BIOLOGICAL NITROGEN REMOVAL FOR PASSIVE ONSITE WASTEWATER TREATMENT SYSTEMS USING SALTWATER TOILET FLUSHING

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ABSTRACT

Incomplete treatment of wastewater is a source of nitrogen pollution that has adverse effects on water bodies, aquatic life, and public health. In addition, fresh water resources continue to become overstressed by growing human needs, prompting some communities to utilize seawater for toilet flushing instead of potable water. This research investigates constraints and solutions towards development of robust and sustainable onsite wastewater treatment systems (OWTS) for systems that use salt water for toilet flushing. Keeping sustainability in mind, a laboratory scale OWTS were designed to make use of passive biological nitrogen removal options, meaning limited to no inputs of energy and chemicals. The systems treated domestic wastewater with added salts to bring the salinity to 1.5% and 3.0% to mimic different OWTS seawater flushing scenarios. A freshwater control consisted of wastewater without salt added. Trickling columns achieved 82% conversion of ammonium to oxidized nitrogen under non-saline conditions and 78% conversion at 1.5% and 3.0% salinity. Microcosms were constructed to evaluate different industrial and agricultural waste products as electron donors for denitrification at 3.0% salinity with freshwater controls. Electron donors used were sulfur pellets, sugarcane bagasse, banana stem, and pine chips, with pine chips and banana stem showing the best nitrogen removal rates. Results show passive biological nitrogen removal systems are a viable sustainable option to treat saline wastewater for OWTS applications.

INTRODUCTION

Nitrogen Pollution

Reactive nitrogen compounds are vital to all life; however, high concentrations of reactive nitrogen in water bodies can lead to eutrophication, degrading water quality, and making them uninhabitable to most aquatic life (USEPA, 1993). Eutrophication can cause a sudden increase in algae populations. As algae grow, they can outcompete and suffocate neighboring organisms. The decomposition of algae biomass consumes dissolved oxygen (DO) in the water. Low DO and high ammonium (NH_4^+) and nitrite (NO_2^-) concentrations can result in fish kills (Florida Department of Agriculture and Consumer Services, 2019). Eutrophication can also negatively impact human health and local economies.

Humans produce about 210 tera grams (Tg) of reactive nitrogen per year (Galloway et al., 2013). This nitrogen enters the surrounding environment through several sources such as fossil fuel and biomass combustion, agricultural and urban runoff, industrial waste, and incomplete wastewater

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treatment (Holmes et al., 2019). For nitrogen that is consumed by humans, about 23 Tg are collectively excreted per year (Smil, 1999).

Onsite Wastewater Treatment

Limited resources are one reason less than 35% of cities in developing countries have any form of wastewater treatment and most wastewater treatment facilities worldwide do little to remove reactive nitrogen (WHRC, 2007). Biological nitrogen removal (BNR) makes use of microorganisms to remove reactive nitrogen from wastewater and is often the least expensive treatment option compared with physical and chemical treatment (Xiao and Roberts, 2010). There are several forms of BNR such as nitrification/denitrification, nitritation/denitritation, partial nitritation/anammox, phototrophic systems, and microbial fuel cells (Winkler and Straka, 2019). The use of nitrification coupled with denitrification in BNR wastewater treatment is one of the most used, studied, and robust BNR methods.

Septic systems have been used as onsite wastewater treatment systems (OWTS) to manage domestic wastewater for over a hundred years. In the United States, there are about 26 million of these systems, accounting for approximately 20% of all homes (USEPA, 2012; USEPA, 2014). In countries with a less developed infrastructure, such as Belize, these systems serve approximately 89% of the population (Grau et al., 2013). A conventional septic system consists of a sewage pipe discharging to a septic tank followed by a drainfield and soil treatment area (Kerr, 1977). These treatment systems can remove solids, fats, oil and grease, but do little for nitrogen removal.

A conventional septic system can be upgraded to incorporate BNR in an engineered treatment process. This requires creating an aerobic treatment unit to promote nitrification, and an anoxic treatment unit with an electron donor to promote denitrification. Passive nitrogen reduction systems (PNRS) have been developed that are similar in their operation and maintenance requirements to conventional septic systems. These systems incorporate an unsaturated biofilter (e.g., a trickling filter) for nitrification and a saturated biofilter containing a solid phase slow release electron donor for denitrification (FOSNRS, 2015). Pine woodchips and sulfur pellets are common and successful solid phase electron donors to promote BNR in PNRS (Greenan et al., 2009; FOSNRS, 2015; He et al., 2018). Sugarcane bagasse and banana stem have been explored in scientific literature (Ueda et al., 2006; Wang et al., 2020; Gao et al., 2022) and are of particular interest as a waste organic product for denitrification in tropical environments.

Saline Domestic Wastewater

Several industries produce wastewater with a high salt concentration, including agro-food, tanneries, textile, paper, pharmaceutical, oil and gas, mining, and desalination (Guo et al., 2022). As the world population increases and natural resources decrease, coastal cities and nations, such as Hong Kong, Avalon, Marshall Islands, and Kiribati, have adopted using seawater for toilet flushing (Yang et al., 2015). Typical ocean seawater has a salinity of around 3%, therefore flushing toilets with seawater results in the production of saline domestic wastewater that needs further treatment.

Potable water has been traditionally used for toilet flushing, so septic systems and PNRS have been studied and designed for treatment of low salinity wastewater. As the salinity of the wastewater increases, BNR becomes more difficult. High salt 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). High salt concentrations can also reduce enzyme activity thereby reducing nitrogen removal efficiency (Uygur and Karg, 2004). Microbial deflocculation due to increased salinity can also result in sludge with poor settling ability (Kim and Ahn, 2019).

Oxygen solubility will also decrease as salinity increases (Xing et al., 2014). For deionized water, oxygen has a solubility near 9 mg/L at 20°C and 1 atm. For the same temperature and pressure, sea water, with a salinity around 3%, will have an oxygen solubility near 7.7 mg/L (Zheng and Mao, 2019), reducing oxygen availability for nitrification.

Goal

Keeping these constraints in mind and the lack of literature on BNR in OWTS at salinities as high as saltwater (~3%), the goal of this research was to develop and implement a PNRS that can effectively and sustainably treat saline wastewater. Figure 1 shows how a conventional septic system can be upgraded to incorporate a PNRS. The red dashed line shows where in the system these reactors would be installed and where the research of this paper focuses.

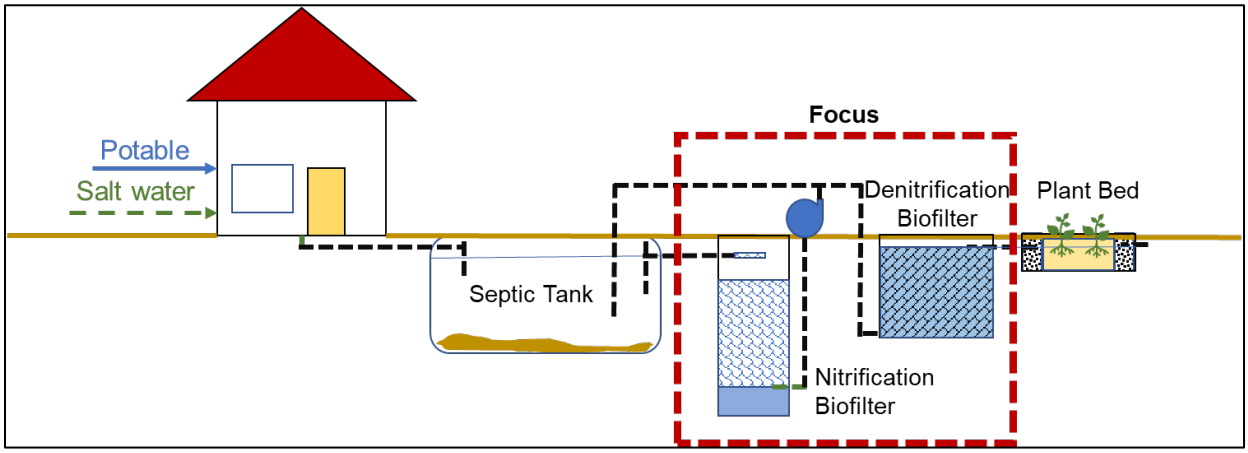

Figure 1. *Onsite Wastewater Treatment System with Biological Nitrogen Removal. Red dashed line shows the focus of this research.*

The research tested the following hypotheses:

1. A trickling filter can provide enough aeration at 3% salinity to allow near complete nitrification of saline wastewater.

2. Organic industrial wastes that are widely available in Caribbean countries can be used as slow-release electron donors for denitrification in OWTS applications where seawater is used for toilet flushing.

MATERIALS AND METHODS

Microcosm Electron Donor Studies

Denitrification microcosm studies were conducted to evaluate several electron donors prior to placing them in a treatment system. Pine woodchips, sulfur pellets, banana stem, and sugarcane bagasse showed promising denitrification results at low salinities and were chosen for further evaluation. The woodchips, banana stem, and bagasse media was cut to a particle size of 1 mm. Sulfur pellets came in a particle size of 0.5 mm. All media materials were washed with tap water three times followed by deionized (DI) water three times and allowed to dry in a 32°C constant temperature room for 48 hours. Effluent wastewater before chlorination from the Northwest Regional Water Reclamation Facility (NRWRF) in Tampa, Florida (about 0.1 % salinity) with added sodium nitrate ($NaNO_3$) to bring the nitrate (NO_3^-) concentration to approximately 55 mg/L-N was used to mimic nitrified wastewater. One set of microcosms was prepared using the wastewater as is, and one set of microcosms was dosed with Instant Ocean™ to bring salinity up to 3%. Five microcosms were constructed for each salinity. The microcosms where run in 500 ml Erlenmeyer flasks. For the first phase of the experiments, ~ 50 g of each electron donor was used since it allowed the less dense electron donors to take up no more than 20% for the flask volume thereby allowing the media to stay below the sample line suction. For the second phase of the experiment, 20 g of electron donor was chosen to test denitrification performance when less than half of the electron donor remains. All the microcosms were inoculated with 50 mL of mixed liquor suspended solids from NRWRF to provide uniformity. All flasks were filled with wastewater to bring the total volume in the flask to 500 mL. The microcosms were constructed to allow no outside air into the system and purged with nitrogen gas (N_2) for 20 minutes prior to start of experiment. They were then connected to gas sample bags to prevent overpressure development due to N_2 gas formation or vacuum caused by sample removal. Samples were collected daily for Total Ammonia Nitrogen (TAN), oxidized nitrogen (NO_x), NO_2^-, pH, conductivity, and salinity. Once all the NO_3^- was consumed, the microcosm was drained of the remaining wastewater and refilled with wastewater having 55 mg NO_3^--N/L and the appropriate salinity.

Laboratory scale PNRS

Two laboratory scale systems were set up in parallel, as shown in Figures 2 and 3. Each system was constructed with a septic tank, nitrification column, and denitrification column. The nitrification column was filled with Lightweight Expanded Clay Aggregate (LECA) and oyster shell media that was sieved to have a particle size distribution of 60%, 30%, and 10% by weight of 4.75-3.35 mm, 3.35-2.36 mm, and 2.36-2 mm respectively. The media was washed 5 times with tap water and 5 times with DI water and allowed to dry in a 32°C constant temperature room for 48 hours. Wastewater was collected from NWRWRF weekly. Wastewater for one parallel side was spiked with Instant Ocean™ to bring the salinity to 1.5%. Wastewater with 1.5% salinity was tested prior to testing 3% saline wastewater to test the experimental setup and to allow for comparison with earlier research using 1.5% salinity. A peristaltic pump with dual heads was connected to a timer to control dosing. This setup was run for 172 days. After day 172, both sides were shut down and cleaned out. The experiment was repeated using only one of the treatment trains and the wastewater salinity was increased to 3% for 77 days. For all

experiments, the pumps and timer were programed to supply one liter per day of wastewater from the storage container to each septic tank. The flow was applied to mimic typical OWTS in accordance with the NSF 40, 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). Columns were operated at an average hydraulic loading rate of 0.25 m/d, which is typical of PNRS applications (Rodriguez-Gonzalez, 2017)

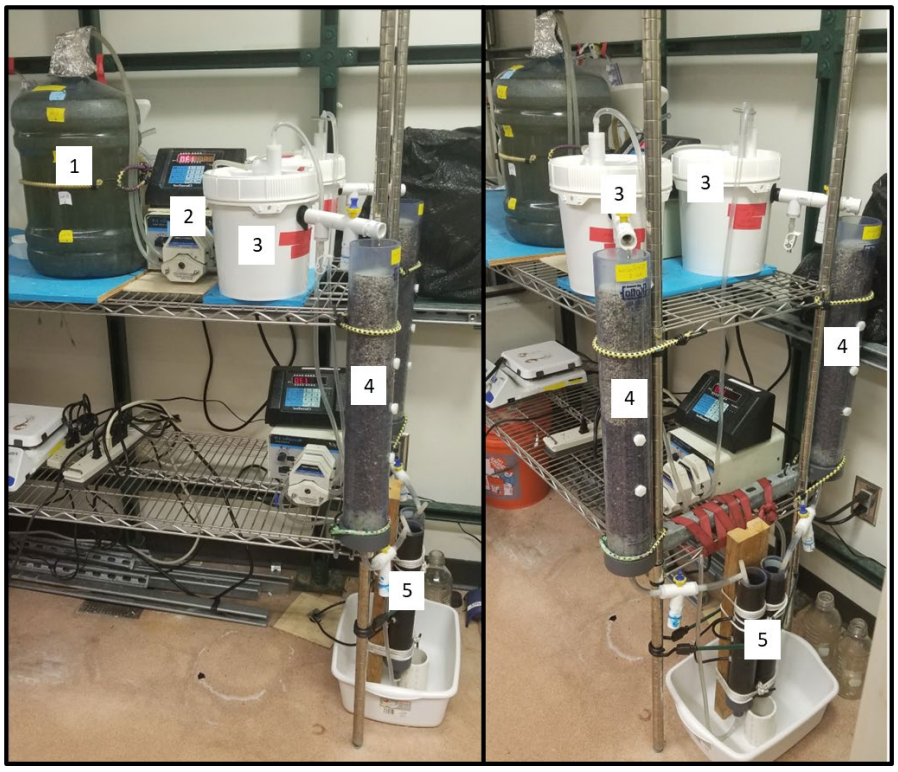

Figure 2. *Photograph of experimental set up, for parallel treatment trains showing 1) wastewater storage container, 2) pumps and timer, 3) septic tank, 4) nitrification column and 5) denitrification column.*

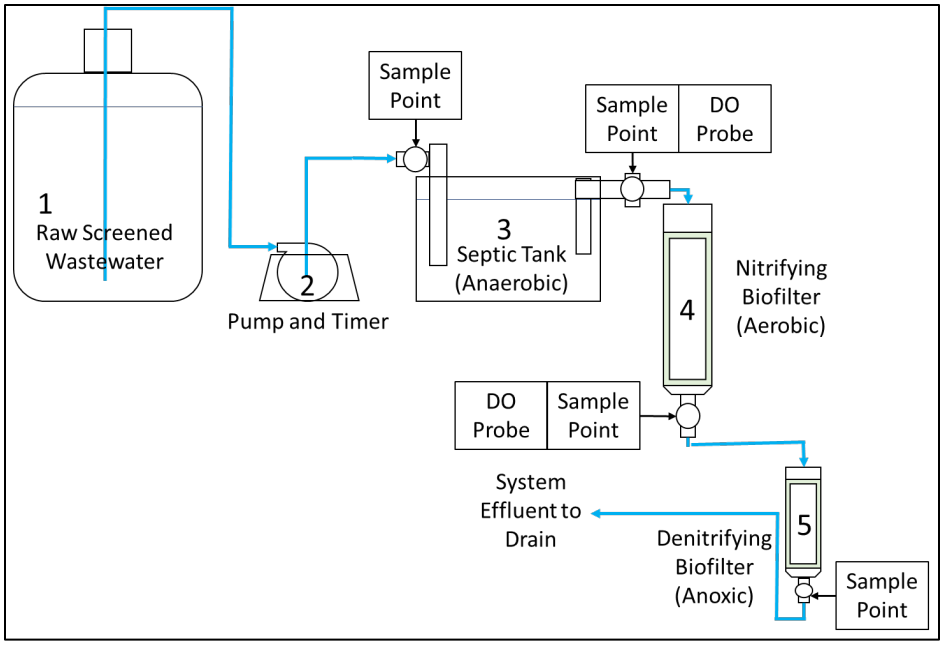

Figure 3. *Schematic diagram of experimental set up showing 1) wastewater storage container, 2) pump and timer, 3) septic tank, 4) nitrification column and 5) denitrification column.*

Samples were collected weekly for TAN, NO_x, NO_2^-, pH, and salinity. DO was measured once per week from sample ports before and after the nitrification biofilters. Total Suspended solids (TSS), Volatile Suspended Solids (VSS) and Chemical Oxygen Demand (COD) was performed bi-weekly.

Analytical Methods

Samples were filtered through a 1.2 µm membrane filters for measurement of TAN, NO_x, NO_2^-, and COD. Unfiltered samples were used to measure pH, conductivity, salinity, TSS and VSS.

TAN and NOx were measured using a Timberline Ammonia Analyzer (TL-2800, Timberline Instrument, Boulder, CO, 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 NO_x-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). An Orion 5 Star (Thermo Scientific Inc., Beverly, MA, USA) meter was used to measure pH, DO, salinity, and temperature. 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 the 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 NO_x, NO_2^-, TSS, and VSS. COD was done as a single sample to minimize use of mercury. Ammonium Chloride (NH_4Cl), Sodium Nitrite ($NaNO_2$), and Potassium Hydrogen Phthalate (KHP) ACS grade salts were used to prepare standards. Sodium Nitrate ($NaNO_3$) was used to regularly check NO_x analysis. NIST

QualityCheck Nutrient Sample A and B ISO 17034 and 1725 were used for initial quality assurance (Agilent, North Kingstown, RI)

Statistical analysis was conducted through RStudio® version 4.2.0 for statistical significance, ANOVA, and t-test and Microsoft Excel for averages, standard deviations, and percent errors.

RESULTS

Microcosm Electron Donor Studies

Figures 4 and 5 show that NO_3^- removal rates were greater at low salinity than at 3% salinity. Initially for the 50 g microcosms, sugarcane bagasse had the highest NO_3^- removal rate out of all the microcosms. It removed nearly all the NO_3^- in just over one day for low salinity microcosm and 80% of the NO_3^- in two days for 3% salinity microcosm. After two days, the sugarcane bagasse microcosm at 3% salinity showed no change in NO_3^- concentrations. Pine wood chips and banana stem behaved similarly with pine wood chips outperforming banana stem in low salinity, and vice versa for 3% salinity. Both pine wood chips and banana stem outperformed sulfur pellets regardless of salinity.

The 20 g microcosm studies were carried out for a longer period. The blue lines in Figure 5 show the day when NO_3^- concentrations reached the detection limit so the microcosms were drained of the remaining wastewater and refilled with wastewater having 55 mg NO_3^--N/L and the appropriate salinity. Figure 5 B shows that under stress (high salinity and low electron donor), microbes were more likely to perform dissimilatory nitrate reduction to ammonium as noted by the increase in NH_4^+ and NO_2^-.

Sugarcane bagasse performed far worse than in the previous set of microcosms, with NO_3^--N concentrations never below 30 mg/L. The poor performance of sugarcane bagasse is likely due to sugar residue in the bagasse leading to fermentation processes in the microcosm, which in turn decreased pH to as low as pH 3. The initial pH for all microcosms was between pH 7 and 8 and no other microcosm showed a consistent decrease in pH. This was not observed in a preliminary experiment (data not shown) with a different source of sugarcane bagasse. Therefore, these experiments are currently being repeated.

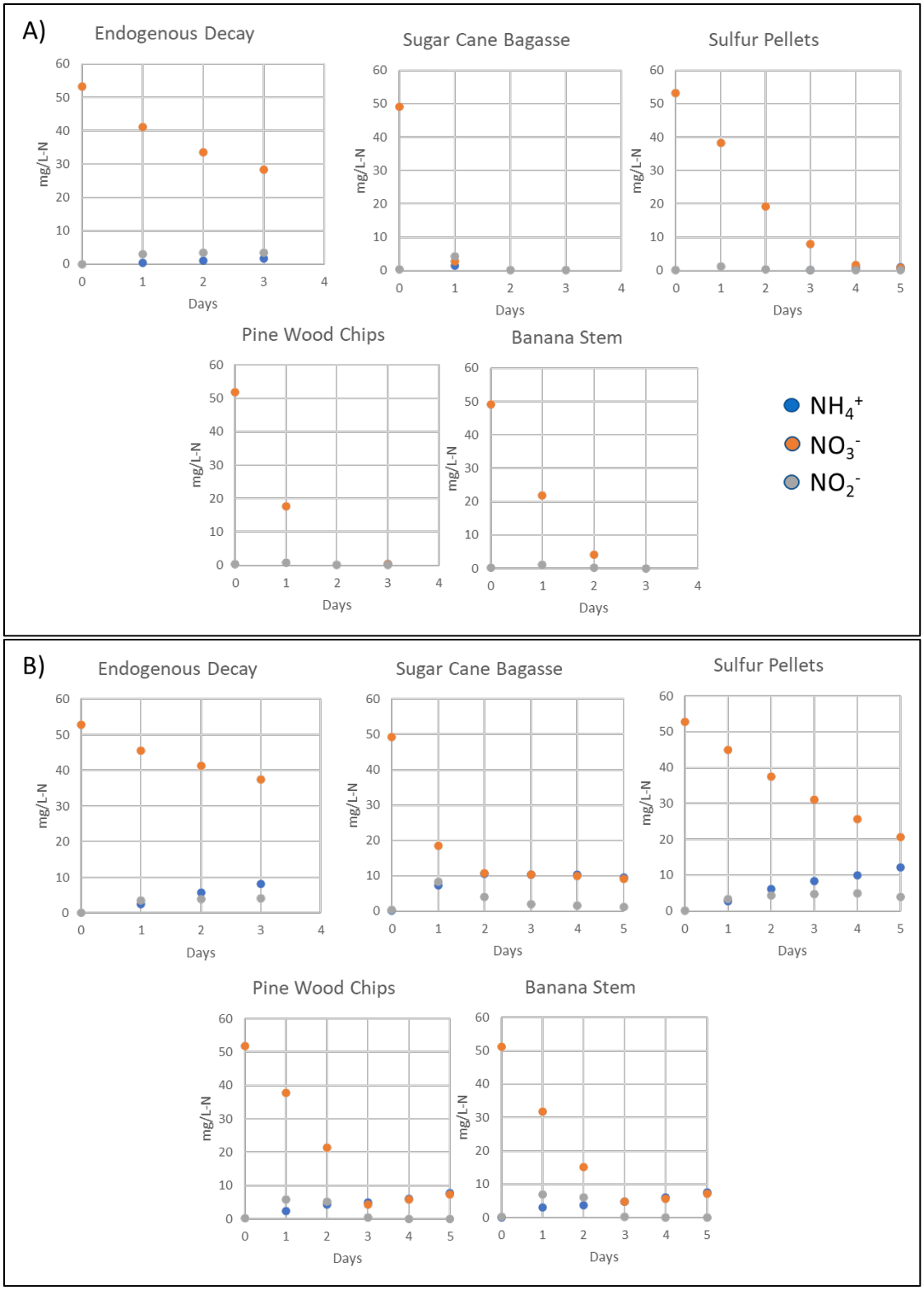

Figure 4. *Total Inorganic Nitrogen species for A) freshwater and B) saltwater microcosm having 50 grams of the following electron donors: pine woodchips, sulfur pellets, banana stem, and sugarcane bagasse.*

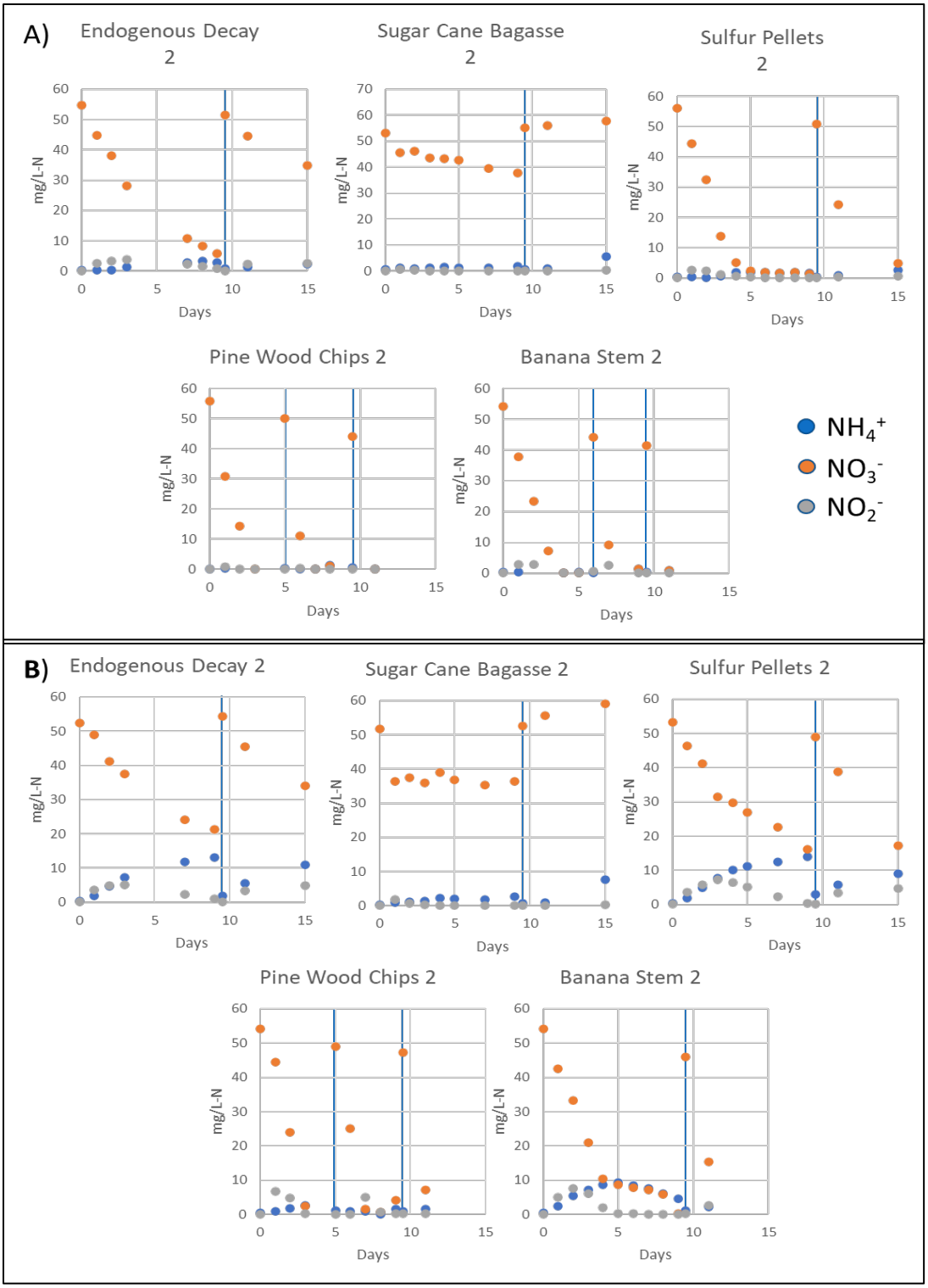

Figure 5. *Total Inorganic Nitrogen species for A) freshwater and B) saltwater microcosm having 20 grams of the following electron donors: pine woodchips, sulfur pellets, banana stem, and sugarcane bagasse. Blue line indicates when the system was recharged to bring nitrate back to 55 mg/L.*

Similar to prior literature (Li et al., 2016; Rout et al., 2017), NO_3^- removal rates showed a stronger correlation to first order than zero order kinetics, based on R^2 values shown in Table 1. Pine wood chips and Banana stem showed a higher R^2 for zero order kinetics with 3% salinity. Rout et al. (2017) noted that zero-order kinetics are observed when exogenous carbon sources are either readily available or critically limiting, and first order kinetics are observed when the exogenous carbon availability changes from readily available to limiting (Reddy and Khanna 2004).

Table 1. *Zero and First order denitrification kinetics for all electron donors. NED = not enough data to perform test.*

	Zero Order		**First Order**	
	R^2	K_0 (mg/L·day)	R^2	K_1 (1/day)
Low Salinity 50 g				
Endogenous Decay	0.966	10.0	0.991	0.232
Sugarcane Bagasse	NED	46.2	NED	2.846
Sulfur Pellets	0.962	15.6	0.992	0.489
Pine Wood Chips	NED	34.2	NED	1.077
Banana Stem	NED	24.9	NED	1.028
Low Salinity 20 g				
Endogenous Decay	0.966	7.2	0.997	0.144
Sugarcane Bagasse	0.833	1.7	0.871	0.067
Sulfur Pellets	0.767	12.6	0.901	0.395
Pine Wood Chips	0.986	21.5	0.995	0.638
Banana Stem	0.999	15.8	0.993	0.483
3% Salinity 50 g				
Endogenous Decay	0.976	6.0	0.989	0.128
Sugarcane Bagasse	0.595	21.0	0.721	0.983
Sulfur Pellets	0.992	17.9	0.998	0.168
Pine Wood Chips	0.833	30.6	0.754	0.529
Banana Stem	0.785	34.0	0.784	0.579
3% Salinity 20g				
Endogenous Decay	0.967	4.4	0.992	0.103
Sugarcane Bagasse	0.307	1.7	0.313	0.123
Sulfur Pellets	0.929	5.7	0.979	0.140
Pine Wood Chips	0.975	18.0	0.919	0.726
Banana Stem	0.999	9.2	0.941	0.309

Laboratory scale PNRS

The average percent removal of TAN was calculated from the septic tank effluent sample to the nitrification column effluent sample and shown on Table 2.

Table 2. *Average percent oxidation of ammonium in wastewater in the nitrification column for wastewater at <0.1%, 1.5%, and 3% salinity.*

Salinity	Average Percent Ammonium Oxidation
<0.1%	82
1.5%	78
3%	78

Figure 6 shows the average concentration of Total Inorganic Nitrogen (TIN) species through the treatment system. There was little to no change in TIN species and concentrations occurring in the septic tank, with the exception of a decrease in ammonium (NH_4^+) concentration for the septic tank operated with wastewater at 1.5% salinity. This may have been due to variations in wastewater NH_4^+ concentrations or biological transformations that were favored at this salinity (e.g., anammox).

In the nitrification column, most of the NH_4^+ was oxidized to NO_3^-. At nitrification columns startup for all salinities, there was a much higher concentration of NO_2^- (data not shown). Column experiments at 3% salinity showed more NO_2^- in the nitrification column effluent than experiments at all other salinities, reaching concentrations as high as 7 mg NO_2^--N/L, while acclimated concentrations were closer to 0.5 mg NO_2^--N /L. As the nitrifying organisms acclimated to the conditions, NO_2^- decreased and NO_3^- increased. This initial startup condition affected the average 3% salinity results more as this column was run for 77 days while the other two experiments were run for 172 days.

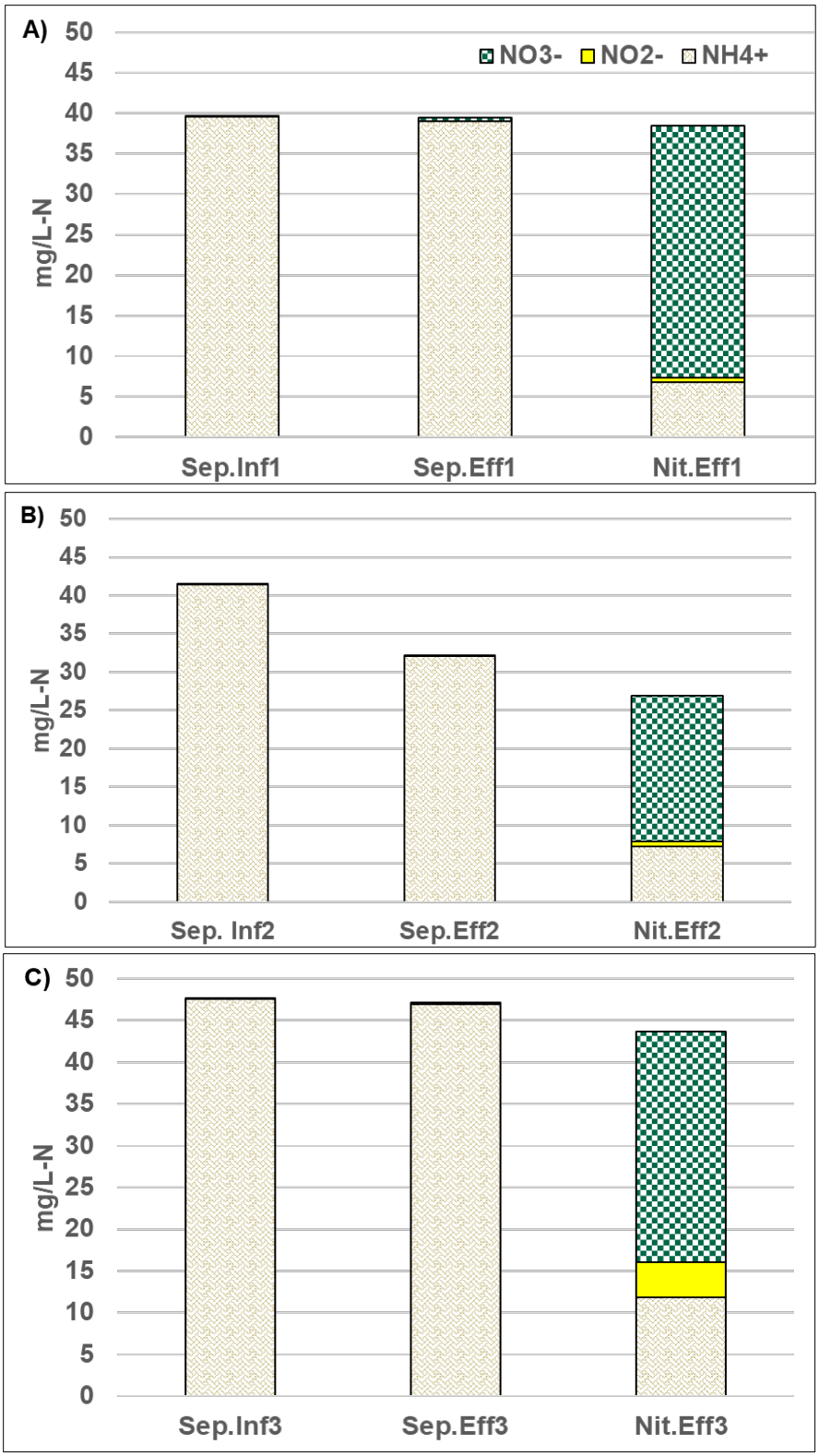

Figure 6. *Total inorganic nitrogen species (TIN) in septic tank influent, septic tank effluent and nitrification column effluent for A) wastewater at <0.1% salinity, B) wastewater at 1.5% salinity, and C) wastewater at 3% salinity.*

CONCLUSIONS

Laboratory scale PNRS showed promising results for implementation of trickling nitrification columns using wastewater with various salinities. It was expected that as the salinity increased, nitrification would become less effective due to lower microbial activity and DO concentrations. Though this was somewhat supported by the results, the effects of salinity were minimal on the percent oxidation of NH_4^+. Additionally, the nitrification column for the 1.5% saline wastewater had a lower influent NH_4^+ concentration, which may result in a lower apparent oxidation efficiency.

Denitrification at higher salinities also had promising results. For the denitrification microcosms conducted at 3% salinity, pine woodchips and banana stems proved to be effective electron donors. Sulfur pellets showed a lower but consistent rate of NO_3^- removal when compared to woodchips and sulfur pellets. Sugarcane bagasse experiments showed that sugarcane bagasse should not be used as an electron donor unless pretreated to remove sugar residue. Under a continuous flow denitrification system, as opposed to the batch setup of this experiment, the sugar residue might not have as much of an effect. Further studies are needed to consider sugarcane bagasse as a potential electron donor for high saline wastewater.

These results show that at salinities as high as typical ocean water salinity (~3% salinity), passive biological nitrogen removal is a viable option for onsite wastewater treatment systems that use saltwater for toilet flushing.

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