Passive Nitrifying Biofilters for Onsite Treatment of Saline Domestic Wastewater

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Passive Nitrifying Biofilters for Onsite Treatment of Saline Domestic Wastewater

by

Daniel Arnulfo Delgado

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Civil Engineering Department of Civil and Environmental Engineering College of Engineering University of South Florida

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Dedication

To the two people that had the greatest hand in making me who I am, my mother and father, Guadalupe Delgado Acosta and Arnulfo Delgado. I love you and appreciate all the effort. We do the best we can.
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# Table of Contents

List of Tables ............................................................................................................................. iii

List of Figures ............................................................................................................................. iv

Abstract ....................................................................................................................................... vi

Chapter 1: Introduction .............................................................................................................. 1
  1.1 Introduction .......................................................................................................................... 1
  1.2 Research Goal, Objectives, and Task ................................................................................. 4
  1.3 Organizational Overview ..................................................................................................... 5

Chapter 2: Background and Literature Review .......................................................................... 6
  2.1 Septic Systems .................................................................................................................... 6
  2.2 Biological Nitrogen Removal (BNR) .................................................................................. 8
  2.3 Passive Nitrogen Removal Systems (PNRS) .................................................................... 10
  2.4 Recirculation in PNRS ....................................................................................................... 12
  2.5 Oyster Shells for Alkalinity and Microbial Attachment ..................................................... 13
  2.6 BNR in Saline Environments ............................................................................................. 14

Chapter 3: Materials and Methods ............................................................................................ 18
  3.1 Inoculum ............................................................................................................................ 18
  3.2 Experimental Program ........................................................................................................ 19
  3.3 Nitrifying Biofilter Media .................................................................................................. 20
  3.4 Nitrifying Biofilters .......................................................................................................... 24
  3.5 Treatment Train ................................................................................................................ 25
  3.6 Septic Tanks ...................................................................................................................... 28
  3.7 Analytical Methods ........................................................................................................... 29
  3.8 Data Analysis .................................................................................................................... 31

Chapter 4: Results and Discussion ............................................................................................. 33
  4.1 Nitrogen Transformations .................................................................................................. 33
  4.2 Phase 1 Single Pass Results .............................................................................................. 39
  4.3 Phase 2 Recirculation Results ........................................................................................... 42
  4.4 Mass Balance .................................................................................................................... 46
  4.5 Passive Saline OWTS Compared to Other Saline Wastewater and PNR Systems ......... 47
  4.6 Diurnal Flow ..................................................................................................................... 48

Chapter 5: Conclusions and Recommendations ......................................................................... 51
References................................................................................................................................. 54

Appendix A: List of Symbols, Acronyms and Abbreviations ......................................................... 61
List of Tables

Table 3.1 Oyster shell and lightweight expanded clay aggregate characteristics in biofilter 1 and 2. 23

Table 4.1 Average percent removal of influent TAN concentrations in phase 1 and phase 2 of the experiment. 36

Table 4.2 Average wastewater parameters for phase 1, single pass wastewater treatment. 39

Table 4.3 Average wastewater parameters for phase 2 with a 0.5:1 recirculation ratio. 42

Table 4.4 Biofilter 1 and 2 performance characteristics for phase 1 and 2 of the experiment. 46

Table 4.5 Saline wastewater treatment systems from other studies. 47
List of Figures

Figure 2.1 Conventional onsite wastewater treatment system with septic tank and drainfield ......6

Figure 2.2 Simplified process diagram of BHS-5.................................................................12

Figure 2.3 Simplified process diagram of pilot scale SANI process .................................16

Figure 2.4 Simplified process diagram of OWTS at Laughing Bird Caye National Park........17

Figure 3.1 Inoculum nitrifying seed reactor with carriers collected from Mote Aquaculture Research Park, Sarasota, Florida ..........................................................19

Figure 3.2 Oyster shell media after sizing, washing and drying.............................................21

Figure 3.3 Lightweight expanded clay aggregate media after sizing, washing and drying........22

Figure 3.4 Nitrification biofilters .........................................................................................24

Figure 3.5 Schematic diagram for experimental set up, phase 1 (single pass) .......................25

Figure 3.6 Photograph of experimental set up, phase 1 .......................................................26

Figure 3.7 Schematic diagram for experimental set up, phase 2 .........................................27

Figure 3.8 Photograph of experimental set up, phase 2 .......................................................28

Figure 3.9 Septic tank, influent, and effluent piping ............................................................29

Figure 4.1 TAN concentrations for raw wastewater, septic tank effluent and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during phase 1 and phase 2 of the experiment .............................................................34

Figure 4.2 Percent conversion of influent TAN concentrations through Biofilter 1 and Biofilter 2 in phase 1 and phase 2 of the experiment .....................................................35

Figure 4.3 NO\textsubscript{2} concentrations for raw wastewater, septic tank effluent and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during phase 1 and phase 2 of the experiment..........................................................37
Figure 4.4 NO$_3^-$ concentrations for raw wastewater, septic tank effluent and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during phase 1 and phase 2 of the experiment..........................................................38

Figure 4.5 Theoretical and actual mg/day needed for complete nitrification of influent NH$_4^+$-N..........................................................................................................................40

Figure 4.6 Nitrogen species profile during phase 1, single pass treatment.................................41

Figure 4.7 Theoretical and actual mg/day needed for complete nitrification of influent NH$_4^+$-N..........................................................................................................................43

Figure 4.8 Nitrogen species profile in septic tank influent and biofilters 1 and 2 influent and effluent during phase 2.................................................................45

Figure 4.9 Nitrogen species profile for raw wastewater (daily average), septic tank effluent, and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during morning (7-7:30am), noon (12:30-1pm), and night (7-7:30pm) dosing...............49
Abstract

The infrastructure to support the growing world population has polluted many water bodies with nitrogen from urban and agricultural runoff and failing wastewater systems, such as improperly sited and maintained septic systems. Septic systems present a low energy and maintenance option to treat domestic wastewater and is a treatment option that can be done far from a centralized grid. This further reduces cost, maintenance, and energy as it reduces the need to build and maintaining sewage piping and the infrastructure to move the water longer distances. However, these systems tend to fail when their upkeep is left to homeowners that may not realize they even have a septic system or that the septic tank needs to be evacuated of sludge every three to five years. One of the biggest shortcomings in these systems and their ability to remove nutrients from wastewater. This failure has more to do with the system design, as a conventional septic system does not have a designated nutrient removal step.

A growing global population and climate change has placed stress on resources such as water and energy. The practicality of using potable water for toilet flushing has come into question as it wastes fresh water and the energy used to treat and convey it. One solution for coastal communities to reduce freshwater and energy stress for wastewater treatment is to use more readily accessible seawater for toilet flushing. Many wastewater treatment processes use microbes to breakdown contaminants, and though halophiles thrive in high salt concentrations, most microbes involved in wastewater treatment are effected negatively by high salt concentrations.
This research evaluated wastewater treatment performance of a Biological Nitrogen Removal (BNR) system for a passive Onsite Wastewater Treatments System (OWTS) using seawater for toilet flushing. The research focused on nitrogen removal and transformations occurring in a septic tank and a nitrification trickling filter using oyster shell media as both a solid phase source of alkalinity addition and a surface for microbial growth of nitrifying bacteria. Two nitrification biofilters were constructed with both oyster shell and Lightweight Expanded Clay Aggregate (LECA) media at different volume percent ratios. Biofilter 1 was 50% oyster shell to 50% LECA by volume and biofilter 2 was 17% oyster shells to 83% LECA by volume. Biofilter performance was evaluated in two phases. Phase 1 was performed with a single pass treatment train and phase 2 was performed with a 0.5:1 recirculation ratio. Nitrified effluent was recirculated to the septic tank to promote pre-denitrification.

For objective one in comparing the performance of Biofilter 1 with 50% oyster shell media and Biofilter 2 with 17% oyster shell media, results showed no significant difference in the nitrogen removal performance of the two biofilters. Biofilter 1 removed 15% TN and 85% TAN while biofilter 2 removed 13% TN and 86% TAN. In phase 2, biofilter 1 removed 75% TAN while biofilter 2 removed 82% TAN. Both systems in phase 2 removed 36% TN. The high alkalinity already presents in the wastewater for both systems made the additional alkalinity in biofilter 1 less of a factor. Objective 2 examined the effects of recirculation on nitrogen performance for the biofilters. Phase 1 with no recirculation showed slightly better nitrification in both biofilters but an average decrease of 12% TN. For Objective 3, comparing this system with prior biological nitrogen removal systems, the 15 ppt salinity did not have a noticeable effect on the systems nitrogen conversion and removal performance. For future work, the salinity should be increased to 30 ppt to further examine salinity effects. A post nitrification
denitrification step should also be a subject for future work. Low COD concentrations in the biofilters nitrified effluent suggested the need for an electron donor for such a denitrification step. The best options for media addition as an electron donor for denitrification in saline wastewater based on sustainable resources available in areas where these systems are found should also be examined.
Chapter 1: Introduction

1.1 Introduction

The advent of industrial nitrogen led to an increase in anthropogenic nutrient pollution; The U.S. National Academy of Engineering has named managing the nitrogen (N) cycle one of the fourteen grand challenges for engineering in the 21st century. Anthropogenic sources of reactive nitrogen compounds include urban and agricultural runoff, failing sewage systems, incomplete wastewater treatment, and industrial discharges (USEPA, 2013; USEPA, 2019). Once reactive nitrogen enters and overpowers a water body several adverse effects to the environment, human health, and local economies follow.

Eutrophication is an overabundance of nutrients in a water body that leads to production of algae populations at a higher level than the environment can support (Glibert et al, 2005). The algae minimize light penetration needed for sea grass and other benthic plants. As the algae die, their decomposition consumes dissolved oxygen (DO) in the water. In addition, some types of algae can produce toxins that can cause rashes, stomach or liver illness, respiratory problems, or neurological effects to humans (USEPA, 2019). High levels of nitrate can cause Methemoglobinemia, also known as blue baby syndrome, resulting in the poorly oxygenated blood of infants. Additionally, ammonia and nitrite can be harmful to fish and other aquatic life (Florida Department of Agriculture and Consumer Services, 2019).

Septic systems are a type of Onsite Wastewater Treatment System (OWTS). Septic systems provide wastewater treatment for 20% of US homes and about the same for other
industrialized countries (Oldfield et al, 2020). In rural areas this percent is far higher due to a lower population density making centralized systems less cost effective (Gorman and Halvorsen, 2006). Though septic systems are widely used, their general design has not changed much since their first use in the 1860’s (Crites and Tchobanoglous, 1998).

This OWTS consists of a septic tank and drain field that provides solids and organics removal. Though some nutrient removal does occur in the septic tank and even in the drain field, a large percent of the nutrients remain in the wastewater and are transported to a receiving water body leading to non-point source pollution. A literature review along with several site studies conducted for the Florida Onsite Sewage Nitrogen Reduction Strategies Study (2015) found that conventional septic systems managed to remove 10% to 50% of the total nitrogen load. The resulting reduction depended on several factors in the soil and water table.

Advanced septic systems, such as Passive Nitrogen Removal Systems (PNRS), introduce processes to this treatment to improve the effluent water quality. PNRS are considered “passive” because they have no compressor for aeration and no more than one effluent pump (Smith et al., 2008; FOSNRS, 2015), thereby requiring little mechanical and electrical input to incorporate nitrification and denitrification processes in the treatment train. Nitrification converts ammonia to nitrate, and denitrification converts nitrate to $N_2(g)$ that can return to the atmosphere. Through its use of nitrification and denitrification, PNRS remove more total nitrogen (TN) from the wastewater and result in far less nitrogen pollution.

PNRS can be designed to recirculate a portion of the wastewater back into the treatment process. Major benefits of recirculation in PNRS are: 1) dilution of BOD in septic tank effluent thereby reducing competition between heterotrophs and nitrifying bacteria in the nitrification reactor to favor nitrifiers, 2) increases total nitrogen removal by bringing the nitrified effluent
back upstream of the nitrification processes where more organic electron donors are present for denitrification, 3) more electron acceptors for nitrification through improved aeration, and 4) less backflushing required due to more regular sheering of biofilm (Solomon et al., 1998; Klees and Silverstein, 1992; Safferman et al., 2004).

Potable water is commonly used to flush toilets, but in areas where freshwater sources are stressed such as in the Belizean Cayes (Kalivoda, 2017), potable water is sometimes replaced by gray water or saltwater. The use of saltwater for toilet flushing introduces issues in the wastewater treatment. Increased salinity will decrease activity coefficient leading to lower apparent rate constant (Lewis and Randall, 1921). For septic systems and PNRS that use microbes to treat wastewater the issues are more severe. The saline water can hinder microbial processes and cause the microbes to lyse (Omil et al., 1995; Lay et al., 2010). Thus, the microbes’ nitrogen removal capability decreases, and the effluent wastewater nitrogen concentrations increase.

The nitrification process requires about 7.14 gram of alkalinity (as CaCO₃) per gram of nitrogen (Xu, 1994; Tchobanoglous et al., 2003). One method to ensure sufficient alkalinity is present in PNRS is to add a solid source of calcium carbonate, such as oyster shells. Oyster shells have shown to be a good source of alkalinity, outperforming other common solid phase alkalinity additives (Sengupta et al., 2007). They have also shown to be a good growth media for denitrifying bacteria in the autotrophic denitrification process largely due to their rough surface, nanosized flake structure, and high surface area (Yoon et al., 2003; Sengupta et al., 2007, Tong et al., 2017).
1.2 Research Goal, Objectives, and Task

The goal of this research was to evaluate PNRS as a low cost, low complexity method for onsite BNR in water scarce regions where seawater is used for toilet flushing. Towards this goal, three specific objectives were identified:

1) Investigate the effect of oyster shell media on unsaturated biofilters performance for treatment of saline wastewater when compared with Lightweight Expanded Clay Aggregate (LECA) media.

2) Monitor the effects of biofilters and system performance for treatment of saline wastewater after recirculating biofilter effluent to the septic tank.

3) Compare prior studies of BNR systems at different levels of salinity to that of the studied systems.

Two biofilters were constructed using different ratios of oyster shells to LECA. Their ability to nitrify the saline wastewater was evaluated under similar operating conditions. The nitrification process consumes alkalinity and oyster shells are composed almost entirely of calcium carbonate, a source of alkalinity. Since improved nitrification performance could be due to increased alkalinity, one biofilter was constructed with just enough oyster shells to provide alkalinity to nitrify wastewater for two years. This equated to 17% oyster shells to 83% LECA by volume. A second biofilter was constructed with 50% oyster shells to 50% LECA.

To accomplish objective two a second phase, phase 2, was added following five months of single pass wastewater treatment, phase 1. In phase 2, half of the nitrified effluent was recirculated to the septic tank (0.5:1 recirculation ratio). During both phases, total nitrogen, total ammonia nitrogen, nitrate, and nitrite, along with other water quality parameters were monitored throughout the treatment train. The changes in the nitrogen species along with other
water quality parameters throughout the treatment train were compared with phase 1 of the experiment. The results were used to compare the system performance to that in the existing literature to complete objective three.

1.3 Organizational Overview

In this thesis, Chapter 2 explores the existing literature on OWTS including septic systems and PNRS. It includes a review of the literature on biological processes involved in nitrification and denitrification. The chapter looks at existing wastewater treatment systems that treat saline wastewater, their system orientation and performance, and the role oyster shells can play in this process.

The methodology involved in answering the research questions is presented in Chapter 3. This includes the steps in construction of a laboratory scale system, how the system was inoculated, how the system was operated to mimic a home septic system, and how the water quality throughout the treatment train was monitored.

The results after operating the two systems for several months are presented in Chapter 4. The results were analyzed to understand the N transformations occurring in the system, how recirculation affected this performance, and how the system compared to other wastewater treatment systems. The chapter is also used to report issues while running and analyzing the system.

The thesis closes with conclusions and recommendations in Chapter 5. The conclusions reflected on the research goals, objectives, tasks, and how the results support or repute the initial assumptions and offers recommendations for future research.
Chapter 2: Background and Literature Review

2.1 Septic Systems

Septic systems are a type of onsite wastewater treatment system (OWTS) that provides wastewater treatment for over 20% of households in the United States, adding up to 26 million individual wastewater treatment systems (USEPA, 2012; USEPA, 2014). The conventional septic system consists of the household sewage pipe discharging to a septic tank, the septic tank effluent is then discharged to a drainfield as shown in Figure 2.1 (Kerr, 1977).

![Figure 2.1 Conventional onsite wastewater treatment system with septic tank and drainfield.](image)

Primary treatment in the septic tank consists of anaerobic biodegradation of organic compounds and removal of settleable solids at the tank bottom and fats, oils, and grease (FOG) at the water surface. The septic tank effluent is discharged into the environment via a series of pipes to a drainfield where soil absorption and further biodegradation by microbes in the soil allows for additional treatment and contributes to “artificial’ groundwater recharge (FOSNRS,
The design of these OWTS, an underground tank and piping in its simplest form, results in wastewater treatment systems that are low cost and simple to operate (USEPA, 1999).

Microbes in the septic tank perform two major organic transformations, anaerobic degradation of organic carbon and ammonification of organic nitrogen (Rodriguez-Gonzalez, 2017). Organic nitrogen enters the septic tank from several sources such as food waste, household products, and urea from human urine and feces. Urea (NH₂CONH₂) is then hydrolyzed to ammonia (NH₃) as shown in Equation 1, and at pH below 9.25 it is further hydrolyzed to ammonium (NH₄⁺) as shown by Equation 2 (Madigan et al., 2010).

\[
\text{NH}_2\text{CONH}_2 + \text{H}_2\text{O} \Rightarrow 2\text{NH}_3 + \text{CO}_2 \quad \text{Equation 1}
\]

\[
\text{NH}_3 + \text{H}_2\text{O} \Rightarrow \text{NH}_4^+ + \text{OH}^- \quad \text{Equation 2}
\]

As the microbes break down and consume organic compounds, they grow in number and the resulting biomass along with settled solids create sludge. The accumulation of FOG and sludge in the septic tank requires the system to be pumped out every three to five years depending on the household size and usage (USEPA, 2002). As this responsibility typically rests on the homeowner, proper routine maintenance is not always accomplished. This results in approximately 10% of septic systems backing up to the ground surface or into the home each year (USEPA, 2003). This is a serious problem in mitigating pollution, as key transformations in the drainfield require an unsaturated zone of soil to complete the treatment of the wastewater to an environmentally safe level prior to discharge into water bodies.

Improper construction and maintenance is believed to make OWTS a major contributor to nutrient and microbial contamination of groundwater (USEPA, 1998). Improper siting of these OWTS can also contribute to groundwater contamination. The depth of the water table is an important factor that can limit their application (FDOH, 2013). Even when the drainfield is sited
and maintained properly, nutrient pollution from these systems is still likely. Unsaturated soil promotes nitrification, converting ammonium (NH₄⁺) to nitrate (NO₃⁻), but NO₃⁻ is highly mobile so the final step, denitrification, is limited without anoxic conditions or organic matter in the soil to convert the NO₃⁻ to nitrogen gas (N₂(g)).

### 2.2 Biological Nitrogen Removal (BNR)

Under ideal conditions, unsaturated soil in the drainfield allows septic effluent to percolate while absorbing oxygen from the air. This creates an aerobic condition allowing nitrifying bacteria to oxidize NH₄⁺ to NO₃⁻. As NO₃⁻ travel in the soil, the concentration of oxygen decreases. Anoxic conditions may favor denitrification if sufficient organic matter is available, creating N₂ gas. Natural nitrification and denitrification is limited due to high water tables, limited oxygen for nitrification, limited electron donor for denitrification, and the speed at which NO₃⁻ travels through the soil. This along with absorption in the soil and assimilation from the microbes reduces some total nitrogen (TN) concentrations in the wastewater effluent.

Nitrification is carried out by autotrophic bacteria that use NH₄⁺ as their inorganic electron donor in a two-step process oxidizing the NH₄⁺ to nitrite (NO₂⁻) followed by the oxidation of NO₂⁻ to NO₃⁻ (Tchobanoglous et al., 2003). The two-step process is combined as a single reaction representing the entire nitrification process (fs = 0.05) in Equation 3 (Crites and Tchobanoglous, 1998):

\[
\text{NH}_4^+ + 1.863 \text{O}_2 + 0.098 \text{CO}_2 \Rightarrow 0.0196 \text{C}_5\text{H}_7\text{O}_2\text{N} + 0.98 \text{NO}_3^- + 0.094 \text{H}_2\text{O} + 1.98 \text{H}^+ \quad \text{Equation 3}
\]

Nitrifiers are slow growing bacteria and more sensitive than many other microorganisms to environmental impacts caused by pH, dissolved oxygen (DO), organic matter, heavy metals, and volatile organic compounds. These factors can severely affect the nitrification rate. If there is
not enough DO, NO$_2^-$ will accumulate and cause the toxicity of the wastewater to further inhibit NO$_2^-$ oxidizing bacteria. Based on the equation above, the oxidation of NH$_4^+$ to NO$_3^-$ requires 4.26 grams of O$_2$ per gram NH$_4^+$-N. Additionally, the nitrifiers synthesize organic carbon from dissolved carbon dioxide (CO$_2$). Spending energy to fix organic carbon from dissolved CO$_2$ puts nitrifiers at a disadvantage when competing with heterotrophs for DO that assimilate organic carbon in the water. This means that heterotrophs can outcompete nitrifiers for oxygen if organic carbon concentrations are too high in the environment. The nitrification process consumes alkalinity as it generates protons. This will cause the pH to drop if the water has insufficient alkalinity. Optimal nitrification occurs between a pH of 7.5 and 8, with a sharp decrease in nitrification rates at a pH below 6.8. About 7.14 gram of alkalinity (as CaCO$_3$) is required per gram of NH$_4^+$-N (Xu, 1994; Tchobanoglous et al., 2003).

Denitrifying bacteria can be heterotrophs that use the organic carbon in the wastewater as their electron donor (Tchobanoglous et al., 2003), or autotrophs that use inorganic compounds such as sulfur (S$^0$), reduced iron, or hydrogen gas as their electron donor (Tong et al., 2017). Many denitrifiers are facultative aerobes, able to use either oxygen, NO$_2^-$, or NO$_3^-$ as electron acceptors, but oxygen is preferred. Some denitrifies can carry out fermentation reactions if neither electron acceptor is present (Tchobanoglous et al., 2003). Since wastewater contains a lot of organic substrate, many denitrifiers involved in these processes carry out heterotrophic denitrification. NO$_3^-$ reduction is carried out in several steps, first NO$_3^-$ is reduced to NO$_2^-$, then nitric oxide (NO), nitrous oxide (N$_2$O), and finally N$_2$ gas. The overall denitrification reaction is as follows:

$$C_{10}H_{19}O_{13}N + 10\text{NO}_3^- \Rightarrow 5\text{N}_2 + 10\text{CO}_2 + 3\text{H}_2\text{O} + \text{NH}_3 + 10\text{OH}^-$$  

Equation 4
Where \( \text{C}_{10}\text{H}_{19}\text{O}_{13}\text{N} \) represents the biodegradable organic matter in wastewater (USEP, 1993) as the electron donor. Organic matter is often measured as Chemical Oxygen Demand (COD) or Biochemical Oxygen Demand (BOD) and approximately 4 grams of BOD are needed per gram of \( \text{NO}_3^- \)-N converted (Barth et al., 1968). From the above equation, heterotrophic denitrification produces alkalinity at about 3.75 grams of alkalinity (as \( \text{CaCO}_3 \)) per gram of \( \text{NO}_3^- \)-N reduced.

### 2.3 Passive Nitrogen Removal Systems (PNRS)

Septic systems can be truly passive and require little in the way of mechanical, electrical, or chemical inputs except for periodic pumping for sludge and FOG removal. For this work, a passive system will be defined as having no compressor for aeration, not more than one effluent pump, and using reactive media for controlled nitrogen removal (Smith et al., 2008; FOSNRS, 2015). An effluent pump enables the system to incorporate recirculation to improve performance (Rodriguez-Gonzalez et al., 2020) or overcome flow restriction due to the systems elevation and tank orientation. Reactive media in the systems, such as oyster shells and wood chips, allows for more complete nitrification and/or denitrification processes to occur in the system and not rely solely on the environmental conditions in soils. Additionally, reactive media, such as zeolite, can act as nutrient buffers during transient loads that absorb ammonia when concentrations are high, and release it when concentrations are low (Rodriguez-Gonzalez et al., 2020).

A report by the Florida Onsite Sewage Nitrogen Reduction Strategies (FOSNRS, 2015) investigated available PNRS and ranked them based on several categories and relevant weighting factors. The weighting factors were established by the project team and a committee consisting of the members from local government, environmental interest groups, state universities, engineering industry, septic tank industry, real estate industry, restaurant industry, home building industry, and consumers (FOSNRS, 2015). Based on the reports research and scoring, it was
found that two-stage biofilters were the most applicable technology, with the simplest operation, and effectiveness at removing nitrogen of all available PNRS when taking into consideration performance above all else, but also cost and adoptability.

Two-stage biofilters consist of an unsaturated biofilter for nitrification, and a saturated biofilter for denitrification. The saturated biofilter contains a solid phase electron donor media to promote denitrification. Pilot scale studies were used to test different biofilter media and system orientations. Seven systems were chosen for home pilot tests and BHS-5, an in-tank two stage biofilter performed better than all the other systems. Stage one nitrification biofilter consisted of expanded clay media and included effluent recirculation. The stage two denitrification biofilter consisted of wood-chips, elemental sulfur, and oyster shell media. It should be noted that denitrification was done in two chambers, with the wood-chip heterotrophic denitrification occurring in chamber one and elemental sulfur and oyster shell mixotrophic denitrification occurring in chamber two.

A simplified process diagram is shown in Figure 2.2. For a 500-gallon septic system, BHS-5 removed TN at 97% with no recirculation and 98% with recirculation. The average influent TN concentration was 75 mg/l and the average effluent TN concentration was 1.8 mg/L (Hazen and Sawyer, 2015). No PNRS investigation was found that achieved better TN removal than BHS-5 (Piluk and Hao, 1989; Anderson et. al., 1998; Hossain et al., 2010; Rodriguez-Gonzalez et al., 2020).
Figure 2.2 Simplified process diagram of BHS-5. Adapted from Florida Onsite Sewage Nitrogen Reduction Strategies Report (FOSNRS, 2015).

2.4 Recirculation in PNRS

As mentioned earlier, recirculating effluent from one tank either back to the same tank or to a tank upstream in the treatment process is a well-studied method to improve wastewater treatment performance (Sorrels and Zeller, 1955). Early studies focused on recirculation to improve BOD removal (Hanumanulu, 1969; Shelef et al., 1978), but several studies since have found multiple benefits in BNR as well (Piluk and Hao, 1989; Hossain et al., 2010; FOSNRS, 2015; Miriyala, 2018; Rodriguez-Gonzalez et al., 2020).

Nitrifiers are slow growing and can be outcompeted for oxygen by heterotrophs if sufficient organic carbon is present. Recirculated effluent with reduced organic carbon can dilute the concentration of organic carbon in the septic tank effluent and provide a more favorable condition for nitrifiers downstream of the septic tank (Klees and Silverstein, 1992). This process is also used to achieve some TN removal through pre-denitrification (FOSNRS, 2015). Combining the nitrified effluent having high NO$_3^-$ concentrations with the septic tank effluent having relatively high BOD concentrations provides the electron donor and acceptor for denitrification, further reducing organic carbon in the nitrification influent.

Additionally, for trickling filters, recirculation can be used to ensure unsaturated conditions in the nitrification reactor and improved aeration with multiple passes through the
filter thereby increasing oxygen and NH$_4^+$ mass transfer (Solomon, 1998, Rodriguez-Gonzalez et al., 2020). The improved mass transfer can be partially attributed to the reduced biofilm layer, especially for NH$_4^+$. The regular sheering of biofilm can also reduce clogging in the biofilter (Rodriguez-Gonzalez et al., 2020)

### 2.5 Oyster Shells for Alkalinity and Microbial Attachment

Oyster shells have been incorporated into biofilters as a method to replace the alkalinity consumed in the nitrification and autotrophic denitrification process (Sengupta et al., 2007; Liu et al., 2010; FOSNRS, 2015; Tong et al., 2017, Rodriguez-Gonzalez et al., 2020). Calcium carbonate (CaCO$_3$) is a common source of alkalinity in natural water bodies (APHA, 2017) and makes up around 96% of oyster shell’s composition (Liu et al., 2010). The remaining composition of the oyster shells is organic carbon and trace elements (Asaoka et al., 2009) that can assist in the initial growth of microorganisms, such as in sulfur oxidizing or perchlorate reducing bioreactors (Conneely, 2011).

Nanosized flakes in the oyster shells structure (Sengupta et al., 2007) is countered by a high crystalline phase of CaCO$_3$ in the oyster shells composition results in a lower dissolution rate when compared to other solid phase alkalinity sources like limestone or marble chips (Asaoka et al., 2009). This reduces the need for backflushing and lowers the effluent suspended solids concentration (Sengupta et al., 2006; Tong et al. 2017). These nanosized flakes and high surface area, anywhere from 1.75 and 2.37 m$^2$/g, along with a rough surface make it a great biofilm carrier (Yoon et al., 2003; Sengupta et al., 2007). Oyster shells also promote greater denitrification rates, less NO$_3^-$ accumulation, and a more stable effluent pH than limestone or marble chips (Sengupta et al., 2006).
Similar work showing improved microbial attachment for nitrifying bacteria was not found but most of the oyster shells physical properties should translate to making it a well-suited biofilm carrier for nitrifying bacteria.

2.6 BNR in Saline Environments

Saline wastewater has become a growing issue for areas trying to conserve freshwater resources by using seawater for toilet flushing. One such area is Hong Kong, where saline wastewater is mixed with other wastewater sources, resulting in wastewater with salt concentrations between 5 and 6 g/L (Wu et al., 2008). Other coastal cities and nations using saltwater for toilet flushing include Avalon, Marshall Islands, and Kiribati (Yang et al., 2015). Though this is a practical solution to energy, water, and population stressors, it does introduce difficulties in wastewater treatment.

Generally, the microbes involved in wastewater treatment are not halophilic microorganisms and become stressed in high salt environments. High saline concentrations can make it difficult for bacteria to perform their metabolic processes and maintain their osmotic pressure, potentially resulting in bacterial plasmolysis (Omil et al., 1995; Vyrides and Stuckey, 2009; Lay et al., 2010). An inability to maintain osmotic pressure along with microbial deflocculation due to increased salinity can also result in sludge with poor settling ability (Kim and Ahn, 2019). Saline concentrations can also reduce enzyme activity of many microorganisms that are not adapted to such conditions, thereby reducing the microbe’s ability remove a desired contaminant (Uygur and Karg, 2004).

As salinity of the water increases, the solubility of oxygen decreases for a given temperature and pressure (Libes, 2009; Xing et al., 2014). For water at 20°C and 1 atm, oxygen solubility is around 9 mg/L for pure water and will drop to about 7.7 mg/L at a salinity of 30 ppt
NaCl (Zheng and Mao, 2019). The decrease in oxygen’s solubility at higher salinities will decrease the DO in the water and subsequently reduce the concentration of electron acceptors available for nitrification.

At 20°C, freshwater has a density of 998.2 kg/m³ and viscosity of 1.002 x 10⁻³ kg/m·s (Crittenden et al., 2012), while seawater has a density of 1024.8 kg/m³ and viscosity of 1.077 x 10⁻³ kg/m·s (IAPWS, 2008; IOC, et al., 2010). Increased density for saline wastewater makes removal of suspended solids through settling more difficult due to an increase in buoyant forces (Lefebvre and Moletta, 2006). Additionally, as the viscosity increases, the flux of the chemical constituents decreases and may decrease treatment efficiency (Crittenden et al., 2012).

Increased salinity will also decrease activity coefficient leading to lower apparent rate constant (Lewis and Randall, 1921). For compounds such as NH₄⁺, not all the total concentration is available to react due to the decrease in effective concentration as a result of a lower activity coefficient (Libes, 2009). Ultimately this leads to a decrease in removal efficiency for the compounds.

Though there are several disadvantages to treating saline wastewater, there are also potential benefits due to the chemical composition in saltwater. A sulfate reduction autotrophic denitrification nitrification integrated (SANI) processes was developed to take advantage of high sulfate (SO₄²⁻) levels found in seawater through the sulfur cycle microbiology (Wu et al., 2016).

The SANI process shown in Figure 2.3 incorporates three biological processes. First organic carbon is oxidized to CO₂ and SO₄²⁻ is reduced to sulfide (S²⁻) by SO₄²⁻ reducing bacteria in an anaerobic environment. Next sulfur oxidizing denitrification is used to oxidizes S²⁻ back to SO₄²⁻ and NO₃⁻ is reduced to N₂ gas in an anoxic environment. Finally, NH₄⁺ is oxidized to NO₃⁻ in an aerobic environment (Lu et al., 2012; Wu et al., 2016).
A large-scale pilot test of the system was conducted at the Sha Tin Sewage Treatment Works in Hong Kong (Wu et al., 2016). The system began with a fine mesh sieve for pretreatment and an anaerobic up-flow reactor for organics removal by biological \( \text{SO}_4^{2-} \) reduction. Two different systems were tested for nitrification and sulfur oxidizing denitrification (SOD), a submerged anoxic/aerobic ring-lace media filter (SAF) and an anoxic/aerobic moving bed biofilm reactor (MBBR) with high density polyethylene floating media. Operation stopped on the SAF system due to low BNR performance. The MBBR system consisted of an anoxic MBBR for SOD and an aerobic MBBR for nitrification with effluent recirculation from the aerobic MBBR to the anoxic MBBR. A simplified process diagram is shown in Figure 2.3. The final MBBR effluent was treated by chemical coagulation and sedimentation in a post-treatment process.

![Simplified process diagram of pilot scale SANI process.](image)

**Figure 2.3** Simplified process diagram of pilot scale SANI process. Adapted from Wang et al. (2009).

From an aerobic reactor influent with an average TN of 48 mg/L to the final MBBR effluent with an average TN of 16 mg/L, the pilot scale SANI processes achieved 67% TN removal. The chemical coagulation and sedimentation step brought the effluent TN down to an average of 8.4 mg/L. This was not much different from the bench scale experiment resulting in 74% TN removal (Wang et al., 2009).

Saline PNRS exist but there has been little work studying their performance. A study by Mark Kalivoda (2017) attempted to model the performance of three different PNRS located on
separate Cayes of the Belize Barrier Reef using water quality reports for the Southern Environmental Association (SEA) of Belize and doctoral research from Dr. Christine Prouty. Two of these systems use salt water for toilet flushing and share a similar biofilter orientation to the SANI process. The PNRS on Laughing Bird Caye National Park has two anaerobic biofilters followed by an aerobic biofilter, as shown in Figure 2.4. With the assistance of recirculation from the aerobic biofilter to the anaerobic biofilters, this system can replicate the SANI process of an anaerobic, anoxic, and aerobic treatment train, incorporating the three biological processes and the benefits of SOD.

Figure 2.4 Simplified process diagram of OWTS at Laughing Bird Caye National Park.
Chapter 3: Materials and Methods

Side-by-side tests were carried out in bench-scale biofilters with varying amounts of oyster shell media for treatment of saline domestic wastewater. The research was conducted in two phases. In phase 1, the two columns were operated as single pass treatment trains with saline wastewater at 15 ppt salinity and a flowrate of 2.1 liters per day. Phase 2 of the research was conducted with a recirculation line from the nitrification biofilter effluent to the septic tank influent, at a recirculation ratio of 0.5:1. All other conditions were the same in phase 1 and 2.

3.1 Inoculum

Inoculum for the experiment was collected from a moving bed bioreactor (MBBR) at Mote Aquaculture Research Park in Sarasota, Florida on 01/20/20. The Mote MBBR treats saline wastewater from a marine recirculating aquaculture system (Boxman et al., 2018). At Mote, 1.5 liters of combined plastic carriers and liquid (~0.4 liters of carriers and 1.1 liters of liquid) were collected from the MBBRs with an ammonia concentration of 0.5-1 mg/L and 15 ppt salinity. The plastic carriers and liquid were brought to the Environmental Engineering laboratories at the University of South Florida, Tampa, Florida and transferred to a 5-liter Erlenmeyer flask, as shown in Figure 3.1. Ammonium Bicarbonate was added to the Erlenmeyer flask to bring NH$_4^+$-N concentrations to 10 mg/L. An Eco Plus ® air stone connected to a Tetra Whisper® 10 air pump (Blacksburg, VA) was installed for aeration. DI water with Instant Ocean was added to bring the salinity to 15 ppt and the final volume in Erlenmeyer flask inoculum reactor to 3.5 liters. Ammonium Bicarbonate was added every 4 days to increase the NH$_4^+$-N concentrations to
10 mg/L. This practice continued for over a month until 03/02/20, when ammonium control switched to removing 2 liters of decanted liquid from the inoculum reactor and replacing it with 2 liters of wastewater from Northwest Regional Water Reclamation Facility (NWRWRF), Tampa, FL. Instant Ocean® Sea Salt (Blacksburg, VA) was added to maintain salinity at 15 ppt.

![Inoculum nitrifying seed reactor with carriers collected from Mote Aquaculture Research Park, Sarasota, Florida.](image)

**Figure 3.1** Inoculum nitrifying seed reactor with carriers collected from Mote Aquaculture Research Park, Sarasota, Florida.

### 3.2 Experimental Program

Phase 1 began on 02/09/20. 500 mL of inoculum was added to each biofilter by securing the biofilter effluent line to prevent drainage while filling the column. After 4 hours the pump was started and began moving wastewater from the storage container to the septic tanks. At this time, the wastewater storage container was located beneath the biofilter effluent line. Once wastewater began exiting the septic tank effluent pipe, the pump speed was reduced to the lowest setting and the nitrification biofilter lines were connected to the wastewater storage container entrance to allow the system to recirculate in a closed loop for one week of acclimatization. After
a week, the biofilter effluent lines were directed to a drain, the pump timer was installed, and the system was operated as a single pass treatment train.

The experiment was restarted on two occasions. On 03/16/20 Covid-19 precautions required that the experiment be shut down until 03/28/20. The biofilters originally had a mesh filter between the media layers to prevent the media from mixing. On 05/11/20 one of these mesh filters clogged between the oyster shells and LECA and prevented flow through the biofilter. At this time the media was removed from the biofilters, and the mesh filters were removed. All media was rinsed with DI 3 times and the experiment was restarted. Each time the systems were restarted the same way as the initial startup: inoculate with 500 mL inoculum, soak for 4 hours, recirculate for one week.

Phase 2 began on 10/05/2020. Once the system configuration was adjusted to that shown in Figures 3.4 and 3.5, the system was refilled prior to the 6am dosing and the recirculation pumps initiated the first recirculation dosing at 6:30am. Pipes and tubing were cleaned to remove biomass, but the nitrification columns and septic tanks were unaltered.

3.3 Nitrifying Biofilter Media

The LECA and oyster shell media was selected and cleaned in the same manner. The media size of 4.75 mm to 2 mm was selected from previous research in BNR systems (Boles et al., 2012; Tong et al., 2017; Rodriguez-Gonzalez, 2017). To select the media size distribution, the oyster shell media was first passed through 4.75 mm, 3.35 mm, 2.36 mm, and 2 mm U.S.A. Standard Sieve Series (Newark Wire Cloth Company, Newark, New Jersey and Fisher Scientific Company U.S.A Standard Test Sieves). An average of 61.5% mass was collected between 4.75 mm and 3.35 mm, 28.7% between 3.35 mm and 2.36 mm, and 9.8% between 2.36 mm and 2
mm. For the experiment, percent mass size distribution was rounded to 60%, 30%, and 10% respectively. This percent mass size distribution was also used for the LECA.

The media was then washed 5 times with tap water and 5 times with DI water. At this amount of washing there were no suspended solids noticeable in the water and the final DI wash had visibly clear water. After washing, the media was left to dry in the laboratory for 24 hours at room temperature (20 to 23 °C). Next, the media was stored in a constant temperature room at 35 °C for 24 hours. Figures 3.2 and 3.3 show close up images of the oyster shell and Lightweight Expanded Clay Aggregate (LECA) media.

![Figure 3.2 Oyster shell media after sizing, washing and drying. A) < 4.75 to 3.35mm B) < 3.35 to 2.36 mm and C) <2.36 to 2mm.](image-url)
Figure 3.3 Lightweight expanded clay aggregate media after sizing, washing and drying. A) < 4.75 to 3.35mm B) < 3.35 to 2.36 mm and C) < 2.36 to 2mm.

The amount of oyster shells added to the nitrification biofilters was different for the two columns to test if nitrification performance due to oyster shell addition was solely due to alkalinity. Media in biofilter 1 was composed of 50% oyster shells and 50% LECA by volume. The oyster shell media in biofilter 2 was calculated to provide enough alkalinity to support nitrification for 2 years, assuming no alkalinity in the water. Using 7.14 g alkalinity consumed as CaCO₃ per one-gram NH₄⁺-N nitrified (Xu, 1994; Tchobanoglous et al., 2003), and assuming an average of 31.1 mg/L NH₄⁺-N in wastewater (Rodriguez-Gonzalez et al., 2020), a flow rate of 2.1 liter per day, a media life of 2 years, and a conservative estimate of oyster shells being 95% CaCO₃, 460 grams of oyster shell was added to Biofilter 2. The remainder of biofilter volume was composed of LECA.
Porosity and bulk density was calculated using a 250, 500, and 1000 ml Pyrex graduated cylinder. The 500 ml graduated cylinder was placed on a scale, zeroed, and filled with 200 ml of dry media and the weight recorded. This was repeated 3 times to get the average bulk density of both media. Similarly, a dry 1000 ml Pyrex cylinder was filled with 200 ml of dry media. A 250 mL graduated cylinder was filled with 250 ml of DI. DI water was added to the 1000 ml gradated cylinder to the 200 mL line. The 200 ml was used as the total volume and the DI used was used as the void volume. The average bulk density for the given media size distribution was found to be 1.32 g/ml. This information was used to determine the volume of LECA to add as both biofilters would have the same media orientation of LECA at the base and oyster shells at the top. The LECA was found to have an average bulk density of 0.66 g/ml. The oyster shells had a porosity of 48% and the LECA had a porosity of 48%. All relative media information is shown on Table 3.1.

Table 3.1 Oyster shell and lightweight expanded clay aggregate characteristics in biofilter 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Oyster Shell Media</th>
<th>Lightweight Expanded Clay Aggregate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percent by volume in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biofilter 1</td>
<td>50%</td>
<td>50%</td>
</tr>
<tr>
<td>Percent by volume in</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biofilter 2</td>
<td>17%</td>
<td>83%</td>
</tr>
<tr>
<td>Particle size distribution</td>
<td></td>
<td>60% &lt;4.75mm to 3.35mm</td>
</tr>
<tr>
<td>Percent by mass</td>
<td></td>
<td>30% &lt;3.35mm to 2.36mm</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10% &lt;2.36mm to 2mm</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>1.32 g/ml</td>
<td>0.66 g/ml</td>
</tr>
<tr>
<td>Porosity</td>
<td>48%</td>
<td>48%</td>
</tr>
</tbody>
</table>
3.4 Nitrifying Biofilters

The nitrifying biofilters were constructed in 2000 mL Koflo Calibration Columns (Koflo Corporation, Cary, IL) with a media height of 45.7 cm, and inside diameter of 7.2 cm, as shown in Figure 3.4. The biofilters were packed with Lightweight Expanded Clay Aggregate (LECA) (Trinity Lightweight, Livingston, AL) and crushed oyster shells (Myco Supply©, Pittsburgh, PA). The bottom of the biofilters was packed with 4.76 cm of K2 Plastic Filter Media (Wholesale Koi Farm, Norco, CA) to bring the LECA and oyster shell media to the calibrated area of the column and function as a screen to prevent the media from clogging the outlet.

Figure 3.4 Nitrification biofilters. A) Biofilter 1, 50% oyster shells, 50% LECA by volume B) Biofilter 2, 17% oyster shells, 83% LECA by volume.
3.5 Treatment Train

Two systems were set up in parallel, each with a septic tank and nitrifying biofilter as shown in Figures 3.5 and 3.6. Both systems were fed from an 18.9-liter plastic storage container. Wastewater was collected from NWRWRF weekly and spiked with Instant Ocean to bring the salinity to 15 ppt. A Cole Parmer® Masterflex® L/S™ Economy Drive pump with dual Cole Parmer® Masterflex® L/S Easy-Load® heads was connected to a ChronTrol® Model XT Table Top timer. The pumps and timer were programmed to supply 2.1 liters per day from the storage container to each septic tank. The flow was applied to mimic typical OWTS, with 35% in the morning (6 doses every 30 minutes between 6am and 8:30am), 25% in the mid-day (8 doses every 30 minutes between 11am and 2:30pm), and 40% in the evening (6 doses every 30 minutes between 6pm and 8:30pm) in accordance with the National Sanitation Foundation Standard 40.

![Figure 3.5](image.png)

**Figure 3.5** Schematic diagram for experimental set up, phase 1 (single pass). 1) wastewater storage container, 2) pumps and timer, 3) septic tank, 4) nitrification biofilters.
Figure 3.6 Photograph of experimental set up, phase 1. 1) wastewater storage container, 2) pumps and timer, 3) septic tank, 4) nitrification biofilters with different ratios of oyster shells and lightweight expanded clay aggregate (LECA).

For phase 2 of the experiment, a recirculation line was incorporated to bring biofilter effluent to the septic tank at a 0.5:1 ratio of Recirculation flow rate to Influent flow rate (Figure 3.7 and 3.8). To do this a second Cole Parmer® Masterflex® L/S™ Economy Drive pump with dual Cole Parmer® Masterflex® L/S Easy-Load® heads were connected to a second ChronTrol® Model XT Table Top timer. The pump timer operated the recirculation pumps similar to the main system pumps to maintain the 0.5:1 recirculation ratio. To ensure a volume of water was present for the recirculation, the recirculation pumps operated at the half hour marks (3 morning doses at 6:32:33am, 7:32:33am, and 8:32:33am; 4 noon doses at 11:31:22am,

**Figure 3.7** Schematic diagram for experimental set up, phase 2 (0.5:1 nitrification biofilter recirculation). 1) wastewater storage container, 2) pumps and timer, 3) septic tank, 4) nitrification biofilters, 5) recirculation column, 6) recirculation pump and timer.
3.6 Septic Tanks

The two bench scale septic tanks were identical 5.68 liter HDPE cylindrical containers (M&M Industries, Inc. Chattanooga, TN) as shown in Figure 3.9. The septic tanks working volume was 3.81 liters with no media in the container. The septic tank influent pipe was attached to the tank lid and rested near the exit of the septic tank, the effluent pipe was attached to the septic tank body with a RainStation Rain Barrel Seal, EarthMinded ®. The inlet pipe rested high enough from the tank bottom, 83 mm, to prevent disturbing the solids. The effluent pipe rested
100 mm from the tank bottom, higher than the influent pipe, to prevent solids carryover, but low enough from the water surface to prevent fats, oil and grease (FOG) carryover. Wastewater flowed from each of the septic tanks to their respective nitrification biofilters by gravity.

![Septic Tank and Effluent Pipe](image)

*Figure 3.9* Septic tank, influent, and effluent piping.

### 3.7 Analytical Methods

Samples were collected twice per week for Total Ammonia Nitrogen (TAN), oxidized nitrogen (NOx), nitrite (NO$_2^-$), pH, conductivity, and salinity. Dissolved oxygen (DO) was measured once per week from sample ports before and after the nitrification biofilters. Once per
week the samples were also measured for Total Suspended solids (TSS), Volatile Suspended Solids (VSS), and Alkalinity. Total Nitrogen (TN) and Chemical Oxygen Demand (COD) were initially measured periodically. By the last month of Phase 1 the sampling frequency for TN and COD was adjusted to once per week and maintained weekly for Phase 2. Samples were filtered through a 0.45 μm membrane filters for TAN, NO$_2^-$, COD, and TN. Unfiltered samples were used to measure pH, conductivity, salinity, alkalinity, TSS and VSS.

TAN and NOx were measured using a Timberline Ammonia Analyzer (TL-2800, Timberline Instrument, USA). NO$_2^-$-N was measured using a combination of Standard Methods 4500 (APHA, 2017) and Strickland and Parsons (1972). NO$_3^-$-N concentrations were calculated by subtracting the NO$_2^-$-N concentration from the NOx-N concentration. COD was measured using HACH method 8000 (3–150 mg/L) adapted from Standard Methods 5220D (APHA, 2017) with addition of 0.5 g of HgSO$_4$ to each vial to eliminate chloride interference (MDL, 3 mg/L COD). TN was measured using HACH method 10071 (5–40 mg/L) adapted from Standard Methods 4500C (APHA, 2017). An Orion 5 Star (Thermo Scientific Inc., Beverly, Massachusetts) meter was used to measure pH, Conductivity, Salinity, and Temperature. Alkalinity was measured using Standard Methods 2320B (APHA, 2017). A portable DO meter (Mettler Toledo, USA) was used to measure DO in accordance with Standard Methods 4500-OG (APHA, 2017). The DO probe was inserted into flow through connections in the lines before and after the nitrification column to allow in-situ DO measurements of flowing wastewater. TSS and VSS were measured using the Standard Methods 2540-D and 2540-E, respectively (APHA et al., 2017).

Samples were measured in triplicate for TAN and NOx, NO$_2^-$, Alkalinity, TSS, and VSS. COD was done as a single sample to minimize use of mercury, and TN was done as a single
sample due to the cost of the HACH kits. Ammonium Chloride Certified ACS (Fisher Chemical, Ottawa, ON), Sodium Nitrite GR ACS (EMD Millipore Corporation, Billerica, MA), Potassium Hydrogen Phthalate, ACS reagent acidimetric standard (Acros Organic, New Jersey USA) were used to prepare standards. Sodium Nitrate (Sigma-Aldrich, St. Louis MO) was used to regularly check NOx concentrations. NIST QualityCheck Nutrient Sample A and B ISO 17034 and 1725 were used for initial quality assurance (Agilent, North Kingstown, Rhode Island)

3.8 Data Analysis

Although Biofilters 1 and 2 used the same pumps, the pump head orientation influenced the final flow rate to each system. Measured flowrate for biofilter 1 was 1.97 L/day and biofilter 2 was 2.10 L/day under the single pass treatment phase. With the addition of recirculation, the flowrate through the biofilters increased by 50%, leading to an increased hydraulic loading rate in both biofilters for phase 2.

The rates of TAN and N removal in the reactor was calculated assuming steady-state conditions in an ideal plug-flow reactor (uniform distribution across the cross-sectional area and no dispersion). It was assumed that whatever was not transported was transformed by the microbes in the reactor. A mass balance was also performed for the two biofilters using Equation 5. For the septic tank and biofilters the mass balance took the form shown in Equation 6.

Accumulation = inflow – outflow – transformation

\[ 0 = \Sigma Q_iS_i - \Sigma Q_eS_e - r_{su}V \]

where: \( r_{su} \) is the rate of substrate utilization, \( Q_i \) is the influent flowrate, \( Q_e \) is the effluent flowrate, \( V \) is the working volume of the reactor(s), \( S_i \) is the influent substrate concentration, and \( S_e \) is the effluent substrate concentration.
In phase 2, biofilter effluent was recirculated to the septic tanks. This meant that the term \( \Sigma Q_i S_i \) for the septic tanks mass balance comprised of the product of the raw wastewater substrate concentrations and raw wastewater flowrate plus the product of the recirculated substrate concentrations and recirculated flowrate.

For phase 1, despite the assumption that there were nearly no transformations of N in the septic tanks, septic tank volumes were included in the mass balance calculation for TN. The alternative of not including the septic tanks in phase 1 made the control volume much bigger for the TN mass balance calculation in phase 2 and misrepresented the results.

The TAN mass balance was performed using only the change in mass through the biofilters. There was little change in the septic tank TAN masses with most of the TAN utilization occurring in the biofilters for both phases. Additionally, similar to TN utilization, including the septic tanks in the control volume for one phase and not the other would have led to a misrepresentation of the data.

Statistical analysis was conducted through RStudio ® for statistical significance, ANOVA, and t-test and Microsoft Excel for averages, standard deviations, and percent errors.
Chapter 4: Results and Discussion

The objectives of this research were to investigate the effect of oyster shell media and recirculation on unsaturated biofilters performance for treatment of saline wastewater and to compare the results with prior studies of N-removing biofilters treating wastewater with varying salinities. Side-by-side tests were carried out in two bench-scale biofilters with varying amounts of oyster shell media and LECA, each with their own septic tank, for treatment of domestic wastewater at 15 ppt salt. The experiments were carried out in two phases, without and with recirculation.

4.1 Nitrogen Transformations

Profiles of septic tank effluent and biofilter effluent TAN concentrations over time for the two biofilters throughout phases 1 and 2 are shown in Figure 4.1. A bold black line on day 144 denotes when the system changed from phase 1 single pass treatment to phase 2 recirculation of biofilter effluent to the septic tank. In phase 1, the septic tank influent was not measured due to minimal N transformations expected in the septic tank. With the addition of recirculation, TN removal through denitrification in the sept tank was monitored by measuring the septic tank influent. Salinity was maintained at approximately 15 ppt and flowrates at 1.97 L/d for biofilter 1 and 2.1 L/d for biofilter 2.
Figure 4.1 TAN concentrations for raw wastewater, septic tank effluent and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during phase 1 and phase 2 of the experiment. Bold line denotes the separation between phase 1 and phase 2 of the experiment.
The two biofilters followed similar trends in both phase 1 and phase 2 of the experiment. In phase 2, recirculation of the nitrified effluent to the septic tank was used to create conditions that favored pre-denitrification using organic matter in the wastewater as a carbon source and electron donor. Recirculation also led to a decrease in biofilter influent TAN concentrations from those seen in phase 1 due to dilution.

The biofilters performance is also shown as a percent removal of TAN in Figure 4.2. Neither biofilter consistently performed better than the other resulting in no significant differences between the two system’s nitrogen removal (p-value=0.1482 at a 95% confidence interval). Similarly, there is not sufficient evidence to prove that there is a difference in the performance between phase 1 and 2 for the two biofilters (p-value=0.209 for biofilter 1 and p-value=0.4371 for biofilter 2).

Figure 4.2 Percent conversion of influent TAN concentrations through Biofilter 1 and Biofilter 2 in phase 1 and phase 2 of the experiment. Bold line denotes the separation between phase 1 and phase 2 of the experiment.
Table 4.1 shows the average percent removal of TAN for both systems in both phases and throughout the experimental run. Figure 4.2 and Table 4.1 are calculated in the same manner. For phase 1, percent removal of TAN was calculated from the septic tank effluent concentrations to the biofilter effluent concentrations. For phase 2, percent removal of TAN was calculated from the raw wastewater concentrations to the biofilter effluent concentrations. Raw wastewater was not sampled for phase 1 due to little Total Kjeldahl Nitrogen (TKN) removal expected in the septic tanks resulting in nearly all the TKN removed occurring in the biofilter for the system lineup. This expectation is supported by Figure 4.1, Table 4.2, and Table 4.3 showing little difference in average septic tank effluent during phase 1, Table 4.2, and average raw wastewater during phase 2, Table 4.3.

Table 4.1 Average percent removal of influent TAN concentrations in phase 1 and phase 2 of the experiment.

<table>
<thead>
<tr>
<th>Period</th>
<th>System 1</th>
<th>System 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase 1</td>
<td>85</td>
<td>86</td>
</tr>
<tr>
<td>Phase 2</td>
<td>75</td>
<td>82</td>
</tr>
<tr>
<td>Average for total Experiment</td>
<td>82</td>
<td>84</td>
</tr>
</tbody>
</table>

Between day 19 and 39 sample ports were installed to measure DO concentrations in situ. Results after port installation indicated decreased oxygen transfer to the biofilters. Figure 4.3 shows the decreasing trend in NO$_2^-$-N concentrations seen in the first 19 days ceased after the port installation. On day 43 vents were installed in each effluent line. Following these changes, the systems returned to pre-sample port installation NOx trends.
Figure 4.3 NO$_2^-$ concentrations for raw wastewater, septic tank effluent and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during phase 1 and phase 2 of the experiment. Bold line denotes the separation between phase 1 and phase 2 of the experiment.
Figure 4.4 NO₃⁻ concentrations for raw wastewater, septic tank effluent and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during phase 1 and phase 2 of the experiment. Bold line denotes the separation between phase 1 and phase 2 of the experiment.
Figure 4.3 and 4.4 also show no significant difference between the two biofilters in NH$_4^+$ and NO$_2^-$ oxidation. Figure 4.4 shows negative values for NO$_3^-$ concentrations on the first 9 days of the analysis. NO$_3^-$ was obtained by the subtraction of NO$_2^-$ from NOx which were obtained from two different methods resulting in a compounding of error. Errors with the ammonia analyzers early operation caused a rapid decrease in the analyzers ability to reduce and measure NOx, causing further error. Figure 4.3 and 4.4 show all NOx results. For all the following results, only NOx showing 5% error or less from a standard of known concentration are shown.

4.2 Phase 1 Single Pass Results

Table 4.2 shows the average wastewater characteristics during phase 1 of the experiment. Biofilter 1 and 2 performed similarly in their ability to remove the measured contaminants (NH$_4^+$-N, TN, TSS, VSS, COD). Salinity was maintained near the desired 15 ppt concentration and temperature remained relatively constant for the wastewater at 22°C.

Table 4.2 Average wastewater parameters for phase 1, single pass wastewater treatment. Standard deviations are given in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Septic Tank 1 effluent</th>
<th>Biofilter 1 effluent</th>
<th>Septic Tank 2 effluent</th>
<th>Biofilter 2 effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.11 (0.30)</td>
<td>7.98 (0.32)</td>
<td>8.09 (0.27)</td>
<td>7.96 (0.26)</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>1.40 (0.35)</td>
<td>3.38 (0.25)</td>
<td>1.49 (0.29)</td>
<td>3.23 (0.31)</td>
</tr>
<tr>
<td>TAN (mg N/L)</td>
<td>37.8 (5.7)</td>
<td>5.7 (5.2)</td>
<td>35.8 (6.5)</td>
<td>5.1 (4.7)</td>
</tr>
<tr>
<td>NO$_2^-$ (mg N/L)</td>
<td>0.2 (0.9)</td>
<td>1.3 (1.3)</td>
<td>0.3 (1.0)</td>
<td>1.3 (1.7)</td>
</tr>
<tr>
<td>NO$_3^-$ (mg N/L)</td>
<td>0.8 (1.9)</td>
<td>25.5 (10.0)</td>
<td>0.8 (2.0)</td>
<td>26.5 (9.9)</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>41 (5)</td>
<td>35 (2)</td>
<td>39 (4)</td>
<td>34 (2)</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO$_3$)</td>
<td>438 (47)</td>
<td>241 (12)</td>
<td>449 (66)</td>
<td>216 (9)</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>92 (20)</td>
<td>57 (29)</td>
<td>81 (33)</td>
<td>58 (31)</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>47 (9)</td>
<td>24 (8)</td>
<td>41 (12)</td>
<td>24 (9)</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>158 (64)</td>
<td>42 (37.54)</td>
<td>133 (27)</td>
<td>30 (12)</td>
</tr>
</tbody>
</table>

As noted in the literature review, major factors that affect nitrification are pH, DO, alkalinity, NO$_2^-$, and COD. DO concentrations <0.50 mg/L will greatly inhibit nitrification rates, with nitrification rates increasing up to a concentration of 3 to 4 mg/L (Tchobanoglous et al.,
From the DO concentrations observed in the septic tank effluent and biofilter effluent, DO concentrations did not inhibit nitrification in the biofilter. For both biofilters, average pH was around the optimal performance range of 7.5 to 8 pH and never dropped below 6.8 pH known to decrease nitrification rates (Tchobanoglous et al., 2003). Alkalinity concentrations present in the wastewater helped maintain pH at a slightly basic level with little change in the influent and effluent concentrations for both biofilters.

The alkalinity requirements for nitrification are 7.14 gram of alkalinity as CaCO$_3$ per gram of NH$_4^+$-N nitrified (Xu, 1994; Tchobanoglous et al., 2003). Using the influent concentrations of TAN, assuming all TAN is NH$_4^+$ -N, and alkalinity as CaCO$_3$ along with the system flowrates, Figure 4.4 shows that there is far more alkalinity present in the biofilter influent than is theoretically needed to for the complete nitrification of the influent NH$_4^+$-N per day in both biofilters.

![Bar chart showing theoretical and actual mg/day needed for complete nitrification of influent NH$_4^+$-N.](image)

**Figure 4.5** Theoretical and actual mg/day needed for complete nitrification of influent NH$_4^+$-N.

Figure 4.5 shows that both biofilters performed well regarding nitrification. Nitrifiers were able to oxidize most of the NH$_4^+$-N, comprising a majority of the TN concentrations in septic tank effluent, to NO$_3^-$-N, comprising a majority of the TN concentrations in the biofilter...
effluent. Some TAN was also removed by assimilation of microbes in the biofilter but the NO$_3^-$-N concentrations in the biofilter effluent support that TAN removal was due to nitrification. NO$_2^-$-N concentrations were far below the 280 mg/L NO$_2^-$-N reported to inhibit NO$_2^-$-N oxidation to NO$_3^-$ (USEPA, 1993).

![Graph showing nitrogen species profile during phase 1, single pass treatment.](image)

**Figure 4.6** Nitrogen species profile during phase 1, single pass treatment.

The biofilters showed some TN removal as well. Though some TN losses were due to microbial assimilation of TKN, a decrease in COD shown in Table 4.2 supported the possibility of simultaneous nitrification and denitrification in the biofilters. Aerobic heterotrophs and not denitrifies could have also led to the observed decrease in COD. The two biofilters removed TN and TAN at nearly the same rate.
4.3 Phase 2 Recirculation Results

On day 144 (10/5/2020) a 0.5:1 recirculation ratio of biofilter effluent was introduced to the system configuration to observe the effects on TN removal. With the addition of recirculation in phase 2, anoxic conditions were created in the septic tank. Because of this, dilution and denitrification were occurring in the septic tank, so raw wastewater entering the septic tank was also monitored. Average wastewater characteristics for the systems are shown in Table 4.3 following the change in the system configuration. From the table it can be seen that the biofilters performed similarly in their ability to remove the measured contaminants (TAN, TN, TSS, VSS, COD). Salinity was maintained at 15 ppt and average wastewater temperatures were relatively constant at 22°C

<table>
<thead>
<tr>
<th></th>
<th>Septic Tank influent</th>
<th>Septic Tank 1 effluent</th>
<th>Biofilter 1 effluent</th>
<th>Septic Tank 2 effluent</th>
<th>Biofilter 2 effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.82 (0.17)</td>
<td>7.84 (0.35)</td>
<td>7.58 (0.27)</td>
<td>7.94 (0.25)</td>
<td>7.60 (0.37)</td>
</tr>
<tr>
<td>DO (mg/L)</td>
<td>NA</td>
<td>1.25 (0.22)</td>
<td>3.14 (0.10)</td>
<td>1.20 (0.20)</td>
<td>3.49 (0.49)</td>
</tr>
<tr>
<td>TAN (mg N/L)</td>
<td>39.0 (5.3)</td>
<td>27.1 (6.5)</td>
<td>9.6 (5.9)</td>
<td>27.0 (5.8)</td>
<td>7.1 (3.4)</td>
</tr>
<tr>
<td>NO$_2^-$ (mg N/L)</td>
<td>BDL</td>
<td>0.1 (0.2)</td>
<td>0.5 (0.3)</td>
<td>BDL</td>
<td>0.6 (0.3)</td>
</tr>
<tr>
<td>NO$_3^-$ (mg N/L)</td>
<td>1.1 (1.5)</td>
<td>0.8 (1.0)</td>
<td>14.2 (2.1)</td>
<td>0.6 (1.0)</td>
<td>16.4 (3.1)</td>
</tr>
<tr>
<td>TN (mg/L)</td>
<td>42 (7)</td>
<td>32 (10)</td>
<td>27 (6)</td>
<td>31 (7)</td>
<td>27 (7)</td>
</tr>
<tr>
<td>Alkalinity (mg/L as CaCO3)</td>
<td>506 (46)</td>
<td>394 (62)</td>
<td>300 (54)</td>
<td>414 (39)</td>
<td>252 (29)</td>
</tr>
<tr>
<td>TSS(mg/L)</td>
<td>47 (2)</td>
<td>51 (15)</td>
<td>29 (14)</td>
<td>35 (11)</td>
<td>17 (5)</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>29 (3)</td>
<td>31 (7)</td>
<td>15 (5)</td>
<td>22 (4)</td>
<td>10 (2)</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>163 (66)</td>
<td>91 (50)</td>
<td>27 (18)</td>
<td>82 (23)</td>
<td>37 (20)</td>
</tr>
</tbody>
</table>

Recirculation did not affect DO concentrations. DO in the recirculated effluent was quickly utilized by microbes in the septic tank resulting in no difference in the septic tank.
effluent DO concentrations between phases 1 and 2. DO concentrations were above the 0.5 mg/L in both biofilters known to decrease nitrification rates.

In phase 1 there was little change in pH through either biofilter, this was not seen in phase 2. Recirculation decreased the alkalinity in both biofilters through dilution resulting in a drop in pH as the nitrifiers consumed what alkalinity remained in the effluent diluted wastewater. This indicates that at higher nitrification rates with recirculation, pH could influence biofilter performance. For the given loadings, pH remained in the optimal nitrification range of 7.5 to 8 pH. If recirculation rates were to increase or alkalinity were to decrease, low pH could affect nitrification rates.

Using Table 4.3 and 7.14 gram of alkalinity as CaCO$_3$ per gram of NH$_4^+$-N nitrified, Figure 4.6 shows the difference in theoretical alkalinity needed to nitrify the influent NH$_4^+$-N, assuming all TAN is NH$_4^+$-N, and the actual alkalinity present in both biofilters. Similar to phase 1, there is far more alkalinity present in the influent than is theoretically needed for nitrification of all the influent NH$_4^+$.

![Figure 4.7](image_url)

**Figure 4.7** Theoretical and actual mg/day needed for complete nitrification of influent NH$_4^+$-N.
One reason oyster shell media was selected for objective 1 of the study was due to its ability to add alkalinity as oyster shells are predominantly CaCO$_3$. Biofilter 1 was comprised of 50% by volume oyster shell media and biofilter 2 was comprised of 17% oyster shell media by volume. The additional oyster shells in biofilter 1 did assist in keeping biofilter 1 effluent alkalinity concentrations higher than biofilter 2 effluent concentrations. Under the experimental set up, the differences in alkalinity were not large enough to cause a difference in biofilter performance.

Average TN concentrations in the septic tank effluent during phase 2 were 18% lower than those in phase 1. Organic carbon, measured as COD, in the raw wastewater and NO$_x$ in the recirculated effluent were used as the electron donor and acceptor respectively resulting in N$_2$ production and a decrease in TN in the wastewater. This can be seen by a decrease in COD concentrations and almost no NO$_2^-$ or NO$_3^-$-N in the septic tank effluent. With predominantly TAN and organic N remaining in the recirculated effluent comprising TN after denitrification, recirculation caused a dilution effect in the septic tank thereby decreasing TN concentration in the septic tank.

The decrease in septic tank effluent COD also had an effect in the biofilters. This can be seen by comparing Figure 4.5 and Figure 4.7. In phase 1, an average decrease of 30% in TN concentrations through the biofilters was partly due to simultaneous nitrification and denitrification occurring in the biofilter. In phase 2, COD concentrations restricted denitrification in the biofilters resulting in an average TN removal of 14% in the biofilters.
Figure 4.8 Nitrogen species profile in septic tank influent and biofilters 1 and 2 influent and effluent during phase 2.

Figures 4.8 shows that a majority of the TAN in the septic tank effluent was converted to NO$_3^-$ through nitrification. Biofilter effluent showed less complete nitrification in phase 2 than in phase 1. Based on measured results in both phase 1 and 2, factors that affect nitrification such as pH, DO, alkalinity, NO$_2^-$, and COD should not have affected nitrification rates. Figure 4.2 does show that the biofilters TAN removal performance did improve several days after installing recirculation. More data may be needed to get a better understanding of the average nitrification ability of the biofilters with recirculation.

Organic N in the biofilters was calculated by subtraction (organic N = TN - TAN - NOx-N). High concentrations of organic N in samples were likely due to an accumulation of error between analysis. This likely explains why organic N appears to increase from the raw wastewater to all other samples.
Biofilter effluent concentrations of TN, TSS, VSS, and COD were lower in phase 2 than phase 1. Though biofilter effluent concentrations of TAN were higher in phase 2, the results along with benefits of recirculation mentioned in the literature review made recirculation appeared to be the better option for wastewater treatment.

4.4 Mass Balance

Measured flowrate for biofilter 1 was 1.97 L/day and biofilter 2 was 2.10 L/day under the single pass treatment phase. With the addition of recirculation, the flowrate through the biofilters increased by 50%, leading to an increased hydraulic loading rate in both biofilters for phase 2. The rates of TAN and N removal in the reactor was calculated assuming steady-state conditions in an ideal plug-flow reactor. It was assumed that whatever was not transported was transformed by the microbes in the reactor. These results are summed up in Table 4.4.

**Table 4.4** Biofilter 1 and 2 performance characteristics for phase 1 and 2 of the experiment.

<table>
<thead>
<tr>
<th></th>
<th>Single Pass</th>
<th>0.5:1 Recirculation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Biofilter 1</td>
<td>Biofilter 2</td>
</tr>
<tr>
<td>HLR (m$^3$/m$^2$·day)</td>
<td>0.48</td>
<td>0.52</td>
</tr>
<tr>
<td>$r_{suTN}$ (mg/L·day)**</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>$r_{suTAN}$ (mg N/L·day)*</td>
<td>65.8</td>
<td>67.1</td>
</tr>
</tbody>
</table>

**Based on overall system volume. *Based on biofilter volume.

Hydraulic loading rates (HLR) in for the two biofilters are higher than what is typical for biofilters used in OWTS, which are normal 0.20 m$^3$/m$^2$·day (Rodriguez-Gonzalez 2017). Increased HLR may have led to a slight decrease in TAN utilization in the biofilters for phase 2. Using the septic tank as a pre-denitrification reactor in phase 2 largely contributed to the doubling of TN utilization in the system.
4.5 Passive Saline OWTS Compared to Other Saline Wastewater and PNR Systems

No prior studies were found analyzing a passive onsite saline wastewater treatment system for N removal either in the laboratory or in the field. There has been prior work modeling real world passive onsite saline wastewater treatment system (Kalivoda, 2017) and several studies looking at forced aeration N removal systems for saline wastewater treatment (Ramos et al., 2007, Wu et al., 2016, Boxman et al., 2018, Wang et al., 2020). Rodriguez-Gonzalez et al. (2020) studied a PNR system with and without recirculation. Prior systems relevant to this study are shown in Table 4.4.

Table 4.5 Saline wastewater treatment systems from other studies.

<table>
<thead>
<tr>
<th>System</th>
<th>Salinity</th>
<th>Loading</th>
<th>Inf</th>
<th>Removal</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Passive Nitrogen Removal Systems for Saline Wastewater (PNRSSW)</td>
<td>15 ppt</td>
<td>0.50 m³/(m²/day)</td>
<td>39 mg/L TN 36.8 mg/L NH₄⁺-N</td>
<td>14% TN 85% TAN</td>
<td>This Study (Laboratory Scale)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.75 m³/(m²/day)</td>
<td>42 mg/L TN 39.0 mg/L NH₄⁺-N*</td>
<td>36% TN* 79% TAN*</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hybrid Adsorption–Biological Treatment System (HABiTS)</td>
<td>0.20 ppt</td>
<td>0.50 m³/(m²/day)</td>
<td>44 mg/L TN 35. 8 mg/L NH₄⁺-N</td>
<td>34% TN 63% NH₄⁺-N</td>
<td>Rodriguez-Gonzalez et al. 2020 (Pilot scale)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HLR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.30 m³/(m²/day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfate Reduction Autotrophic Denitrification Nitrification Integrated (SANI)</td>
<td>6 ppt</td>
<td>12.5hr HRT</td>
<td>48 mg/L TN 37 mg/L TAN-N</td>
<td>67% TN 96% TAN</td>
<td>Wu et al. 2016 (Pilot scale)</td>
</tr>
</tbody>
</table>

Notes: percent removals were based on adjusted system boundaries to compare the systems most accurately. For the PNRSSW, the system boundary was whole system. For HABiTS, from the septic tank effluent to aerated biofilter effluent. For SANI, from the sulfate reduction up-flow sludge bed influent to the aerobic moving bed biofilm reactor effluent. * = with 0.5:1 recirculation.
The PNRSSW in this study performed similar to the HABiTS. In single pass treatment, PNRSSW performed slightly worse in TN removal but far better in TAN removal. The two systems performed nearly identical with recirculation. This indicates that at 15 ppt salinity, the salt concentrations are having little effect on the nitrifying bacteria.

The PNRSSW performed worse than the SANI system. The SANI system had forced aeration to assist in nitrification and recirculated the nitrified effluent to an anoxic chamber at a much greater rate (200-400% recirculation).

4.6 Diurnal Flow

Pumps were connected to a timer to regulate the flow from the wastewater storage container to the septic tanks. The timer and pumps were set up to mimic typical home septic systems, with 35% in the morning (6am and 8:30am), 25% in the mid-day (11am and 2:30pm), and 40% in the evening (6pm and 8:30pm). In real world applications, this diurnal flow patterns along with temperature changes throughout the day effect the ability of nitrifiers to oxidize NH₄⁺ in the incoming wastewater. All the results shown till this point were from samples collected during the evening dosing period. To get a better understanding of how the biofilters performed throughout the day, samples were collected during the morning, noon, and evening dosing periods during a single day. Figures 4.9 show the results of this sampling for both biofilters.
Figure 4.9 Nitrogen species profile for raw wastewater (daily average), septic tank effluent, and biofilter effluent for A) Biofilter 1 and B) Biofilter 2 during morning (7-7:30am), noon (12:30-1pm), and night (7-7:30pm) dosing. Raw wastewater changed little throughout the day.
Raw wastewater and septic tank effluent N concentrations show little change throughout the day. A slight increase in NO$_3^-$-N can be seen in the septic tank effluent between the morning dosing and the noon and night dosing. This was most likely due to a longer time for denitrification from the previous night’s NO$_3^-$-N dosing to the septic tank.

Throughout the day, most of the incoming TAN in the septic tank effluent was converted to NO$_3^-$ through nitrification. The lowest effluent TAN concentrations are in the noon dosing where flow is the lowest, and the highest effluent TAN concentrations are in the night dosing where flow is the highest. The lower flowrates result in a longer hydraulic residence time of the wastewater in the biofilter, giving the nitrifiers time to convert more of the NH$_4^+$ in the wastewater.
Chapter 5: Conclusions and Recommendations

The objectives of this study were to investigate the effect of oyster shell media on unsaturated biofilters performance for treatment of saline wastewater, the effect of recirculation on treatment of saline wastewater, and to compare prior studies of N removal systems for treatment of wastewater at varying saline concentrations to that of a laboratory scale passive saline wastewater treatment system. To achieve these objectives, two biofilters were constructed using two ratios of oyster shell media to LECA media. Biofilter 1 was constructed with 50% by volume oyster shell to 50% by volume LECA media. Biofilter 2 was constructed with 17% oyster shells to 83% LECA media by volume. The systems were operated in two phases, without recirculation for phase 1, and with recirculation for phase 2.

With respect to objective 1, biofilter 1 and biofilter 2 showed no significant difference in their performance through the entirety of this experiment. In phase 1, biofilter 1 removed 15% TN and 85% TAN while biofilter 2 removed 13% TN and 86% TAN. In phase 2, biofilter 1 removed 75% TAN while biofilter 2 removed 82% TAN. Both systems in phase 2 removed 36% TN. The results show that increased oyster shell media fraction had no significant effect on the performance of the two biofilters under the given conditions. The high alkalinity already present in the wastewater for both systems made the additional alkalinity in biofilter 1 irrelevant.

For objective 2, biofilter effluent was recirculated to the septic tank at a 0.5:1 ratio for phase 2. Biofilter effluent concentrations decreased by 22% for TN but increased by 55% for TAN. Effluent TAN concentrations did decrease to similar concentrations to those seen in phase 1 several weeks after recirculation was initiated. Effluent TSS, VSS, and COD also decreased in
phase 2 by 60%, 48%, and 7% respectively. Based on the results, recirculating PNRS would still be a better option than a single pass system. Future work looking at recirculation effects on saline wastewater treatment with a post denitrification step are needed to definitively assess the cost to benefits of recirculation.

For objective 3, Table 4.4 was constructed to compare the performance of the passive OWTS in this study to similar wastewater treatments systems for N removal. HABiTS in a single pass configuration performed better at TN removal but worse at TAN removal. The two systems performed nearly identical when recirculation was added. This indicates that at 15 ppt salinity there is little effect on the nitrifying bacteria under these conditions. The SANI system performed better at both TN and TAN removal. This is likely due to the recirculation setup in the SANI system more so than the forced aeration.

For this study, the passive OWTS used organic carbon in the septic tank as the electron donor for denitrification. There was far less COD in the biofilter effluent than was present in the septic tank. There may not be enough organic carbon in the biofilter effluent for a denitrification step post nitrification. Future work would look at the addition of an electron donor or multiple electron donors for denitrification. Future work would also be identifying additional media options for both the nitrification and denitrification reactors given what is most practical for typical areas where saline septic systems would be deployed.

The real-world application of this study is to construct a passive N removal system for OWTS that use ocean water or brine for toilet flushing. The current salinity of 15 ppt is lower than would be seen for a system in this scenario. In future studies, salinity would be increased to the average ocean salinity of 30 ppt. Also relevant for real world applications is the study of
increased flowrates to observe and quantify the change in TN removal due to change in volumetric flowrate for a given system volume.
References


Appendix A: List of Symbols, Acronyms and Abbreviations

BNR = Biological Nitrogen Removal
BOD = Biochemical Oxygen Demand
CaCO$_3$ = Calcium Carbonate
COD = Chemical Oxygen Demand
DO = Dissolved Oxygen
HABiTS = Hybrid Adsorption–Biological Treatment System
HLR = Hydraulic Loading Rate
HRT = Hydraulic Retention Time
MBBR = Moving Bed Biofilm Reactor
N = Nitrogen
NH$_3$ = Ammonia
NH$_4^+$ = Ammonium
NO$_2^-$ = Nitrite
NO$_3^-$ = Nitrate
NO = Nitric Oxide
N$_2$O = Nitrous Oxide
N$_2$ = Nitrogen gas
OWTS = Onsite Wastewater Treatment
PNRSSW = Passive Nitrogen Removal Systems for Saline Wastewater
PNRS = Passive Nitrogen Removal Systems
SA NI = Sulfate Reduction Autotrophic Denitrification Nitrification Integrated
SOD = Sulfur Oxidizing Denitrification
TN = Total Nitrogen
TAN = Total Ammonia Nitrogen
TKN = Total Kjeldahl Nitrogen
TSS = Total Suspended Solids
VSS = Volatile Suspended Solids