Metrohm 881 Compact IC Pro (Herisau, Switzerland) ion chromatography system. MDLs for NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-} and NH_4^+ were 0.01, 0.04, 0.01, 0.02 and 0.07 mg L^{-1}, respectively.

3.2.5 Trace Analyses

GSM and MIB analysis where performed by Solid Phase Micro-extraction (SPME) and GC/MS. Samples were prepared using a modification of method 6040D (APHA et al., 2012). Preparation consisted of adding 9 g of salt, previously calcinated for 5 hours at 550°C, 30 mL sample and 3 µL of the IBMP solution to a 45 mL glass vial containing a PTFE stirrer and PTFE septa cap. The vial was placed in a water bath with temperature range of 60-65°C to ensure volatilization of the desired compounds. A divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) fiber was injected into the vial with 0.8 cm exposed to the headspace for 30 minutes. After retraction the fiber was fully exposed in the GC/MS injector for 3 minutes. Samples were diluted when necessary to ensure readings within the detection range of 1ng L^{-1} to 40 ng L^{-1}. Stock solutions of 100 mg L^{-1} for both GSM and MIB were purchased from Sigma Aldrich (St. Louis, MO). A 1ug L^{-1} 3-isobutyl-2-methoxypyrazine (IBMP) (Sigma Aldrich,) solution was used as an internal standard. Compound separation and detection was performed using a Perkin Elmer (Waltham, MS) Clarus 580 GC/MS equipped with a 30 m x 0.25 mm x 0.25 µm HP-5MS Agilent column (Santa Clara, CA). The injector temperature was set to 250°C. The initial oven temperature was set at 50°C, with a ramp of 15°C min^{-1} and a maximum temperature of 280°. For the first 2 minutes split-less mode was activated, and for the remaining 15 minutes the split was set at 50 ml/min He with 1mL min^{-1} carrier flow. The MS method used a solvent delay of 3 minutes and selected ion recording for 124/151 (IBMP), 95/107/135 (MIB) and 112/116 m z^{-1} (GSM).

3.2.6 Statistical Analysis

Statistical analyses were performed to compare the performance of RAS with and without the UV-TiO_2 treatment. A paired t-test assuming equal variances was performed in Excel 2011 for GSM, MIB and N species results from both phases with α=0.05.

3.3 Results

3.3.1 Batch Reactor Applications

Average water quality data for all HES's treatment units and UV-TiO_2 reactor performance during treatment cycles 1 to 13 are shown in Table 3.1. pH remained neutral in all treatment units including the UV-TiO_2. DO concentrations were highest in the fish tank, as expected, due to the pure oxygen injection prior to the recirculation. In the UPBR, DO concentrations were higher than expected since denitrification is an anaerobic treatment process. This could have hindered denitrification as evident by the high NO_2^--N concentrations in the effluent of the UPBR and the low removal of nitrate (25%). In the effluent of the UV-TiO_2, slightly significant increases were observed in NO_2^--N concentrations when compared to the MBBR effluent, but concentrations remained lower than 1 mg L^{-1}. No significant changes were observed in NH_4^+-N and NO_3^--N concentrations.

The highest GSM and MIB concentrations were found in the drum filter effluent (solids slurry), reaching average concentrations of 101 and 235 ng L^{-1}, respectively (Figure 3.3). Concentrations in the fish tank averaged 29 and 41 ng L^{-1} for GSM and MIB, respectively, and were not significantly different than those in the MBBR. Concentrations detected in HES's RAS were somewhat lower than expected since previous monitoring by HES had shown concentrations as high as 50 ng L^{-1}. The UPBR achieves some removal of the compounds, as shown by the significantly lower concentrations in the UPBR effluent compared to the MBBR effluent. For both

GSM and MIB the UV-TiO_2 reactor showed significant removal resulting in concentrations as low as 10 and 20 ng L^{-1}, respectively. On average, 72% of GSM and 62.6% of MIB were removed after 6 hours of contact time.

Table 3.1: Average water quality for HES's RAS and UV-TiO_2 reactor.

	Ground water	Fish tank	Drum filter	UPBR	MBBR	UV-TiO_2
pH	7.62 $\pm 0.06_5$	6.59 $\pm 0.1_5$	6.76 $\pm 0.07_4$	6.57 $\pm 0.09_5$	6.65 $\pm 0.05_5$	7.88 $\pm 0.17_5$
DO (mg L^{-1})	7.07 $\pm 0.25_5$	10.23 $\pm 0.6_5$	8.19 $\pm 0.55_4$	2.53 $\pm 0.33_5$	7.22 $\pm 0.34_5$	7.66 $\pm 0.24_5$
Conductivity (mS cm^{-1})	1.3 $\pm 0.06_5$	1.62 $\pm 0.16_5$	1.64 $\pm 0.1_4$	1.56 $\pm 0.01_5$	1.41 $\pm 0.32_5$	1.68 $\pm 0.12_4$
NH_4^+-N (mg L^{-1})	0.25 $\pm 0.07_5$	0.48 $\pm 0.26_5$	1.88 $\pm 0.63_4$	0.88 $\pm 0.59_5$	0.21 $\pm 0.14_5$	0.39 $\pm 0.12_4$
NO_2^--N (mg L^{-1})	0.05 $\pm 0_5$	0.09 $\pm 0.00_5$	0.46 $\pm 0.22_4$	3.11 $\pm 0.35_5$	0.08 $\pm 0.01_5$	0.11 $\pm 0.01_4$
NO_3^--N (mg L^{-1})	0.82 $\pm 0.06_5$	30.3 $\pm 2.44_5$	29.7 $\pm 4.3_4$	22.78 $\pm 6.19_5$	29.55 $\pm 6.64_5$	34.95 $\pm 28.49_4$
Alkalinity ($CaCO_3$ mg L^{-1})	145.53 $\pm 1.52_3$	94 $\pm 6.41_3$	216.48 $\pm 24.46_2$	174.77 $\pm 6.67_3$	78.13 $\pm 20.93_3$	77.28 $\pm 21.76_2$

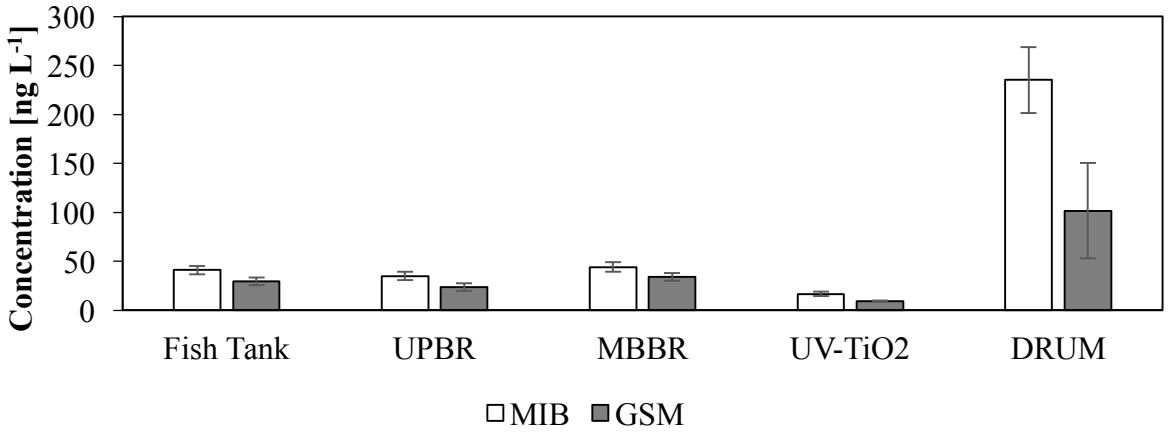


Figure 3.3: GSM and MIB concentrations in RAS treatment units and the effluent of the UV-TiO_2 batch reactor.

UV-TiO_2 reactor performance over time was investigated by collecting a final sample corresponding to Cycle 311. At this point the UV-TiO_2 reactor had been in operation for 104 days.

A comparison of percent reductions between several cycles are shown Figure 3.4. Highest performance was observed during cycle 8, where GSM and MIB concentrations reached 40 ng L^{-1} and 57 ng L^{-1} for GSM and MIB, respectively. A 10% reduction in removal performance was observed from cycle 1 to 311.

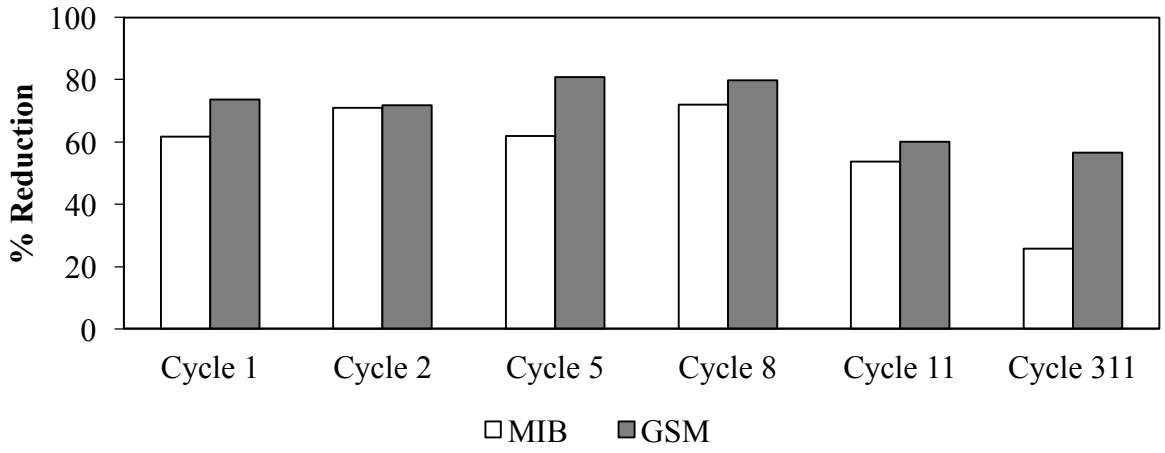


Figure 3.4: UV-TiO_2 percent degradation performance for Cycles 1, 2, 5, 8, 11 and 311.

3.3.2 Continuous Flow Application

Preliminary experiments showed that the production of GSM and MIB within the bench-scale RAS were greatly reduced with operation times longer than 2 months. An initial short-term experiment was performed by injecting a GSM and MIB solution into the bench-scale RAS (data not shown). After stabilization for 100 minutes (HRT of UPBR) concentrations were still very low, at approximately 10 ng L^{-1}. Immediately after stabilization the UV-TiO_2 reactor was installed in the main line as described in section 3.2.3. Within 48 hours of run time, MIB concentrations in the UV-TiO_2 effluent were below detection limits (1ng L^{-1}) while GSM decreased to 3 ng L^{-1}. After 72 hours of continuous treatment, GSM and MIB concentrations remained constant throughout the RAS. GSM concentrations were higher than MIB, differing from Phase I. Although GSM and MIB removal was excellent in this configuration, nitrification inhibition was observed with the application of the UV-TiO_2 system in the main line resulting in high NH_4^+-N

concentrations (>10 mg L^{-1}; not shown). Average NO_2^--N concentrations were negligible for all treatment units, including UPBR effluent differing from conditions in Phase I. Alkalinity increases in the UV-TiO_2 were slight, around 2 mg L^{-1}, but increases in alkalinity were another indication that nitrification was limited.

To overcome nitrification inhibition, the UV-TiO_2 reactor was reduced to half its size and applied as a side stream treatment (Q= 90 mL min^{-1}). During this period the HES wastewater added to the system had a higher concentration of GSM and MIB and did not require additional injections. Average results for an observation period of 25 days prior to installation of UV-TiO_2 and 15 days after installation are shown in Table 3.2 and Figure 3.5. The pH for RAS remained neutral for all treatments with and without the UV-TiO_2 reactor. DO concentrations were above 4 mg L^{-1} after the MBBR and decreased by about 0.5 mg L^{-1} with the UV-TiO_2 treatment. The main N species in the bench scale RAS was NO_3^--N, and no significant changes were observed with the addition of UV-TiO_2. Similar results were observed for NO_2^--N in the MBBR, although the NO_2^--N concentration in the UPBR increased considerably with UV-TiO_2 indicating incomplete denitrification. NH_4^+-N concentrations remained below 1 mg L^{-1} for the MBBR with and without the UV-TiO_2 treatment showing no inhibition of nitrification. Some production of NH_4^+-N was observed in the UPBR. Although averages show decreased GSM and MIB concentrations, due to the high variability statistical analysis revealed no significant differences when the RAS was operated with or without the UV-TiO_2 treatment.

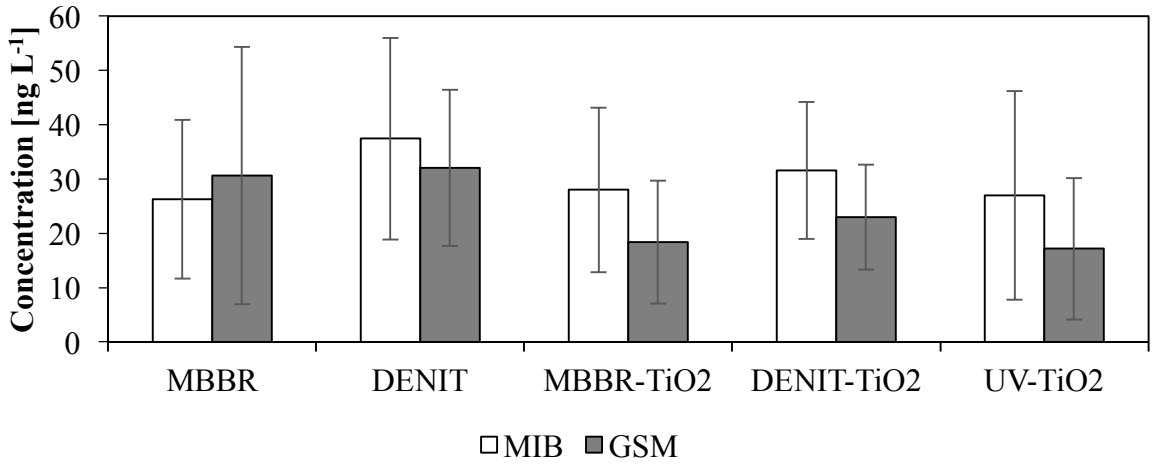


Figure 3.5: UV-TiO_2 concentrations in continuous flow applications on the bench-scale RAS

Table 3.2: Average water quality for the bench scale RAS with and without the influence of UV-TiO_2.

	FEED	MBBR	MBBR+ UV-TiO_2	DENIT	DENIT+ UV-TiO_2	UV-TiO_2
pH	7.45 $\pm 0.34_{10}$	7.42 $\pm 0.27_{10}$	7.31 $\pm 0.08_{7}$	7.06 $\pm 0.23_{10}$	6.79 $\pm 0.18_{7}$	7.34 $\pm 0.10_{7}$
DO (mg L^{-1})	0.87 $\pm 1.15_{16}$	4.35 $\pm 1.32_{10}$	3.75 $\pm 0.43_{7}$	1.65 $\pm 0.85_{10}$	1.01 $\pm 0.18_{7}$	3.67 $\pm 0.66_{7}$
Conductivity (mS cm^{-1})	1.42 $\pm 0.04_{16}$	1.41 $\pm 0.17_{10}$	1.44 $\pm 0.12_{7}$	1.40 $\pm 0.16_{10}$	1.44 $\pm 0.13_{7}$	**1.43 ±0.14**
NH_4^+-N (mg L^{-1})	76.83 $\pm 7.74_{14}$	0.20 $\pm 0.09_{7}$	0.14 $\pm 0.04_{7}$	3.02 $\pm 3.12_{7}$	0.80 $\pm 0.78_{7}$	**0.16 $\pm 0.10_{8}$**
NO_2^--N (mg L^{-1})	2.20 $\pm 1.26_{14}$	0.26 $\pm 0.26_{7}$	0.37 $\pm 0.25_{7}$	0.40 $\pm 0.26_{7}$	1.63 $\pm 1.88_{7}$	**0.38 $\pm 0.35_{8}$**
NO_3^--N (mg L^{-1})	0.02 $\pm 0.06_{13}$	33.07 $\pm 8.66_{8}$	28.93 $\pm 4.46_{7}$	13.45 $\pm 12.40_{7}$	14.78 $\pm 13.80_{7}$	**29.10 $\pm 4.64_{8}$**
Alkalinity ($CaCO_3$ mg L^{-1})	201.75 $\pm 23.99_{16}$	74.90 $\pm 21.43_{10}$	64.31 $\pm 18.54_{7}$	105.53 $\pm 27.43_{10}$	87.57 $\pm 27.28_{7}$	59.04 $\pm 11.29_{6}$

* Number of samples are shown as subscripts.

The possible detrimental effect of bacterial growth and age on the activity of the TiO_2 plate was investigated by comparing removal performance in batch mode between plates utilized for 30 days and a freshly coated plate (Figure 3.6). Reactor conditions were similar to continuous flow

applications with the exception of total recirculation of the water treated. Treatment consisted of recirculation of 2 L of a 50 ng/L solution of GSM and MIB in di-ionized water for 6 hours within the UV-TiO_2 reactor. GSM and MIB concentrations in the bulk liquid versus treatment time are shown in Figure 3.6 for both plates. Both the fresh and used plates showed good removal, with approximately 70% removal for GSM and 40% removal for MIB. The results were surprising due to the visible growth of biofilms in parts of the used plates, which were expected to interfere with UV irradiation. No significant differences were observed between the two plates.

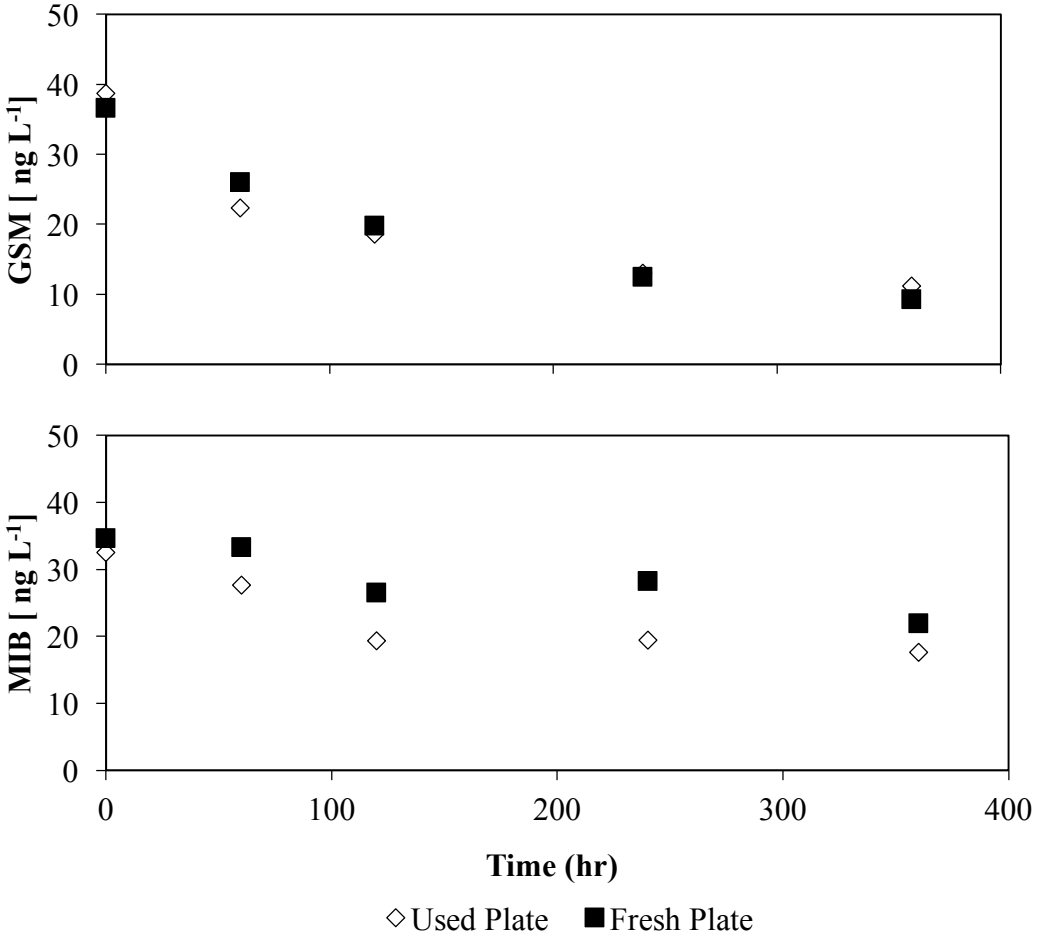


Figure 3.6: Comparison between fresh and used UV-TiO_2 plates for degradation of GSM and MIB

3.4 Discussion

3.4.1 GSM and MIB Removal

GSM and MIB are a hindrance in the aquaculture industry due to their negative effect on fish quality and the complex and expensive methods needed for their removal. Studies have shown the production of these compounds under high organic and aerobic conditions (Guttman and van Rijn, 2009), which are predominant in RAS. At HES, the highest GSM and MIB concentrations were found in the slurry from the drum filter (Figure 3.3), where high organic conditions exist due to the concentrated waste. Furthermore, the solids in the filter are removed by spraying the wastewater onto the drum, oxygenating the water and increasing DO concentrations. MIB concentrations in the fish tank and MBBR, were somewhat lower than expected. Previous monitoring by HES had encountered concentrations as high as 50 ng L^{-1} (unpublished data). Hamlin et al. (2008) studied the same aquaculture systems and saw concentrations as high as 10 ng L^{-1} for GSM and 70 ng L^{-1} for MIB. Lower concentrations were also observed in the effluent of the UPBR, which differs from the results in the above mentioned study. Removal of the off-flavor compounds is possible through uptake into the anaerobic sludge within the UPBR. Guttman and van Rijn (2009) saw similar results in a marine RAS where the anaerobic sludge could both absorb and biodegrade GSM and MIB by 71% and 89%, respectively.

The UV-TiO_2 reactor in HES showed significant removal for both GSM and MIB (Figure 3.3). On average, 72% of GSM and 62.6% of MIB were removed after 6 hours of contact time. This removal was higher than that observed in previous laboratory studies with de-ionized water spiked with the off-flavor compounds (Pettit et al., 2014) where 60% of GSM and 50% MIB were removed after 8 hours of contact time. The water quality in the location of reactor placement could have been a factor in improved performance (Table 3.1). The UV-TiO_2 reactor was placed after

the MBBR, where nitrification takes place and alkalinity is consumed. Lower alkalinity positively impacts degradation by limiting scavenging of hydroxyl radicals by carbonates present in the wastewater (Kutschera et al., 2009). In addition, the high DO in the wastewater provides desired conditions for degradation as observed by Pettit et al. (2014), where improved degradation was achieved when an aerator was added to the synthetic solution. Furthermore the pH levels in HES's RAS at the location of UV-TiO_2 were favorable for GSM degradation according to a study by Kutschera et al. (2009), where the highest degradation rate constant was observed at pH levels close to 7.

Comparison between different cycles of the UV-TiO_2 treatment (Figure 3.4) gives a snapshot of the resilience of the treatment. Cycle 1 showed the best performance, with over 72% and 62% removal of GSM and MIB, respectively. The performance was slightly reduced in Cycle 11 and was reduced further in Cycle 311. The difference between the first and following cycles could be attributed to adsorption of the off-flavor compounds to the catalyst that occurs at the startup of the reactor. This phenomenon was also observed in previous laboratory studies with de-ionized water spiked with the off-flavor compounds (Pettit et al., 2014). Adsorption to other surfaces within the reactor, such as tubing, connectors and the glass bottle could have also occurred and resulted in an improved performance for Cycle 1. A study by Elhadi et al. (2004) showed that significant losses of GSM and MIB can occur on glass and non-Teflon surfaces. In this study, 40 and 30% losses for GSM and MIB, respectively, occurred in a glass column with Teflon tubing within a 20 day period. Surprisingly, samples taken within the glass feed bottles had 8% loss of the compounds within 4 days (Elhadi et al., 2004). Similar mechanisms could have occurred in the first cycle of the UV-TiO_2 reactor to a smaller extent. Decreased performance over time could also be caused by bacterial growth on the plates that may limit UV penetration and diminished UV

intensity. Loss of the catalyst could be an alternate explanation for the decrease in performance; however, previous studies on the TiO_2 coated plates showed negligible loss of the catalyst when submitted to 7 day de-ionized water flush at a rate of 2.5 mL s^{-1} (Zhao et al., 2015). The comparison between freshly coated TiO_2 plates and plates utilized for a 30 day period with visible growth showed no difference in performance but it is unknown how this value would change with longer periods of operation.

In the bench-scale RAS, GSM and MIB concentrations were lower than those at HES possibly due to differences in microbial diversity due to the synthetic aquaculture wastewater feed and lack of fish fecal waste. This was apparent in the low concentrations observed in preliminary studies. Preliminary studies with the UV-TiO_2 reactor in the main line and spikes of GSM and MIB to increase baseline concentrations resulted in nitrification inhibition. A possible cause of inhibition was the inactivation of nitrifying bacteria by carry-over of oxidants produced in the UV-TiO_2 reactor to the MBBR. Robertson et al. (2005) studied the effect of UV and UV-TiO_2 slurry for inactivation of pathogens. Utilizing a UV lamp with 330 to 450 nm spectra, 4 log removal of pathogens was achieved by both UV and UV-TiO_2 treatment. Although nitrifiers are not pathogenic, due to their sensitivity to water quality changes and slow growth, there could have been some inactivation effects.

The results for the bench-scale RAS with side stream UV-TiO_2 treatment showed insignificant reduction of GSM and MIB (Figure 3.5). The limited performance compared with the batch process could have been due to multiple factors. First, the reduction of the reactor size reduced UV irradiation and catalyst surface, which can affect mass transfer between the contaminants and the catalyst (Pestana et al., 2014; McCullagh et al., 2011). The reduction of the flowrate to achieve the same HRT (within the reactor) as the batch reactor application reduced the

turbulence within the reactor, also affecting mass transfer and oxygenation of the liquid (Zhang et al., 2013). The higher pH in the system (Table 3.2), when compared to HES's RAS, could have also affected the degradation performance of the UV-TiO_2 reactor as observed by Kutschera et al. (2009) where the degradation rate constants of both GSM and MIB decreased when pH increased from 7 to 9. Furthermore, the lower concentrations of GSM and MIB possibly affected UV-TiO_2 performance, which is expected to increase with increasing concentration due to concentration dependent reaction rates (Pettit et al, 2014; Lawton et al. 2013).

3.4.2 Effects of UV-TiO_2 on Water Quality

Aside from the removal of solids, the major concern in RAS is the removal of N compounds NH_4^+-N and NO_2^--N due to high toxicity for aquatic species at concentrations above 1 mg L^{-1} and 0.3 mg L^{-1}, respectively (Timmons et al., 2002; Hamlin et al., 2008). At HES, NH_4^+-N concentrations were well below 1 mg L^{-1} for the fish tank, MBBR and UV-TiO_2 effluent. Higher NH_4^+-N concentrations were observed in the effluent of the UPBR, possibly due to dissimilatory nitrate reduction to NH_4^+-N and the ammonification of organic N in the molasses. High NO_2^--N concentrations in the effluent of the UPBR indicated incomplete denitrification. The slight, but significant increase of NO_2^--N concentrations in the effluent of the UV-TiO_2 was not unexpected. NO_3^--N photolysis has been found by many authors to occur in treatments utilizing UV/VUV, which result in increased NO_2^--N concentrations (Kutschera et al., 2009; Gonzalez and Braun, 1995). On the other hand, NO_3^--N can be produced as well since hydroxyl radicals produced in UV-TiO_2 reactor can oxidize NO_2^--N to NO_3^--N (Gonzalez and Braun, 1995). An unexpected result was the slight increase in alkalinity. It is known that carbonates are scavengers of hydroxyl radicals and can affect GSM and MIB reduction rates (Kutschera et al., 2009). In any case, the differences observed are not expected to affect fish health nor biological processes in the RAS.

3.4.3 Reactor Configuration and Applications

An obstacle to the implementation of full-scale UV-TiO_2 can be the impracticality of a batch reactor (Pestana et al., 2014) as well as the possible high cost of land, construction and operation of an additional treatment process that would allow the desired contact time. A continuous flow application would be possible at HES by replacing the UV lamps used for disinfection in the oxygenation basin prior to recirculation (Figure 3.1) with lamps that allow for a spectra range sufficient for catalyst activation and a TiO_2. Coated glass could be placed above (and/or below) the UV lamps, potentially reducing the energy cost for UV treatment. Further research would be needed to determine whether this strategy effectively reduces off flavor compound concentrations in the RAS.

An alternative for the location of the UV-TiO_2 reactor is application as a batch process at the depuration stage prior to harvesting. Depuration of mature fish occurs in high clarity and quality water (Burr et al., 2012) that is only partially recirculated and treated by ozonation and activated carbon. Depuration can require anywhere from 2-8 weeks depending on the gender of the fish as well as the degree of GSM and MIB taint. Application of the UV-TiO_2 at this stage could allow for greater water recirculation rates, reducing or even eliminating freshwater inputs. Improved results are expected with this operational strategy due to batch operation and more favorable pH and DO concentrations.

3.5 Conclusions

An immobilized TiO_2 and UV reactor was applied as a batch reactor in an operating RAS (Phase I) and as a continuous reactor in a bench-scale RAS (Phase II). Improved performance on the removal of GSM and MIB was observed as a batch reactor since it allowed longer treatment without the effect of constant production of the compounds in the biological treatment processes.

No harmful effects were observed on other water quality parameters when the UV-TiO_2 reactor was operated as a batch or side stream process. Treatment performance of UV-TiO_2 was affected by GSM and MIB concentrations and dissolved oxygen. Due to the possible production of NO_2^--N and scavenging of carbonates, location of the UV-TiO_2 reactor is suggested at a point where NO_3^--N is low and prior to any addition of chemicals for alkalinity control. By lowering concentrations in the RAS, depuration times are expected to be reduced resulting in lower demand for highly treated water, improved product quality and cost savings.

CHAPTER 4: SIMULTANEOUS DENITRIFICATION AND OFF-FLAVOR COMPOUND REMOVAL IN A TIRE SULFUR HYBRID ADSORPTION DENITRIFICATION (T-SHAD) REACTOR

4.1 Introduction

Recirculating Aquaculture Systems (RAS) present an alternative for high fish production rates with reduced ecological impacts (i.e lower freshwater inputs and wastewater outputs) that are associated with traditional fish farming (Hamlin et al., 2008; Martins et al., 2010; Gonçalves and Gagnon, 2011). Typical RAS treatment is focused on the removal of suspended solids and NH_4^+ that result from fish feces, biomass and uneaten food (Timmons et al., 2002). The removal of NH_4^+ is of particular importance due to the high toxicity for aquatic species at levels above 1 mg L^{-1} (Timmons et al., 2002; Hamlin et al., 2008, reference 2). In RAS, NH_4^+ is primarily removed by aerobic biological oxidation to NO_2^- and subsequently to NO_3^-. Due to the toxicity of NO_2^- at levels above 0.3 mg L^{-1} (Timmons et al., 2002; Hamlin et al., 2008), enough oxygen is provided to achieve complete nitrification to NO_3^-. The accumulation of NO_3^- often occurs, particularly in low water exchanges environments (3-10%) characteristic of many RAS (van Rijn, 2008). Prior studies indicate a relationship between chronic health effects and NO_3^--N concentrations above 100 mg L^{-1} in RAS water. These effects, which range from abnormal behavior to increased mortality, (Hamlin, 2006; Davidson et al., 2014) as well as the potential for eutrophication of RAS waste receiving waters, highlight the importance of the mitigation of all inorganic N species in RAS.

The most common NO_3^- removal technique in the aquaculture industry is heterotrophic denitrification where NO_3^- is utilized as an electron acceptor and is reduced to inert N_2 gas and released to the environment. This biological process requires a source of organic carbon as an electron donor, which can be a limiting factor in RAS (Eq 4.1). Biologically available organic carbon is significantly removed in the sedimentation basin (Tsukuda et al., 2015) as well as the aerobic stage for nitrification in RAS. Limited organic carbon availability can result in incomplete denitrification and release of NO_2^- into the system causing toxicity. In such case, the addition of external sources of organic carbon, such as methanol, acetate or sucrose would be required and can be costly (Park and Yoo, 2009; Hamlin et al., 2008). Furthermore, the addition of external carbon sources has to be closely monitored to achieve the desired C/N ratio and avoid carry-over that could hinder other biological processes within RAS.

$$NO_3^- + 0.132C_{12}H_{22}O_{11} + 1.0H^+ \rightarrow$$
$$0.058C_5H_7O_2N + 0.471N_2 + 1.293CO_2 + 1.748H_2O \qquad \text{Equation 4.1}$$

An alternative to heterotrophic denitrification is autotrophic denitrification, where an inorganic electron donor, such as H_2 or elemental sulfur, is utilized. Sulfur oxidizing denitrification (SOD; Eq. 4.2) is particularly popular due its high nitrate removal rates and lower biomass production compared to heterotrophic denitrification (Sengupta et al., 2007). This metabolic process results in SO_4^{2-} production as well as alkalinity destruction and requires an alkalinity source to ensure that the pH remains within the optimal range of treatment (Park and Yoo, 2009; Oh et al., 2001).

$$NO_3^- + 1.1S^0 + 0.4CO_2 + 0.76H_2O + 0.08NH_4^+ \rightarrow$$
$$0.08C_5H_7O_2N + 1.1SO_4^{2-} + 0.5N_2 + 1.28H^+ \qquad \text{Equation 4.2}$$

The majority of the studies on denitrification in RAS have employed heterotrophic denitrification in fluidized media beds (Tsukuda et al., 2015, Kim et al., 2004) and upflow packed bed reactors (UPBRs) (Hamlin et al., 2008; Singer et al., 2008; Saliling et al., 2007). Fluidized media beds and UPBRs have been found to provide high treatment efficiency with a small footprint when using media materials as carriers for denitrifying biomass (Tsukuda et al., 2015). A variety of carbon sources and media materials have been studied. Saliling et al., (2007), for example, tested an UPBR with wood chips and wheat straw and found high removal rates of synthetic aquaculture water with flowrate of 15 mL min^{-1}. Hamlin et al., (2008) studied the denitrification rates of three different carbon sources in an UPBR with plastic carriers. Removal rates were found to be 670 mg L^{-1} d^{-1} for methanol, acetate and molasses.

Most studies of SOD applications have been for treatment of domestic wastewater and NO_3^- contaminated groundwater and have proven successful under varying concentration ranges (Sengupta et al., 2007; Krayzelova et al., 2014; Tong et al., 2016). Limited research has been done on SOD for RAS applications. Particularly promising is the study by Christianson et al. (2015), where granular sulfur packed fluidized biofilters achieved removal rates as high as 800 mg NO_3^--N L^{-1}-d^{-1}. Studies investigating the effects of SOD on RAS water quality as well as the removal of off-flavor compounds are lacking.

Off-flavor compounds Geosmin (GSM) and 2-methylisoborneol (MIB) are non-toxic secondary metabolites of cyanobacteria and some actinomycetes (Guttman and van Rijn, 2008) that cause an earthy musty flavor that can be detected in water at concentrations as low as 10 ng L^{-1} (Drikas et al., 2009). In aquaculture systems, GSM and MIB are problematic due to their accumulation in the lipid-rich tissue that cause an off-flavor in the fish, particularly catfish, salmon and sturgeon (Howgate, 2004). The current removal approach for these compounds is to purge

them from the fish prior to harvesting, which requires large amounts of highly treated water (Burr et al., 2012). Advanced treatment technologies are required for removal of GSM and MIB from RAS. Powdered and granular adsorbents, such as activated carbon, are the most common removal technology for GSM and MIB, with varying results due to the variability in the base material, adsorption capacity of the media (Yu, et al., 2007; Cook et al., 2000) and interactions with natural organic matter (Matsui et al., 2013; Newcombe et al., 2002). However, removal of GSM and MIB to below the detection threshold requires large dosages of activated carbon (Matsui et al., 2013), resulting in high capital and operational costs (Bamuza-Pemu and Chirwa, 2012).

The overall goal of this study is to investigate the performance of a novel UPBR for RAS treatment. The Tire Sulfur Hybrid Adsorption Denitrification (T-SHAD) process was developed by Krayzelova et al. (2014) and found effective for removal of NO_3^- from wastewater. T-SHAD utilizes recycled tire mulch that serves as both a biofilm carrier as well as an adsorbent due to its high capacity for NO_3^- (Lisi et al., 2004). The combination of SOD and adsorption processes allows for consistent performance under variable loadings and concentrations (Krayzelova et al., 2014). The specific objectives of this study were to (1) determine the adsorption capacity of tire mulch for GSM and MIB, (2) assess denitrification and off-flavor compound removal performance of T-SHAD in different reactor configurations in a bench-scale RAS and (3) compare T-SHAD to heterotrophic denitrification utilizing molasses as an organic electron donor and carbon source.

4.2 Materials and Methods

4.2.1 Materials

The materials utilized in the study were previously presented by Krayzelova et al. (2014). Scrap tire mulch were obtained from Liberty Tire Recycling (Rockledge, Florida) and hand-sorted to obtain a particle size between 1.0 and 1.5 cm. The tire mulch was rinsed with de-ionized water

and dried for 4 hours at 105°C. Elemental sulfur pastilles (0.4-0.6 cm) were obtained from Martin Midstream Partners (Seneca, Illinois). Crushed oyster shells were added as an alkalinity source and were purchased from Myco Supply (Pittsburgh, Pennsylvania) and sieved to remove fines smaller than 0.6 cm.

4.2.2 Adsorption Studies

Adsorption isotherms were performed on the tire mulch to determine off-flavor adsorption capacity. The tire mulch was further pre-treated and placed in a vial with 200 mL di-ionized water for a minimum of 24 hrs at 100 rpm to reduce leachates that were interfering with off-flavor detection. Varying amounts of pre-treated tire mulch (0-12 g) were added to 50 mL amber glass vials filled with 30 mL of approximately 100 ng L^{-1} GSM and MIB solution. Kinetic studies were performed with a tire mass of 2 g and vials were sacrificed after 15, 30, 60, 120, 480 and 1440 mins. The vials were placed in a Labquake™ tube shaker rotator and samples were collected after varying contact times. Samples were stored in amber vials at 4°C and analyzed according to section 4.2.5.

Two models were fit to the data and used to predict maximum adsorption capacity. The Langmuir model follows Equation 4.3:

$$q_e = \frac{Z_T \cdot b \cdot c_e}{1 + b \cdot c_e} \qquad \text{Equation 4.3}$$

where q_e is the milliequivalents (meq) of adsorbate per grams of adsorbent, C_e is the equilibrium concentration in meq L^{-1}, b is the Langmuir adsorption constant (L meq^{-1}) and Z_T is the maximum adsorption capacity of the adsorbent (meq g^{-1}). The Freundlich model follows Equation 4.4:

$$q_e = k_L \cdot C_e^{\,1/n} \qquad \text{Equation 4.4}$$

where k_L is the Freundlich constant for adsorption capacity ((meq g^{-1})(L mg^{-1})$^{n-1}$) and 1/n is an exponent related to surface heterogeneity (dimensionless). In both cases q_e can be calculated using Equation 4.5.

$$q_e = \frac{(C_i - C_e)V}{W} \qquad \text{Equation 4.5}$$

where C_i is the initial concentration of the adsorbate (meq L^{-1}), V is the volume (L) and W is the mass of the adsorbent (g). For all models, the least squares method was used to optimize the fit using the Solver function in Excel (Brown, 2001).

4.2.3 Bench-Scale RAS

The laboratory scale RAS (Figure 4.1) was presented previously in Chapter 3. Briefly, the system was composed of a 2 L glass feed container with synthetic wastewater, a moving bed bioreactor (MBBR) and a heterotrophic UPBR. The wastewater was pumped into the system at a flow rate of 1.3 L d^{-1} using a Masterflex C/L Dual Channel Pump (Cole Palmer; Vernon Hills, IL). The synthetic wastewater consisted of a solution of 0.25 g L^{-1} NH_4Cl and 0.4 g L^{-1} $NaHCO_3$ and dissolved fish feed pellets (1.58 g L^{-1}) from the feeders at Healthy Earth's RAS in Sarasota, Florida. The main line recirculated water at a rate of 360 L d^{-1} using a Masterflex L/S peristaltic pump (Cole Palmer; Vernon Hills, IL). Two Cole-Parmer Valved Variable Area Acrylic flow meters were used for flow measurement and control into the MBBR and UPBR. The MBBR consisted of a glass bottle with a working volume of 3 L, with 55% filled with plastic media collected from the MBBR in Healthy Earth's RAS. A compressor and air diffuser (Tetra Whisper®, Blacksburg, VA) provided the DO needed for nitrification. A side-stream was connected after the MBBR where 10.8 L d^{-1} were pumped to an UPBR consisting of an acrylic column (Koflo; Cary, IL) of approximately 0.75 L with 53% of its volume filled with plastic media from Healthy Earth's UPBR. A single-syringe infusion pump (Cole Palmer; Vernon Hills, IL) was connected to the side

stream to provide molasses ($C_{12}H_{22}O_{11}$; Hamlin et al., 2009) at a flow rate of 4 μL hr^{-1}. The effluent from this column was pumped back to the mainline and recirculated to the MBBR.

4.2.4 T-SHAD Column

The T-SHAD column utilized was constructed and studied previously by Krayzelova et al. (2014). A 0.75 L acrylic column (KOFLO; Cary, IL) was packed with a mixture of tire (250 g), sulfur pastilles (40 g) and oyster shells (13 g). The T-SHAD column was tested in two operational phases within RAS. During Phase I (Figure 4.1a) the T-SHAD column served as a polishing treatment for the bench-scale RAS described in Chapter 3. The T-SHAD column was fed from a reservoir that collected both the MBBR (nitrified) and the heterotrophic UPBR (denitrified) effluent. The T-SHAD column was operated for 44 days under this configuration and treated RAS water both with and without the addition of the photocatalysis treatment described in Chapter 3, section 3.2.3). The flowrate into the T-SHAD column was 1.3 L d^{-1} with empty bed contact time (EBCT) of 12hrs. For Phase II (Figure 4.1b), the T-SHAD column replaced the heterotrophic UPBR as the denitrification side treatment in the RAS (EBCT=3.1 hrs). The addition of the external electron donor (molasses) was eliminated since it was needed. Prior to Phase II, the T-SHAD column had been in intermittent operation for close to two years, and was amended with oyster shells (13 g) due to low observed pH in the effluent.

4.2.5 Analytical Methods

Approximately 50 mL samples were collected from bench scale RAS and T-SHAD to determine water quality parameters. pH (Oakton Acorn Series pH/ion/C, Orion 5 Star ThermoScientific), Conductivity (Orion 5 Star ThermoScientific), DO (Hach HQ40d Portable, Orion 5 Star ThermoScientific) were measured using calibrated electrodes. A portion of the samples was filtered through a 0.45 μm mixed Cellulose Esters filter (FisherScientific, Waltham,

MA). Concentrations of anions (NO_2^-, NO_3, PO_4^{-3}, SO_4^{-2}) and cations (NH_4^+) were measured in the filtrate using a Metrohm 881 Compact IC Pro (Herisau, Switzerland) ion chromatography system. MDLs for NO_3^-, NO_2^-, SO_4^{2-}, PO_4^{3-} and NH_4^+ were 0.01, 0.04, 0.01, 0.02 and 0.07 mg L^{-1}, respectively. Chemical oxygen demand (COD) was measured for filtered samples with the Vario Tube Test (Loveland Co) COD LR test kits (MDL: 0-150 mg L^{-1}), according to the Standard Methods (APHA et al., 2012).

4.2.6 Trace Analyses

Off flavor compounds GSM and MIB analysis was performed by Solid Phase Micro-extraction (SPME) with Divinylbenzene/ carboxen/ polydimethylsiloxane (DVB/CAR/PDMS) fibers and GC/MS detection. Samples were prepared using a modification of method 6040D (APHA et al., 2012). Briefly, 9 g of salt, previously calcinated for 5 hours at 550°C, 30 mL sample and 3 µL of the 3-isobutyl-2-methoxypyrazine (IBMP) solution where added to a 45 mL glass vial containing a PTFE stirrer and PTFE septa cap. The vial was placed in a water bath with temperature range of 60-65°C to ensure volatilization of the desired compounds. The fiber was injected into the vial with 0.8 cm exposed to the headspace for 30 minutes. After retraction the fiber was fully exposed in the GC/MS injector for 3 minutes. Samples were diluted when necessary to ensure readings within the detection range of 1ng L^{-1} to 40 ng L^{-1}. Stock solutions of 100 mg L^{-1} of the off-flavor compounds and IBMP were purchased from Sigma Aldrich (St. Louis, MO). The IBMP stock was diluted in methanol to achieve a 1µg L^{-1} solution that was utilized as an internal standard. Compound separation and detection was performed using a Perkin Elmer (Waltham, MS) Clarus 580 GC/MS equipped with a 30 m x 0.25 mm x 0.25 µm HP-5MS Agilent column (Santa Clara, CA). The injector temperature was set to 250°C for the GC method. The initial oven temperature was set at 50°C, with a ramp of 15°C min^{-1} and a maximum temperature of 280°. For the first 2

minutes split-less mode was activated, and for the remaining 15 minutes the split was set at 50 mL min^{-1} He with 1 mL min^{-1} carrier flow. The MS method used a solvent delay of 3 minutes and selected ion recording for 124/151 (IBMP), 95/107/135 (MIB) and 112/116 m/z (GSM).

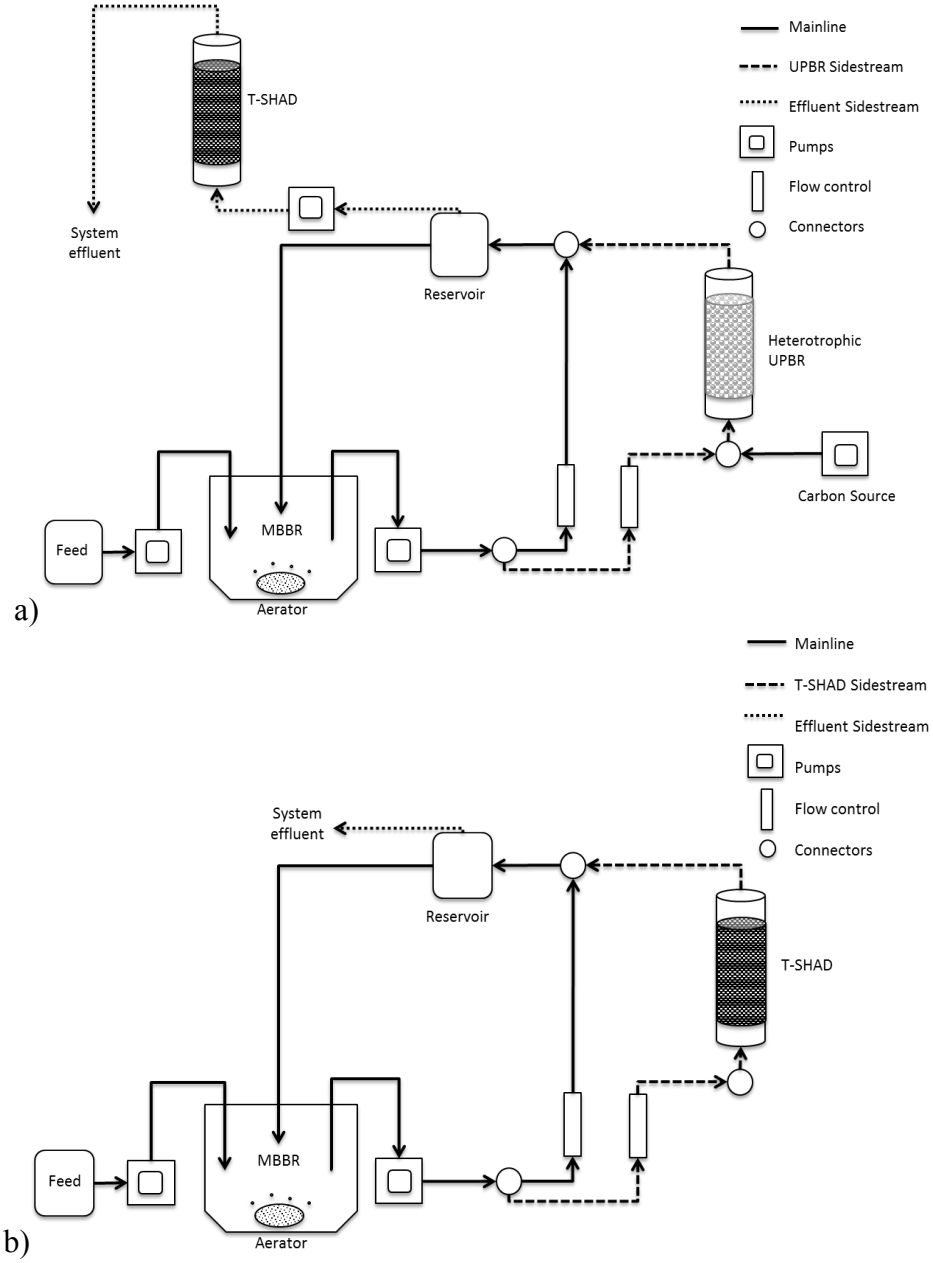


Figure 4.1: Bench scale RAS with T-SHAD for effluent NO_3^- polishing (a) and T-SHAD as denitrification side stream treatment (b). Mainline and side stream flows are showed in different line styles (Not to scale).

4.2.7 Statistical Analysis

Statistical analyses were performed to evaluate the performance of the T-SHAD column. A paired t-test assuming equal variances was performed in Excel 2011 for the concentrations and removal rates of GSM, MIB and N species results from both phases with α=0.05.

4.3 Results

4.3.1 Off-Flavor Compound Adsorption

The results for the tire adsorption isotherms, kinetics and Freundlich and Langmuir isotherm model fits to the average data are shown in Figure 4.2. High GSM removal was achieved with tire doses above 2 g (Figure 4.2a). From the Langmuir model (Figure 4.2c), maximum adsorption capacity for tire mulch is 2982 and 27.106 ng g^{-1} for GSM and MIB, respectively. The model provided a good fit to the data, with R^2 values of 1.0 and 0.98 for GSM and MIB, respectively. The Freundlich model (Figure 4.2d) also provided a good fit, with R^2 values of 0.99 for GSM and 0.98 for MIB. Adsorption kinetic results are shown in Figure 4.2b. For GSM, 94% of the removal occurred within 8 hours of contact time while only 76% of MIB removal was observed even after 24 hrs.

4.3.2 T-SHAD Application in RAS

4.3.2.1 Phase I-T-SHAD Effluent Polish

Water quality results for all treatment units within the bench-scale RAS are shown in Table 4.1. pH in the system remained neutral and showed no significant difference when compared to the influent. As expected, DO was significantly lower after the T-SHAD, due to anoxic conditions that developed in the reactor. NH_4^+-N concentrations were on average 0.19±0.09 mg L^{-1} in the influent of Phase I and increased significantly in the effluent of T-SHAD to 0.85± 0.57 mg L^{-1}. No

significant differences were observed between the influent and effluent of T-SHAD for NO_2^--N, with concentrations at approximately 0.32 ±0.26 mg L^{-1}.

NO_3^--N concentrations were significantly different, with T-SHAD removing on average 97% of NO_3^--N in the systems effluent. No significant differences were observed for PO_4^{3-}-P concentrations in any of the phases, whereas SO_4^{2-} increased significantly in the effluent of T-SHAD. On average, NO_3^--N removal rates for Phase I were 51.93 ±9.03 mg NO_3^--N $L^{-1}d^{-1}$ with a production of 133.77 ±138.14 mg SO_4^2 $L^{-1}d^{-1}$.

4.3.2.2 Phase II-T-SHAD Denitrification Side-Treatment

Water quality results for Phase II for all treatment units within the bench-scale RAS are also shown in Table 4.1. Similar to Phase I, pH in the system remained neutral and showed no significant difference when compared to the influent. DO was significantly lower in the T-SHAD column effluent were higher than Phase I possibly due to the high DO concentrations from the MBBR and lower EBCT. Levels of NH_4^+-N and NO_2^--N were higher during Phase II when compared to Phase I, but overall no significant difference was observed from the influent and concentrations in the RAS remained low. On average, only 21% of NO_3^--N was removed in T-SHAD for Phase II. No significant differences were observed for PO_4^{3-}-P but significantly higher concentrations of SO_4^2 were observed in the T-SHAD effluent.

Removal and production rates for T-SHAD during Phase II are also shown in Table 4.2. The production rates during this phase were compared to those of the heterotrophic UPBR in Phase I due to similar operating conditions within the RAS. In both T-SHAD and the heterotrophic UPBR some production of COD was observed, however, due to high variability this was not significantly different from the influent. The variability in the effluent COD concentrations of the UPBR could be influenced by overload of the carbon source (molasses) as can often occur in heterotrophic

denitrification reactors (Metcalf and Eddy, 1979). The high variability of COD in T-SHAD Phase II was possibly influenced by the high COD released when the column was repacked with oyster shells, as some biomass was removed. Eliminating this data point results in a production rate of only 2.56 ±88, similar, albeit much more variable, to the effluent of the T-SHAD column in Phase I. The average NO_3^--N removal rate for this phase was 96.26 ±124.48 mg $L^{-1}d^{-1}$, significantly higher and more variable than the NO_3^--N removal rate during Phase I (Table 4.3).

Table 4.1: Average water quality results for the influent and T-SHAD for Phase I and Phase II.

	Phase I-Effluent polish (EBCT=12 hrs)			Phase II-Side treatment (EBCT=3.08 hrs)		
	RAS FEED	INFLUENT (MBBR+H-UPBR)	T-SHAD	RAS FEED	MBBR	T-SHAD
pH	7.45 ±0.34	7.37 ±0.22	**6.24 ±0.97**	7.64 ±0.28	6.04 ±0.48	6.14 ±0.55
DO (mg L^{-1})	0.82 ±1.15	4.10 ±1.07	**1.21 ±0.40**	1.97 ±2.10	5.31 ±0.41	**2.46 ±1.29**
COD (mg L^{-1})	114 ±94.3	32.94 ±17.37	34.55 ±16.52	93.50 ±67.61	51.50 ±32.67	67.50 ±52.00
NH_4^+-N (mg L^{-1})	60.72 ±16.72	0.19 ±0.09	**0.85 ±0.57**	47.34 ±18.76	0.31 ±0.85	1.51 ±2.75
NO_2^-N (mg L^{-1})	0.76 ±0.41	0.29 ±0.22	0.28 ±0.31	0.37 ±0.35	0.32 ±0.26	0.78 ±1.07
NO_3^-N (mg L^{-1})	0	30.96 ±6.81	**1.38 ±2.50**	0.01 ±0.02	56.96 ±7.07	**44.42 ±16.01**
PO_4^{3-} P (mg L^{-1})	0	4.96 ±1.67	4.25 ±1.25	0.62 ±1.00	4.68 ±1.74	4.73 ±1.89
SO_4^{2-} (mg L^{-1})	54.10 ±25.86	188.32 ±119.20	**265.49 ±106.40**	15.85 ±10.34	206.45 ±134.31	**271.67 ±172.49**
GSM (ng L^{-1})	-	26.70 ±19.97	5.45 ±1.78	-	5.13 ±4.95	6.34 ±2.02
MIB (ng L^{-1})	-	28.22 ±13.86	21.84 ±6.22	-	9.91 ±9.48	9.40 ±5.06

*Values in bold represent significant difference compared to the influent

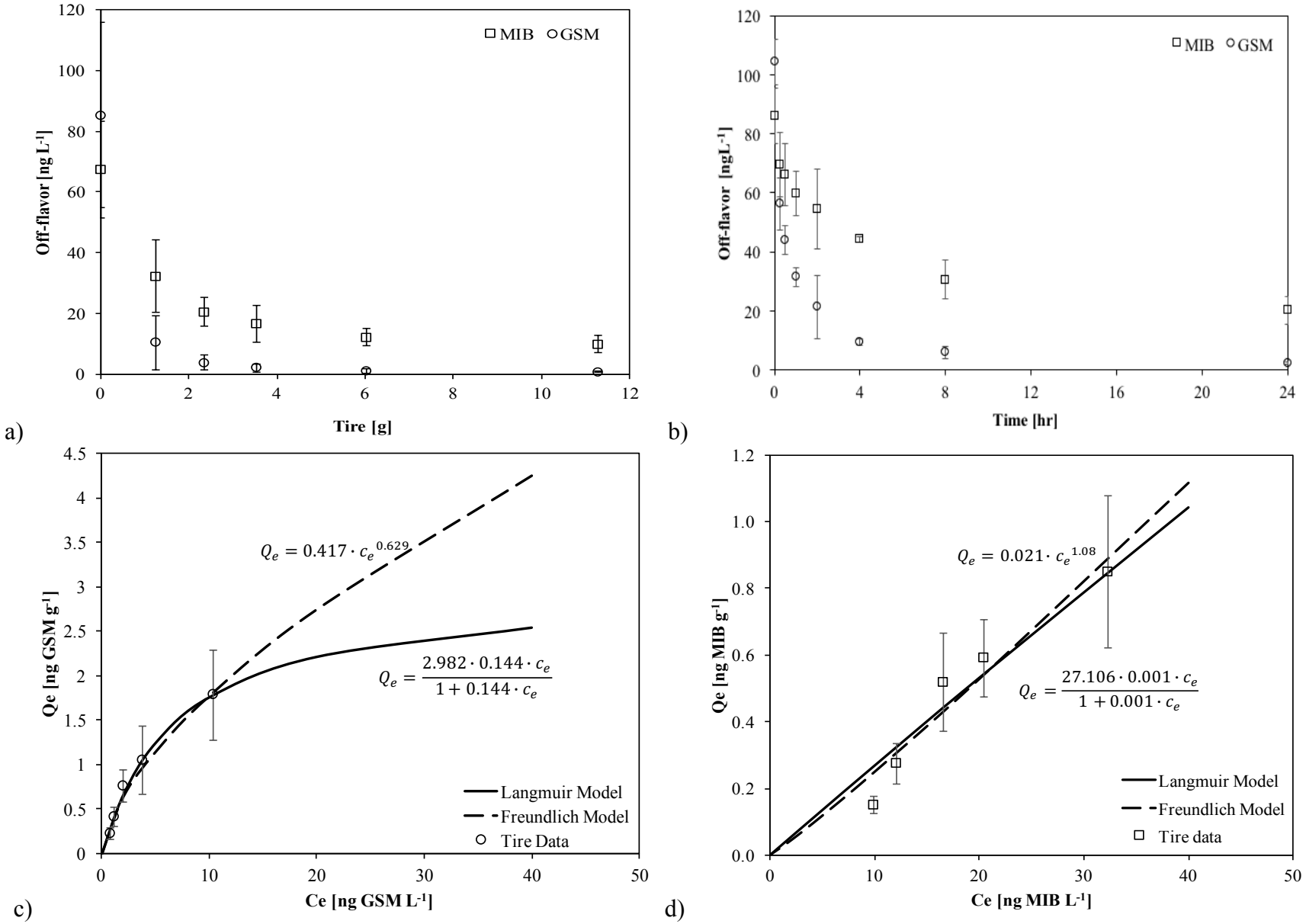


Figure 4.2: Off-flavor adsorption isotherm (a), kinetics (b) and models for GSM (c) and MIB (d).

Table 4.2: Removal and production rates (+) for T-SHAD and H-UPBR in Phase I and T-SHAD in Phase II.

	T-SHAD (EBCT=12 hrs)	T-SHAD (EBCT=3.08 hrs)	H-DENIT UPBR (EBCT=3.08 hrs)
COD ($mg\ L^{-1}d^{-1}$)	(+)2.55 ±16.29	(+)138.24 ±367.89	(+)148.89 ±282.94
NH_4^+-N ($mg\ L^{-1}d^{-1}$)	**(+)1.14 ±1.02**	(+)12.26 ±24.26	**(+)16.64 ±19.36**
NO_2^-N ($mg\ L^{-1}d^{-1}$)	(+)0.02 ±0.51	(+)3.47 ±9.77	**(+)3.62 ±5.47**
NO_3^-N ($mg\ L^{-1}d^{-1}$)	**51.93 ±9.03**	**96.26 ±124.48**	**138.16 ±112.42**
PO_4^{3-} P ($mg\ L^{-1}d^{-1}$)	1.23 ±2.79	(+)0.43 ±13.68	(+)9.71 ±18.06
SO_4^{2-} ($mg\ L^{-1}d^{-1}$)	**(+)133.77 ±138.14**	**(+)500.91 ±0.49**	45.15 ±264.33
MIB ($ng\ L^{-1}d^{-1}$)	15.80 ±25.35	3.83 ±54.02	(+)63.29 ±165.03
GSM ($ng\ L^{-1}d^{-1}$)	**38.03 ±34.44**	(+)8.55 ±47.11	(+)21.16 ±149.54

*Values in bold represent significant difference compared to the influent

*Removal rate calculated by: $\frac{(Influent-Effluent)\cdot Q}{V}$

The high variability of NO_3^--N removal rate in Phase II corresponded with higher NO_3^--N concentrations in the influent as well as higher and more variable DO in the effluent. These factors could have influenced denitrification within T-SHAD. No significant difference was observed in the removal or production of N species and PO_4^{3-}-P between the heterotrophic UPBR and T-SHAD in Phase II. On the other hand, SO_4^{2-} concentrations were significantly higher for T-SHAD than the heterotrophic UPBR as it is produced during SOD.

The results for off-flavor compounds in the influent (MBBR) and effluent of the T-SHAD during Phase II are shown Figure 4.3. GSM and MIB concentrations in the RAS system climbed steadily to around 10 and 30 ng L^{-1}, respectively, within 7 days of startup. Also during this time,

lower GSM and MIB were observed for the T-SHAD effluent. The lower effluent T-SHAD concentrations possibly influenced concentrations in the RAS and after 7 days of installation, the concentrations started to decrease to 5 ng L^{-1} and remained at those levels until the end of the experiment. However, average GSM and MIB concentrations for the whole study period showed no significant differences from the influent.

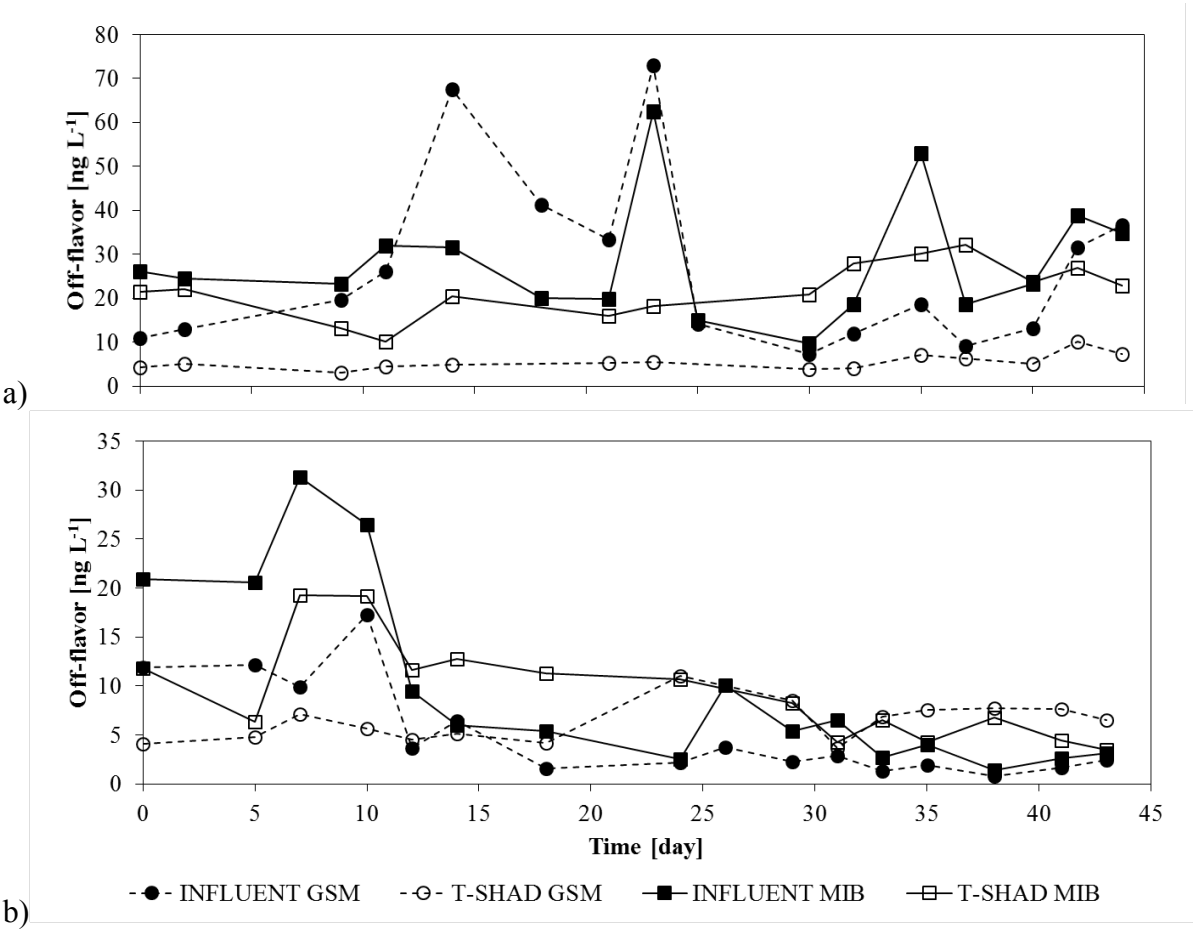


Figure 4.3: Off-flavor compound concentrations vs time in T-SHAD effluent polish (a) and T-SHAD side stream (b).

4.4 Discussion

4.4.1 Adsorption of Off-Flavor Compounds

Adsorption of GSM and MIB is often achieved with powdered or granular activated carbon (Matsui et al., 2012; Summers et al., 2013; Elhadi et al., 2006). The adsorption capacity of the

carbons is very variable and dependent on the base material of the carbon, surface area, dose, contact time and presence of competing adsorbates, such as dissolved organic matter (Matsui et al., 2013; Elhadi et al., 2006, Cook et al., 2000). The results of the adsorption isotherms for GSM and MIB with the tire mulch are promising. A dose of approximately 66.7 g L^{-1} achieved removal of 94% and 64% of GSM and MIB, respectively within 480 hours of contact time. From the Langmuir model, maximum adsorption capacity for GSM and MIB was 3.42 and 10.13 ng g^{-1} tire (Figure 4.2a and 4.2b). Although the model showed a good fit to the data for MIB but was not able to capture the significant removal achieved with 1 g of tire as shown in Figure 4.2a. The Freundlich model provided a better fit with K and n^{-1} constants of 0.379 and 0.674 for GSM and 0.016 and 1.16 for MIB. The preferential removal of GSM over MIB has also been seen in studies with powdered activated carbon (Lalezary et al., 1986) due to it higher hydrophobicity.

Although no other studies have looked at the adsorption of off-flavor compounds on tire mulch, Kelly et al., (2006) investigated the reduction of off-flavor compounds with natural rubber and found removals of 19.4 % and 30.1% for GSM and MIB, respectively, with a dose of 1.43 g L^{-1} and an 8 hr contact time. Normalizing the removal efficiency by dose results in ten times as much removal in the natural rubber when compared to the recycled tire mulch utilized in this study. In comparison, a dose of 20 mg L^{-1} of powdered activated carbon removed over 90% of GSM and MIB with a contact time of 3 hrs (Lalezary et al., 1986). It is important to note that the same tire mulch utilized in the column experiments, with sizes varying from 1-1.5 cm, was used for the adsorption isotherm. This variation in size, as well as the presence of fibers within the tire pieces, could have caused the high variations observed (Figure 4.2) and possibly affected removal performance. Studies with powdered and granular activated carbons have shown enhanced

treatment with decreasing particle size (Matsui et al., 2013). The impact of the fibers on adsorption capacity was not investigated in this study.

4.4.2 T-SHAD in RAS

4.4.2.1 Effect on Water Quality and N Removal

The removal of N species is in RAS essential to ensure fish health in high density systems. Treatment systems within RAS would usually include a solids removal process and aerobic treatment to oxidize NH_4^+-N and subsequently NO_2^--N in the water. These processes result in high NO_3^--N concentrations that must be addressed. The control of other system parameters, such as pH and DO, are crucial for the removal of N species. In this study, a novel hybrid treatment process combining commercially available tire mulch, sulfur pellets and oyster shells was used to remove NO_3^--N and off-flavor compounds, GSM and MIB, in RAS. The T-SHAD column effects on the water quality were minimal (Table 4.1). The slight decrease in pH occurring at Phase I (EBCT = 400 min) is not unexpected due to the H^+ producing nature of the SOD process when compared to other heterotrophic denitrification processes (Park and Yoo, 2009; Oh et al., 2001). The prior use of the T-SHAD column for synthetic wastewater treatment (Krayzelova et al., 2014) and the intermittent use thereafter within RAS could have also influenced pH and alkalinity as noted before the start of Phase II (not shown) when the column was repacked with fresh oyster shells. The addition of the fresh oyster shells caused slight increases in the pH of the RAS in Phase II, making up for the acidic conditions before the startup. Regardless of the effluent pH of the T-SHAD column, the pH of the bench scale RAS was within the optimum pH for sulfur-oxidizing bacteria (6-8), *Thiobacillus denitrificans* as suggested by Holt et al. (1994).

DO concentrations in the effluent of T-SHAD were relatively high, particularly for an anaerobic process. This high DO concentration probably affected denitrification performance.

Moreover, aerobic elemental sulfur oxidation may have occurred resulting in increased SO_4^{2-} in the effluent of T-SHAD. This phenomenon is discussed later together with SO_4^{2-}-S productivity. No significant difference were observed in COD in the influent and effluent of the T-SHAD column showed in both phases. This indicates SOD was the predominant NO_3^--N removal process in T-SHAD. The previous study with T-SHAD (Krayzelova et al., 2014) indicated high COD leaching by the tires that can be utilized as the electron donor in heterotrophic denitrification. The study also showed this COD leaching decreased considerably with time under flow through conditions. Because of the long and intermittent use of the T-SHAD column, COD leaching from the tire is expected to be insignificant.

The slight increase in NH_4^+-N in both phases was unexpected since according to Eq. 2, NH_4^+ is consumed in the SOD process. This increase could potentially be attributed to dissimilatory NO_3^- reduction to NH_4^+. In contrast, a similar study that employed SOD in a UPBR with sulfur granules, sand and oyster shells showed significant removal of 7 mg L^{-1} NH_4^+-N (Tong et al., 2016). Regardless of this significant increase in T-SHAD effluent, NH_4^+-N concentrations in the RAS remained below the 1mg L^{-1} level recommended by Timmons et al., (2002). NO_2^--N remained low for RAS and T-SHAD during both phases and close to the recommended threshold of 0.3 mg L^{-1} (Timmons et al., 2002; Hamlin et al., 2008). NO_3^--N removal rates seemed to have increased with decreasing contact time, the opposite is true for removal efficiency (Table 4.1) when it dropped from 96% to 22% in Phase I and II respectively. The increase in NO_3^--N removal rates corresponded to an increase in SO_4^{2-}-S concentrations and productivity in the effluent of T-SHAD. Productivity for Phase I was approximately 0.86 mg SO_4^{2-}-S mg^{-1} NO_3^--N while it considerably increased to 1.74 during Phase II. For Phase II, the productivity was higher than the stoichiometric relationship shown in Equation 4.2. This phenomenon has been encountered in prior

SOD studies and has been mostly attributed to the aerobic oxidation of elemental sulfur (Tong et al., 2016; Boles et al., 2012).

The high productivity of SO_4^{2-}-S in T-SHAD presents concerns due to possible accumulation in low exchange RAS. Although SO_4^{2-} concentrations were as high as 600 mg L^{-1} at the beginning of the study they tapered down to approximately 110 mg L^{-1} at the end of the study. This decrease was influenced by the variability of DO, NO_3^--N removal as well as the dilution by the low SO_4^{2-} RAS feed supplied at 1.3 L d^{-1}. Another concern with T-SHAD would be the possibility of heavy metal leachates that could affect fish health. In the T-SHAD study by Krayzelova et al. (2014) metal concentrations in the leachate after 72 hours of treatment were 0.82, 0.11, 0.013, 0.064 and 0.021 mg L^{-1} of Zn, Se, Sb, Mn and Co, respectively. Other metals, such as Pb, Fe and As were below detection limits (Krayzelova et al., 2014). The release of Zn from the tires could be of concern due to high toxicity to some aquatic species (Skidmore, 1964). Zn concentration was not measured in this study but it was expected to be lower than 0.82 mg L^{-1} due to the length of operation of the T-SHAD column.

Table 4.3 summarizes the data from this study and select studies comparable to the T-SHAD column. NO_3^--N removal rates for T-SHAD were on the lower range for both SOD and heterotrophic denitrification applications. This low removal can be attributed to the significantly higher DO concentrations in this study, particularly for Phase II. The acidic conditions prior to Phase II as well as the repacking of the column with oyster shells may have also caused loss of denitrifying biomass decreasing NO_3^--N removal rates. The comparison between removal rates of the T-SHAD column in Phase II and the heterotrophic UPBR showed few significant differences. Significantly lower pH in T-SHAD versus the heterotrophic UPBR is easily explained by Eq. 4.1 and 4.2, where H^+ is consumed in the first and produced in the second. The significantly higher

SO_4^{2-} production was also expected from Eq. 2. The effluent of the heterotrophic UPBR showed significant increases in NH_4^+-N and NO_2^--N when compared to the influent showing incomplete denitrification possibly due to DO concentrations and dissimilatory NO_3^- reduction to NH_4^+. Increased NO_2^--N concentrations were also observed in the study by Sailing et al., (2007) but with much higher NO_3^--N removal rates of 1340 mg $L^{-1}d^{-1}$.

4.4.2.2 Off-Flavor Compound Removal

GSM and MIB are a hindrance in RAS systems due to their presence at trace concentrations and their accumulation in the lipid tissue of fish (Howgate, 2004) resulting in off-flavor. The removal of these compounds is often complex and expensive, requiring advanced treatment technologies (Ho et al., 2012) not within RAS. Studies have found production of GSM and MIB under the high organic and aerobic conditions that are common in RAS (Guttman and van Rijn, 2009; Schrader and Summerfelt, 2010). Biological removal of these compounds has been attributed to anaerobic sludge chemical and physical uptake where 93% and 79% removal of GSM and MIB, respectively, was achieved in 12 day batch tests with sludge from a mariculture digestion basin (Guttman and van Rijn, 2009). In this study, significant removal of GSM (79.6%) was achieved during Phase I (Table 4.1, Figure 4.2a) with EBCT of 720 min in low DO conditions, while no significant removal of MIB was observed. Little variation in GSM concentrations were also observed in the effluent of the T-SHAD during this phase.

The application of T-SHAD in the bench scale RAS resulted in a significant decrease of the EBCT (185 min) and no significant differences in the concentrations of the off-flavor compounds. Similar results were observed in the heterotrophic UPBR installed in the bench-scale RAS during Phase I. This result is also supported by the findings of Hamlin et al., (2008) were no significant difference of GSM and MIB was observed in the effluent of heterotrophic UPBRs with

varying carbon sources. The high removal rates of T-SHAD under high EBCT possibly indicate adsorption to the tire as seen in the adsorption isotherm results. The EBCT of 720 min in Phase I should have resulted in higher removal of GSM and MIB than that seen in the adsorption studies due to higher dosing of the tire (333 g L^{-1}). The decreased removal of GSM and MIB during Phase I in T-SHAD could have been attributed to effects of competing dissolved organic matter as observed in studies with powder and granular activated carbon by Summers et al., (2015); Newcombe et al. (2002) and Chen et al. (1997). This could have also had an effect on off-flavor removal in T-SHAD during Phase II when the EBCT was reduced to 185 min. Other factors, such as temperature changes, hydraulic loading rates and the transient nature of the compounds could have also affected removal of the off-flavor compounds (Elhadi et al., 2006) in the bench-scale RAS.

4.5 Conclusions

A novel UPBR employing Tire Sulfur Hybrid Adsorption Denitrification (T-SHAD) was applied in a bench-scale RAS for the removal of NO_3^--N and off flavor compounds, GSM and MIB. When applied as a polishing step and operated under high EBCT (720 min) removal of 96.6% of NO_3^--N and 69.6% of GSM was achieved with no significant removal of MIB. The application of T-SHAD within RAS as denitrification side treatment for NO_3^--N removal at a lower EBCT (185 min) resulted in limited NO_3^--N removal (21%) and showed no significant difference for off-flavor compounds. The results for T-SHAD within RAS under 185 min EBCT were not significantly different than a RAS with a molasses fed heterotrophic UPBR, with the exception of high productivity of SO_4^{2-} that resulted from SOD processes. Adsorption studies showed the tire has significant adsorption capacity for the off-flavor compounds but can be limited by EBCT and, possibly, the presence of competing organic matter in RAS. Further studies are required to assess

toxicity of the tire mulch to aquatic species for the successful application of T-SHAD in full scale RAS.

Table 4.3: Summary of selected studies of SOD and off-flavor compound removal technologies.

Influent NO_3^--N (mg L^{-1})	Reactor	Media	Application	Volume (L)	EBCT (min)	NO_3^--N removal (mg L^{-1} d^{-1})	SO_4^{2-}-S Productivity(m g mg^{-1} NO_3^--N)	Off-flavor removal	Study
30	UPBR	Tire,S, oyster shells	Bench-scale RAS	0.75	720	51.9	0.86	69.6 % GSM NSD MIB	This study Phase I
50	UPBR	Tire, S, oyster shells	Bench-scale RAS	0.75	185	96.3	1.74	NSD	This study Phase II
50	UPBR	Tire, S, oyster shells	Bench-scale synthetic wastewater	0.75	375	177	1	NA	Krayzelova et al. (2014)
7.6-17	FBR	S grains	Full-scale RAS	206	3.2-4.8	800	NSD	NA	Christianson et al. (2015)
14.42	FBR	Sand	RAS	285	15	270	NSD	NA	Tsukuda et al., (2015)
50	UPBR	S, oyster shell, sand	Bench-scale synthetic wastewater	0.75	210	158.4	1.66	NA	Tong et al., (2016)
30	UPBR	Plastic carriers, molasses	Bench-scale RAS	0.75	100	138.2	NSD	NSD	This study Phase I
28	UPBR	S granules	RAS seawater	30	640	48	3.1	NA	Simard et al., (2015)
55	UPBR	Plastic carriers, molasses	RAS	1000	185	670	NA	NSD	Hamlin et al., (2008)
NA	Aerobic PBR	Sand	Bench-scale lake water	0.6	173	NA	NA	93.3% GSM 63.7% MIB	Hsieh et al., (2010)

*NA-Not applicable

*NSD-No significant difference

*Removal rate calculated by: $\frac{(Influent - Effluent) \cdot Q}{V}$

CHAPTER 5: PASSIVE ENHANCEMENT OF ONSITE WASTEWATER TREATMENT FOR TOTAL INORGANIC NITROGEN REMOVAL WITH HYBRID ADSORPTION AND BIOLOGICAL TREATMENT SYSTEMS (HABiTS)

5.1 Introduction

Centralized wastewater treatment involves collecting and conveying sewage from individual residences to large wastewater treatment systems. However, in many rural and suburban areas, where there are low population densities and large distances between households, the cost of centralized treatment is prohibitive. Onsite wastewater treatment systems (OWTS) are an alternative to centralized systems that have been used for centuries worldwide. In the United States, for example, OWTS treat close to a third of the wastewater produced (USEPA, 2002). Similar numbers are reported for other developed countries, such as France (20%), and OWTS are even more prevalent in developing countries (Petitjean et al., 2016; Libralato et al., 2012). The cost effectiveness of OWTS is attributed to their simple operation, low energy and maintenance requirements and little to no chemical use (USEPA, 1999). Regardless of these benefits, there are still major challenges in OWT application, including limitations due to high water table elevations and proximity to drinking water supplies and environmentally sensitive areas (FDOH, 2013) with stringent regulations. In particular, the limited nitrogen (N) removal in conventional OWTS (USEPA, 1999) can result in nutrient contamination of ground and surface water (Liu et al, 2009). Furthermore, variable water usage and long idle times (e.g. during vacations) result in highly variable loading rates, which can negatively affect biological treatment process within OWTS (USEPA, 2002).

Conventional OWTS consist of a septic tank for primary treatment and solids separation and a soil infiltration system for additional biological treatment and pathogen removal. A number of technologies have been developed to provide enhanced treatment in OWTS. Moussavi et al. (2010) investigated an upflow septic tank as opposed to the conventional horizontal flow system. Improved removal of total suspended solids (TSS) and chemical oxygen demand (COD) was observed at retention times as low as 24 hours. However, these modifications had little effect on N removal. Oh et al. (2014) studied the use of recycled rubber particles as filter media for the treatment of septic tank effluent and observed 93% removal of TSS and 90% removal of NH_4^+-N. Chang et al. (2010) studied a modification of the conventional drainfield design that included a vertical flow area for nitrification and a horizontal flow area with a combination of sand, tire crumbs and sawdust for denitrification. Greater removal of total N (TN) was observed in the modified drainfield (70%) compared with a conventional drainfield (50%). Passive N removal systems that are incorporated into the conventional OWTS treatment train have also been studied. Conventional nitrifying biofilters with sand media have been shown to improve TSS and TKN removal (Anderson et al., 1998, USEPA, 2002). Passive aeration through the biofilter has been shown to provide sufficient dissolved oxygen (DO) for nitrification; however, transient loadings can result in variable N concentrations in the effluent (Petitjean et al., 2016).

This study investigated a Hybrid Adsorption and Biological Treatment System (HABiTS), which is a modification of passive N removal OWTS (Figure 5.1). HABiTS employs a combination of ion exchange (IX) and biological N removal (BNR) to enhance the performance of OWTS. HABiTS has the potential to overcome variable loading rates by adsorbing N loads in excess of the system biodegradation capacity during high loading rate periods. During low loading

rate periods, N-containing ions (NH_4^+ or NO_3^-) are desorbed and can be subsequently utilized by the microbial population.

Zeolite materials have the ability to adsorb positively charged ions, such as NH_4^+ (Wen et al., 2006; Rodriguez-Gonzalez et al. 2015; Smith & Smith, 2015). Lahav and Green (1999) used zeolite as an adsorbent in a two-stage nitrification system, where adsorption of NH_4^+ took place in one stage and bioregeneration of NH_4^+ laden regenerant brine took place in a second stage. In a prior study in our laboratory, the zeolite material, chabazite, was added to a sequencing batch reactor (Zeo-SBR) treating high NH_4^+ strength wastewater from anaerobic digestion of swine manure (Aponte-Morales et al., 2016). Addition of chabazite reduced the inhibition of free ammonia to nitrifying bacteria and allowed for complete N removal with the addition of an electron donor during the denitrification stage. Clinoptilolite, a lower cost zeolite material, has also been studied for the enhancement of N removal in passive N removing OWTS (Hirst et al. 2013; Rodriguez-Gonzalez et al., 2015; Smith & Smith, 2015); however, no prior studies have compared HABiTS with conventional packed bed nitrification reactors under transient loading conditions.

It has been shown that tire mulch has the ability to remove NO_3^--N from water (Lisi et al., 2004). In prior research, Krayzelova et al. (2014) demonstrated the HABiTS concept using scrap tire chips as an NO_3^- IX medium in sulfur oxidizing denitrification (SOD) bioreactors. Batch studies with the tire show a maximum adsorption capacity of 0.66 mg NO_3^--N g^{-1} tire but adsorption kinetics were slow and only 80% of the initial NO_3^--N was removed within 30 days with a 200 g L^{-1} dose. Combining the tire and SOD in column experiments resulted in improved NO_3^--N removal under transient loadings. Furthermore, the tire mulch was found to leach carbon that can be utilized as an electron donor for denitrification. The combination of heterotrophic denitrification and SOD, or mixotrophic denitrification, has potential benefits of COD removal,

reduced SO_4^{2-} production, and reduced requirements for external alkalinity source for SOD (Oh et al., 2001; Sengupta et al., 2007; Sahinkaya and Dursun, 2012; Krayzelova et al., 2014).

Parting from the studies by Hirst et al. (2013) and Krayzelova et al. (2014), this study's innovation is the application of a two-stage HABiTS for the removal of total inorganic N (TIN) from transient loads of OWTS. The specific objectives of this work include (1) determine NH_4^+ adsorption capacity, hydraulic properties, cost and availability of various IX media for application in HABiTS, (2) compare the performance of HABiTS enhanced OWTS with nitrification/denitrification biofilters without an adsorptive medium under transient loading conditions, and (3) compare the hourly performance of HABiTS with nitrification/denitrification biofilters without an adsorptive medium under transient loading conditions.

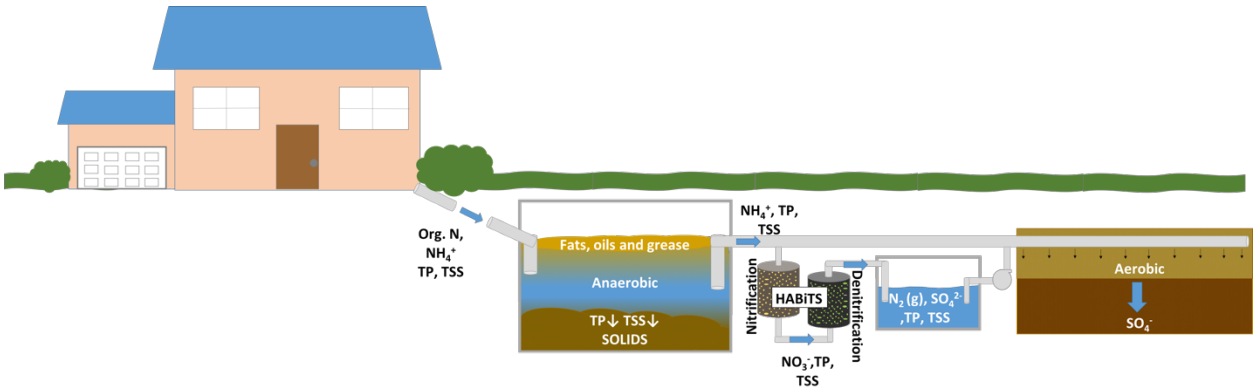


Figure 5.1: HABiTS enhanced OWTS

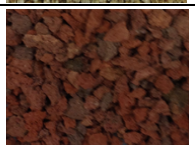
5.2 Materials and Methods

5.2.1 Abiotic Batch Adsorption Studies

Batch NH_4^+ adsorption studies for Stage 1 HABiTS nitrification were performed on a variety of materials utilized in OWTS and BNR applications (Table 5.1) using USEPA protocols (USEPA, 1992). Flasks were filled with 100 mL of synthetic septic tank effluent and 2 g of media and set in a shaker at 100 rpm at room temperature (22-25°C). The synthetic septic tank effluent simulated cation and anion concentrations based on Hirst et al. (2013) and was prepared with

NH_4Cl 0.16 g L^{-1}, NH_4SO_4 0.235 g L^{-1}, $MgCl_2$ 0.142 g L^{-1}, $KHCO_3$ 0.013 g L^{-1}, Na_2CO_3 0.15 g L^{-1}, $CaCO_3$ 0.132 g L^{-1}, KH_2PO_4 0.28 g L^{-1} and 15 mL of 0.2 N H_2SO_4 to achieve a pH of about 6.7. The adsorption studies were performed in triplicate and samples were collected after 24 hrs. The best performing medium was subsequently utilized for adsorption isotherm and kinetic studies with an NH_4Cl solution and synthetic septic tank effluent with approximately 100 mg L^{-1} NH_4^+-N. Adsorption studies on the selected material were performed with and without presence of competing cations. The kinetic studies were performed with 2g of the selected material and the synthetic septic tank effluent solution described previously.

Table 5.1: Characterization of media used for initial batch reactor experiments

Media	Particle size (mm)	Surface area (m^2 g^{-1})	Provider	Cost ($ m^{-3})	Material pictures
Sand	1-2	0.1*	Seffner Rock & Gravel	23.92	
Vermiculite	1-2	0.901	Therm-O-Rock West Inc., Chandler, AZ.	374.64	
Lava rock	1-2	5.863	Vergolo	275	
Expanded Clay	1-2	0.662	Big River, Alpharetta, GA.	46.79	
Clinoptilolite	1-2	40*	Zeox Mineral Materials Corp, Cortaro, AZ.	392.86	
Extruded Plastic Media	14.5	>252.7	Pentaire Aquatic Ecosystems, Apopka, FL.	1,255	

*Value from similar product (NV-Na Ash Meadows Clinoptilolite) offered from St. Cloud Mining.

Three models were fit to the isotherm data and used to predict maximum adsorption capacity. The Langmuir model follows Equation 5.1:

$$q_e = \frac{Z_T \cdot b \cdot c_e}{1 + b \cdot c_e} \quad \text{Equation 5.1}$$

where q_e is the milliequivalents (meq) of adsorbate per grams of adsorbent, C_e is the equilibrium concentration in meq L^{-1}, b is the Langmuir adsorption constant (L meq^{-1}) and Z_T is the maximum adsorption capacity of the adsorbent (meq g^{-1}). The Freundlich model follows Equation 5.2:

$$q_e = k_L \cdot C_e^{\,1/n} \quad \text{Equation 5.2}$$

where k_L is the Freundlich constant for adsorption capacity ((meq g^{-1})(L $mg^{-1})^{n-1}$) and 1/n is an exponent related to surface heterogeneity (dimensionless). In both cases q_e can be calculated using Equation 3.

$$q_e = \frac{(C_i - C_e)V}{W} \quad \text{Equation 5.3}$$

where C_i is the initial concentration of the adsorbate (meq L^{-1}), V is the liquid volume (L) and W is the mass of the adsorbent (g). Finally, the IX model follows Equation 5.4:

$$q_e = \frac{Z_T \cdot k \cdot C_e}{C_x + k \cdot C_e} \quad \text{Equation 5.4}$$

where q_e is expressed in meq g^{-1}, C_e is the liquid phase equilibrium concentration (meq L^{-1}), k is the ion exchange affinity (dimensionless), C_x is the concentration of the exchanged ion (meq L^{-1}) and Z_T is the adsorption capacity of the adsorbent (meq g^{-1}). To simplify the equation, C_x can be related to C_e as described in Equation 5.5:

$$C_x = C_{X_i} + C_{e_i} - C_e \quad \text{Equation 5.5}$$

where C_{xi} and C_{ei} are the initial concentrations for the exchanged ion and the equilibrium concentration of the target ion (m_{eq} L^{-1}). For all models, the least squares method was used to optimize the fit using the Solver function in Excel (Brown, 2001).

5.2.2 HABiTS and Control Biofilter Studies

5.2.2.1 Materials

Zeolitic material clinoptilolite was acquired from Zeox Mineral Materials Corp (Cortaro, AZ) and sieved to desired particle size range (1-2.38mm). Expanded clay was obtained from Trinity Lightweight (Riverlite, Big River, Alpharetta, GA) and sieved to desired particle size range (1-2.38mm). Both materials were pre-treated prior to use. Pre-treatment consisted of 15 min deionized (DI) water rinses and 24 hr drying at 105°C ± 3. Scrap tire mulch was obtained from Liberty Tire Recycling (Rockledge, Florida) and hand-sorted to obtain a particle size between 1.0 and 1.5 cm. The tire mulch was rinsed with DI water and dried for 4 hours at 105°C (Krayzelova et al., 2014). Elemental sulfur pastilles (0.4-0.6 cm) were obtained from Southern Ag (Palmetto, FL). Crushed oyster shells were purchased form a local agricultural supplier (Shells, Tampa, FL) and sieved to remove fines (1-2 mm).

5.2.2.2 Influent Wastewater

Effluent from a 30 L bench-scale septic tank (hereafter referred to as influent) was used to feed the columns described below. The influent was applied at varying rates according to the National Sanitation Foundation Standard 40 for variable loading, where 35%, 25% and 45% of the daily volume was distributed between the morning period (6 to 8am), afternoon period (11 to 2pm), and evening period (6 to 8pm), respectively. The variable loading was achieved with a Masterflex L/S peristaltic pump (Cole Palmer, Vernon Hills, IL) connected to a ChonTrol four-circuit programmable timer (Cole Palmer; Vernon Hills, IL). The timer was programmed to allow a single dose every hour of the dosing periods described above. The HRT in the tank varied from 3 to 14 days depending on the operational phase (See section 5.2.2.3) The septic tank was fed with screened raw sewage from the Falkenburg Advanced Wastewater Treatment plant in Tampa, FL.

Due to the low concentration (~30 mg L^{-1}) of NH_4^+-N in the sewage during the initial part of the study that coincided with the wet season in Florida, urea was added to the sewage to increase NH_4^+-N to a target concentration of 100 mg L^{-1}, which lies in the high end of the range of septic tank effluent concentrations recorded by Lowe et al. (2009). Due to the variability of the wastewater collected from the treatment plant there was significant variability in the influent composition, similar to real OWTS. Due to high pH conditions which developed in the septic tank after 120 days of operation, the septic tank was modified on day 170 to an 8 L bottle resulting in an average HRT of 3 days.

5.2.2.3 HABiTS and Control Biofilter Column Operation

Two side-by-side biofilter systems were constructed (Figure 5.2). One was designated as the control column and the second was HABiTS. The first stage of treatment occurred in unsaturated packed acrylic columns (88.9 mm diameter, 609.6 mm length; KOFLO, Cary, IL) to allow for aerobic conditions that favored nitrification. Packing materials for both columns are described in Table 5.2. The clinoptilolite dose in HABiTS Stage 1 was calculated to be able to withstand typical NH_4^+ loads over a 14 day period with no nitrification (e.g. due to biofilm die-off after a long idle period or after shock loading of a toxicant) based on the measured adsorption capacity. The materials used in the control treatment were based on its low cost and prior research using these materials in OWTS (Smith, 2012; Hirst et al., 2013) as well as its low capacity for NH_4^+ adsorption (Rodriguez-Gonzalez et al. 2015). The materials were mixed prior to packing in the column to ensure even distribution along the length of the biofilter. An expanded clay layer of 41 mm was included at the end of both columns for drainage purposes. A porous 30 micron nylon mesh fabric (SEFAR NITEX®, Heiden, Switzerland) was installed between the mixed layer and the expanded clay underdrain to avoid settling of the smaller media.

Stage 1 biofilters were constructed and operated prior to the installation of Stage 2. The columns were flushed with deionized water to remove fines and then flushed with 15 L of local groundwater to reduce some of the Na^+ loads from the clinoptilolite that could hinder nitrification (Aponte-Morales et al., 2016). At the end of the groundwater flush, effluent Na^+ concentrations from the columns were 100.36 and 22.4 mg L^{-1} for HABiTS and control columns, respectively.

The second stage biofilters were upflow packed bed reactors (60.35 mm diameter, 406 mm length; KOFLO, Cary, IL) containing an inorganic electron donor (S^0) to allow for anoxic conditions and denitrification. The media packed into each Stage 2 column are also described in Table 5.2. Both Stage 2 columns were packed with S^0 and oyster shells to support SOD and provide an alkalinity control. The adsorptive medium in Stage 2 HABiTS (tire mulch) was selected based on the results of Krayzelova et al. (2014). The plastic carriers used in Stage 2 control did not have an adsorptive capacity for NO_3^--N (data not shown).

Table 5.2: Media distribution in HABiTS and control biofilter treatments.

	Stage 1-Nitrification		Stage 2- Denitrification	
HABiTS	Expanded Clay (2-2.38mm)	528 g	Tire (10-15cm)	250 g
	Expanded Clay (1-1.38mm)	352 g	S^0 pastilles (0.4-0.6 cm)	40 g
	Clinoptilolite (2-2.38mm)	220 g	Oyster shells (1-2 mm)	10 g
CONTROL	Expanded clay (2-2.38 mm)	660 g	Plastic Carriers (14.5 mm)	110 g
	Expanded clay (1-1.38 mm)	440 g	S^0 pastilles (0.4-0.6 cm)	40 g
			Oyster shells (1-2 mm)	10 g

During column operation, the influent was distributed over the top of the unsaturated columns with a Masterflex L/S peristaltic pump (Cole Palmer, Vernon Hills, IL) connected to a timer and dosed as described in section 5.2.2.2. according to the NSF Standard 40 described above.

The columns were studied in four different phases described in Table 5.3 as follows. In the first phase the columns were fed at a rate of 2.1 L d^{-1} resulting in a hydraulic loading rate (HLR) of 0.34 m^3 m^{-2} d^{-1}. In this phase the biofilters were monitored to observe the effect of IX and the length of time required for nitrification start-up. Phase II began several weeks after Phase I at the same loading rate. Phase III began after Phase II when a lower flowrate was applied, 1.3 L d^{-1} (HLR = 0.21 m^3 m^{-2} d^{-1}) to improve nitrification. At this point, Stage 2 denitrification columns were also installed. During Phase IV, urea spiking in the sewage was discontinued and NH_4^+-N concentrations were allowed to return to natural levels.

Backwashing of Stage 1 columns for both control and HABiTS treatment was performed on day 37 during the Phase II study period to improve column hydraulics. Backwashing was performed with a mixture of groundwater and septic tank effluent to avoid any major desorption of NH_4^+ from the HABiTS columns that would give an advantage over the control column. The columns were filled from the bottom, drained twice, overflowed for 5 minutes at a rate of 250 mL min^{-1}, drained again, and then immediately connected back to the influent pumps. During Phase III the nylon mesh described above was removed to reduce clogging and the Stage 1 column media were re-distributed on day 87. The columns were backwashed again on day 140 using the protocol described above. All backwashes were performed on Stage 1 columns. At the beginning of Phase IV Stage 2 columns were inverted to redistribute biomass along the column. No other maintenance was performed until the end of the experiment.

Hourly sampling studies were performed in triplicate during Phases III and Phase IV of biofilter operation. Studies were conducted for each stage on different days and samples were collected at every dosing, while influent samples were only collected at the beginning of every period. Idle studies were performed periodically to determine biofilter performance recovery after

short or long periods without any influent. Two major idle studies were performed, a 56-day idle study between Phase I and II, and a 31-day idle study after Phase IV.

Table 5.3: Operational phases for HABiTS and control biofilter studies.

Phase	Operational Phase	HLR $m^3\ m^{-2}\ d^{-1}$	Target NH_4^+-N $mg\ L^{-1}$	EBCT (hrs)		Length of study (d)
				Stage 1	Stage 2	
I	Startup- Stage 1	0.34	100	43.2	-	31
II	High Loading –Stage 1	0.34	100	43.2	-	52
III	Moderate Loading-Two-Stage treatment	0.21	100	69.84	13.92	252
IV	Low loading-Two-stage treatment	0.21	40	69.84	13.92	117

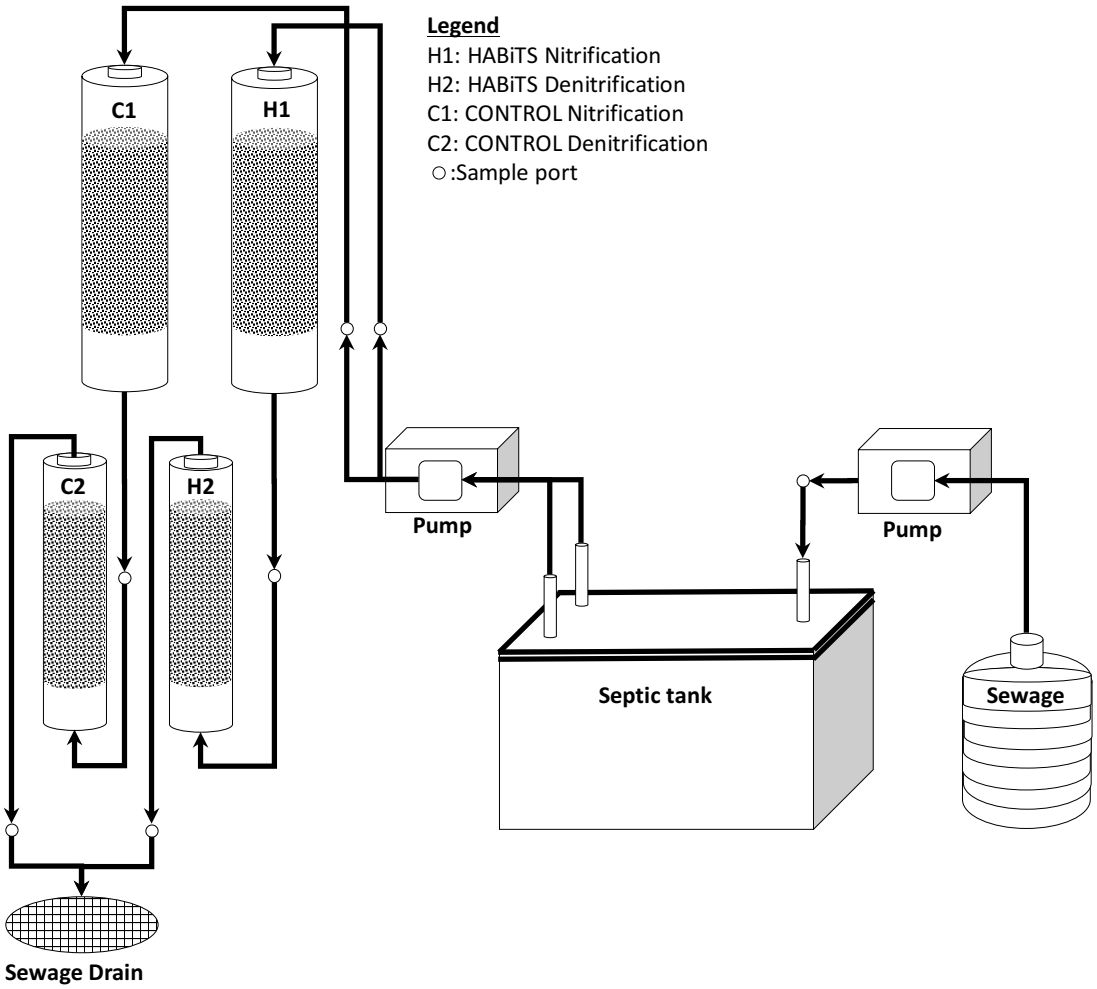


Figure 5.2: Experimental setup schematic for HABiTS and control treatment.

5.2.3 Analytical Methods

Samples were collected at least three times per week during the noon dosing period. During the hourly studies samples were collected during every dosing period. A portion of the samples was filtered through a 0.45 μm mixed cellulose esters filter (FisherScientific, Waltham, MA). Concentrations of anions (NO_2^-, NO_3, Cl^-, PO_4^{-3}, SO_4^{-2}) and cations (NH_4^+, Ca^{2+}, Mg^{2+}, Na^+, K^+) were measured in the filtered samples using a Metrohm 881 Compact IC Pro (Herisau, Switzerland) ion chromatography system. Method detection limits (MDLs) for NO_3^-, NO_2^-, SO_4^{2-} and PO_4^{3-} were 0.01, 0.04, 0.01, and 0.02 mg L^{-1}, respectively. MDLs for Na^+, K^+, Mg^{2+}, Ca^{2+} and NH_4^+ were 18.50, 0.07, 0.09, 0.27. 0.20 mg L^{-1}, respectively. pH and dissolved oxygen (DO) were measured using an Oakton Acorn Series meter (Orion 5 Star ThermoScientific) and calibrated electrodes. Total Nitrogen (TN) and Chemical oxygen demand (COD) were measured periodically for filtered and less frequently in unfiltered samples. COD was measured with the Vario Tube Test (Loveland, Co) COD LR test kits (MDL: 0-150 mg L^{-1}), according to the Standard Methods (5220 C; APHA et al., 2012;). TN was measured with HACH TNT test tubes, method 10071 DR80 (HACH, Loveland, Colorado).

5.2.4 Statistical Analysis

Statistical analyses were performed to compare the performance of the two studied columns. A two-sample t-test assuming equal variances was performed in Excel 2011 for N species results from both columns with α=0.05.

5.3 Results and Discussion

5.3.1 Abiotic Adsorption Studies

Percentage of cation concentrations in the synthetic septic tank effluent as well as in the bulk liquid after 24 hr adsorption for each candidate material tested are shown in Figure 5.3.

Clinoptilolite removed 83% of the NH_4^+. Removal of K^+ (36%) and Ca^{2+} (53%) was also observed. The removal of K^+ was expected as zeolites have shown a higher affinity to K^+ than NH_4^+ (Ames et al., 1960). The order of affinity for zeolites as described by Ames et al., is as follows: $Cs^+ > Rb^+ > K^+ > NH_4^+ > Ba^{2+} > Na^+ > Ca^{2+} > Fe^{3+} > Mg^{2+}$. Although there is a higher selectivity for Na^+ than Ca^{2+}, the clinoptilolite is naturally loaded with Na^+ and significant exchange will occur due to the high concentration of Ca^{2+} in the synthetic wastewater. Vermiculite achieved 42% removal of NH_4^+, while also removing Ca^{2+} (29%) and very little K^+ (2%). Vermiculite has been found to remove about 55% of NH_4^+ in absence of competing cations (Lv et al., 2013). Cations exchanged within clinoptilolite and vermiculite were Na^+ (260% and 74% increase, respectively) and Mg^+ (5% and 41% increase, respectively). Other materials tested showed low removal of cations (<1%), while none of the materials showed significant removal of anions (not shown). Based on its distinctive IX performance, ease of handling, availability and moderate price, clinoptilolite was selected for further study.

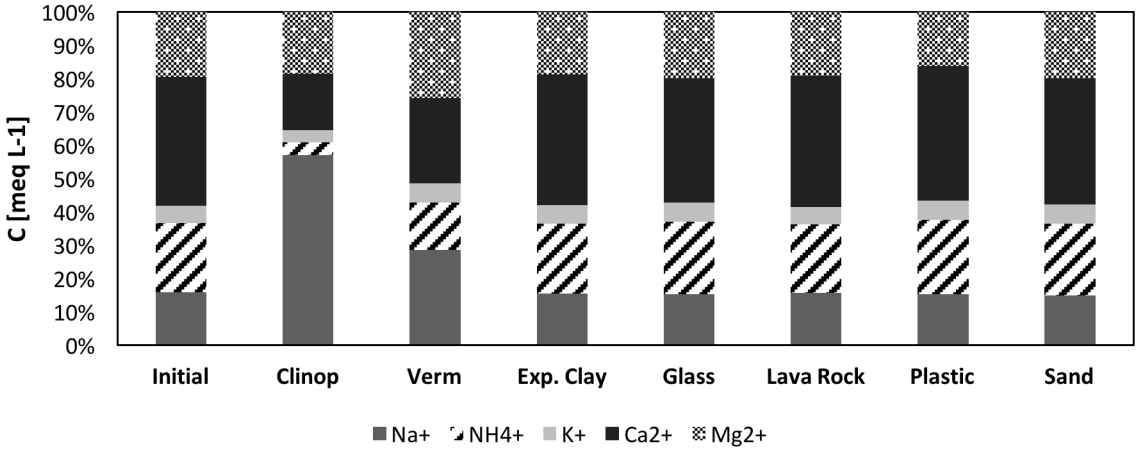


Figure 5.3: Cation concentrations in the bulk liquid after 24 hrs of adsorption with varying media materials.

The results from the adsorption isotherm studies with and without competing cations are shown in Figure 5.4. A dose of 20 g L^{-1} of clinoptilolite removed 94% of NH_4^+ in the absence of

competing ions. In the presence of competing cations, the removal of NH_4^+ with the same dose was reduced to 87.8%. The results for both experiments show the major ion exchanged is Na^+ (Figure 5.4) and was used as C_x for the IX model fit. The fit for the Langmuir, Freundlich and IX model is shown in Figure 5.5 and Table 5.4. Based on the correlation coefficients, the Langmuir and IX model provided a better fit (0.97) than the Freundlich (0.94) for the studies without competing anions. Maximum adsorption capacity was calculated at 25.58 and 19.52 mg NH_4^+-N g^{-1} clinoptilolite for the Langmuir and IX model.

A better fit for all models (R^2= 0.99) was observed for the adsorption isotherms with competing cations. The maximum adsorption capacity was reduced to 14.35 and 11.69 mg NH_4^+-N g^{-1} clinoptilolite for the Langmuir and IX model respectively. The reduced capacity is possibly due to exchange of other cations present in the synthetic septic tank effluent. With a dose of 20 g L^{-1} of clinoptilolite, removal of K^+ (40.34%), Ca^{2+} (58.77%) and Mg^{2+} (30.89%) was achieved (not shown). The reduced capacity is important to consider when designing the Stage 1 biofilter to withstand NH_4^+-N loads during periods without significant biological removal. During regular operation, however, nitrification would be a significant process in the removal of NH_4^+ from the bulk liquid and would be the limiting factor for IX. This was also observed by Aponte-Morales et al. (2016) with the zeolite material chabazite. The results from the kinetic study (Figure 5.6) show that the majority of the IX occurs within the first 2 hours of contact time removing around 64.49% of NH_4^+-N. At this contact time removal of K^+ (13.12%) is observed while Ca^{2+} and Mg^{2+} are released. Maximum removal of NH_4^+-N (81.6%) was observed at 16 hr contact time. This contact time resulted in 20.16 % and 15.81% removal of K^+ and Ca^{2+}, respectively (not shown).

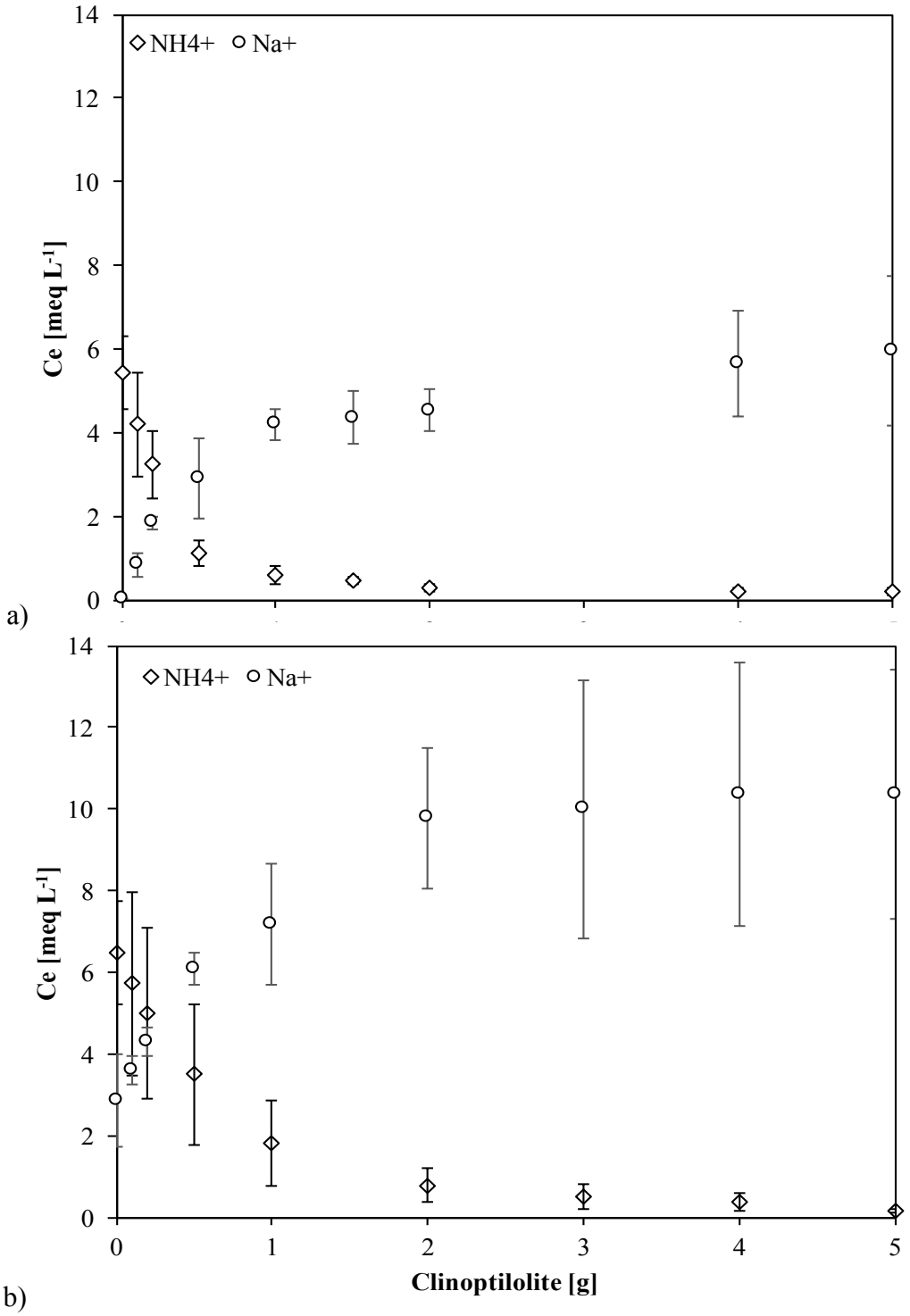


Figure 5.4: NH_4^+ concentrations in the bulk liquid after 24 hr adsorption with varying clinoptilolite doses without (a) and with (b) the influence of competing cations

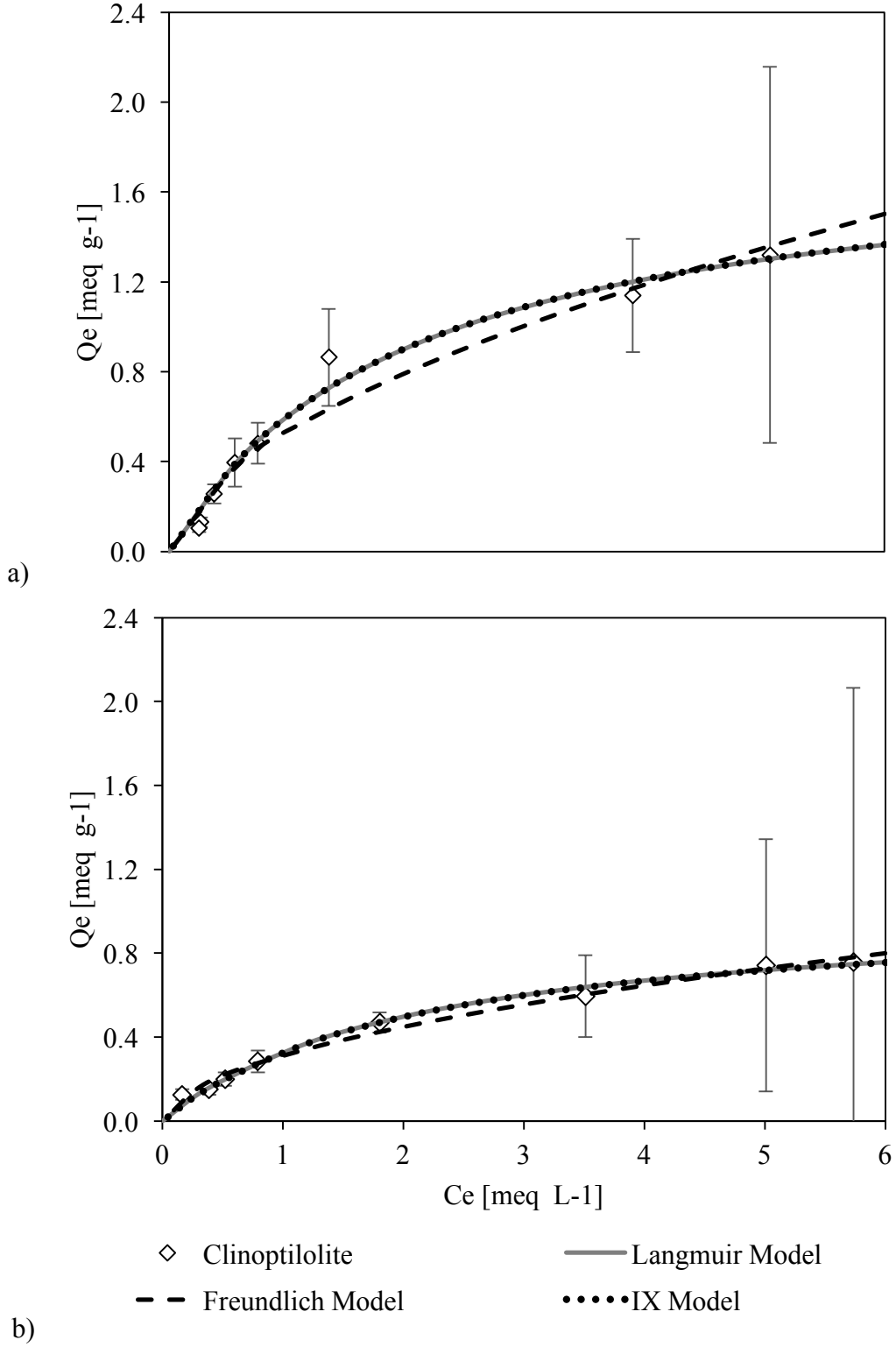


Figure 5.5: Langmuir, Freundlich and IX models for the clinoptilolite adsorption isotherms without (a) and with (b) the influence of competing cations.

Table 5.4: Langmuir, Freundlich and IX model fit for clinoptilolite adsorption isotherms in the absence and presence of competing cations.

Solution	Model	Equation	R^2
NH_4Cl in DI	Langmuir	$q_e = \frac{1.827 \cdot 0.593 \cdot c_e}{1 + 0.593 \cdot c_e}$	0.97
	Freundlich	$q_e = 0.595 \cdot C_e^{0.575}$	0.94
	IX	$q_e = \frac{1.394 \cdot 4.225 \cdot C_e}{C_x + 4.225 \cdot C_e}$	0.97
Synthetic septic tank effluent	Langmuir	$q_e = \frac{1.025 \cdot 0.471 \cdot c_e}{1 + 0.471 \cdot c_e}$	0.99
	Freundlich	$q_e = 0.311 \cdot C_e^{0.527}$	0.99
	IX	$q_e = \frac{0.835 \cdot 5.411 \cdot C_e}{C_x + 5.411 \cdot C_e}$	0.99

*qe expressed in milliequivalent g^{-1} clinoptilolite
*Ce expressed in milliequivalents L^{-1}

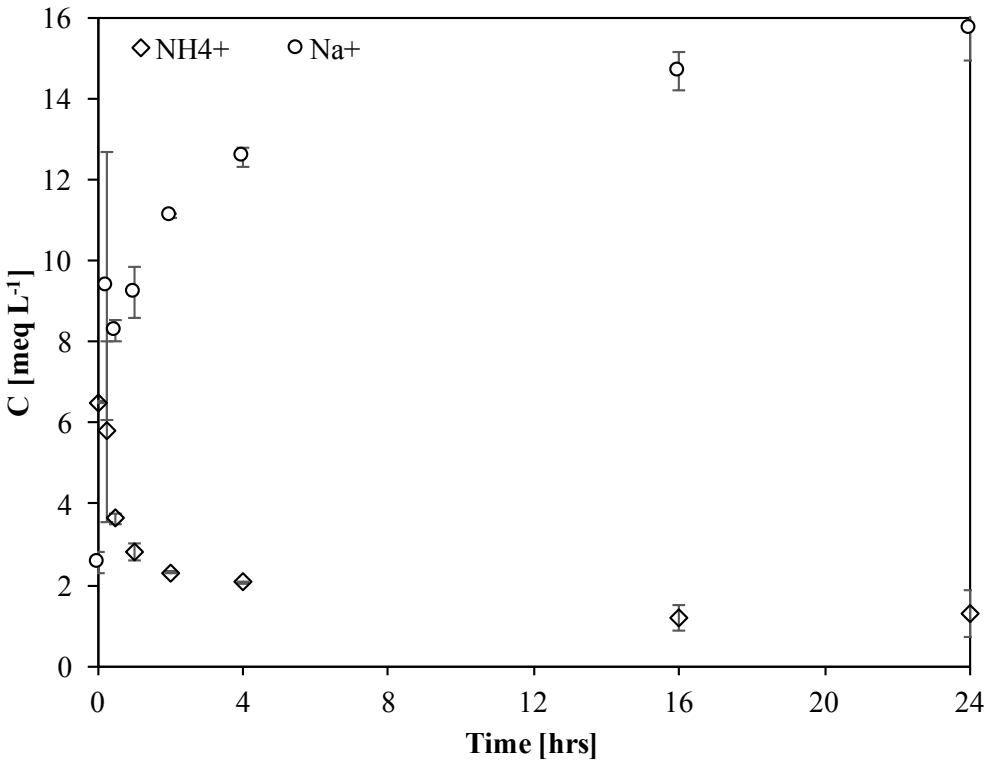


Figure 5.6: Adsorption kinetics for clinoptilolite with the influence of competing cations.

5.3.2 HABiTS and Control Biofilter Studies

5.3.2.1 Phase I-Startup Stage 1 Biofilter under High NH_4^+-N Loading Rates

Influent and effluent NH_4^+-N, Na^+, NO_2^--N, and NO_3^--N concentrations during Phase I for the nitrification stage of HABiTS and the control treatment are shown in Figure 5.7. During the start-up phase, removal of NH_4^+-N in HABiTS ranged from 75 to 85%, which was significantly greater than the control. As shown in Figure 5.7b, the ion exchanged with NH_4^+ was Na^+; however, the effluent Na^+ concentration only reached a maximum of 210 mg L^{-1} on day 2 and decreased to the influent value within 12 days. High Na^+ concentrations are of concern due to the inhibitory effect of Na^+ on nitrifying bacteria. However, Na^+ concentrations observed in this study were much lower than the value of 8,000 mg L^{-1} reported by Sanchez et al. (2004) to inhibit nitrification. The groundwater flush prior to Phase I reduced the load of Na^+ from the clinoptilolite, as was observed by Aponte-Morales et al., (2016). Other cations present, such as Mg^{2+}, Ca^{2+} and K^+ showed little difference from influent (not shown). In contrast, in the control effluent NH_4^+-N concentrations only started to decrease after day 7 (Figure 5.7a), which coincided with increases in effluent NO_2^--N concentrations (Figure 5.7c). Some NO_2^--N was also observed in HABiTS but to a lesser extent. Start-up of nitrification within both biofilters occurred much faster than in gravel/sand filters studied by Petitjean et al. (2016), where nitrification was observed after 12 days at a HLR of 0.117 m^3 m^{-2}-d^{-1}. It is important to note that neither of the columns was seeded with active nitrifying biomass, indicating that some nitrifying microorganisms were likely present in the influent feed. Transient accumulation of NO_2^--N during start-up was consistent with literature showing that NH_4^+-N oxidation kinetics are faster than NO_2^--N oxidation (Almutairi & Weatherley, 2015).

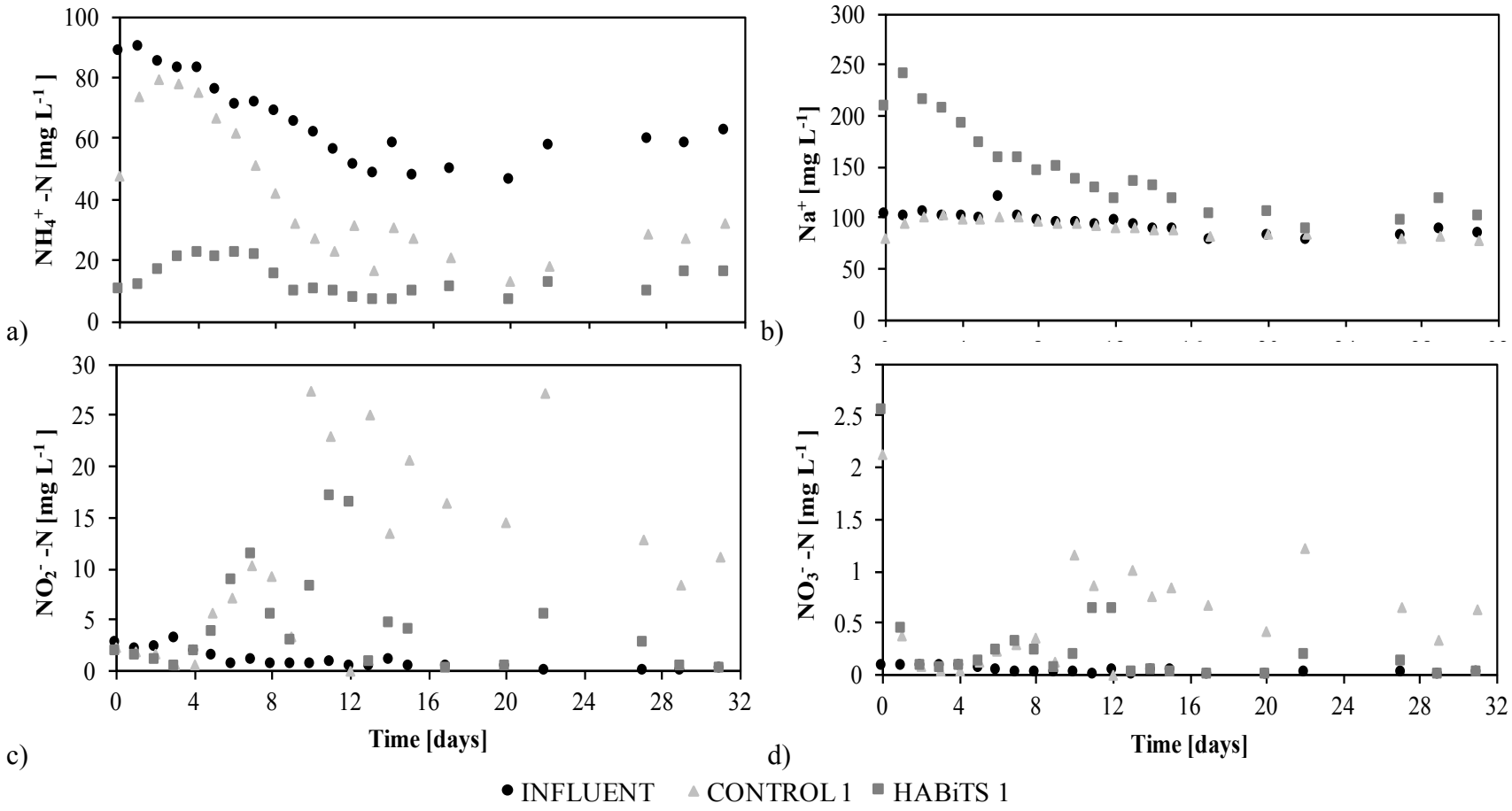


Figure 5.7: NH_4^+-N (a), Na^+ (b), NO_2^--N (c), and NO_3^--N (d) daily variation in the influent, control and HABiTS columns effluent for Phase I under HLR of 0.34 m^3 m^{-2}-d^{-1} (Rodriguez-Gonzalez et al. 2016).

Table 5.5: Average N species results for influent, control and HABiTS Stage 1 biofilters during Phase I.

	Phase I		
	Influent	Control	HABiTS
NH_4^+-N (mg L^{-1})	65.39±13.83	41.09±21.79	13.48±5.43
NO_2^--N (mg L^{-1})	0.96±0.90	11.59±8.88	4.54±5.01
NO_3^--N (mg L^{-1})	0.03±0.03	0.59±0.50	0.28±0.54

Based on the amount of clinoptilolite added, saturation of the HABiTS media and breakthrough should have occurred within 15 days of start-up. The low effluent NH_4^+-N concentrations in HABiTS after 14 days showed that nitrifying biofilms were bio-regenerating the clinoptilolite. Meladonic & Weatherley (2008) also saw considerable retardation of NH_4^+-N breakthrough due to nitrification in clinoptilolite columns treating synthetic wastewater. In the case of HABiTS Stage 1, however, little production NO_2^--N and NO_3^--N was observed. Based on the average effluent NO_2^--N and NO_3^--N concentrations, only 7.4 % and 44% of the NH_4^+-N removed was due to nitrification in the Stage 1 of HABiTS and control treatment, respectively. Simultaneous nitrification/denitrification in anoxic zones within the columns could have possibly occurred, resulting in lower NO_2^--N and NO_3^--N in the effluent. This phenomenon, however, was not observed in Phases II and III and was not studied further. Significantly lower concentrations of NH_4^+-N and NO_2^--N (p-value <0.05), were observed for HABiTS during start-up phase when compared to the control treatment. This result also highlights the efficiency of HABiTS to reduce start-up periods by maintaining low N concentrations while nitrifying biofilms are being established. Almutairi &Weatherly (2015) observed similar results when testing multiple IX columns, with and without the addition of external aeration and nitrifying biomass. Huang et al. (2015) tested clinoptilolite for NH_4^+-N removal in groundwater and also reported on the robustness

of the treatment in the case of insufficient biological activity. Increasing the clinoptilolite percentage in the HABiTS column would likely have resulted in greater removal of NH_4^+ and longer periods of sustained IX but would have defeated the goal of keeping these systems cost-effective and promoting hybrid IX and biological treatment. For example, although high NH_4^+-N removal was observed in pilot scale nitrifying biofilters with 100% clinoptilolite media, the material was not selected for full scale tests due to cost constraints and comparable performance to conventional media biofilters. (FOSNRS, 2015).

5.3.2.2 Phase II-Stage 1 Nitrification Biofilter under High NH_4^+-N Loading Rates

Influent and effluent N species concentrations during Phase II are shown in Figure 5.8. A summary of the N results and water quality parameters measured in Phase II are shown in Table 5.6. During Phase II, NH_4^+-N concentrations (Figure 5.8a, Table 5.6) in the influent were highly variable (57.49 ±19.78), which affected the variability of the effluent of the control column (15.78±9.24). However, the average HABiTS effluent NH_4^+-N concentration was less than 15 mg L^{-1}, with little variation (11.03±3.45) and was significantly lower than effluent concentrations in the control column (p-value <0.05). Hirst et al. (2013) reported that little effluent NH_4^+-N variability was observed in a nitrifying biofilter with a clinoptilolite medium. No significant differences were observed in NO_2^--N and NO_3^--N effluent concentrations from the columns (Figure 5.8b-c). This indicated that IX continued to occur in the HABiTS column and possibly bio-regeneration of the clinoptilolite. Another indication of the effect of IX in HABiTS was the high NO_3^- concentrations recovered from HABiTS when compared to the control column within the first flush (day 0) due to nitrification of stored concentrations of NH_4^+ during Phase I. No significant difference was observed in the other cations and anions from both biofilter treatments (Table 5.5).

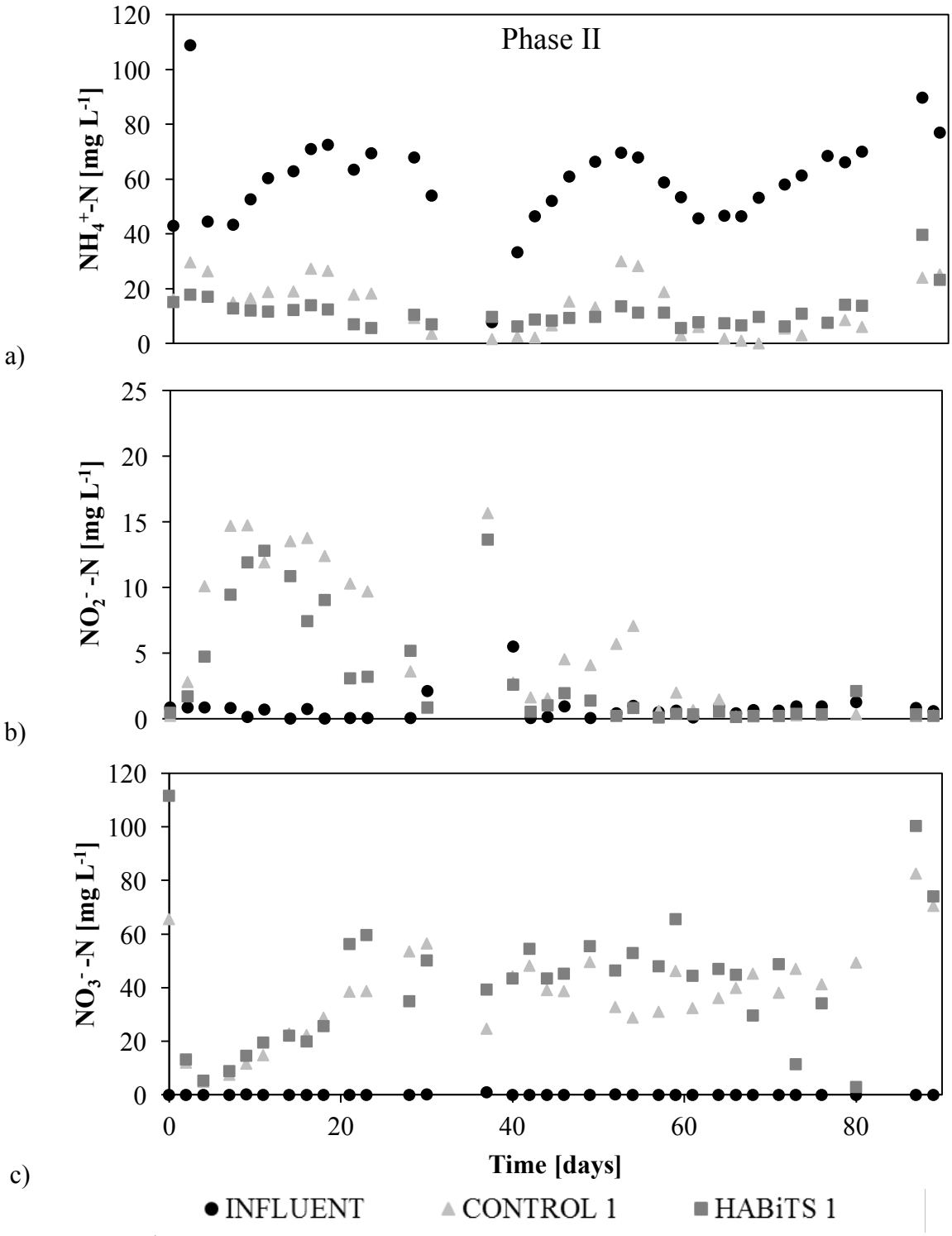


Figure 5.8: NH_4^+-N, NO_2^--N, and NO_3^--N daily variation in the influent, control and HABiTS columns effluent for Phase II under variable HLR.

A 7-day gap in results between days 30 and 37 was due to column and equipment maintenance as well as a required system backwash due to clogging and increased head loss within

the columns possibly due to the high HLR. Based on NH_4^+-N removal, there was no significant impact on the treatment performance due to backwashing for either column, with the exception in variability in TSS/VSS concentrations (not shown), most likely due to release of biomass during the first flush after backwash. Although not desirable, backwashing after over two months of intermittent treatment is not uncommon. In studies by Petitjean et al. (2016) clogging of a sand column was observed within 45 days of operation under varying HLR (0.07-0.117 $m^3\ m^{-2}\text{-}\ d^{-1}$). In this study the particle size was about half that of Petitjean et al. (2016) and the HLR during Phase I was about three times higher. Increasing particle size can reduce clogging but could potentially affect treatment within the biofilter due to reduced surface area. Reducing the HLR could also reduce clogging potential and was investigated in Phase III.

Table 5.6: Average water quality results for influent, control and HABiTS Stage 1 biofilters during Phase II.

	PHASE II (HLR = 0.34 $m^3\ m^{-2}\text{-}d^{-1}$)		
	Influent	**Control Stage 1**	**HABiTS Stage 1**
pH	7.20 ±0.40	6.68 ±0.38	6.59 ±0.41
DO (mg L^{-1})	0.81 ±0.39	4.06 ±0.65	4.36 ±0.57
NH_4^+-N (mg L^{-1})	57.49 ±19.78	15.78 ±9.24	**11.03 ±3.45***
NO_2^--N (mg L^{-1})	2.60 ±8.36	7.72 ±5.42	5.10 ±4.60
NO_3^--N (mg L^{-1})	0.10 ±0.23	32.78 ±17.43	38.53 ±24.40
Org. N (mg L^{-1})	11.52 ±19.24	4.86 ±2.11	7.79 ±4.70
Na^+ (mg L^{-1})	79.62 ±3.19	77.78 ±6.37	84.09 ±13.16
K^+ (mg L^{-1})	22.07 ±5.16	21.70 ±5.62	30.32 ±4.98
Ca^{2+} (mg L^{-1})	138.74 ±6.48	128.00 ±14.29	127.81 ±24.52
Mg^{2+} (mg L^{-1})	40.19 ±5.33	35.32 ±5.10	33.87 ±4.89
Cl^- (mg L^{-1})	87.19 ±4.61	85.41 ±6.72	85.77 ±6.95
PO_4^{3-}-P (mg L^{-1})	19.85 ±4.05	13.66 ±3.41	13.24 ±4.13
SO_4^{2-}-S (mg L^{-1})	82.12 ±39.10	130.23 ±13.10	133.00 ±16.61
COD (mg L^{-1})	122.50 ±68.85	43.50 ±11.99	42.29 ±6.05

*Values in bold are significantly different from the comparing treatment.

5.3.2.3 Phase III- Two-Stage Treatment under Moderate NH_4^+-N Loading Rate

Phase III (Figure 5.9, Table 5.7) commenced after 52 days of treatment and included a reduction of the HLR to 0.21 m^3 m^{-2}- d^{-1} to enhance nitrification and reduce clogging. Similar NH_4^+-N removal performance was observed in both columns for Stage 1 nitrification under the lower HLR condition where effluent NH_4^+-N concentrations were comparable to that of Phase II (Figure 5.9a). This result was different from that of Luo et al. (2014) where decreased NH_4^+-N removal was observed with increasing HLR in a system that combined soil and clinoptilolite. The increased effluent NH_4^+-N concentration was most likely due to an increase in influent NH_4^+-N concentrations (+30 mg L^{-1}) around day 90. In this phase, nitrification completely dominated the removal processes of NH_4^+ for both control and HABiTS biofilters as opposed to the IX treatment. This was supported by the observed reduction in effluent pH as well as high effluent NO_3^--N concentrations (Figure 5.9c). Although some heterotrophic degradation occurred in the columns based on more than 50% removal of COD (Table 5.7), average effluent DO concentrations were > 4 mg L^{-1} showing that lack of oxygen was probably not the cause for incomplete nitrification.

The integration of Stage 2 denitrification for both biofilters was done on day 95, during Phase III. Acclimation of Stage 2 was observed between day 95 and 113. Transient NO_2^--N production was observed for the effluent of HABiTS Stage 2 but not for the control Stage 2 column during this period. In HABiTS, leaching of carbon from the tire mulch, as reported by Krayzelova et al. (2014), could have allowed partial heterotrophic denitrification to occur resulting in high NO_2^--N production. In time and due to the high availability of S^0, SOD startup was observed and NO_2^--N concentrations dropped to below 1 mg L^{-1}. Samples collected on day 113 showed that HABiTS Stage 2 removed 97% of the NO_3^--N while only 64% of it was removed in the control Stage 2 column, indicating faster denitrification in HABiTS possibly due to the partial

heterotrophic denitrification as discussed previously. The high removal of NO_3^--N correlated with increase SO_4^{2-}-S in the effluent resulting in a productivity of 2.30 mg SO_4^{2-}-S mg^{-1} NO_3^--N. This calculated productivity is higher than that from the stoichiometric value of 1.6 mg SO_4^{2-}-S mg^{-1} NO_3^--N reported by Sengupta et al., (2007) and could indicate aerobic oxidation of elemental sulfur which has been reported in several studies of SOD (Tong et al., 2016; Boles et al., 2012). This is also supported by the high and variable DO concentration in the effluent of Stage 2 (3.34 ±1.68 mg L^{-1}). The productivity for control column Stage 2 was only 0.79 mg SO_4^{2-}-S mg^{-1} NO_3^--N and could be caused by slow SOD startup and possibly SO_4^{2-}-S reduction within the column (Boles et al., 2012). The averages of all N species and SO_4^{2-}-S were not significantly different between biofilters during this period.

Another notable period during Phase III occurred between days 140 and 170. The long HRT in the septic tank resulted in high pH and volatilization of NH_3 effectively reducing the NH_4^+-N concentration in the influent to approximately 16.5±4.7 mg L^{-1}. During this period NH_4^+-N concentrations decreased to 2.46±1.79 and 0.03±0.06 mg L^{-1} for HABiTS and control Stage 1, respectively, and were significantly higher for the HABiTS biofilter. Significantly higher concentrations of NH_4^+-N were also observed for Stage 2 HABiTS compared to Stage 2 in the control column. NO_3^--N was also significantly higher for HABiTS Stage 1 (43.45±7.20) than the control (28.9±3.18). These results indicate desorption of the stored NH_4^+-N in the clinoptilolite and subsequent nitrification due to the previous high loading. Similar results were observed by Miladonic & Weatherly (2008) when testing clinoptilolite columns at varying loading rates. Effluent NO_3^--N concentrations from HABiTS Stage 2 were significantly lower (0.88± 1.90) than those in the control column (6.42 ± 4.15). This resulted in a productivity of 0.55 mg SO_4^{2-}-S mg^{-1} NO_3^--N for HABiTS Stage 2 and 0.87 mg SO_4^{2-}-S mg^{-1} NO_3^--N for Stage 2 of the control treatment.

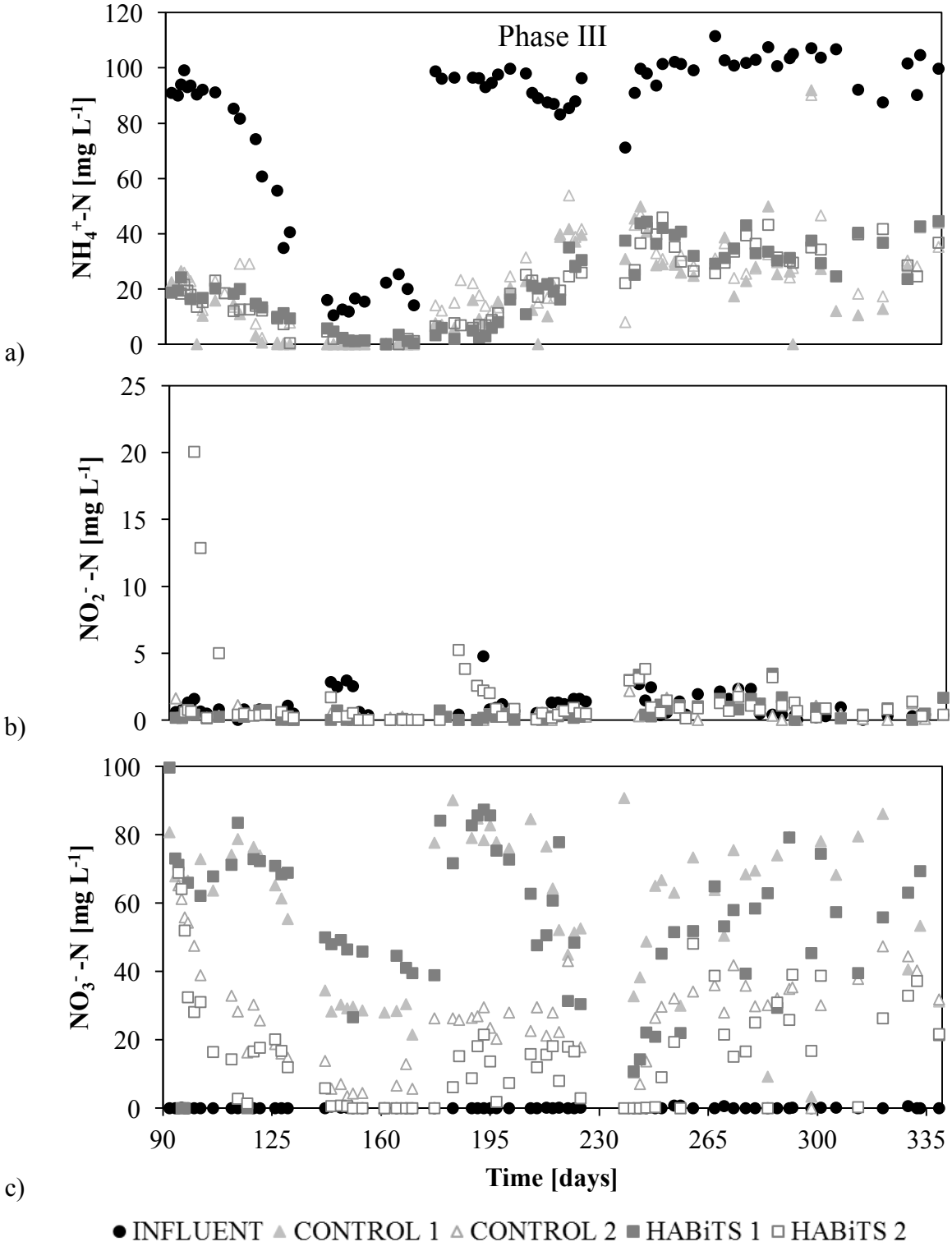


Figure 5.9: NH_4^+-N, NO_2^--N, and NO_3^--N daily variation in the influent, control and HABiTS columns effluent for Phase III under variable HLR.

Recovery after the low influent concentrations occurred between day 177 and 201. During this period the septic tank was modified (as described in section 5.3.1) and NH_4^+-N in the influent increased to an average of 80.56 ±30.66 mg L^{-1}. Average Stage 1 effluent NH_4^+-N concentrations were 5.80±4.4 mg L^{-1} and 10.76 ±5.17 mg L^{-1} for HABiTS and control, respectively, and were significantly different ($p<0.05$). Significantly lower concentrations of NH_4^+-N were also observed for HABiTS Stage 2 whereas a significant increase in NO_2^--N concentrations was observed. No significant difference was observed for NO_3^--N production. These results indicate that IX continued to be a significant removal mechanism within HABiTS, particularly when a high rate loading period occurs after a low loading rate period. For Stage 2, significant differences were observed for NO_3^--N removal, where HABiTS removed 89.55% compared to 67.84% in the control treatment. Productivity of SO_4^{2-}-S, on the other hand, was significantly lower for HABiTS (0.6 mg SO_4^{2-}-S mg^{-1} NO_3^--N) than the control treatment (1.1 mg SO_4^{2-}-S mg^{-1} NO_3^--N). This lower productivity indicates that another NO_3^--N removal mechanism was occurring in HABiTS Stage 2, possibly heterotrophic denitrification using the COD from the carbon leachate or adsorption to the tire (Krayzelova et al., 2014). Although the effects of NO_3^--N adsorption to the tire mulch in the HABiTS Stage 2 was not observed in other periods of Phase III, it was observed in the study by Krayzelova et al., (2014) in both batch experiments and column experiments with synthetic wastewater. However, the contact time required for adsorption in Krayzelova's study was much higher than that in this study. The limited contact time could have reduced the removal of NO_3^--N by adsorption.

In terms of overall performance, nitrification in both treatments was not significantly different, while significantly lower NO_3^--N concentrations were found in HABiTS Stage 2. Overall TIN removal for HABiTS was 53.54%, versus 40.97% for the control treatment. The overall

performance of HABiTS could have been affected by slow desorption of the retained NH_4^+ during the previous high loading period in Phase II. Regardless, nitrification in this phase (Phase III) was improved when compared to Phase II resulting in lower NO_2^--N (< 2 mg L^{-1}) and higher production of NO_3^--N.

5.3.2.4 Phase IV- Two Stage Biofilter Treatment under Low NH_4^+-N Loading Rate

N species and water quality parameters for Phase IV are shown in Figure 5.10 and Table 5.7. During this phase, addition of urea to the sewage was discontinued and the influent NH_4^+-N concentration decreased. Although no backwash was performed during this phase, Stage 2 biofilters were inverted several times to re-distribute the solids within the layers of the column and reduce the accumulation of the solids in the biofilter inlet. NH_4^+-N concentrations in the Stage 1 of control treatment dropped rapidly and reached levels below 1 mg L^{-1} within 11 days (Figure 5.10a). In HABiTS, however, the rate of NH_4^+-N removal was much slower and only decreased to 2.41 mg L^{-1}, 35 days after the beginning of Phase IV. Average results for N species show significantly higher NH_4^+-N concentrations for both stages of HABiTS. This is also true for NO_3^--N production, where HABiTS produced 46.78 ±11.83 mg L^{-1} while the control column only reached 37.40 ±12.40.

High NO_3^--N concentrations were observed in the Stage 2 effluent of both treatments during the first 10 days of Phase IV (day 339 to 350) possibly due to washout of denitrifying biomass after redistribution of the solids. Due to the higher load into HABiTS Stage 2, significantly higher NO_3^--N concentrations in the effluent were also observed. Overall, HABiTS was able to remove only 28.7 % of TIN while the control treatment was able to remove 62%.

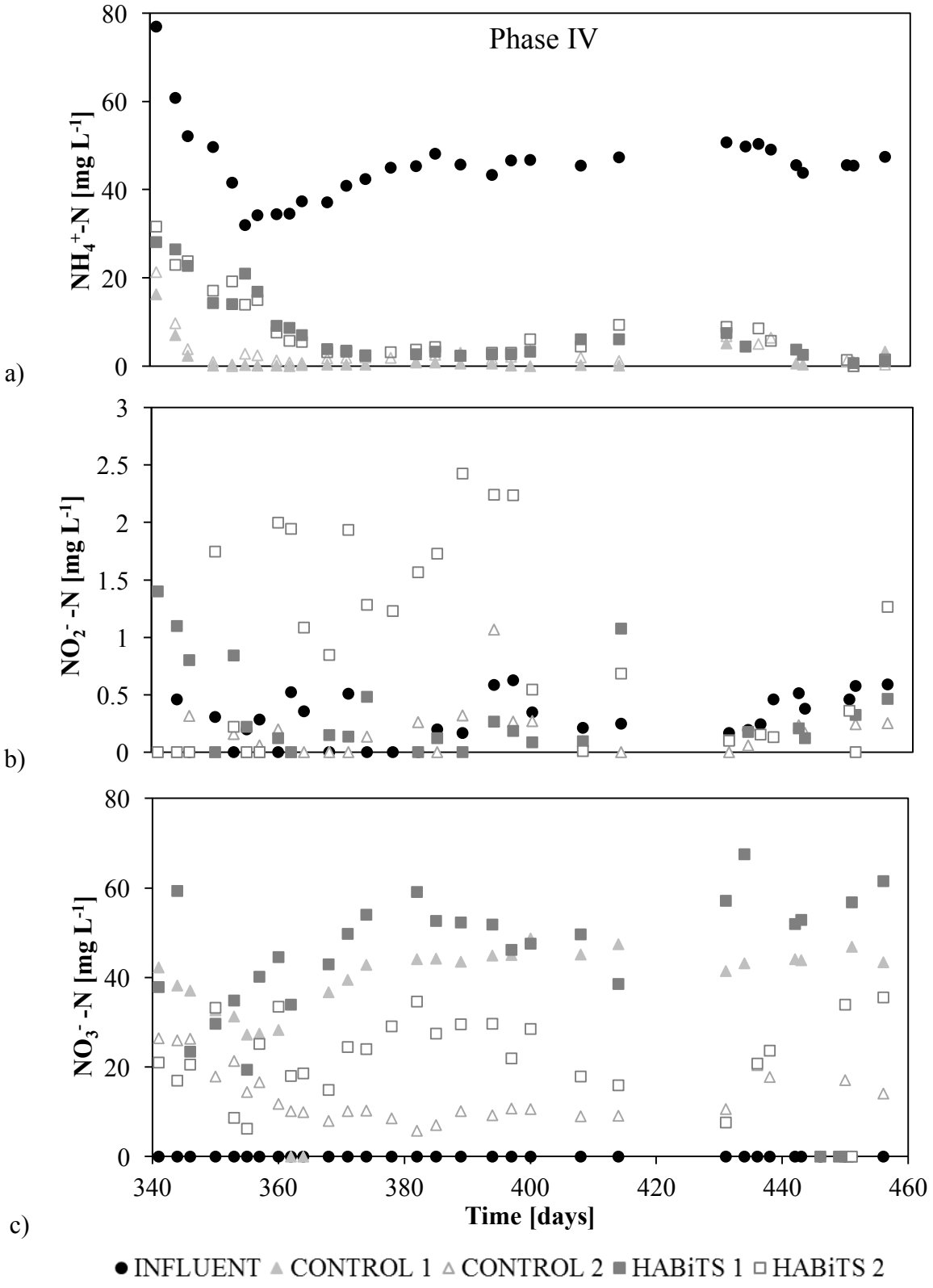


Figure 5.10: NH_4^+-N, NO_2^--N, and NO_3^--N daily variation in the influent, control and HABiTS columns effluent for Phase IV under variable HLR.

Table 5.7: Average water quality results for influent, control and HABiTS column during Phases III and VI.

	PHASE III (HLR = 0.21 m^3 m^{-2}-d^{-1})					**PHASE IV (HLR = 0.21 m^3 m^{-2}-d^{-1})**				
	Influent	**Control Stage 1**	**Control Stage 2**	**HABiTS Stage 1**	**HABiTS Stage 2**	**Influent**	**Control Stage 1**	**Control Stage 2**	**HABiTS Stage 1**	**HABiTS Stage 2**
pH	7.52 ±0.41	6.21 ±0.77	6.54 ±0.32	5.91 ±1.29	6.64 ±0.33	6.98 ±1.36	5.87 ±2.06	6.10 ±1.74	5.87 ±0.94	6.37 ±0.39
DO ($mg\ L^{-1}$)	0.85 ±0.49	5.06 ±1.06	3.53 ±1.45	4.87 ±1.51	3.34 ±1.68	0.58 ±0.37	4.56 ±1.77	3.30 ±1.63	4.92 ±0.86	3.05 ±1.55
NH_4^+-N ($mg\ L^{-1}$)	80.56 ±30.66	19.79 ±17.07	21.82 ±16.08	21.51 ±13.99	20.25 ±12.90	45.65 ±8.42	1.69 ±3.41	3.38 ±4.26	**8.42 ±8.05***	**8.76 ±7.95***
NO_2^--N ($mg\ L^{-1}$)	0.97 ±0.91	0.50 ±0.54	0.67 ±0.74	0.57 ±0.75	1.49 ±2.98	0.28 ±0.21	0.15 ±0.72	0.33 ±1.06	0.33 ±1.28	**0.95 ±2.81***
NO_3^--N ($mg\ L^{-1}$)	0.07 ±0.17	58.96 ±21.86	25.68 ±15.12	58.60 ±23.47	**16.17 ±15.96***	0.00 ±0.00	37.40 ±12.40	13.70 ±6.06	**46.78 ±11.83***	**23.04 ±8.26***
Na^+ ($mg\ L^{-1}$)	88.65 ±12.47	90.19 ±11.44	89.06 ±11.39	88.53 ±10.68	88.39 ±6.26	104.22 ±10.99	102.80 ±11.36	99.50 ±15.61	97.17 ±11.88	97.02 ±11.99
K^+ ($mg\ L^{-1}$)	29.13 ±10.32	28.85 ±9.60	29.74 ±10.24	33.68 ±6.66	35.11 ±6.92	30.76 ±26.73	30.48 ±26.59	29.43 ±26.39	21.55 ±6.90	23.94 ±6.31
Ca^{2+} ($mg\ L^{-1}$)	138.03 ±35.20	109.12 ±27.73	132.34 ±27.43	112.88 ±35.33	132.50 ±30.40	152.12 ±13.51	110.56 ±11.33	118.81 ±18.12	90.17 ±15.61	111.70 ±13.98
Mg^{2+} ($mg\ L^{-1}$)	36.82 ±10.25	31.22 ±9.80	31.76 ±9.64	29.79 ±6.37	31.60 ±8.97	35.43 ±4.91	27.99 ±3.29	27.45 ±4.04	24.92 ±3.43	26.67 ±3.29
Cl^- ($mg\ L^{-1}$)	91.73 ±30.53	90.48 ±28.98	93.20 ±30.93	90.27 ±29.69	91.88 ±30.36	111.05 ±25.42	91.39 ±41.00	105.52 ±27.10	97.78 ±32.94	109.47 ±25.54
PO_4^{3-}-P ($mg\ L^{-1}$)	3.24 ±1.81	1.70 ±1.40	1.57 ±1.75	2.05 ±1.52	1.16 ±1.08	2.21 ±1.51	0.00 ±1.08	0.67 ±0.92	0.81 ±1.16	0.56 ±0.86
SO_4^{2-}-S ($mg\ L^{-1}$)	22.48 ±16.22	42.31 ±16.79	74.14 ±23.62	43.48 ±9.02	70.90 ±22.48	22.95 ±18.61	46.79 ±24.46	87.67 ±19.91	51.82 ±14.64	73.98 ±26.10
COD ($mg\ L^{-1}$)	88.75 ±33.50	22.00 ±14.79	42.59 ±26.42	28.40 ±7.27	45.44 ±24.06	74.50 ±54.42	-	23.00 ±32.53	-	34.00 ±29.70

*Values in bold are significantly different from the comparing treatment.

5.3.2.5 Hourly and Idle Studies

Average N species for the three dosing periods as well as the daily average for hourly studies during Phase III are shown in Figure 5.11 and 5.12. The results supported the overall findings for Phase III. No significant differences were observed for Stage 1 (Figure 5.11a, 5.11b, 5.11c) overall or at any of the dosing periods during the day. For Stage 2 (Figure 5.12c), NH_4^+-N concentrations were significantly higher in HABiTS (27.07±0.31 mg L^{-1}) than the control treatment (23.96±0.35 mg L^{-1}) during the evening dosing, possibly due to the higher loading during the morning period. NO_2^--N concentrations were approximately 1.09±0.17 mg L^{-1} significantly higher for HABiTS Stage 2 overall, and at the morning and evening period. No significant removal was observed for NO_3^--N while SO_4^{2-}-S was significantly lower for HABiTS during the evening dose (80.08±3.64 mg L^{-1}), indicating limited denitrification.

Phase IV hourly studies results for average N species for the three dosing periods as well as the daily average are shown in Figure 5.13 and 5.14. Significantly higher daily NO_3^--N concentrations were observed in HABiTS Stage 1 (Figure 5.13d; 49.99±6.32 mg L^{-1}) when compared to the control (Figure 5.13d; 41.46±4.28 mg L^{-1}). This was also true for the averages of the morning and noon periods (Figure 5.13a, 5.13b). For the morning period, about 10 hrs had passed from the last dose allowing for desorption of NH_4^+-N and subsequent nitrification. The noon dosing is the lowest loading of the day and would allow desorption of the exchanged NH_4^+-N as supported by the significantly higher NH_4^+-N concentrations during this dosing period (Figure 5.13a; 2.36±0.13 mg L^{-1}). Stage 2 results (Figure 5.14d) showed significantly higher daily NO_3^--N concentrations for HABiTS when compared to the control treatment. This could have been caused by the higher loading received from Stage 1, as discussed previously. Significantly lower daily productivity of SO_4^{2-}-S was observed for HABiTS Stage 2 when compared to 1.21 mg SO_4^{2-}

-S mg^{-1} NO_3^--N produced from the control treatment. This result supports the lower NO_3^--N removal of HABiTS (45.7%) compared to the control treatment (53.6%).

The hourly studies show limited performance of HABiTS during continuous operation and reduction of NH_4^+-N loads. Enhanced removal within HABiTS is expected during continuous operation with increasing loading rates as discussed in the recovery from a low loading period during Phase III. The results for the idle studies also support this conclusion (Figure 5.15). After the first flush of an idle period of 30 days (Figure 5.15a), HABiTS Stage 1 was able to remove 39 mg d^{-1} of NH_4^+-N while producing 0.99 mg d^{-1} of NO_2^--N and releasing 220.1 mg d^{-1} of NO_3^--N. Similar results were observed for NH_4^+-N removal in the control column (44 mg d^{-1}) while much less NO_2^--N (0.06 mg d^{-1}) and NO_3^--N (111 mg d^{-1}) were produced showing the small amount of clinoptilolite in HABiTS is able to store 2.7 times as much TIN as the control biofilter. A similar trend was observed for the 56-day idle study (Figure 5.15b) were HABiTS produced about 2.1 times as much TIN as the control. These results highlight the effect of desorption and subsequent nitrification during no loads within the HABiTS Stage 1 biofilter. It is expected that biofilms should be sustained longer within the HABiTS column due to the availability of N stored in the clinoptilolite. However, long idle periods could limit this effect to an extent since the Stage 1 biofilter would eventually dry out and possibly result in the desiccation of the biofilms. Biofilter dryness and its effect on performance was not monitored in this study, but the reduced sample volume (about one fifth of the volume expected under continuous operation) collected after the 31-day idle stud, could be an indicator of dry conditions within both biofilters. Results for Stage 2 showed complete denitrification for both treatments during the idle period of 31 days.

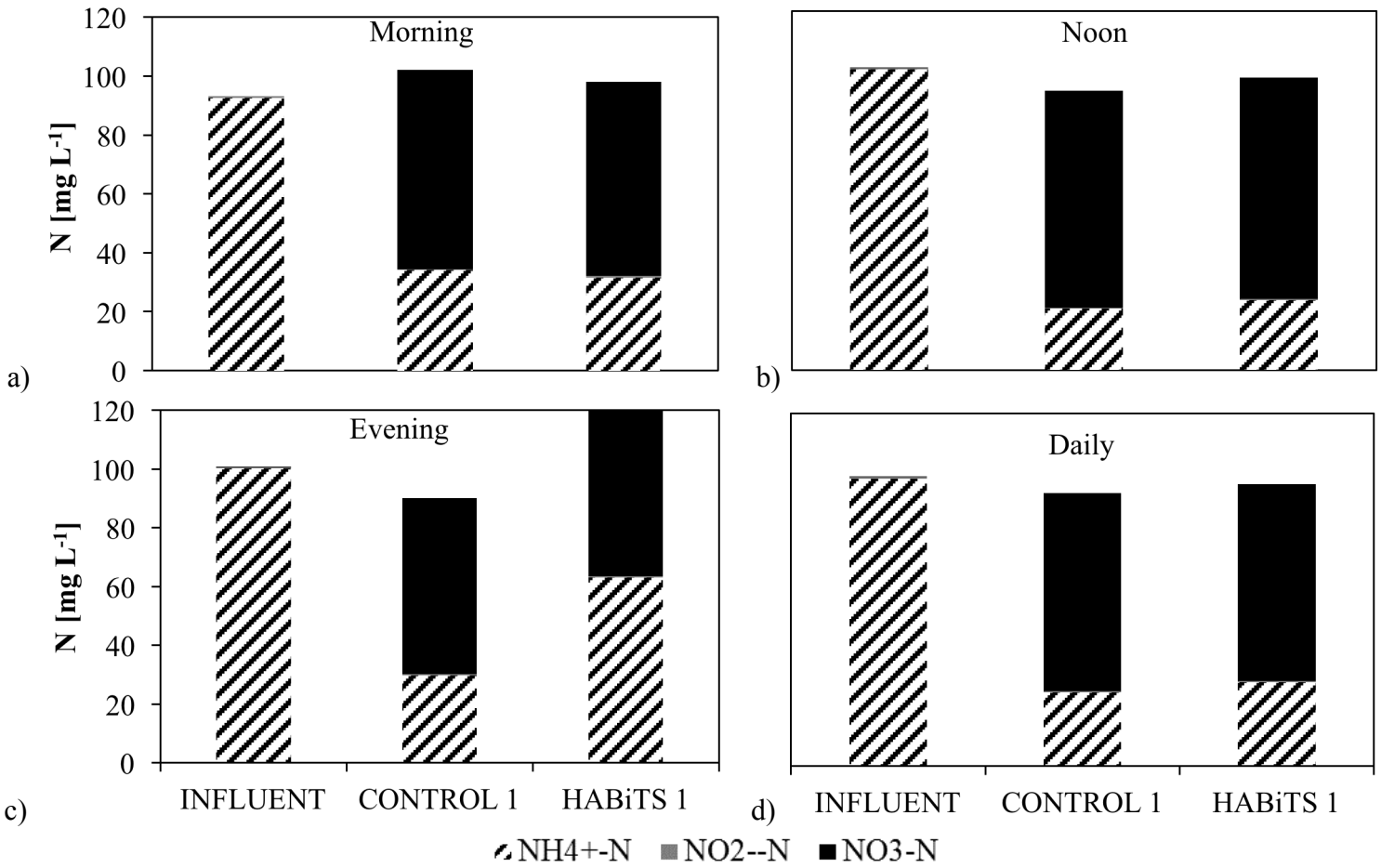


Figure 5.11: N species concentrations for 6am (a), 12pm (b), 6pm (c) and daily average (d) for Phase III hourly studies for Stage 1 for both Control and HABiTS biofilter treatments.

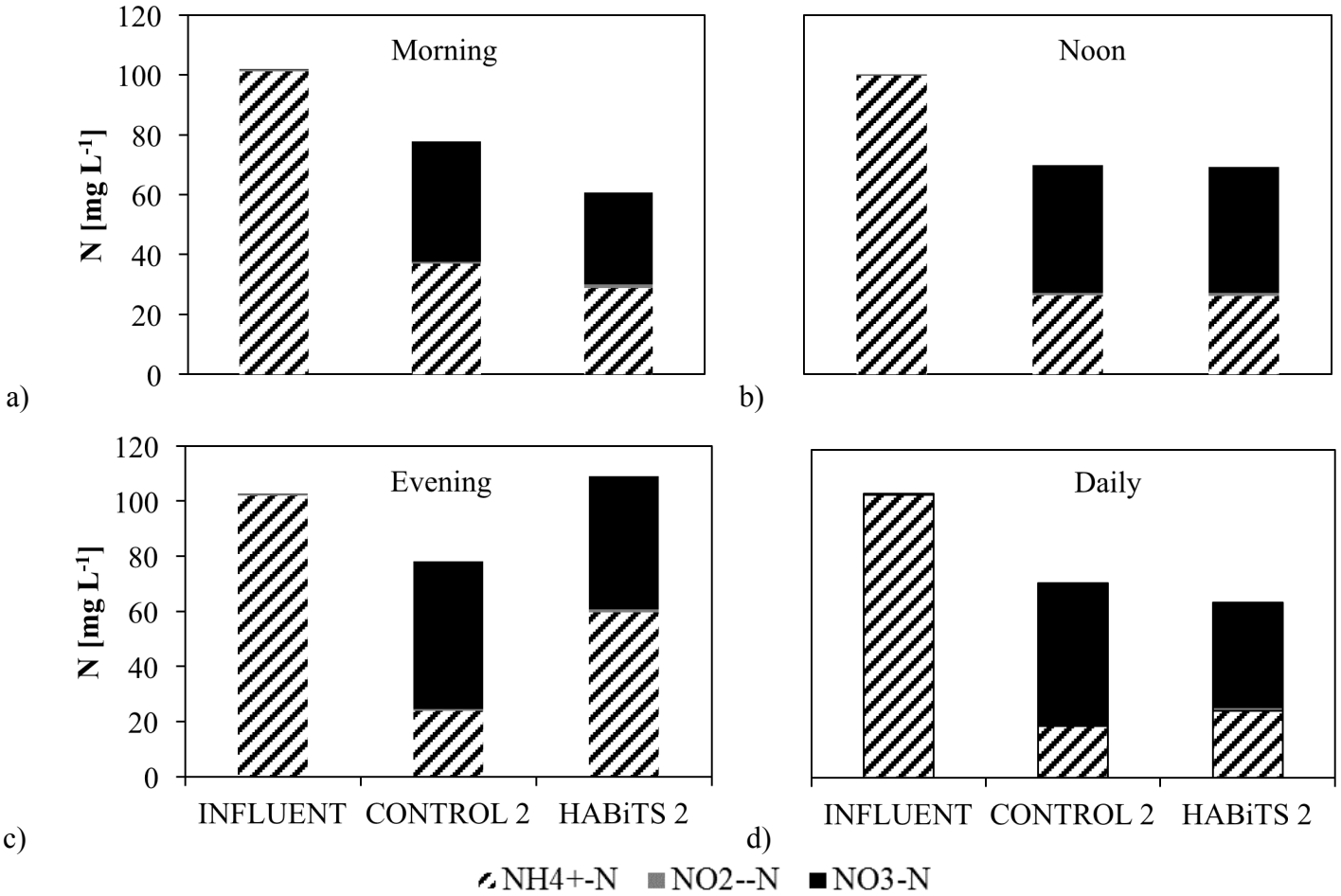


Figure 5.12: N species concentrations for 6am (a), 12pm (b), 6pm (c) and daily average (d) for Phase III hourly studies for Stage 2 for both Control and HABiTS biofilter treatments.

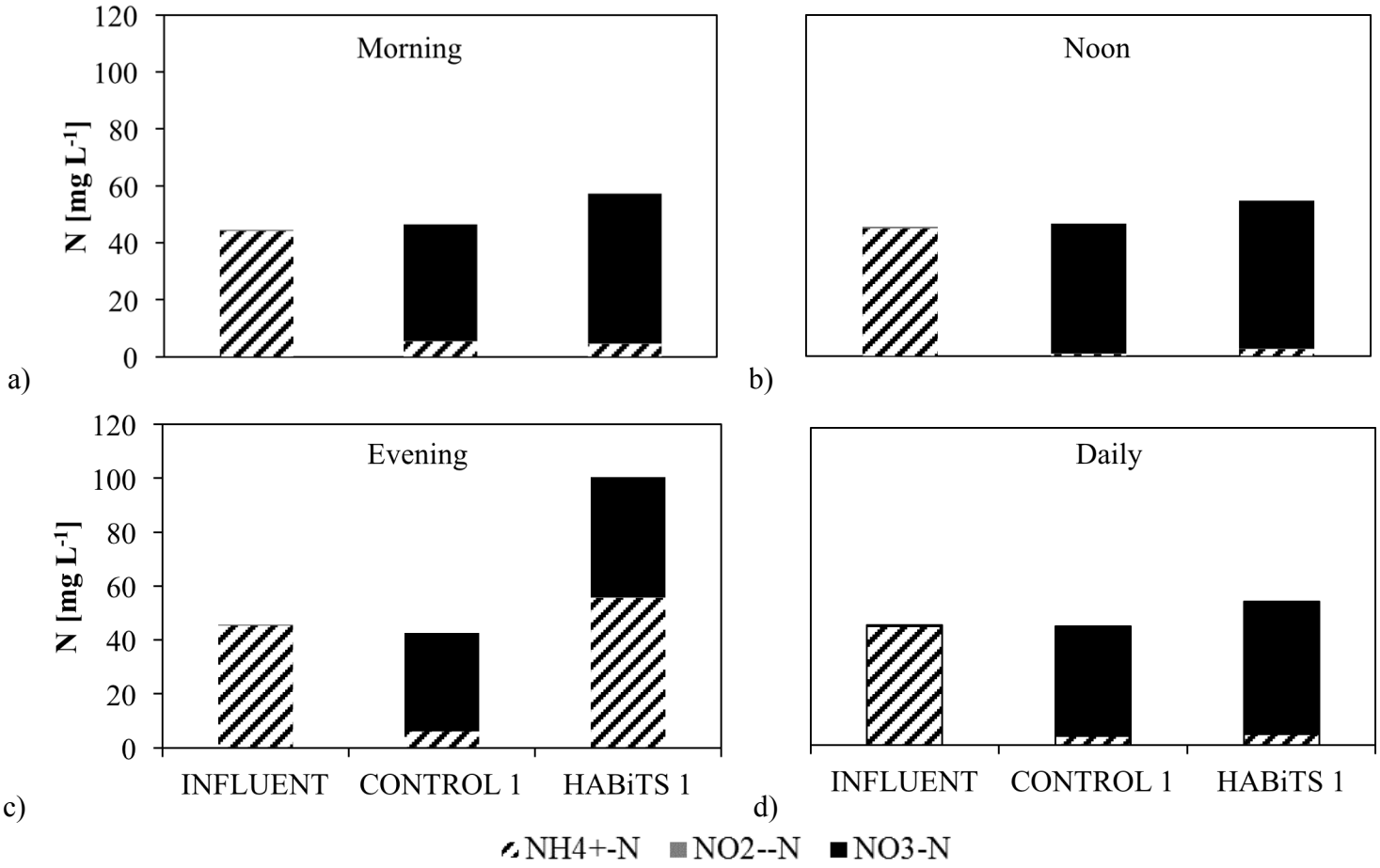


Figure 5.13: N species concentrations for 6am (a), 12pm (b), 6pm (c) and daily average (d) for Phase IV hourly studies for Stage 1 for both Control and HABiTS biofilter treatments.

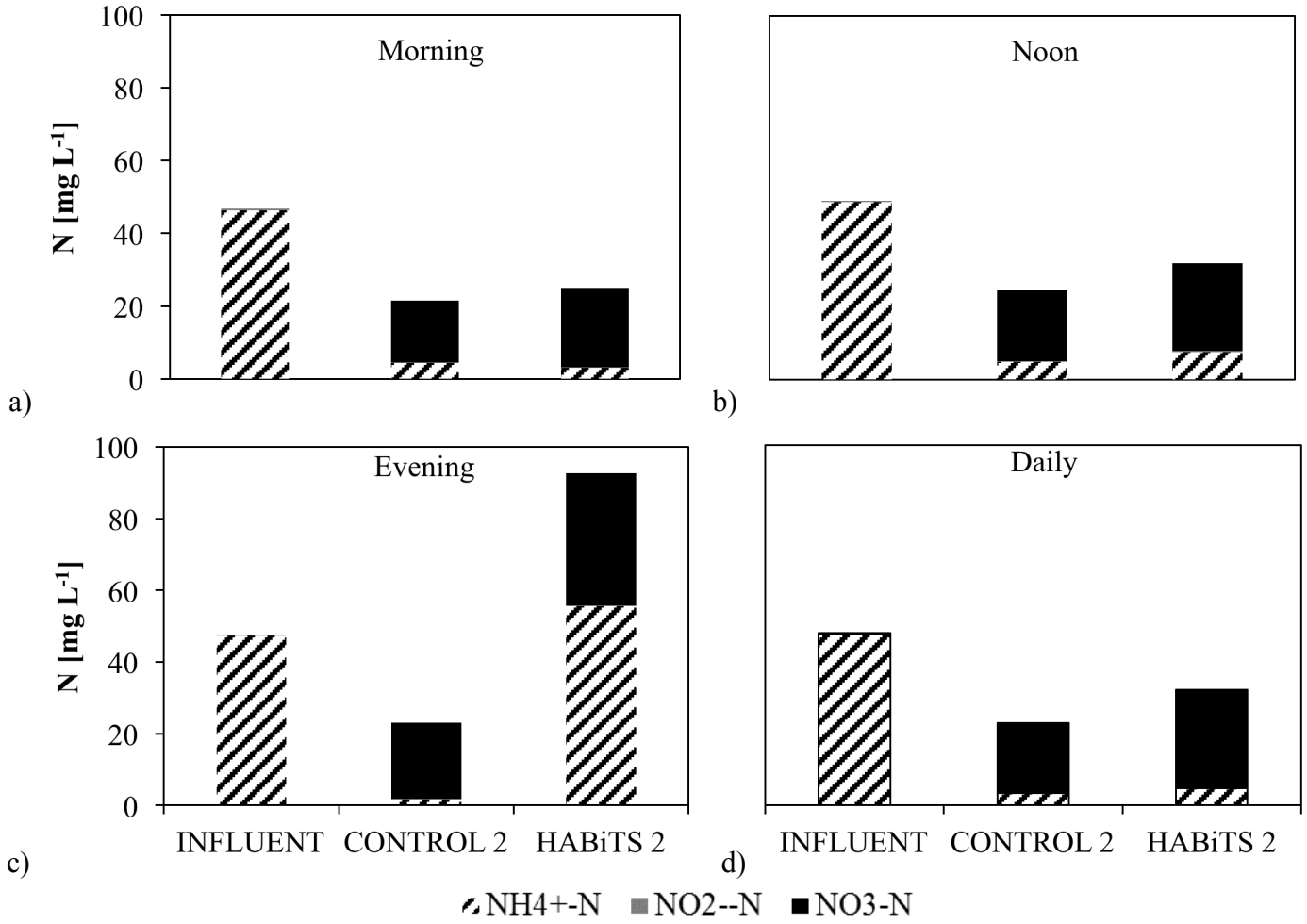


Figure 5.14: N species concentrations for 6am (a), 12pm (b), 6pm (c) and daily average (d) for Phase IV hourly studies for Stage 2 for both Control and HABiTS biofilter treatments.

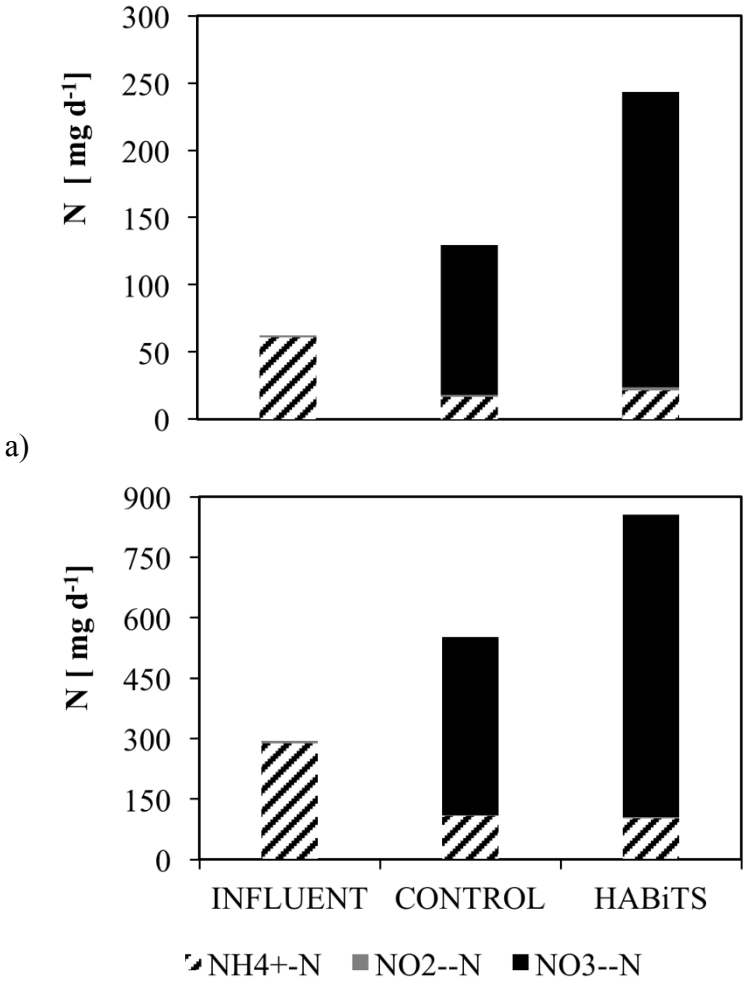


Figure 5.15: Stage 1 TIN concentrations at first flush after 31 days idle (a) and 56 days idle (b) periods.

5.3.2.6 Overall Performance of HABiTS and Control Column

The results for the cumulative removal (compared to the septic tank effluent) of N species and TIN for all phases are shown in Table 5.8. For Stage 1, the HABiTS control column showed higher removal of NH_4^+-N, lower production of NO_2^--N and higher production of NO_3^--N when compared to the control column. These results indicate enhanced nitrification possibly due to the incorporation of the IX media. The results for Stage 2 show similar NH_4^+-N removal for both columns, while higher NO_2^--N production and lower NO_3^--N production was observed for HABiTS when compared to the control. Evaluating the treatments in terms of overall TIN removal

shows better performance was achieved for HABiTS removing approximately 1.41 g of N more than the control treatment. Under the current operation of 1.3 L d^{-1} and average concentrations of 50 mg L^{-1} NH_4^+-N in the influent, this difference results in approximately 22 days of loads over the 487 day study period that where mitigated by HABiTS and not the control treatment.

Table 5.8: Cumulative removal of N species and TIN during all phases for HABiTS and control treatment.

	Phase	Control Stage 1	HABiTS Stage 1	Control Stage 2	HABiTS Stage 2
NH_4^+-N (g)	I	1.58	3.38		
	II	4.56	5.07		
	III	21.58	21.21	18.13	18.81
	IV	6.37	5.43	6.23	5.45
	Total	**34.09**	**35.09**	**24.36**	**24.25**
NO_2^--N (g)	I	-0.69	-0.23		
	II	-0.56	-0.27		
	III	0.10	0.14	0.10	-0.17
	IV	0.01	-0.01	-0.01	-0.10
	Total	**-1.14**	**-0.38**	**0.09**	**-0.27**
NO_3^--N (g)	I	-0.04	-0.02		
	II	-3.57	-4.20		
	III	-20.32	-20.30	-8.39	-5.27
	IV	-5.78	-6.62	-2.07	-3.32
	Total	**-29.70**	**-31.13**	**-10.46**	**-8.59**
TIN (g)	I	0.85	3.13		
	II	0.43	0.60		
	III	1.36	1.04	9.84	13.37
	IV	0.60	-1.20	4.15	2.03
	Total	**3.25**	**3.58**	**13.99**	**15.40**

*Removal rate calculated by: $(Influent - Effluent) \cdot Q \cdot d$
*- represent production of the compounds

5.4 Conclusions

Hybrid Adsorption and Biological Treatment System (HABiTS) are promising alternative for passive N removal in OWTS. Clinoptilolite was found to be the best medium for the nitrification stage of HABiTS due to its high IX capacity for NH_4^+. Langmuir, Freundlich, and IX

models showed a good fit for clinoptilolite adsorption isotherms. From the IX model it was found that clinoptilolite has a 11.69 mg g^{-1} NH_4^+-N exchange capacity even when in competition with other cations present in septic tank effluents. Results from the biofilter studies showed that the combined IX and nitrification in HABiTS can allow for faster startup, sustain variable loading, and achieve over 80% removal of NH_4^+ concentrations at loading rates higher than 0.34 m^3 m^{-2}-d^{-1} when compared to the conventional media column with 73% removal. Continuous operation under this loading rate, however, can result in clogging and will require more frequent backwashing than is generally considered practical for homeowners. Under lower loading rate conditions the biological treatment was enhanced and dominated the NH_4^+ removal processes in both columns. The addition of a denitrification stage decreased TIN by 53.54% and 40.97% under moderate loading rates 0.21 m^3 m^{-2}-$d^{-1.}$ Further decrease in NH_4^+-N loading rates resulted in high desorption of exchanged NH_4^+ in the clinoptilolite, resulting in lower TIN removal efficiencies (28.7%) when compared to the conventional control treatment (62%). The combined results for all phases show enhanced TIN removal in HABiTS, which was able to mitigate approximately 22 days of loads over the control treatment.

Based on these results the application of HABiTS for the enhancement of OWTS is expected to:

- Buffer transient loading into the system, where N is adsorbed during high loading rates, slowly released during low loading rates
- Sustain longer periods of idle time due to the slow release of N
- Allow for faster startup and potentially faster recovery from idle periods that result in bioregeneration of the clinoptilolite and restore the high adsorption capacity

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

Non-point sources (NPS) of pollution, are non-discernable, diffuse sources of pollution that are often difficult to localize and in turn mitigate. NPS can include stormwater runoff, agricultural/aquaculture wastes and wastes from small decentralized wastewater treatment systems such as conventional septic systems. In some watersheds, the mitigation of these NPS is imperative to reduce their detrimental effects on the water environment. This dissertation focused on two of the above mentioned NPS, aquaculture wastes and conventional septic systems and addressed novel treatment technologies for the mitigation of nutrients and trace organics produced by these sources. The research was divided in three chapters; their corresponding research questions, objectives and major findings and recommendations were:

1. *Chapter 3: Can the application of a UV-TiO_2 reactor reduce off-flavor compounds in RAS?*

- Investigate the performance of the UV-TiO_2 treatment under batch and continuous flow reactor configurations for the removal of GSM and MIB in RAS.

An immobilized TiO_2 and UV reactor was applied as a batch reactor in an operating RAS and as a continuous reactor in a bench-scale RAS. Improved performance on the removal of GSM and MIB was observed in the batch reactor since it allowed longer treatment time without the effect of constant production of the compounds in the biological treatment processes. Treatment performance of UV-TiO_2 was affected by GSM, MIB and dissolved oxygen concentrations.

- Evaluate and discuss the effect of the UV-TiO_2 treatment on water quality parameters and the possible impacts on biological wastewater treatment processes in RAS.

No harmful effects were observed on other water quality parameters when the UV-TiO_2 reactor was operated as a batch or side stream process.

- Recommendations

Due to the possible production of NO_2^--N and scavenging of carbonates, full-scale application of the UV-TiO_2 reactor is suggested at a point where NO_3^--N is low and prior to any addition of chemicals for alkalinity control. Furthermore the application of the UV-TiO_2 in an oxygen saturated area would positively affect the performance. The full-scale application of UV-TiO_2 is expected to reduce GSM and MIB in RAS, and in turn reduce depuration times resulting in lower demand for highly treated water, improved farmed fish product quality and cost savings. For successful application as a full-scale system in RAS, research into the intermediates produced in the degradation of GSM and MIB as well as their effect on fish health needs to be performed.

2. *Chapter 4: Does the application of T-SHAD in RAS improve nutrient and off-flavor compound removal when compared to conventional heterotrophic denitrification?*

- Determine the adsorption capacity of tire mulch for GSM and MIB.

Adsorption studies showed that the tire mulch has significant adsorption capacity for the off-flavor compounds but can be reduced by the presence of competing organic matter in RAS. A minimum EBCT of 8 hours is needed for removal of over 90% and 60% of GSM and MIB, respectively.

- Assess denitrification and off-flavor compound removal performance of T-SHAD in different reactor configurations in a bench-scale RAS.

When applied as a polishing step and operated under high EBCT (720 min) removal of 96.6% of NO_3^--N, 69.6% of GSM and no removal of MIB was achieved. The application of T-SHAD within RAS as denitrification side treatment for NO_3^--N removal at a lower EBCT (185

min) resulted in reduced NO_3^--N removal to 21% and showed no significant removal for off-flavor compounds.

- Compare T-SHAD to heterotrophic denitrification utilizing molasses as an organic electron donor and carbon source.

The T-SHAD results under 185 min EBCT showed no significant differences to the molasses fed heterotrophic UPBR with the exception of high productivity of SO_4^{2-} that resulted from SOD processes.

- Recommendations

High EBCT are recommended for T-SHAD to ensure both denitrification and removal of GSM and MIB. Due to the production and the possible impact of the accumulation of SO_4^{2-}-S, it is recommended that T-SHAD be combined with other heterotrophic denitrification treatments, possibly with treatment utilizing fish waste was electron donor. This combination could allow for increased EBCT in T-SHAD to remove GSM and MIB, reduced COD in RAS, low NO_3^--N concentrations and limit the SO_4^{2-}-S production. Further studies are required to assess toxicity of the tire mulch to aquatic species for the successful application of T-SHAD in full scale RAS.

3. *Chapter 5: What IX/adsorption medium best balances both NH_4^+ removal and cost effectiveness for application in OWTS?*

- Determine NH_4^+ adsorption capacity, hydraulic properties, cost and availability of various IX media for application in HABiTS.

Clinoptilolite was found to be the best medium for the nitrification stage of HABiTS due to its high IX capacity for NH_4^+. Langmuir, Freundlich and IX models showed a good fit for clinoptilolite adsorption isotherms. From the IX model it was found that clinoptilolite has a 11.69 mg g^{-1} NH_4^+-N exchange capacity even when in competition with other cations present in septic

tank effluents. The cost of clinoptilolite can be high, compared to other materials such as sand or expanded clay. However, due to the high exchange capacity only a fraction of the total mass of the biofilter would need to be filled with clinoptilolite, enough to withstand a 2 week OWTS loading while keeping concentrations of NH_4^+-N low.

4. *Chapter 5: How is the BNR process within HABiTS affected by IX?*
    - Compare the performance of HABiTS enhanced OWTS with nitrification/denitrification biofilters without an adsorptive medium under transient loading conditions.

Results from the biofilter studies showed that the combined IX and nitrification in HABiTS can allow for faster startup, sustain treatment under variable loadings, and achieve over 80% removal of NH_4^+ concentrations at loading rates higher than 0.34 m^3 m^{-2}-d^{-1} when compared to the conventional media column with 73% removal. However, clogging can occur under constant operation under this loading rate. Under lower loading rates the biological treatment was not limited and dominated the NH_4^+ removal processes in both columns. The addition of a denitrification stage decreased TIN by 53.54% and 40.97%, for the HABiTS and control treatment, respectively, under moderate loading rates 0.21 m^3 m^{-2}-d^{-1}. Further decrease of NH_4^+-N loading rates results in high desorption of exchanged NH_4^+ in the clinoptilolite, resulting in lower TIN removal efficiencies (28.7%) when compared to the conventional control treatment (62%).

5. *Chapter 5: Does the proposed hybrid system enhance the removal of TIN in OWTS under transient loading conditions?*
    - Compare the hourly performance of HABiTS with nitrification/denitrification biofilters without an adsorptive medium under transient loading conditions.

It was found that the performance of HABiTS varies with daily and hourly loads, particularly when recovering from periods of very low loading to high loadings and vice versa.

When recovering from low N loading periods, IX was observed for HABiTS and the biofilter out performed the conventional treatment in overall TIN removal. However, recovery from a high loading period resulted in release of NH_4^+-N stored in the clinoptilolite and increased production of NO_3^--N that could affect the performance of the denitrification stage.

- Recommendations

For full-scale applications, larger particle size for both the clinoptilolite and expanded clay would reduce the frequency of clogging observed at the beginning of this study. The addition of an alkalinity source in the nitrification stage of HABiTS, particularly in areas with low alkalinity water, is also recommended to reduce the consumption of the alkalinity source in the denitrification stage that could require replacement of the alkalinity source sooner than expected. Moreover, higher EBCT is recommended for the denitrification stage of HABiTS to mitigate the higher NO_3^--N loading from the enhanced nitrification in Stage 1.

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APPENDIX A LIST OF ACRONYMS

BNR- Biological Nitrogen Removal

DWTS- Decentralized Water Treatment Systems

EBCT- Empty Bed Contact Time

FDAC- Florida Department of Agriculture and Consumer Services

FDOH- Florida Department of Health

GSM- Geosmin

HABiTS- Hybrid Adsorption and Biological Treatment Systems

HES-Healthy Earth Systems

H-UPBR- Heterotrophic Upflow Packed Bed Reactor

IX- Ion Exchange

MIB- 2-methylisoborneol

MBBR- Moving Bed Bioreactor

N-Nitrogen

NPDES- National Pollution Discharge Elimination System

NPS- Non-point sources of pollution

OWTS- Onsite Wastewater Treatment Systems

P-Phosphorus

RAS- Recirculating Aquaculture Systems

SOD- sulfur oxidizing denitrification

TIN-Total Inorganic Nitrogen

TKN- Total Kedhjal Nitrogen

T-SHAD- Tire Sulfur Hybrid Adsorption Denitrification

USDA- United States Department of Aquaculture

USEPA- United States Environmental Protection Agency

UV- Ultra-violet

APPENDIX B SUPPLEMENTARY DATA FOR CHAPTER 5

The following sections include an additional experiment (B.1) and supplementary data for Chapter 5. The daily data showed in sections B.2, B.3, B.4 and B.5 was used to calculate average concentrations presented in Chapter 5.

B.1 Abiotic Column Adsorption Studies

An additional experiment was performed for the characterization of the clinoptilolite material to be utilized in HABiTS. The purpose of this experiment was to determine NH_4^+ breaktrough in an unsaturated clinoptilolite layered column. A 60 mm diameter column (KOFLO, Cary, Illinois) was packed with clinoptilolite (h= 6 cm) and expanded clay (h=5 cm). Synthetic septic tank effluent was distributed over the surface at a rate of 4 mL min^{-1}. After breakthrough, NH_4^+ was removed from synthetic septic tank effluent and flowed through to the column to observe desorption. Samples were collected periodically to determine adsorption capacity of the media layer. Average retention time within the column was determined by monitoring Cl^- concentrations in the effluent.

Effluent variation of cation concentrations from the abiotic clinoptilolite packed column during NH_4^+ adsorption are shown in Figure B.1. Even after 48 hrs NH_4^+ concentrations in the effluent were below 10 mg L^{-1}. Breakthrough was observed after 6 days of column run resulting in a total adsorption of over 3 g of NH_4^+. It is expected that accounting for biological processes in the model will likely retard the breakthrough of NH_4^+ when real septic tank effluent is used. Desorption kinetics were slower than adsorption (Figure B.2). After 24 hrs NH_4^+ concentrations where still > 80 mg L^{-1}. The desorption rate of NH_4^+ from clinoptilolite is expected to increase

due to BNR regeneration (Lahav and Green, 1997; Aponte-Morales et al., 2016).The average retention within the column was 51 mins.

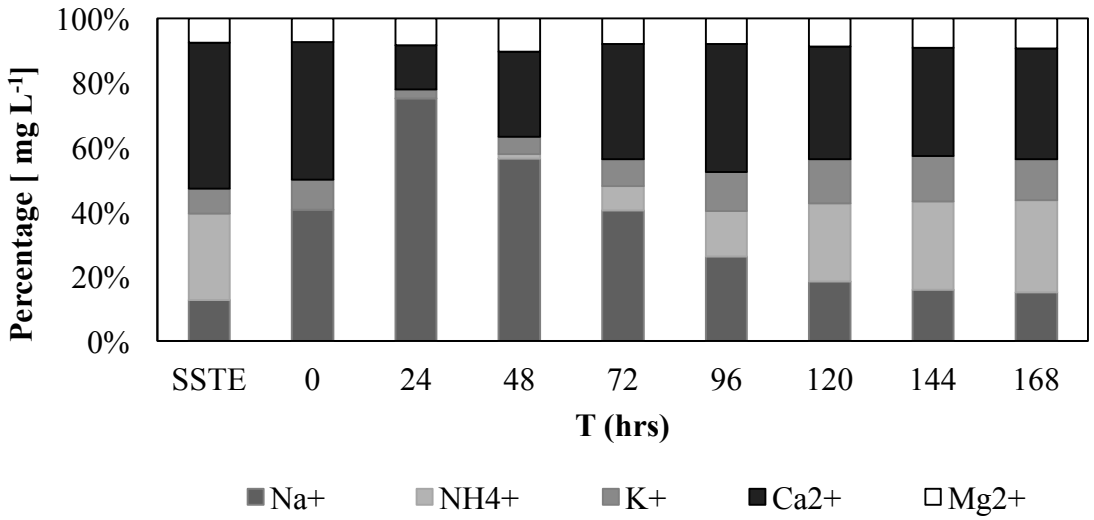

Figure B.1: Synthetic septic tank effluent initial concentration and variation of cations concentration in the effluent of the clinoptilolite-expanded clay column until breakthrough of NH_4^+.

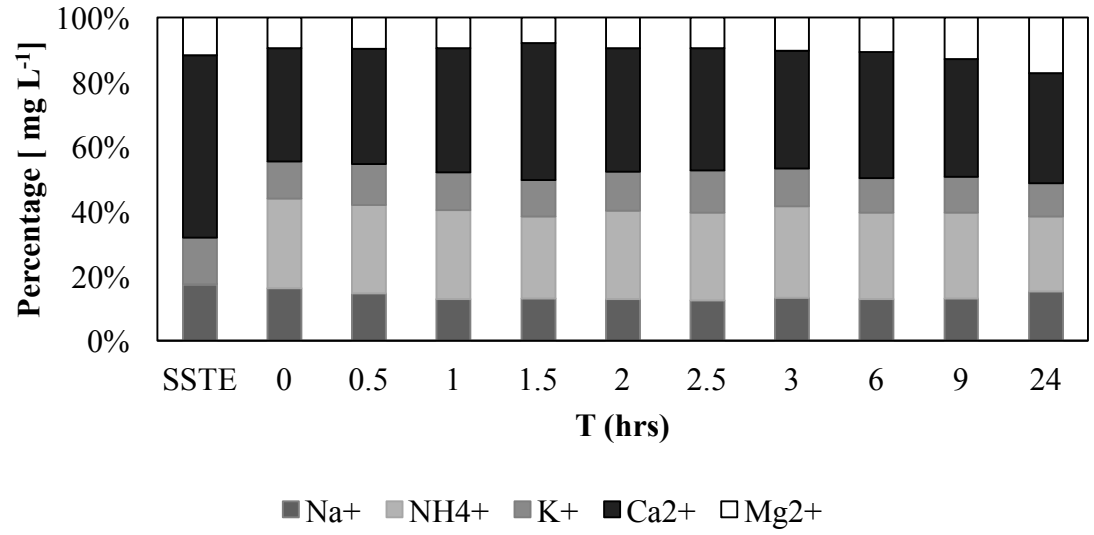

Figure B.2: Synthetic septic tank effluent initial concentration and variation of cations concentration in the effluent of the clinoptilolite-expanded clay column for desorption of NH_4^+.

B.2 Phase I Data

Table B.1: Phase I daily water quality data for the influent of HABiTS and control column.

Day	Na^+ (mg L^{-1})	NH_4^+-N (mg L^{-1})	K^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Cl^- (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	SO_4^{2-}-S (mg L^{-1})
0	103.56	88.43	20.78	184.44	30.97	120.10	2.64	0.09	3.76	29.21
1	101.61	89.85	19.47	185.05	30.60	117.99	2.14	0.09	3.97	34.39
2	104.44	85.08	21.35	189.98	33.31	117.93	2.30	0.08	3.91	35.27
3	100.85	82.64	19.55	182.59	31.81	110.70	3.08	0.08	3.51	28.43
4	100.05	82.70	19.14	173.28	31.04	117.21	1.99	0.08	3.01	33.18
5	98.40	75.74	19.31	164.66	31.94	113.89	1.49	0.06	4.22	33.96
6	119.79	71.20	19.72	155.80	31.86	73.26	0.61	0.03	1.41	17.06
7	100.60	71.37	19.55	177.05	33.02	74.33	1.03	0.02	1.88	19.50
8	97.13	68.83	17.24	111.39	32.50	73.84	0.53	0.02	1.87	16.89
9	94.10	65.31	17.90	117.71	34.74	70.62	0.55	0.03	1.77	16.98
10	94.99	61.47	17.57	99.22	33.39	71.17	0.55	0.02	1.89	19.09
11	92.17	55.80	16.83	87.05	30.93	67.26	0.76	0.00	1.84	18.49
12	96.08	51.48	17.08	132.98	30.06	68.35	0.46	0.04	1.50	19.99
13	92.08	48.61	15.61	138.18	26.18	65.17	0.41	0.00	1.45	16.85
14	88.92	58.28	17.73	149.14	33.59	62.07	1.01	0.04	1.61	20.29
15	88.33	47.62	15.69	121.97	30.99	60.42	0.46	0.04	1.42	11.54
17	78.10	49.60	15.39	139.59	31.10	52.07	0.49	0.00	1.94	12.91
20	81.29	46.45	15.47	128.20	33.84	53.00	0.32	0.00	1.77	18.44
22	77.40	57.33	22.20	122.43	36.35	55.35	0.08	0.01	1.72	24.15
27	82.57	59.49	34.67	148.04	39.15	53.73	0.07	0.02	1.33	19.23
29	87.91	58.50	35.65	155.84	36.95	55.78	0.07	0.00	1.75	21.50
31	84.72	62.70	42.94	168.19	36.20	57.00	0.11	0.02	2.46	27.02

Table B.2: Phase I daily water quality data for the effluent of the control column Stage 1.

Day	Na^+ (mg L^{-1})	NH_4^+-N (mg L^{-1})	K^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Cl^- (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	SO_4^{2-}-S (mg L^{-1})
0	79.55	47.52	21.35	210.95	21.78	97.60	2.24	2.12	2.47	35.38
1	94.55	73.42	21.52	197.65	27.80	114.84	1.79	0.38	3.18	33.93
2	100.72	79.24	21.19	191.33	29.87	118.37	1.74	0.08	3.36	34.19
3	102.37	77.82	20.62	176.85	29.12	115.08	0.68	0.05	3.05	
4	98.99	75.01	19.72	169.87	28.79	117.44	0.68	0.05	3.07	
5	98.36	66.97	21.44	165.07	29.41	118.16	5.70	0.13	1.85	
6	100.51	61.95	19.80	165.24	30.25	118.85	7.23	0.23	1.80	31.17
7	101.49	51.10	20.37	157.60	29.12	119.63	10.41	0.29	1.84	31.62
8	96.63	41.75	18.47	138.95	26.10	114.54	9.25	0.36	1.88	29.55
9	95.11	32.27	18.55	135.54	27.29	112.29	3.35	0.13	1.42	29.24
10	95.20	27.14	23.62	127.40	25.92	110.40	27.38	1.15	1.71	30.85
11	92.92	23.08	17.57	128.41	25.32	111.95	22.97	0.87	1.92	30.70
12	89.68	31.51	18.14	136.08	26.41					
13	91.16	16.70	17.16	130.15	25.42	105.12	25.03	1.00	1.61	32.80
14	87.20	30.81	17.98	128.76	28.00	97.14	13.49	0.75	1.86	29.46
15	88.33	27.48	18.39	135.77	28.44	99.05	20.68	0.85	1.77	30.39
17	82.53	21.02	16.20	138.79	29.53	61.07	16.43	0.67	1.63	23.29
20	83.11	12.86	15.07	138.79	29.88	88.29	14.52	0.43	1.60	33.83
22	83.07	18.11	19.44	139.79	28.81	80.71	27.17	1.23	2.40	35.72
27	78.80	28.93	33.30	145.21	32.52	83.23	12.97	0.66	2.10	43.57
29	82.69	27.44	34.03	158.36	34.74	81.65	8.43	0.34	1.95	41.87
31	78.18	31.90	39.37	159.43	32.60	86.18	11.28	0.63	2.55	45.99

Table B.3: Phase I daily water quality data for the effluent of the HABiTS column Stage 1.

Day	Na^{+} (mg L^{-1})	NH_4^{+}-N (mg L^{-1})	K^{+} (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Cl^{-} (mg L^{-1})	NO_2^{-}-N (mg L^{-1})	NO_3^{-}-N (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	SO_4^{2-}-S (mg L^{-1})
0	207.66	10.65	18.82	117.84	19.69	90.03	1.84	2.56	2.72	35.07
1	240.72	12.12	22.34	159.61	24.21	115.60	1.47	0.45	3.43	33.69
2	214.09	16.78	16.85	153.42	25.48	113.12	1.02	0.08	3.28	32.77
3	206.23	21.26	25.28	157.89	26.43	124.08	0.48	0.07	3.61	45.33
4	191.30	22.60	26.67	160.11	27.49	115.95	1.83	0.09	2.53	32.84
5	171.48	20.84	27.57	148.21	26.54	110.29	3.74	0.13	2.97	31.15
6	158.34	22.20	29.04	148.78	27.18	113.67	8.77	0.23	1.65	30.11
7	157.45	21.46	29.13	150.55	28.55	118.82	11.46	0.32	1.96	31.87
8	145.55	15.66	22.96	132.59	24.35	109.73	5.53	0.24	1.82	27.56
9	148.59	9.90	25.01	122.48	25.92	112.55	2.93	0.06	1.55	28.90
10	137.43	10.07	24.19	118.95	24.73	111.40	8.15	0.18	1.80	31.13
11	127.20	9.99	22.96	112.75	23.56	109.79	17.14	0.62	1.88	30.07
12	116.75	7.48	23.04	114.03	22.75	100.32	16.51	0.62	1.65	32.14
13	134.23	6.66	21.57	124.45	20.33	106.18	0.79	0.03	1.34	33.17
14	129.43	6.60	23.86	119.45	26.65	100.52	4.73	0.05	1.44	30.35
15	117.09	10.01	22.06	124.76	27.05	98.50	3.98	0.02	1.59	31.61
17	103.51	11.20	23.49	131.18	30.74	80.36	0.26	0.00	2.10	32.94
20	105.42	6.77	25.19	145.02	29.88	82.72	0.43	0.00	1.88	30.06
22	88.32	12.66	20.98	123.96	27.68	68.77	5.57	0.19	2.30	29.10
27	96.56	9.77	31.76	133.86	29.42	96.63	2.69	0.12	2.10	49.37
29	116.80	15.66	30.78	152.28	33.63	81.31	0.49	0.00	2.93	38.43
31	101.77	16.21	29.00	154.39	31.62	83.84	0.18	0.02	2.05	41.52

B.3 Phase II Data

B.3.1 Phase II Data for the Influent of HABiTS and Control Column

Table B.4: Influent daily nutrient concentrations for Phase II.

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
0	42.93	0.86	0.00	43.79	50.66	94.46	3.02	3.84
2	108.93	0.87	0.04	109.84		49.88	2.96	3.75
4	44.55	0.85	0.05	45.46			2.86	
7	43.30	0.82	0.03	44.15			2.44	
9	52.61	0.12	0.13	52.86	3.82	56.67	3.02	3.37
11	60.29	0.69	0.03	61.01			2.89	
14	62.90	0.03	0.03	62.96			3.06	
16	71.01	0.72	0.02	71.76	1.90	73.65	3.25	3.77
18	72.52	0.00	0.04	72.56			3.70	
21	63.38	0.06	0.04	63.48			3.00	
23	69.39	0.06	0.04	69.48	2.05	71.53	3.55	3.51
28	67.85	0.06	0.06	67.97			3.47	
30	54.04	2.10	0.10	56.24			2.87	
37	7.83	37.73	1.07	46.63			3.97	
40	33.22	5.51	0.07	38.80			2.32	
42	46.49	0.04	0.02	46.55			2.54	
44	51.96	0.13	0.03	52.12	5.40	57.52	4.11	4.42
46	60.87	0.94	0.06	61.88			4.51	
49	66.22	0.06	0.07	66.35			4.62	
52	69.59	0.40	0.09	70.08	5.27	75.35	2.56	4.00
54	67.84	0.96	0.02	68.82			2.93	
57	58.80	0.50	0.03	59.32			3.16	
59	53.42	0.63	0.03	54.08			3.25	
61	45.55	0.09	0.03	45.66			3.00	
64	46.61	0.93	0.03	47.57			3.49	
66	46.48	0.40	0.03	46.91			2.25	
68	53.13	0.65	0.09	53.86	1.96	55.82	2.76	4.42
71	58.04	0.64	0.03	58.71			4.00	
73	61.35	0.93	0.00	62.27	6.67	68.94	4.30	4.71
76	68.46	0.95	0.04	69.45			4.24	
78	66.17			66.17				
80	69.98	1.28	0.02	71.28		57.48	4.68	4.71

Table B.5: Influent daily cations and anions concentrations for Phase II.

Day	Na^+ (mg L^{-1})	K^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Cl^- (mg L^{-1})	SO_4^{2-}-S (mg L^{-1})
0	86.33	36.74	142.47	37.29	98.08	135.60
2	79.29	26.46	144.39	37.43	91.99	134.12
4	83.68	28.61	144.18	37.53	92.25	94.64
7	83.46	28.61	140.04	37.30	92.86	28.89
9	84.46	25.23	144.96	38.59	91.34	61.91
11	83.30	24.47	148.42	39.52	90.68	64.08
14	81.41	21.88	139.59	36.56	88.66	50.68
16	79.01	23.11	140.87	37.54	87.29	44.67
18	78.55	22.68	145.33	38.88	85.02	104.02
21	74.57	21.20	141.20	37.31	81.11	20.09
23	77.74	19.78	142.34	37.91	84.07	22.52
28	76.34	18.18	135.00	36.34	83.32	18.72
30	77.80	18.30	135.54	37.46	81.40	112.15
37	76.34	19.91	121.77	33.10	85.23	108.84
40	80.01	20.59	129.20	39.55	88.96	110.59
42	79.95	18.37	131.56	43.02	88.16	111.99
44	76.41	16.44	138.13	46.60	83.78	107.12
46	78.85	16.50	137.71	46.98	84.53	106.64
49	78.26	17.30	131.11	53.14	83.75	96.35
52	76.72	16.93	141.06	51.75	81.35	108.87
54	80.79	16.69	139.55	45.66	85.23	107.46
57	85.73	17.18	138.53	42.08	91.15	81.54
59	87.61	17.61	146.54	41.41	93.02	108.72
61	84.08	31.52	134.13	36.13	90.88	132.14
64	88.76	57.38	145.75	35.94	94.70	140.45
66	88.79	64.51	146.52	34.82	96.32	171.18
68	90.10	74.89	152.15	35.24	96.89	149.67
71	90.51	80.72	150.58	33.50	97.25	145.31
73	83.30	66.47	145.69	31.04	89.88	63.80
76	86.67	63.77	149.28	32.67	92.44	116.64
78	84.99	55.23	147.94	33.61		
80	83.83	49.45	149.36	35.16	88.79	35.47

B.3.2 Phase II Data for the Effluent of the Control Column Stage 1

Table B.6: Control column Stage 1 daily effluent nutrient concentrations for Phase II.

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
0	16.14	0.21	65.70	82.05	3.06	85.12	1.07	1.71
2	29.61	2.79	12.13	44.53	2.80	47.33	1.81	2.28
4	26.24	10.10	4.98	41.32			2.08	
7	14.85	14.71	7.50	37.05			1.96	
9	16.54	14.73	11.65	42.92		40.33	2.32	2.26
11	18.75	11.90	14.79	45.44			1.97	
14	18.99	13.50	22.91	55.41			2.18	
16	27.33	13.78	22.30	63.41	4.30	67.71	2.38	2.82
18	26.47	12.39	28.87	67.73			2.68	
21	17.90	10.28	38.46	66.64			2.72	
23	18.27	9.70	38.68	66.65	6.58	73.23	2.57	2.58
25								
28	9.25	3.59	53.48	66.33			2.20	
30	3.47	0.82	56.53	60.82			1.54	
32								
35								
37	1.67	15.65	24.64	41.96			1.32	
40	2.63	2.75	44.21	49.58			2.35	
42	2.26	1.64	48.30	52.20			2.11	
44	6.70	1.54	39.20	47.44	7.53	54.98	2.43	1.75
46	15.37	4.55	38.68	58.60			3.30	
49	13.13	4.08	49.68	66.90			3.27	
52	30.03	5.71	32.84	68.58		57.95	2.31	3.37
54	28.17	7.08	28.82	64.08			2.50	
57	18.73	0.62	31.11	50.46			2.26	
59	3.01	1.99	46.22	51.21			2.26	
61	5.95	0.67	32.34	38.96			2.22	
64	1.72	1.48	36.14	39.34			2.07	
66	0.97	0.20	39.93	41.10			1.77	
68	0.09	0.19	45.26	45.53		36.34	1.90	3.11
71	5.37	0.21	38.21	43.80			2.88	
73	2.84	0.27	47.11	50.22	5.50	55.72	3.08	3.53
76	7.60	0.30	41.41	49.31			2.98	
78	8.55			8.55				
80	6.12	0.33	49.41	55.86	25.87	81.73	3.24	3.32

Table B.7: Control column Stage 1 daily effluent cation and anion concentrations for Phase II

Day	Na^+ (mg L^{-1})	K^+ (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Cl^- (mg L^{-1})	SO_4^{2-}-S (mg L^{-1})
0	78.26	28.18	170.00	36.60	82.01	55.18
2	82.47	29.11	147.25	35.42	91.45	45.56
4	83.40	28.06	141.36	35.83	92.38	46.55
7	83.03	26.58	132.79	34.16	90.15	47.84
9	83.99	29.66	133.69	35.05	91.19	46.42
11	72.73	25.02	126.71	32.76	80.73	42.86
14	82.43	25.89	132.46	35.17	89.97	44.22
16	80.35	26.63	133.60	34.90	87.19	48.17
18	77.55	24.59	133.90	34.81	84.60	45.13
21	78.67	23.11	127.25	34.28	86.29	45.96
23	77.89	22.43	126.86	33.62	84.35	42.31
25						
28	76.24	18.24	121.62	32.25	84.10	39.50
30	75.84	17.44	117.07	31.39	83.81	43.31
32						
35						
37	54.60	12.20	118.96	23.86	62.09	40.00
40	79.32	17.07	110.60	31.69	89.59	39.73
42	79.01	15.41	110.99	34.55	88.88	39.79
44	72.00	14.78	106.84	34.72	78.02	35.46
46	77.32	15.70	120.89	41.33	85.65	39.41
49	81.45	16.87	118.60	46.76	90.39	42.36
52	78.95	16.93	128.49	47.26	85.32	39.72
54	80.32	16.50	126.44	43.13	84.33	39.00
57	85.08	16.50	126.50	40.15	90.34	39.50
59	85.30	14.28	124.33	37.14	91.28	39.40
61	84.96	21.75	122.92	33.90	89.91	42.83
64	87.42	44.60	125.47	32.11	93.13	52.78
66	87.36	56.95	130.80	30.88	94.87	57.99
68	88.95	62.11	128.67	30.30	95.44	61.56
71	89.88	76.92	130.51	29.46	97.34	62.98
73	85.99	72.37	126.77	28.39	92.51	59.55
76	83.33	61.80	122.95	27.40	88.64	54.63
78	87.61	59.35	131.55	30.11		
80	84.39	54.37	122.80	28.50	88.42	52.67

B.3.3 Phase II Data for the Effluent of the HABiTS Column Stage 1

Table B.8: HABiTS column Stage 1 daily effluent nutrient concentrations for Phase II.

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
0	15.07	0.47	111.72	127.26	7.10	134.36	1.18	1.61
2	17.85	1.71	13.25	32.81	0.94	33.75	1.72	2.21
4	17.11	4.73	5.27	27.11			1.62	
7	12.84	9.44	8.98	31.25			2.20	
9	12.00	11.91	14.64	38.55		33.88	2.25	2.36
11	11.60	12.78	19.52	43.90			2.14	
14	12.17	10.88	22.07	45.12			1.90	
16	13.95	7.44	19.98	41.37	6.39	47.76	2.76	3.00
18	12.43	9.06	25.78	47.28			2.74	
21	7.00	3.07	56.42	66.48			2.03	
23	5.59	3.19	59.71	68.49	6.86	75.35	2.03	2.09
25								
28	10.48	5.18	34.97	50.64			1.97	
30	7.04	0.85	50.28	58.17			1.65	
32								
35								
37	9.63	13.66	39.30	62.59			1.00	
40	6.27	2.61	43.40	52.28			2.13	
42	8.66	0.55	54.51	63.73			1.84	
44	8.29	1.02	43.49	52.81	10.24	63.04	2.62	2.15
46	9.40	1.95	45.22	56.56			2.97	
49	9.77	1.38	55.64	66.79			4.08	
52	13.53	0.20	46.47	60.20	15.15	75.35	2.36	3.35
54	11.32	0.82	52.96	65.11			2.60	
57	11.30	0.11	48.06	59.47			2.14	
59	5.66	0.38	65.56	71.60			1.88	
61	7.80	0.33	44.44	52.57			1.17	
64	7.31	0.54	47.12	54.97			2.01	
66	6.56	0.13	44.91	51.60			1.27	
68	9.76	0.23	29.66	39.65	4.72	44.36	1.81	3.03
71	6.15	0.20	48.91	55.26			1.88	
73	10.90	0.36	11.50	22.76			2.28	3.05
76	7.62	0.35	34.24	42.21			2.34	
78	14.08							
80	13.79	2.10	2.95	18.84	22.07	40.91	3.76	4.15

Table B.9: HABiTS column Stage 1daily effluent concentrations of cations and anions for Phase II.

Day	Na^{+} (mg L^{-1})	K^{+} (mg L^{-1})	Ca^{2+} (mg L^{-1})	Mg^{2+} (mg L^{-1})	Cl^{-} (mg L^{-1})	SO_4^{2-}-S (mg L^{-1})
0	122.41	34.95	208.11	39.37	89.26	59.31
2	99.76	32.92	146.59	31.67	90.80	49.26
4	96.02	30.21	144.81	33.40	92.64	47.99
7	88.04	31.20	133.39	32.52	87.89	48.57
9	87.89	31.57	129.67	32.53	89.02	47.98
11	90.77	33.84	138.24	34.89	91.11	50.75
14	89.55	35.99	136.65	33.91	92.59	48.94
16	90.36	37.60	141.92	34.50	88.13	47.44
18	87.69	40.31	143.84	34.82	85.97	45.43
21	80.07	31.62	117.52	30.70	85.94	43.32
23	78.73	30.01	112.61	29.87	84.89	41.41
25						
28	80.50	33.53	122.07	31.69	83.54	37.96
30	75.41	29.40	114.97	30.36	80.68	39.23
32						
35						
37	57.37	22.50	101.05	23.41	59.92	41.30
40	72.14	25.21	96.77	31.18	87.29	39.47
42	73.48	23.48	103.84	30.41	88.83	40.38
44	74.16	23.89	104.01	34.56	81.14	38.01
46	78.60	25.74	115.55	39.44	85.50	39.55
49	79.70	25.98	115.52	43.74	86.61	42.48
52	79.07	26.41	129.09	44.47	83.58	39.16
54	79.26	25.49	120.32	39.57	87.13	40.39
57	77.63	25.06	114.23	36.82	90.18	39.15
59	76.38	22.23	97.04	33.43	91.47	40.20
61	77.53	24.82	106.67	33.23	90.70	38.38
64	84.08	26.23	111.71	31.58	94.53	55.62
66	85.58	27.58	120.61	31.01	94.44	57.55
68	85.58	26.54	133.56	31.09	92.93	55.70
71	89.32	27.52	132.47	30.11	86.25	56.77
73	91.38	27.58	149.90	29.11	89.01	56.66
76	91.07	29.49	143.26	27.54	88.18	54.20
78	99.30	34.77	167.78	31.42		
80	95.97	33.73	171.33	31.07	90.11	52.21

B.4 Phase III Data

B.4.1 Phase III Data for the Influent of HABiTS and Control Column

Table B.10a: Influent daily nutrient concentrations for Phase III (day 87 to day 163)

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
87	89.69	0.81	0.00	90.49	17.69	108.18	4.49	9.75
89	76.99	0.57	0.00	77.57			3.53	
92	90.97	0.60	0.00	91.56			4.76	
94	90.00	0.76	0.03	90.78		83.93	4.05	5.12
95	94.05	0.78	0.00	94.84	5.41	100.24	4.57	5.22
96	99.14	1.29	0.09	100.52			5.60	
97	93.13	1.34	0.04	94.50			4.91	
98	93.70	1.60	0.00	95.30			4.22	
100	90.48	0.65	0.00	91.13			3.78	
102	92.15	0.49	0.00	92.64	8.49	101.13	4.78	4.98
106	91.23	0.80	0.00	92.02		73.79	8.57	5.10
109								
112	85.18	0.03	0.08	85.29			6.33	
114	81.65	0.78	0.00	82.43	13.40	95.84	2.31	5.02
117								
119	74.16	0.81	0.00	74.97			4.57	
121	60.75	0.55	0.00	61.30			3.74	
123								
126	55.69	0.37	0.00	56.06			4.98	
128	34.84	1.07	0.03	35.94			3.84	
130	40.65	0.48	0.02	41.15			3.28	
133								
135								
137								
140								
142	15.98	2.82	0.00	18.81	6.10	24.90	2.73	3.05
144	10.58	2.50	0.54	13.61			2.11	
147	12.70	2.95	0.11	15.76			2.55	
149	11.87	2.51	0.09	14.47			2.94	
151	16.68	0.59	0.08	17.35			1.06	
154	15.41	0.35	0.00	15.76			3.25	
156					24.73	24.73		4.64
158								
161	22.30	0.00	0.00	22.30			4.04	
163								

Table B.10b: Influent daily nutrient concentrations for Phase III (day 165 to day 248)

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
165	25.31	0.00	0.00	25.31			3.37	
168	19.97	0.00	0.00	19.97			3.98	
170	14.24	0.00	0.00	14.24	5.72	19.97	3.53	4.42
172								
175								
177	98.72	0.28	0.00	99.00			2.41	
179	96.18			96.18				
181								
183	96.52	0.42	0.00	96.94	0.66	97.60	4.10	4.00
185								
187								
189	96.51	0.00	0.00	96.51			5.48	
191	96.24	4.78	0.00	101.02			2.34	
193	93.02	0.78	0.00	93.80			2.21	
195	94.56	0.91	0.00	95.47			1.52	
197	97.54	1.19	0.04	98.78			3.56	
199								
201	99.74	0.66	0.00	100.41		99.80	6.07	3.96
203								
206	97.95			97.95				
208	90.92	0.50	0.00	91.42			5.29	
210	88.99	0.51	0.04	89.54			5.14	
213	87.60	1.32	0.09	89.02			5.14	
215	87.06	1.32	0.00	88.38		84.81	0.00	4.25
217	83.17	1.19	0.10	84.47			0.00	
220	85.44	1.59	0.00	87.03			0.00	
222	87.96	1.58	0.00	89.54			0.00	
224	96.32	1.39	0.12	97.84			0.00	
227								
229								
231								
234								
236								
238	71.11			71.11				
241	91.01	2.68	0.00	93.69			1.91	
243	99.66	1.46	0.00	101.12			2.09	
245	97.97	2.46	0.00	100.43			1.28	
248	93.62	0.35	0.00	93.97			2.68	

Table B.10c: Influent daily nutrient concentrations for Phase III (day 250 to day 336)

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
250	101.48	0.58	0.07	102.12			4.56	
254	102.14	1.40	0.72	104.25			4.00	
256	101.48	0.42	0.81	102.70			0.00	
260	99.16	1.92	0.00	101.08			1.28	
267	111.58	2.14	0.07	113.79			1.77	
270	102.81	1.59	0.64	105.04			1.59	
273	100.84	2.34	0.00	103.18			3.91	
277	101.76	2.34	0.00	104.10			2.38	
280	102.95	0.48	0.00	103.43			3.88	
284	107.46	0.42	0.00	107.89			2.03	
287	100.66	0.42	0.00	101.08			4.58	
291	103.43	0.32	0.00	103.75			2.14	
292	105.03	0.00	0.15	105.18			4.90	
293								
294								
298	107.11	0.22	0.04	107.38			5.27	
299								
301	103.75	0.30	0.10	104.15			5.00	
305								
306	106.78	0.96	0.09	107.83			5.73	
308	0.00	0.00	0.00				0.00	
313	92.07	0.00	0.00	92.07			2.59	
321	87.47	0.33	0.00	87.79			2.37	
329	101.57	0.30	0.65	102.52	12.72	115.23	0.00	4.65
332	90.24	0.45	0.00	90.69			2.07	
333	104.74	0.41	0.00	105.14			3.35	
336								

B.4.2 Phase III Data for the Effluent of the Control Column Stage 1

Table B.11a: Control column Stage 1 daily effluent nutrient concentrations for Phase III (day 87 to day 163).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
87	24.00	0.23	82.55	106.78	20.36	127.14	1.01	1.43
89	25.10	0.24	70.65	95.98			2.57	
92	22.45	1.64	80.83	104.92			2.37	
94	22.26	0.18	67.80	90.24	13.53	103.77	2.57	3.07
95	26.60	0.36	69.51	96.48		84.81	2.10	2.54
96								
97								
98	17.34	0.59	66.67	84.60			2.39	
100								
102	10.43	0.16	72.91	83.50			2.51	
106	15.80	0.34	63.65	79.78			4.82	
109								
112	14.52	1.12	74.10	89.74			7.01	
114	10.97	0.39	78.71	90.07			2.06	
117								
119	3.07	0.60	76.43	80.10			2.85	
121	0.66	0.65	73.99	75.30			3.03	
123								
126	0.08	0.02	65.19	65.29			2.76	
128	0.17	0.02	61.36	61.55			1.89	
130	0.12	0.02	55.33	55.47			3.05	
133								
135								
137								
140								
142	0.00	0.47	34.40	34.87			2.74	
144	0.00	0.46	28.38	28.84			2.04	
147	0.00	0.34	30.35	30.69			3.24	
149	0.00	0.40	29.20	29.60			2.97	
151	0.00	0.12	29.67	29.79			1.92	
154	0.00	0.08	28.57	28.65			3.41	
156								
158								
161	0.00	0.18	27.96	28.14			3.75	
163								

Table B.11b: Control column Stage 1 daily effluent nutrient concentrations for Phase III (day 165 to day 248).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
165	0.15	0.26	28.46	28.86			1.01	1.43
168	0.00	0.09	30.52	30.61			2.57	
170	0.12	0.00	21.54	21.67			2.37	
172							2.57	3.07
175							2.10	2.54
177	7.60	0.27	77.69	85.56				
179	7.18			7.18				
181							2.39	
183	6.01	0.00	90.12	96.13				
185							2.51	
187							4.82	
189	16.08	0.03	78.99	95.11				
191	9.12	0.00	84.83	93.95			7.01	
193	7.89	0.43	78.45	86.77			2.06	
195	7.06	0.30	82.72	90.08				
197	15.40	0.76	77.81	93.98			2.85	
199							3.03	
201	20.48	0.00	75.91	96.39				
203							2.76	
206	22.81			22.81			1.89	
208	12.41	0.41	84.54	97.36			3.05	
210		0.00	29.55	29.55				
213	10.08	0.00	76.65	86.74				
215	21.23	0.69	64.25	86.17				
217	38.88	0.60	52.13	91.60				
220	41.64	0.37	44.93	86.94			2.74	
222	37.10	0.38	51.38	88.85			2.04	
224	39.64	0.27	52.55	92.45			3.24	
227							2.97	
229							1.92	
231							3.41	
234								
236								
238	30.87	2.18	90.81	123.86			3.75	
241	45.51	0.27	32.71	78.49				
243	49.89	0.35	38.37	88.61			1.01	1.43
245	40.77	0.31	48.82	89.90			2.57	
248	28.70	0.89	65.06	94.65			2.37	

Table B.11c: Control column Stage 1 daily effluent nutrient concentrations for Phase III (day 250 to day 336).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
250	28.96	1.67	66.75	97.38			1.23	
254	28.78	1.03	63.11	92.92			0.00	
256	25.85	0.13	30.03	56.02			0.00	
260	24.80	0.00	73.40	98.20			1.43	
267	29.62	1.55	63.76	94.94			2.67	
270	38.66	0.78	50.42	89.86			0.00	
273	17.35	2.33	75.54	95.22			1.93	
277	22.86	1.31	68.46	92.62			0.95	
280	27.97	1.22	69.55	98.74			1.45	
284	49.94	0.32	9.26	59.52			1.32	
287	25.39	0.00	74.06	99.45			2.39	
291	26.33	1.20	79.30	106.83			0.00	
292								
293								
294								
298	91.92	1.08	3.41	96.41			1.55	
299								
301	27.25	0.89	78.21	106.35			0.00	
305								
306	12.10	0.43	68.23	80.76			1.55	
308								
313	10.51	0.08	79.51	90.09			0.00	
321	12.78	0.00	86.18	98.96			2.94	
329	28.28	1.27	40.65	70.21			0.00	
332								
333	42.23	0.10	53.37	95.71			1.90	
336								

B.4.3 Phase III Data for the Effluent of the Control Column Stage 2

Table B.12a: Control column Stage 2 daily effluent nutrient concentrations for Phase III (day 87 to day 163).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
87								
89								
92								
94								
95	22.48	0.64	65.24	88.35			7.56	
96	26.02	0.51	61.19	87.72			6.80	
97	24.42	0.38	55.82	80.61			4.92	
98	22.96	0.64	54.27	77.87			5.03	
100	16.17	0.24	47.48	63.90			2.30	
102	12.49	0.16	38.99	51.64	16.42	68.06	2.76	2.91
106	22.82			22.82	25.84	48.67		2.87
109	18.56			18.56				
112	13.67	0.15	32.93	46.75			2.76	
114	29.27	0.25	28.35	57.86		51.75	2.21	2.70
117	29.27	0.30	16.25	45.81			2.36	
119	7.54	0.65	30.27	38.46			1.82	
121	2.30	0.50	25.68	28.48			1.54	
123								
126	0.66	0.00	18.82	19.49			2.60	
128	0.32	0.03	16.07	16.42			2.15	
130	7.81	0.14	14.72	22.67			0.78	
133								
135								
137								
140								
142	0.00	0.00	13.78	13.78		20.63	2.80	3.03
144	0.00	0.00	5.70	5.70			2.12	
147	0.00	0.36	7.02	7.38			2.98	
149	0.00	0.00	3.53	3.53			3.38	
151	0.00	0.23	4.37	4.59			2.65	
154	0.07	0.27	4.51	4.86			3.15	
156								4.19
158								
161	0.37	0.00	0.00	0.37			2.88	
163								

Table B.12b: Control column Stage 2 daily effluent nutrient concentrations for Phase III (day 165 to day 248).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
165	1.04	0.00	6.67	7.71			3.22	
168	0.10	0.54	12.91	13.55			3.52	
170	0.00	0.39	5.70	6.09	2.55	8.64	3.27	4.15
172								
175								
177	14.08	0.24	26.27	40.59			1.74	
179	12.24							
181								
183	15.06	0.58	26.31	41.94	4.96	46.90	1.45	1.71
185	23.25	0.10	25.84	49.19			1.21	
187								
189	22.22	0.00	26.50	48.72			0.80	
191	17.76	0.00	26.89	44.65			0.84	
193	13.89	0.00	29.60	43.50			0.00	
195	12.04	0.11	23.53	35.69			0.00	
197	13.49	0.26	20.27	34.02			0.00	
199								
201	24.70	0.03	27.94	52.67	9.66	62.33	1.79	1.60
203								
206	31.53							
208	23.15	0.14	22.63	45.92			0.00	
210	15.09	0.00	29.55	44.63			0.00	
213	16.74	0.60	21.19	38.53			0.00	
215	16.85	0.51	28.02	45.38		44.26	0.00	
217	39.80	0.85	22.32	62.96			0.00	
220	53.97	0.75	43.06	97.78			0.00	
222	38.86	0.56	16.75	56.17			0.00	
224	41.79	0.87	17.86	60.53			0.00	
227								
229								
231								
234								
236								
238	8.05	1.88	0.00	9.93			1.76	
241	43.27	1.20	10.49	54.96			0.00	
243	47.12	3.08	7.16	57.36			0.78	
245	44.29	3.52	13.66	61.48			1.25	
248	32.98	2.35	26.40	61.73			0.00	

Table B.12c: Control column Stage 2 daily effluent nutrient concentrations for Phase III (day 250 to day 336).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
250	30.94	0.96	29.68	61.58			0.00	
254	29.99	0.95	32.19	63.13			0.00	
256	32.20	0.27	0.00	32.47			0.00	
260	28.47	2.25	34.10	64.83			0.00	
267	31.06	1.59	36.02	68.66			0.00	
270	36.59	1.79	27.96	66.34			0.00	
273	23.98	1.55	41.88	67.42			2.13	
277	25.64	1.06	35.91	62.61			1.15	
280	27.44	1.21	29.90	58.55			0.00	
284	32.67	1.33	30.22	64.22			1.59	
287	29.77	1.17	32.11	63.05			0.00	
291	24.27	1.19	34.98	60.44			0.00	
292	27.63	1.16	35.46	64.24			0.00	
293								
294								
298	90.29	0.29	0.04	90.61			5.79	
299								
301	46.73	0.89	30.21	77.84			2.08	
305								
306								
308								
313	18.36	0.51	37.92	56.79			0.00	
321	17.36	0.74	47.38	65.48			0.00	
329	30.22	1.08	44.46	75.76		75.56	0.00	4.73
332	28.20	0.00	40.27	68.47			2.23	
333								
336								

B.4.4 Phase III Data for the Effluent of the HABiTS Column Stage 1

Table B.13a: HABiTS column Stage 1 daily effluent nutrient concentrations for Phase III (day 87 to day 163).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
87	39.64	0.34	100.53	140.52	8.22	148.74	0.98	1.24
89	23.23	0.23	74.22	97.68			2.26	
92	18.80	0.18	99.70	118.68			2.93	
94	19.55	0.18	73.13	92.85		77.32	2.19	2.68
95	24.24	0.42	71.19	95.84	1.67	97.51	1.81	2.35
96								
97								
98	16.46	0.39	66.03	82.87			3.00	
100								
102	16.80	0.14	62.15	79.09			3.66	
106	20.29	0.26	67.83	88.39			8.21	
109								
112	18.28	0.34	71.25	89.86			5.11	
114	19.96	0.25	83.60	103.81			1.70	
117								
119	14.70	0.72	72.83	88.25			2.54	
121	13.37	0.76	72.25	86.37			3.36	
123								
126	9.99	0.04	70.90	80.94			4.57	
128	11.28	0.04	68.49	79.81			4.22	
130	9.35	0.03	68.85	78.23			3.39	
133								
135								
137								
140								
142	5.74	0.00	49.96	55.70			1.40	
144	4.67	0.72	47.93	53.32			1.81	
147	2.42	0.00	49.18	51.60			4.40	
149	1.51	0.00	46.53	48.04			2.45	
151	1.21	0.00	26.65	27.87			0.91	
154	1.36	0.00	45.78	47.14			1.65	
156								
158								
161								
163								

Table B.13b: HABiTS column Stage 1 daily effluent nutrient concentrations for Phase III (day 165 to day 248).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
165	3.46	0.07	44.54	48.08			0.55	
168	1.31	0.00	41.01	42.32			0.69	
170	0.47	0.00	39.50	39.97			1.44	
172								
175								
177	3.36	0.73	38.87	42.96			0.89	
179	6.06	0.24	84.15	90.44				
181								
183	1.94	0.00	71.61	73.56			2.90	
185								
187								
189	5.03	0.00	82.73	87.75			3.14	
191	2.65	0.15	85.62	88.42			1.73	
193	2.89	0.43	87.40	90.72			1.61	
195	5.95	0.23	85.68	91.87			3.14	
197	8.04	0.48	75.30	83.82			1.79	
199								
201	16.29	0.05	72.80	89.15			0.00	
203								
206	10.90							
208	21.16	0.07	62.69	83.92			3.32	
210	20.22	0.06	47.65	67.93			0.00	
213	21.79	0.28	50.61	72.67			0.00	
215	19.18	0.30	60.75	80.24				
217	16.28	0.49	77.77	94.55				
220	35.10	0.17	31.39	66.67				
222	27.96	0.23	48.46	76.64				
224	30.48	0.43	30.46	61.37				
227								
229								
231								
234								
236								
238	37.48	3.01	148.90	189.40			0.00	
241	25.21	3.40	10.82	39.43			0.95	
243	43.79	0.42	14.35	58.56			1.76	
245	44.36	0.27	22.14	66.78			1.15	
248	36.36	1.13	20.90	58.40			0.00	

Table B.13c: HABiTS column Stage 1 daily effluent nutrient concentrations for Phase III (day 250 to day 336).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
250	42.20	0.83	45.28	88.31			3.04	
254	39.63	1.11	51.56	92.30			0.00	
256	40.73	0.21	21.97	62.91			0.00	
260	31.97	1.02	51.89	84.88			0.00	
267	29.20	1.59	64.94	95.73			1.61	
270	31.30	0.99	53.24	85.52			2.16	
273	34.76	0.79	58.00	93.55			3.82	
277	42.96	1.59	39.34	83.90			2.71	
280	32.98	1.24	58.41	92.63			1.72	
284	33.51	3.47	62.98	99.95			1.39	
287	30.27	1.72	29.45	61.44			1.42	
291	31.32	0.00	79.15	110.47			2.41	
292								
293								
294								
298	37.60	0.87	45.38	83.86			3.02	
299								
301	29.33	0.83	74.43	104.60			3.40	
305								
306	24.64	0.15	57.35	82.14			0.91	
308								
313	40.34	0.39	39.55	80.28			0.00	
321	36.77	0.89	55.86	93.52			1.49	
329	23.64	0.00	63.11	86.75			2.36	
332								
333	42.70	0.49	69.36	112.55			1.78	
336								

B.4.5 Phase III Data for the Effluent of the HABiTS Column Stage 2

Table B.14a: HABiTS column Stage 2 daily effluent nutrient concentrations for Phase III (day 87 to day 163).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
87								
89								
92								
94								
95	18.38	0.61	68.97	87.96			1.10	
96	21.51	0.75	64.13	86.39			2.10	
97	19.57	0.65	51.92	72.13			2.28	
98	17.87	20.06	32.43	70.36			1.95	
100	13.64	12.86	28.16	54.66			1.75	
102	15.20	0.19	31.08	46.47	14.53	61.01	2.33	2.39
106	23.18	5.01	16.42	44.61	11.55	56.16	2.97	3.34
109	18.58							
112	12.06	0.39	14.32	26.77			3.63	
114	12.58	0.50	2.78	15.86			1.88	2.31
117	12.58	0.34	1.39	14.30			1.73	
119	13.96	0.38	16.66	31.00			1.88	
121	12.19	0.43	17.67	30.29			2.00	
123								
126	9.35	0.60	20.14	30.10			1.67	
128	7.39	0.30	16.82	24.51			0.80	
130	0.45	0.18	12.04	12.66			2.51	
133								
135								
137								
140								
142	4.67	1.71	5.91	12.29	7.19	19.48	0.00	0.75
144	2.03	0.23	0.62	2.88			0.00	
147	2.42	0.30	0.74	3.47			0.91	
149	1.31	0.51	0.61	2.43			0.87	
151	1.16	0.00	0.07	1.24			0.00	
154	1.36	0.00	0.00	1.36			1.03	
156					6.26	6.26		1.86
158								
161	0.15	0.00	0.00	0.15			1.21	
163								

Table B.14b: HABiTS column Stage 2 daily effluent nutrient concentrations for Phase III (day 165 to day 248).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
165								
168	1.90	0.00	0.00	1.90			0.00	
170	1.21	0.00	0.00	1.21	5.62	6.83	0.00	1.38
172								
175								
177	6.53	0.00	0.00	6.53			0.00	
179								
181								
183	7.70	5.25	6.23	19.18	6.70	25.87	2.41	2.48
185	6.84	3.81	15.27	25.92			0.00	
187								
189	6.06	2.55	8.77	17.38			0.00	
191	7.17	2.22	18.19	27.57			1.29	
193	6.99	2.00	21.61	30.60			0.00	
195	8.76	0.83	13.70	23.30			0.00	
197	11.26	0.26	1.92	13.45			0.00	
199								
201	18.31	0.83	7.42	26.57	5.13	31.69	0.00	0.48
203								
206	25.20							
208	23.18	0.04	15.92	39.14			0.00	
210	17.23	0.51	11.95	29.69			0.00	
213	21.71	0.18	15.70	37.59			0.00	
215	22.10	0.42	18.18	40.70	7.96	48.67		2.08
217	19.39	0.67	7.93	27.99				
220	24.53	0.90	17.98	43.41				
222	28.35	0.52	16.62	45.49				
224	25.99	0.49	2.90	29.38				
227								
229								
231								
234								
236								
238	22.07	2.96	0.00	25.02			4.63	
241	26.82	3.13	0.00	29.95			0.68	
243	36.52	3.82	0.00	40.35			1.45	
245	42.08	0.95	0.15	43.18			1.22	
248	39.85	0.56	0.29	40.70			2.04	

Table B.14c: HABiTS column Stage 2 daily effluent nutrient concentrations for Phase III (day 250 to day 336).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
250	45.84	1.46	9.11	56.42			1.05	
254	35.33	0.93	19.31	55.58			0.00	
256	29.99	0.12	0.00	30.10			0.00	
260	26.47	0.87	48.21	75.54			0.00	
267	25.79	1.27	38.82	65.88			2.57	
270	29.59	0.67	21.61	51.88			0.00	
273	33.47	1.73	15.01	50.22			1.29	
277	39.53	1.07	16.62	57.23			1.15	
280	36.44	0.78	25.08	62.29			2.37	
284	43.17	3.20	0.00	46.37			1.49	
287	31.39	1.09	30.98	63.46			1.97	
291	30.15	1.29	25.83	57.27			0.00	
292	29.59	0.68	39.01	69.28			1.60	
293								
294								
298	35.03	0.21	16.79	52.03			1.50	
299								
301	34.22	0.86	38.74	73.82			0.00	
305								
306								
308								
313	39.77	0.34	0.26	40.37			1.46	
321	41.65	0.80	26.27	68.73			1.46	
329	28.63	1.40	32.89	62.92	11.75	74.68	2.05	6.21
332	24.67	0.28	37.28	62.23			0.00	
333								
336								

B.5 Phase IV Data

B.5.1 Phase IV Data for the Influent of HABiTS and Control Column

Table B.15: Daily influent nutrient concentrations for Phase IV (day 339 to day 456).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
339	99.63	0.35	0.00	99.99	3.79	103.77	3.81	9.58
341	76.95	0.00	0.00	76.95	7.87	84.81	3.83	8.57
344	60.79	0.46	0.00	61.24			0.00	
346	52.07	0.00	0.00	52.07	12.47	64.54	3.79	
348								
350	49.72	0.31	0.00	50.02			3.46	
353	41.59	0.00	0.00	41.59			3.99	
355	32.02	0.20	0.00	32.22			2.20	
357	34.15	0.29	0.00	34.43			2.50	
360	34.43	0.00	0.00	34.43			0.00	
362	34.53	0.52	0.00	35.06			0.00	
364	37.38	0.35	0.00	37.73			1.92	
368	37.11	0.00	0.00	37.11			2.48	
371	40.93	0.51	0.00	41.43			3.67	
374	42.38	0.00	0.00	42.38			3.83	
378	44.94	0.00	0.00	44.94			0.00	
382	45.36	0.00	0.00	45.36			4.36	
385	48.08	0.20	0.00	48.28	1.93	50.21	0.00	9.50
389	45.68	0.17	0.00	45.85			0.00	
394	43.32	0.58	0.00	43.90			0.00	
397	46.59	0.63	0.00	47.22			2.09	
400	46.73	0.35	0.00	47.08			0.00	
408	45.46	0.21	0.00	45.67			2.01	
414	47.29	0.25	0.00	47.54			4.82	
431	50.69	0.17	0.00	50.86			2.59	
434	49.80	0.20	0.00	50.00			2.26	
436	50.36	0.24	0.00	50.61			2.66	
438	49.05	0.46	0.00	49.51			3.07	
442	45.53	0.52	0.00	46.04			2.50	
443	43.81	0.38	0.00	44.19			2.15	
446								
449								
450	45.55	0.46	0.00	46.01			3.26	
451	45.44	0.58	0.00	46.02			2.23	
456	47.48	0.59	0.00	48.07			2.96	

B.5.2 Phase IV Data for the Effluent of the Control Column Stage 1

Table B.16: Control column Stage 1 daily effluent nutrient concentrations for Phase III (day 339 to day 456).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
339	43.96	0.37	31.19	75.53			0.00	
341	16.23	0.00	42.23	58.45			0.00	
344	7.05	0.00	38.21	45.26			1.37	
346	2.35	0.32	37.02	39.69			0.00	
348								
350	0.13	0.00	32.68	32.81			0.00	
353	0.40	0.16	31.27	31.82			0.00	
355	0.18	0.00	27.22	27.40			0.00	
357	0.15	0.06	27.53	27.74			0.00	
360	0.09	0.20	28.27	28.56			0.00	
362	0.00							
364	0.20							
368	0.35	0.00	36.73	37.09			0.00	
371	0.35	0.00	39.55	39.91			2.35	
374	0.29	0.14	42.89	43.32			0.00	
378								
382	0.97	0.26	44.10	45.33			0.00	
385	0.76	0.00	44.23	44.98			0.00	7.29
389	0.52	0.32	43.59	44.43			0.00	
394	0.53	1.07	44.89	46.50			0.00	
397	0.10	0.27	45.01	45.38			0.00	
400	0.00	0.27	48.78	49.05			1.30	
408	0.26	0.10	45.17	45.52			1.54	
414	0.16	0.00	47.45	47.61			1.70	
431	5.13	0.00	41.44	46.57			3.39	
434	4.40	0.06	43.15	47.60			2.09	
436								
438								
442	0.54	0.23	44.11	44.89			1.80	
443	0.32	0.17	43.93	44.42			2.08	
446								
449								
450								
451	0.90	0.24	46.87	48.01			2.36	
456	3.29	0.25	43.40	46.95			2.33	

B.5.3 Phase IV Data for the Effluent of the Control Column Stage 2

Table B.17: Control column Stage 2 daily effluent nutrient concentrations for Phase IV (day 339 to day 456).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
339	35.25	0.28	31.82	67.34			1.41	
341	21.30	0.12	26.46	47.88			1.91	
344	9.75	0.00	25.98	35.73			2.35	
346	3.84	0.62	26.32	30.78			1.59	
348								
350	0.97	0.18	17.90	19.05			1.59	
353		0.00	21.32	21.32			0.00	
355	2.85	0.08	14.49	17.43			0.00	
357	2.50	0.14	16.64	19.28			1.79	
360	1.27	0.00	11.76	13.03			0.00	
362	0.86	0.23	10.16	11.25			2.02	
364	0.73	0.46	9.92	11.11			0.00	
368	1.69	0.00	7.95	9.64			0.00	
371	1.82	0.86	10.17	12.85			0.00	
374	1.92	0.72	10.33	12.97			0.00	
378	1.88	0.32	8.54	10.74			0.00	
382	0.79	0.00	5.76	6.55			0.00	
385	2.54	0.69	7.10	10.33	9.06	19.39	0.00	6.79
389	3.07	0.74	10.22	14.04			0.00	
394	1.63	0.47	9.29	11.40			0.00	
397	2.14	1.03	10.73	13.90			0.00	
400	3.50	0.93	10.62	15.05			0.00	
408	1.99	0.32	8.98	11.29			1.50	
414	1.16	0.02	9.12	10.30			2.16	
431	6.83	0.03	10.59	17.45			0.00	
434								
436	5.00	0.10	20.50	25.60	-26.31	-0.71	1.76	0.04
438	6.49	0.23	17.81	24.53			0.00	
442								
443								
446								
449								
450	1.13	0.23	17.09	18.45				
451								
456	0.33	0.48	14.10	14.90				

B.5.4 Phase IV Data for the Effluent of the HABiTS Column Stage 1

Table B.18: HABiTS column Stage 1 daily effluent nutrient concentrations for Phase III (day 339 to day 456).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
339	44.56	1.67	21.42	67.65			2.06	
341	28.13	1.40	37.93	67.46			1.32	
344	26.51	1.10	59.39	87.00			0.00	
346	22.73	0.80	23.43	46.96			0.00	
348								
350	14.34	0.00	29.71	44.05			0.00	
353	14.05	0.84	34.87	49.76			0.00	
355	20.99	0.22	19.42	40.64			0.00	
357	16.82	0.00	40.20	57.03			0.00	
360	9.18	0.12	44.59	53.89			0.00	
362	8.68	0.00	33.92	42.60			0.00	
364	6.99							
368	3.82	0.15	42.97	46.93			0.00	
371	3.48	0.14	49.75	53.37			1.21	
374	2.41	0.48	54.07	56.96			0.00	
378								
382	2.72	0.00	59.08	61.80			3.24	
385	3.27	0.12	52.73	56.11	2.69	58.81	0.00	6.56
389	2.33	0.00	52.29	54.62			0.00	
394	2.66	0.27	51.89	54.82			0.00	
397	2.69	0.18	46.24	49.11			0.00	
400	3.24	0.09	47.62	50.95			0.00	
408	6.11	0.10	49.69	55.90			1.44	
414	6.07	1.08	38.59	45.74			0.00	
431	7.48	0.11	57.13	64.71			3.29	
434	4.43	0.18	67.56	72.16			1.89	
436								
438								
442	3.77	0.21	51.98	55.96			1.80	
443	2.61	0.12	52.91	55.65			1.69	
446								
449								
450								
451	0.64	0.32	56.85	57.82			1.86	
456	1.16	0.47	61.56	63.19			3.36	

B.5.5 Phase IV Data for the Effluent of the HABiTS Column Stage 2

Table B.19: HABiTS column Stage 2 daily effluent nutrient concentrations for Phase IV (day 339 to day 456).

Day	NH_4^+-N (mg L^{-1})	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	TIN (mg L^{-1})	Org. N (mg L^{-1})	TN (mg L^{-1})	PO_4^{3-}-P (mg L^{-1})	TP (mg L^{-1})
339	36.78	0.39	21.65	58.83			2.57	
341	31.60	0.00	21.06	52.66			2.24	
344	22.97	0.00	16.94	39.91			2.13	
346	23.72	0.00	20.55	44.26			0.70	
348								
350	17.04	1.75	33.31	52.10			0.00	
353	19.24	0.22	8.67	28.13	9.34	37.47	2.30	7.41
355	13.94	0.00	6.28	20.22			0.00	
357	15.00	0.00	25.23	40.23			0.00	
360	7.64	2.00	33.53	43.17	9.46	52.63	0.00	3.72
362	5.71	1.94	18.06	25.71			0.00	
364	5.53	1.09	18.63	25.25			0.00	
368	3.16	0.85	14.91	18.92	8.46	27.37	0.00	5.36
371	3.25	1.93	24.50	29.68			0.00	
374	2.34	1.28	24.08	27.70			1.30	
378	3.17	1.23	29.13	33.53			0.00	
382	3.70	1.57	34.65	39.92			0.00	
385	4.35	1.73	27.52	33.60			0.00	
389	2.29	2.43	29.55	34.26	4.92	39.19	0.00	5.32
394	3.06	2.24	29.68	34.98			0.00	
397	2.98	2.24	21.90	27.12			0.00	
400	6.03	0.55	28.57	35.15			0.00	
408	4.43	0.01	17.94	22.38			0.00	
414	9.37	0.68	15.91	25.96			0.00	
431	8.85	0.10	7.64	16.60			2.03	
434								
436	8.57	0.15	20.79	29.51			1.53	0.04
438	5.79	0.13	23.64	29.55			1.66	
442								
443								
446								
449								
450	1.38	0.36	33.95	35.68			0.00	
451								
456	1.49	1.26	35.53	38.28			1.33	

B.6 Data for Hourly Studies

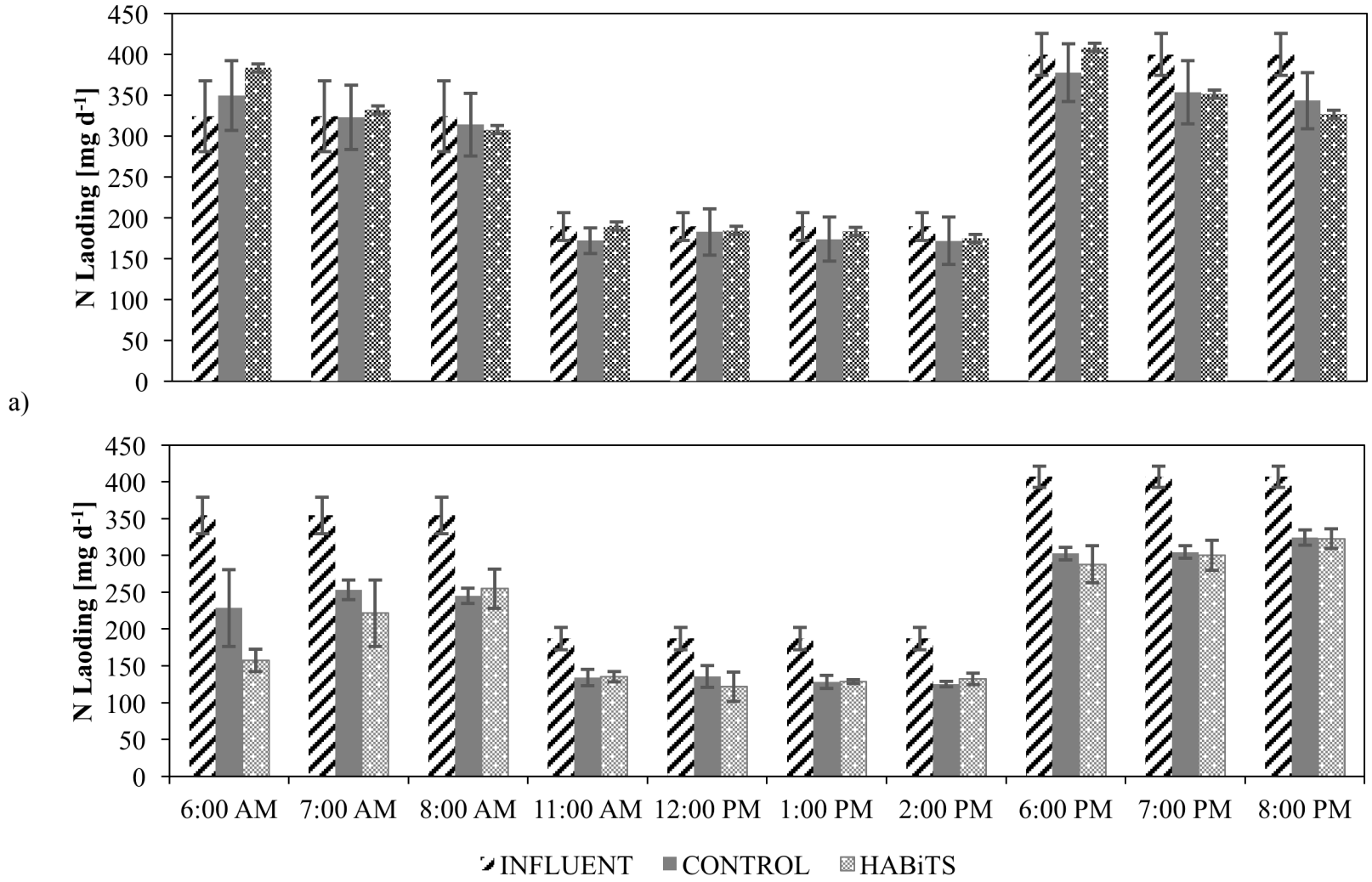

Figure B.3: N loading for Phase III hourly studies for Stage 1 (a) and Stage 2 (b) for both Control and HABiTS biofilter treatments

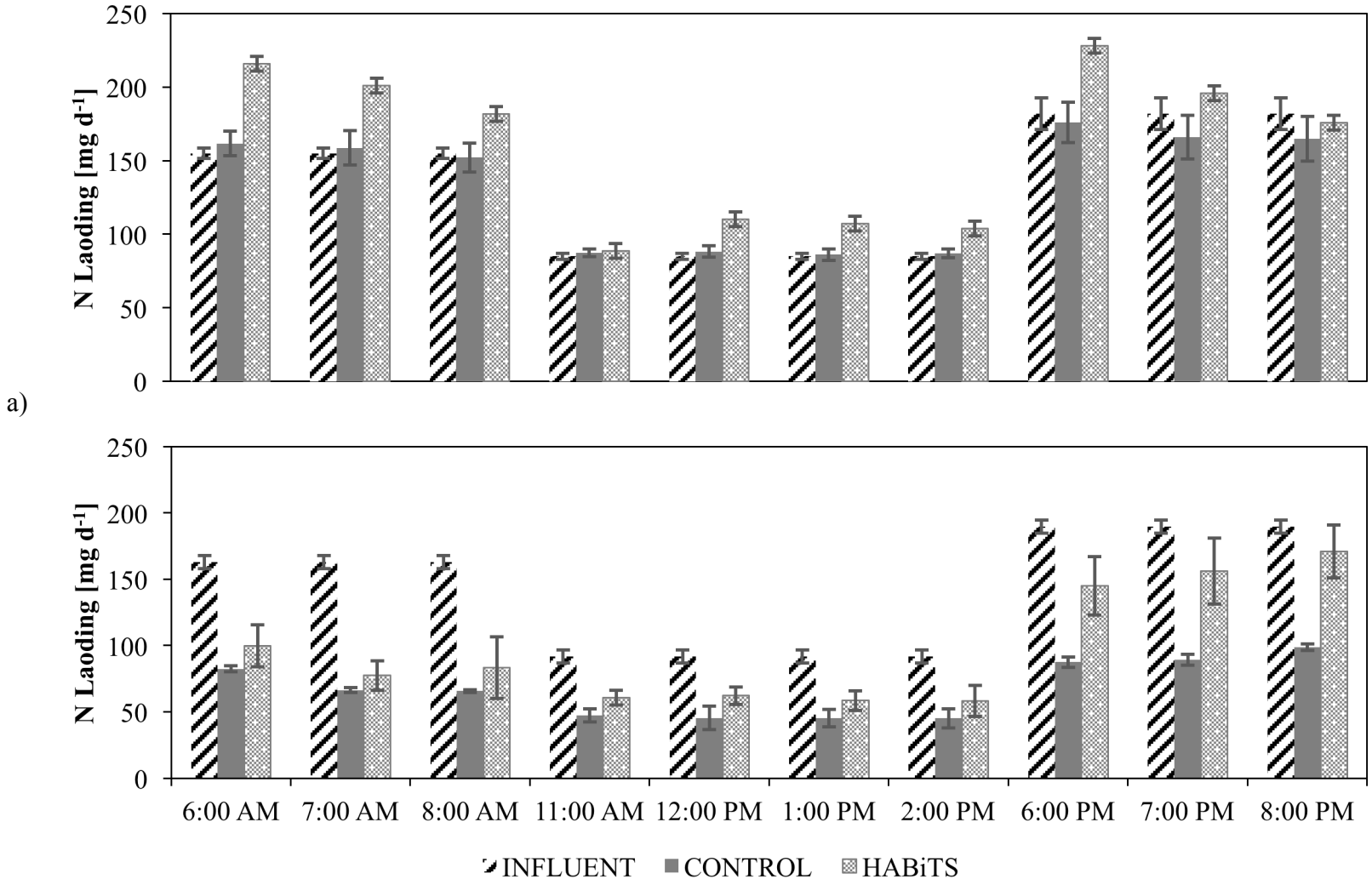

Figure B.4: N loading for Phase IV hourly studies for Stage 1 (a) and Stage 2 (b) for both Control and HABiTS biofilter treatment

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