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Abstract

With growing populations in the coastal areas globally, the amount of freshwater resources has become an issue of increasing concern. In Guam, a multi-year drought could detrimentally impact the capacity of 90% of the freshwater resource coming from the Northern Guam Lens Aquifer (NGLA). Using seawater for flushing toilets is a way to save freshwater in coastal areas, however, seawater can influence the efficiency of wastewater treatment if it directly flows into wastewater treatment plants. The objective of this study is to evaluate the nitrification rate of wastewater under different salinity concentrations. To meet this objective, two conducting bench-scale, continuous-flow bioreactors using PVA-gel beads biocarrier were operated under the controlled conditions, such as temperature, pH, and dissolved oxygen, with the exception that seawater bioreactor influent was mixed with stepwise increasing sea salt. Key nitrification parameters (NH_4^+, NO_3^-, and NO_2^-) were monitored for the determination of nitrification rates and efficiencies of ammonium removal. In light brackish water (0% to 30% of sea salt compared to seawater), the salinity did not affect the nitrification rate. In brackish water (30% to 80% of sea salt), the nitrification process was slightly inhibited with the stepwise increasing salinity. In saline water (80% to 100% of sea salt), the transient decrease of nitrification was observed due to increase of the inactivity of bacteria. To examine bacteria activity, freshwater (0% sea salt) was supplied again after 100% of sea salt condition. The result showed that bacteria resumed activity within several days, and nitrification rate was returned to the rate observed prior to the addition of sea salt in this experiment.

Keywords: nitrification, seawater, salinity, wastewater treatment process, bioreactor

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1. Introduction

With growing populations in the coastal areas globally, the amount of freshwater resources has become an issue of increasing concern. Worldwide, nearly 2.4 billion people, representing 37% of the global population, live within 100 km of the coast (Smith, 2017). According to a report of the National Oceanic and Atmospheric Administration (NOAA), in the United States alone a total of 123.3 million people, or 39% of the nation's population, lived in low-elevation areas near the shoreline in 2010; furthermore, this number was predicted to increase an additional 8% by 2020 (NOAA, 2018). In Guam, 90% of potable water sources come from the Northern Guam Lens Aquifer (NGLA) (Simard, 2015). This lens consists of a natural rainwater impoundment in a karst limestone stratum supported by the denser underlying saltwater-saturated zone. Potentially, a multi-year drought could detrimentally impact the capacity of this invaluable freshwater resource. Therefore, finding ways of saving freshwater consumption in Guam is very important to protect the NGLA.

One strategy of replacing freshwater use is to use seawater for flushing toilets. Hong Kong has shown a successful history of switching freshwater to seawater for flushing toilets (Leung et al., 2012). It is currently supplying 750,000 m^3 of seawater per day to 80% of its seven million inhabitants, thereby saving $27 million in production and distribution expenses annually (M.C.M., 2012). However, seawater can influence the efficiency of wastewater treatment if it directly flows into wastewater treatment plants (WWTPs). The biological process is a main contaminant removal process in WWTPs. Microbes consume carbon, nitrogen, phosphorus, and other nutrient sources. Depending on aeration conditions, microbes generate either methane or carbon dioxide. The increased population of microbes and other settled waste is collected and removed. In this process, salt, especially chloride in seawater, can act as an inhibitor for nitrifying bacteria (Omil et al., 1995; Moussa et al., 2006). The metabolisms of various nitrifying species can also be inhibited by high levels of salinity, resulting in decreased removals of ammonium (Yu et al., 2002; Bernhard et al., 2007; Moussa et al., 2006). Thus, before determining the use of seawater as toilet flushing water, the effect of salinity on microbial activity, especially focusing on nitrifying bacteria, should be carefully taken into consideration.

1.1. Wastewater Treatment Process

The wastewater treatment process includes the primary clarifier, biological treatment, the secondary clarifier, and UV disinfection. Following the treatment process, the treated wastewater is then discharged to rivers or the ocean (Figure 1). The use of biological treatment is a standard method of wastewater treatment from an economic viewpoint and has been used to treat domestic and industrial wastewater. During the process, organic matter, mainly in soluble form, is converted into water (H_2O), carbon dioxide (CO_2), ammonium (NH_4^+), Nitrite (NO_2^-), nitrate (NO_3^-) and biological cells (Visvanathan C., 2000). The most important of these nutrients are carbon, nitrogen, and phosphorus. The content of the individual nutrients in wastewater should correspond to the needs of the bacteria in the activated sludge (Winkler, 2012). Domestic wastewater has the Carbon: Nitrogen: Phosphorus (C: N: P) ratio of 100: 5: 1 (Metcalf & Eddy, 2003). Recent research showed that biofilm systems could remove many water quality parameters without the need for further treatment processes (Dohare and Trivedi, 2014). High saline concentrations have negative effects on organic matter, nitrogen, and phosphorus removal

(Intrasungkha et at., 1999). Nitrogen (N) is one of the key nutrients in wastewater treatment. Increased levels of nitrogen in the environment can result in the contamination of groundwater. When excess level of nitrogen is discharged to rivers or lakes, eutrophication can occur that adversely affects benthic ecosystem (Rabalais et al., 2009). It can also affect the coral reef and impair the ocean ecosystem (Howarth, 2008). A common method to remove nitrogen in the wastewater treatment is nitrification and denitrification by nitrifying bacteria. Nitrification is generally the bottleneck for nitrogen removal in wastewater treatment plants due to the slow growth rates of the microorganisms involved.

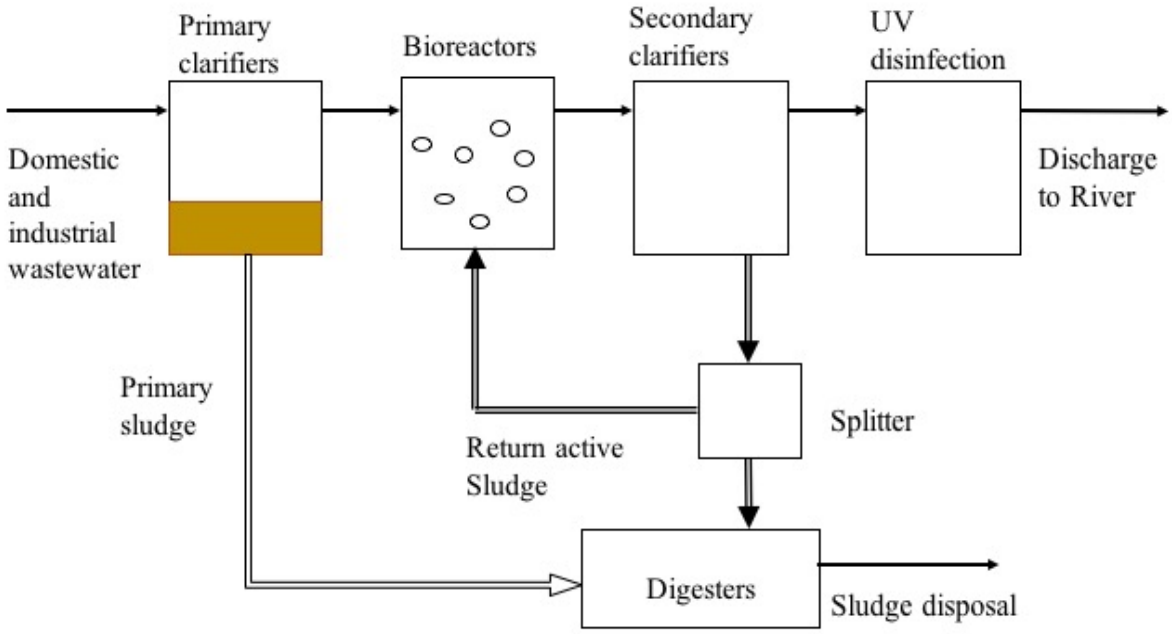

Figure 1. Wastewater treatment plant process.

1.2. Nitrification Process

Nitrification is a biological process consisting of oxidation of the nitrogenous compound ammonium via nitrite to nitrate (Eq. 1 and 2). It is an essential component of the nitrogen cycle, which occurs in soils, natural water bodies, and wastewater treatment systems.

$$2\ NH_4^+ + 3\ O_2 \rightarrow 2\ NO_2^- + 4\ H^+ + 2\ H_2O \quad (1)$$

$$2\ NO_2^- + O_2 \rightarrow 2\ NO_3^- \quad (2)$$

Ammonium nitrogen is one of the dominant contaminants in the wastewater system. The concentration of ammonium can vary easily by pH. By Eq. 3, the Ka of ammonium ion can be expressed as Eq. 4. The pKa value is one method to indicate the strength of an acid. The pH is the sum of the pKa value and the log of the concentration of the conjugate base (NH_3) divided by the concentration of the weak acid (NH_4^+) (Eq. 5) (Bhagavan, 2002). The pKa value of Eq. 3 is 9.25, so the concentration of NH_4^+ is expected to be relatively higher between pH 7 ~ 8, which is the normal pH range for the bioreactor system (Ashtari et al., 2016).

$$NH_4^+ \rightleftharpoons H^+ + NH_3 \quad (3)$$

$$Ka = \frac{\{H^+][NH_3]}{[NH_4^+]} \quad (4)$$

$$\text{pH} = \text{pKa} + \log\frac{[NH_3]}{[NH_4^+]} \quad (5)$$

The natural processes in the nitrogen cycle include fixation and uptake, by which N is incorporated into the organic matter, ammonification, by which N is released from organic matter, nitrification, and denitrification (USGS, 2011). These processes are performed by bacteria. The microorganisms involved in nitrification require oxygen for metabolism. Nitrification is performed by two functionally defined groups of microbes, referred to together as nitrifiers: Nitrosomonas is a well-known genus of ammonium oxidizing bacteria (AOB), and Nitrobacter is a well-known genus of nitrite oxidizing bacteria (NOB) (Horan, 2003; Robertson and Groffman, 2006). Followed by denitrifying bacteria under anoxic conditions (very low amount of free oxygen), these organisms collectively provide a natural pathway for the transformation of organic nitrogenous contaminants to dinitrogen gas, which diffuses into the atmosphere, thus being eliminated in an efficient, environmentally friendly manner.

Recent studies suggest that microbial Nitrogen could transform by nitrifier denitrification and anaerobic ammonia oxidation (Anammox) process (Nannipieri and Eldor, 2009). Nitrifier denitrification carried out by AOB is the oxidation of NH_4^+ to NO_2^- followed by the reduction of NO_2^- to nitrogen oxide (NO), nitrous oxide (N_2O), and nitrogen gas (N_2) (Norton, 2008). Anammox involves oxidation of ammonia to N_2 with the reduction of nitrite (Jetten, 2001). Anammox bacterial species can reduce nitrite to hydroxylamine, which can condense with ammonium to hydrazine (N_2H_4); the formed hydrazine is eventually oxidized to N_2 and the released electrons are used to reduce nitrite (Strous et al., 1997).

In addition, nitrifying bacteria in the nitrification process are sensitive to environmental factors such as temperature (Sousa et al.,2012; Hoang, 2013), dissolved oxygen level (Sharma and Ahlert, 1997), pH (Antoniou et al., 1990), and salinity (Moussa et al., 2006). Salt is a common factor that affects the microbial communities in wastewater treatment plants. It is well known that high salinity in wastewater inhibits the metabolic activity of many species of bacteria (Omil et al., 1995).

1.3. Objectives and Scope

The influence of saltwater on microbial activities is a relatively new field of study. Accordingly, research into applications of adaptive methodologies to enhance the performances of the processes and systems challenged by the impact of high salt concentrations is urgently needed.

The objective of this study is to evaluate the nitrification rate of wastewater under different salinity concentrations and determine the optimal environmental conditions for the design of seawater-based systems. Two conducting bench-scale, continuous flow treatment using moving-bed biofilm reactors were used to evaluate the influence of seawater on biological nitrification as applicable to municipal wastewater treatment in an effort to define the optimal conditions for design and operation. A well-characterized porous biocarrier was employed for immobilization and retention of effective biomass. The biological treatment system to be studied consisted of two parallel unit processes: wastewater treatment bioreactor without salt addition (bioreactor A)

and wastewater treatment bioreactor with salt addition (bioreactor B). They were operated under the same conditions, with the exception of bioreactor B which was subject to stepwise increases in salinity up to complete seawater inclusion. The other unit process was served as a background control to allow for comparison of results and assessment of impacts. Attention was given to monitoring key nitrogenous parameters in the ionic form [ammonium nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^-N), and nitrate nitrogen (NO_3^-N)] to allow for the determination of treatment rates and efficiencies. To estimate salinity, chloride concentrations and conductivity were also measured. Other control factors, such as temperature, pH, and dissolved oxygen (DO), were also measured periodically.

2. Methods

2.1. Bioreactor Design

Plexiglass bench-scale bioreactors (Figure 2) with liquid volumes of 1.7 L were used in continuous flow mode with peristaltic pumps (Masterflex L/S, Cole-Parmer). Aeration requirements were met by using a Blue Stone Air Pump (AP-0025B, General Hydroponics) with diffuser stones, which also served to maintain thorough mixing of the PVA-gel beads. The bioreactors had a water depth of 24 cm and a diameter of 12 cm and were considered to function as complete-mixed units. A small mesh screen with 2-mm openings was fashioned internally to the outflow pipe of each bioreactor to prevent the loss of the PVA-gel beads. 30.0 L of influent tanks were prepared. A sparger was installed to provide continuous aeration. The schematic diagram of the bioreactor design is shown in Figure 3.

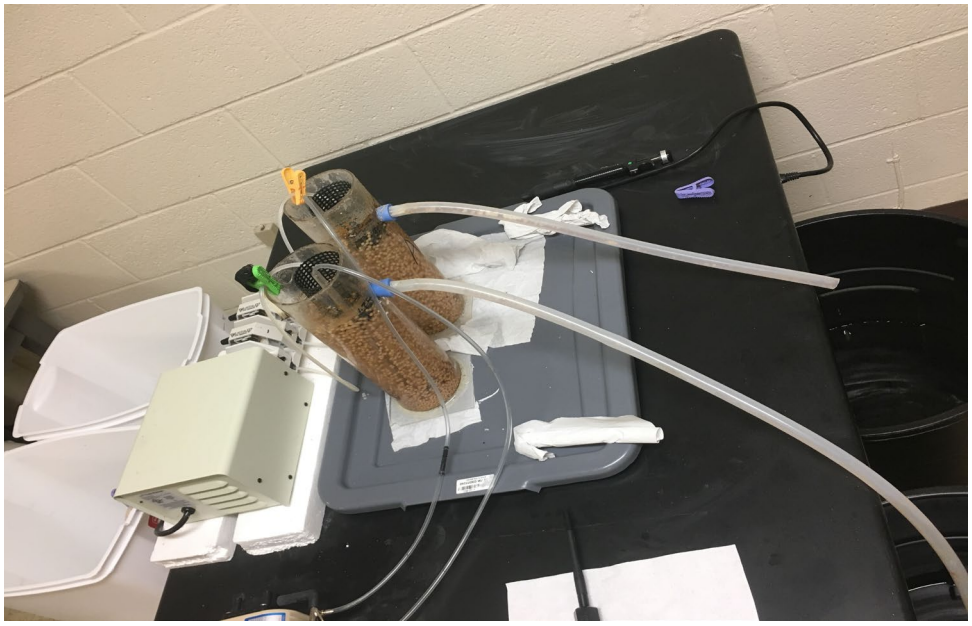

Figure 2. A photo of plexiglass bench-scale bioreactors.

Biocarriers consisting of polyvinyl alcohol (PVA) gel has demonstrated effectiveness in wastewater treatment (Hoa et al., 2006; Rouse et al., 2005). PVA-gel beads (Figure 4) constitute

an example of a biocarrier having a porous matrix that allows for microorganisms to attach and form a biofilm inside and outside the gel beads. This provides favorable conditions for the retention and cultivation of slowly growing microorganisms by preventing them from being flushed out of the unit process. The PVA-gel beads have a diameter of 4 mm with a specific gravity of 1.025 (Kuraray Aqua Co., 2008), making them easy to suspend in water. They are hydrophilic in nature and have a porous structure with a continuum of passages that are 10 to 20 μm in diameter throughout each bead. Biofilm cultures are known to offer protective niches for biomass, thus allowing some degree of protection to harmful effects in the environment, which may also assist in abating the effects of salt. At 8% volume, PVA-gel beads were added in two bioreactors containing 1.7-L wastewater. Bacteria used to develop a nitrifying culture for this study originated from the aerated, influent-holding pond of the Umatac-Merizo sewage treatment plant in southern Guam (Figure 5). Pond samples drawn in plastic bottles were transported to the bioreactor laboratory at WERI, UOG, and used as the seed in bench-scale reactors containing a biocarrier material.

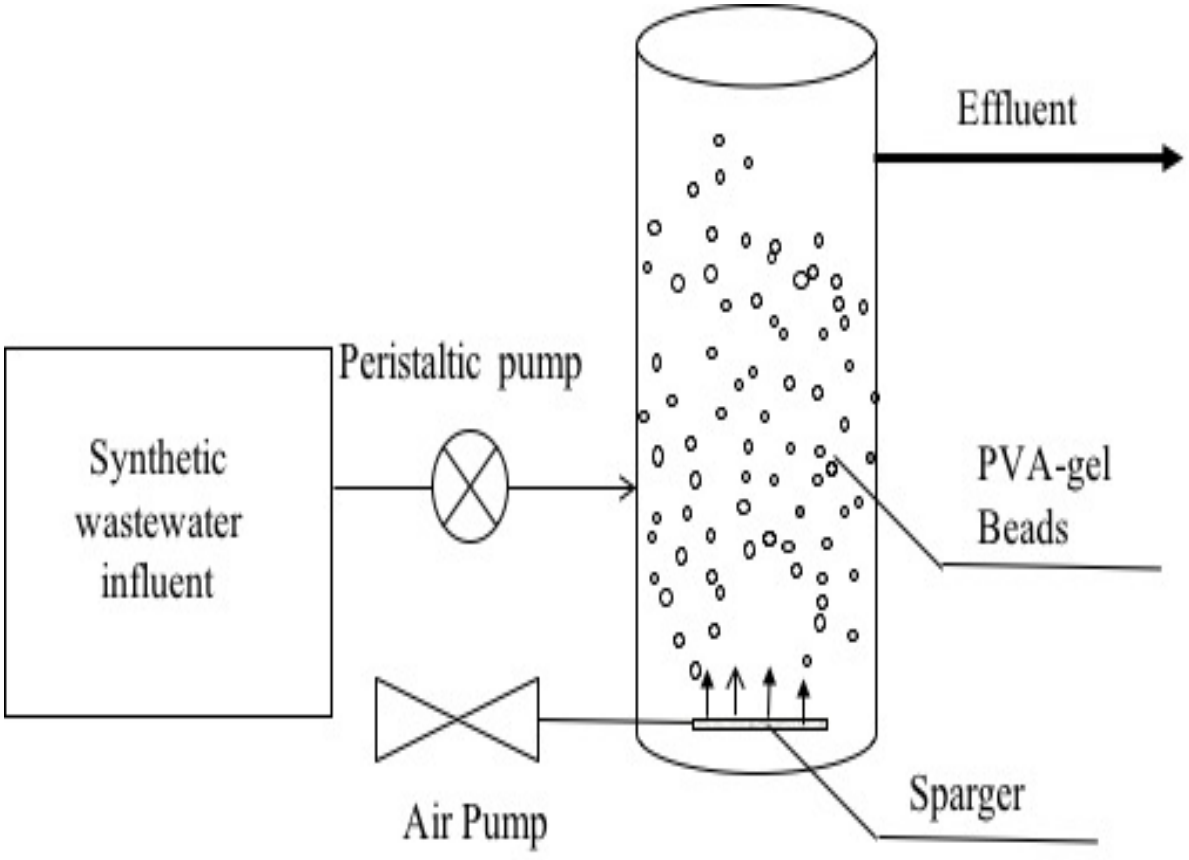

Figure 3. Schematic diagram of bioreactor design.

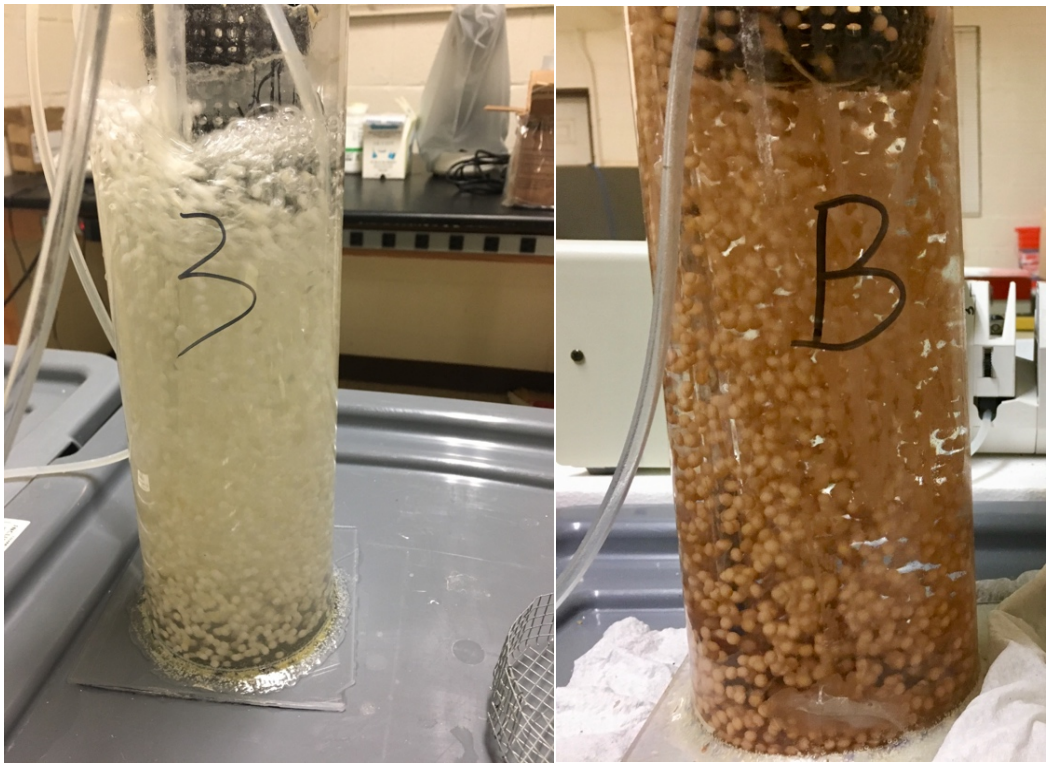

Figure 4. PVA-gel beads biocarrier use in this study (left: new beads; right: beads with bacteria).

Figure 5. Ponding for cultivating nitrifying bacteria in Umatac-Merizo sewage treatment plant.

2.2. Determination of Optimum Hydraulic Retention Time (HRT)

The preliminary experiment was performed to determine optimum hydraulic retention time (HRT) in relation to ammonium removal under synthetic wastewater flow conditions. Firstly, ammonium sulfate (RICC) was selected to prepare synthetic wastewater that mimics a level of common municipal wastewater. Ammonium sulfate ($(NH_4)_2SO_4$) is an inorganic salt with a number of commercial uses. The most common use is as a soil fertilizer (Speight, 2017). 141.4 mg/L of ammonium sulfate, which is equal to 30 mg/L of NH_4^+-N, was added to the influent. In this experiment, tap water from the NGLA was used as a wastewater source. The tap water was analyzed to check background constituents, such as chloride, NO_3^--N, and NO_2^--N, which can influence nitrification. Chloride (Mercuric Thiocyanate Flow injection method, SM4500-Cl.G) was analyzed by FIA Injection Analyzer (Lachat, Quichem 8500 Series 2). NO_3^--N (cadmium reduction method, SM4500-NO3.1) and NO_2^--N (diazotization method, SM4500-NO3.I) were analyzed by a Flow Inject Analysis machine (Lachat, Quikchem 8500 Series 2). NH_4^+-N concentrations in the experiment were also analyzed by the Flow Inject Analysis machine (automated phenate method, SM4500-NH3.G). Tap water samples used in this study included Chloride (42~50 mg/L), NH_4^+-N (< 0.01 mg/L), NO_3^--N (0.7 ~ 4.7 mg/L), and NO_2^--N (< 0.005 mg/L). During the experiment, temperature, pH, and DO were also measured to examine experiment conditions. In this experiment, each value was regulated at: 7.0 ± 0.5 of pH and 9.0 ± 0.9 mg/L of DO for influent, and 25 ° C of room temperature. In the case of pH, 1.0 N of acid (Potassium Phosphate Monobasic, Aqua Solutions) or 1.0 N of base buffer solution (Sodium Hydroxide, Sigma-Aldrich) was added if pH in the effluent was lower or higher than 6.5 ~ 7.5. A YSI Pro 1020 meter was used for pH and temperature measurements. A YSI Pro 2030 meter was used for DO. In this experiment, samplings were conducted every 48 hours to analyze NH_4^+-N, NO_3^--N and NO_2^--N.

The operation was then conducted at various influent flow rates corresponding to hydraulic retention times (HRTs) ranging from 1.0 h to 6.0 h to observe the effect of HRT on nitrification efficiency. Loading rate and removal rate of nitrogen-ammonium are expressed as:

$$\text{Loading rate } R_L = \frac{C_i}{t} \quad (6)$$

where C_i is the concentration of NH_4^+-N in the influent container, and t is the HRT.

Removal rate of NH_4^+-N is shown below:

$$\text{Removal rate } Rr = \frac{C_i - C_r}{t} \quad (7)$$

where C_r is the concentration of NH_4^+-N in the bioreactor.

Therefore, removal efficiency of NH_4^+-N over the course of nitrification can be defined as:

$$\text{Removal efficiency (\%)} = \frac{R_r}{R_L} \quad (8)$$

2.3. The Effect of Sea Salt

Under the HRT=3.0 hr condition, two bench-scale bioreactors were operated. One bioreactor (bioreactor A) was operated under synthetic wastewater conditions without adding sea salt. The other bioreactor (bioreactor B) was operated under the increment of sea salt (Instant Ocean®) concentration with the same synthetic wastewater condition. Real seawater generally contains 31 ~ 38 g/L of salt (Hankins et al., 2016). Seawater concentrations vary depending on locations. In this experiment, it was assumed that seawater around Guam contains 36.0 g/L of salt. Table 1 shows the seven salt increment steps applied to the experiment. For example, 100% salt means that 36.0 g/L of salt was added.

The two bioreactors were operated for about 198 days. Operation days of each salt increment in bioreactor B were also shown in Table 1. Chloride concentrations in influent were measured about 3 — 6 times for each step. Conductivity values were measured many times with the conductivity meter (Thermo Orion Star A215 pH/Conductivity Benchtop Multiparameter Meter) to confirm the salinity of the influent. Figure 6 shows the relationship between conductivity and chloride concentrations measured at the same sampling period. The R^2 value showed 0.993 that confirmed conductivity values were reliable to estimate salinity. With measured chloride concentration data, salinity values were calculated by Eq. 9.

$$\text{Salt (\%)} = [1{,}806.55 \times \text{chloride (mg/L)}] / 19{,}800 \text{ mg/L} \quad (9)$$

where 19,800 mg/L is an assumed chloride value in real seawater.

Table 1. The addition of sea salt to different water types.

Method		Freshwater	Brackish water					Saline water
Salt added	g/L	-	7.2	10.8	18.0	25.2	28.8	36.0
Salt (%)	[1]estimated	0	20	30	50	70	80	100
	[2]calculated	0	19	35	52	69	80	103
Operation days at each step[3] (days)		49	32	22	30	25	22	18

[1]estimated % (salt added (g) / 36.0 g) × 100
[2]calculted % by Eq. 9.
[3]total cumulative operation days until 100% saltwater is 198 days

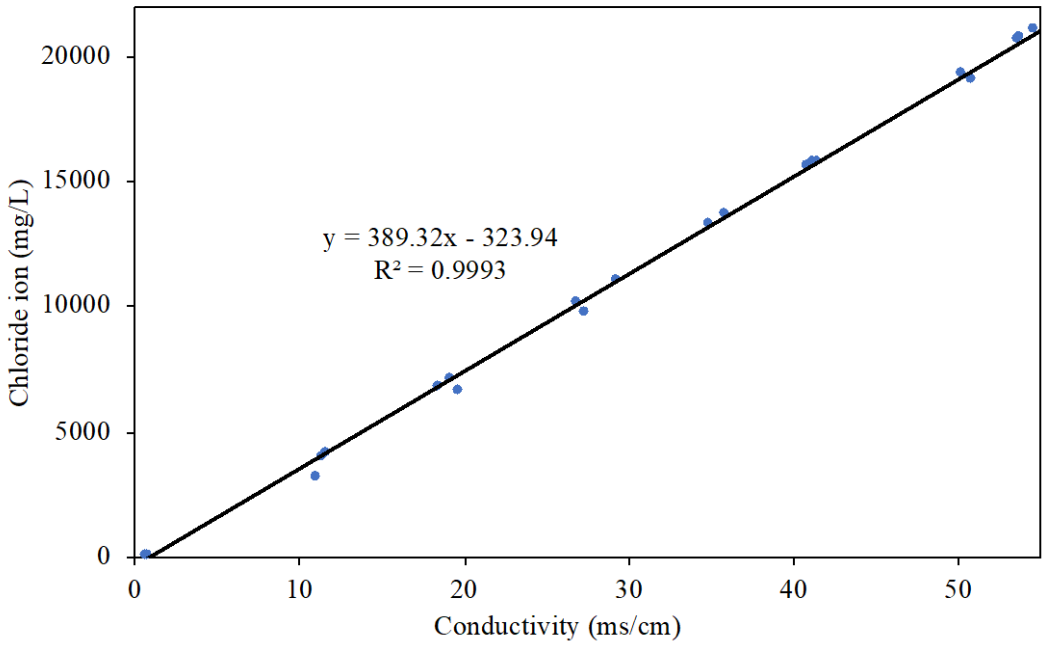

Figure 6. Linear regression results between chloride ion and conductivity concentration.

Calculated salinity values were compared with salt added values to examine any difference between added salt (%) and calculated salt (%) based on measured chloride ions. Salt (%) showed a ± 5% error. Recall that 30.0 L of the influent tank was used, the volume of the bioreactor is 1.7L, and HRT applied to this experiment was 3 hrs. Thus, 30.0 L of wastewater was consumed in about two days; therefore, every two days, wastewater containing salt was prepared. This repeated manual step might result in slightly different chloride and salt concentrations. After completing the saltwater experiment, fresh tap water was supplied again for about 14 days to examine whether the bacteria resumed activity.

Over the course of this experiment, NH_4^+-N, NO_3^-N, and NO_2^-N were measured. In the case of bioreactor B, the same sampling procedure with bioreactor A was followed to compare any biological inhibition by added synthetic wastewater. Temperature, pH, and DO were also measured. The pH was controlled by buffer solutions. Analysis methods and pH control were described earlier.

3. Results

3.1. Determination of Optimum Hydraulic Retention Time

Two bioreactors (one as duplicate) were operated to investigate HRTs. HRTs were set up 1, 2, 3, 4, 5, and 6 hours. Loading rate, removal rate, and removal efficiency of ammonium were introduced in the method section (Eq. 6, 7, and 8). Figure 7 shows the change in ammonium removal efficiency over HRTs. As shown in Figure 7, at HRTs of 3 hr through 6 hr, the efficiency was consistently at about 96%; then at HRTs of 1.0 hr and 2.0 hr, the efficiency dropped off to about 48% and 88%, respectively. At HRT = 3 hr, the ammonium loading rate of

11.04 mg/L*hr is a reasonably high industrial rate for a nitrification unit process. In that respect, an HRT of 3 hr might be considered reasonable for this study.

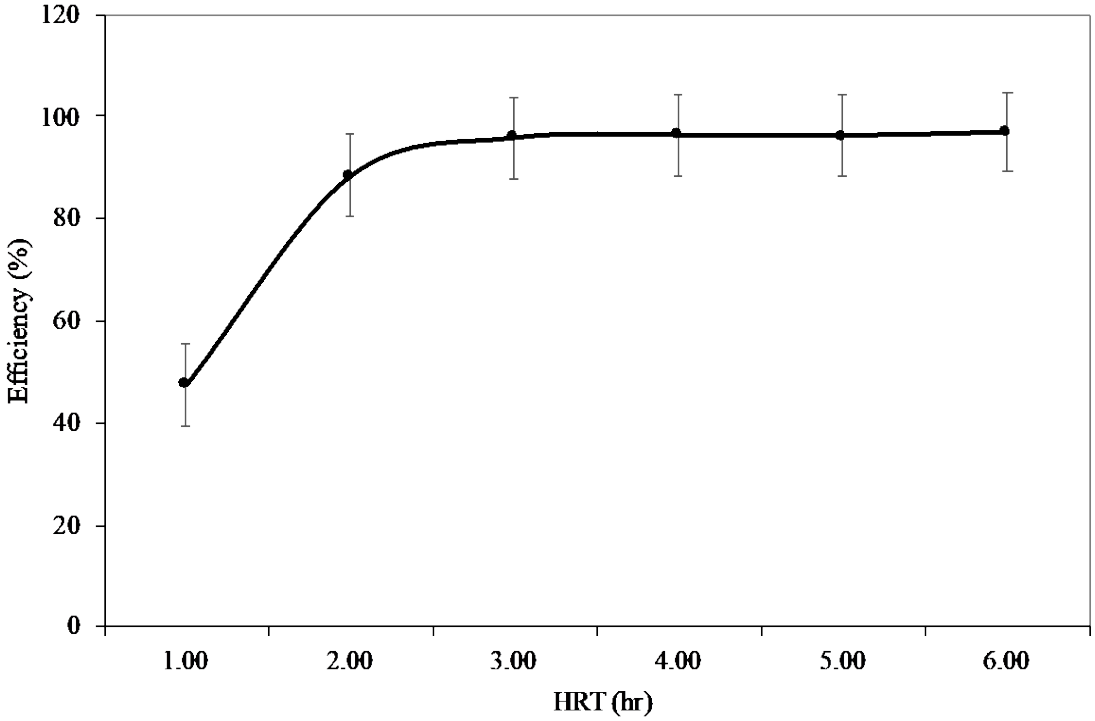

Figure 7. Ammonium removal efficiency over HRTs.

The purpose of this study, though, is to evaluate the impact of salt concentration on nitrification rate/efficiency rather than to achieve the highest possible nitrification rate, which could only be effectively improved upon by increasing the amount of biomass in the process, which, in turn, could only be done by adding and enriching additional biocarrier material (i.e., PVA-gel beads). Furthermore, at HRT = 3.0 hr, the system is near the upper limits of its efficiency, where the introduction of an environmental stress factor (e.g., salt) would be most readily detectable (i.e., a relatively small drop in efficiency), thus serving the purpose of this study to be sensitive to the effect of salt.

3.2. Water Qualities

Since nitrification is a microbiological process, the nitrifying bacteria are sensitive to environmental factors such as temperature (Sousa et al.,2012; Hoang, 2013), DO (Sharma and Ahlert, 1997), pH (Antoniou et al., 1990), and salinity (Moussa et al., 2006). According to a previous study, the optimum temperature is between 15°C and 35 °C (Halling-Sørensen and Jørgensen, 1993). The microorganisms involved in nitrification are aerobic (requiring free oxygen for metabolism). The level of DO is important for the growth of nitrifiers. Wilen et al. (2010) suggested that DO concentration was usually maintained higher than 2 mg/L in the wastewater treatment plant to prove oxygen depletion. In this experiment, the independent effect of salinity on nitrification was observed. Table 2 shows the average value of the physical-

chemical parameters (temperature, pH, and DO) measured in two bioreactors A and B (influent and effluent) at different estimated salt percentages. The temperature in the bioreactors was kept between 25.2 °C and 25.7 °C. DO in the bioreactors was provided by an air pump, which was kept between 6.7 mg/L to 7.8 mg/L, which would not inhibit the activity of bacteria in the reactor.

Table 2. Operational parameters (average values) of experiments in the bioreactors during the experiments.

Parameters	Bioreactor A	Bioreactor B						
Experiment	Without sea salt addition	0% sea-water	20% sea-water	30% sea-water	50% sea-water	70% sea-water	80% sea-water	100% sea-water
Temperature (°C) influent water	25.4	25.7	25.2	25.5	25.5	25.4	25.6	-
pH influent water	7.6	7.1	7.3	7.3	7.3	8.2	7.6	8.0
Temperature (°C) effluent water	25.8	26.0	25.6	25.7	25.9	25.6	25.8	25.2
pH effluent water	6.8	6.8	7.0	6.8	6.6	7.3	6.8	8.0
DO effluent water (mg/L)	7.1	6.7	7.1	7.0	6.8	7.7	7.6	7.8

The range of pH in the influent was 7.1 - 8.2, which reflected the range of pH in the GNLA 6.97 – 8.05 (GWA, 2017). In the wastewater treatment, the maximum nitrification occurred at a pH level of approximately 7.8 (Antoniou et al., 1990). As shown in Eq. 1, the first step of nitrification from ammonium (NH_4^+) to nitrate (NO_3^-) will produce H^+, which will lead to an increase in acidity and a decrease in pH. This pH decrease is consistent with what we observed during our experiment. At bioreactor A (without salt addition) and low salinity in bioreactor B, pH in the effluent (6.6 – 7.3) was always lower than the influent (7.1 – 8.2), which means the nitrification process occurred. At 100% percent seawater in bioreactor B, the pH in the effluent showed no change compared with pH in the influent, which indicated the nitrification rate was inhibited at high salinity.

3.3. Performances of Bioreactor A (without sea salt addition)

Bioreactor A was operated over 200 days under synthetic wastewater conditions. The concentration of NH_4^+-N was controlled to 30.0 mg/L by adding 141.4 mg/L of ammonium sulfate. In reality, the influent concentration of NH_4^+-N in bioreactor A was 26.3 ± 3.4 mg/L and influent concentration of NO_3^--N was between 2.8 mg/L to 5.0 mg/L, which is mostly from the groundwater origins in Guam (0.7 mg/L to 4.7 mg/L). The concentration of NH_4^+-N in the effluent was from 0.3 mg/L to 5.8 mg/L during the whole period. Nitrate and nitrite are products during the nitrification process. The concentration of NO_3^--N in the effluent was from 26.1 mg/L to 33.1 mg/L, and the concentration of NO_2^--N was always lower than 0.5 mg/L. As a consequence, the average ammonium removal rate was 91.9%, and the average nitrite and nitrate

production rate was 90.1%, which were almost identical from the beginning to the end of the experiment (Figure 8 & 9). Due to other ways of nitrogen transformation, such as Anammox process and nitrifier denitrification (Nannipieri and Eldor, 2009; Norton, 2008), some amount of ammonium might convert to N_2 which is released by gas. This demonstrated that the production rate was lower than the removal rate. Nitrate is the main product in the nitrification process (Figure 10). This indicates almost full nitrogen converted to nitrate in the end, and the biofilm reactor worked well under these experimental conditions.

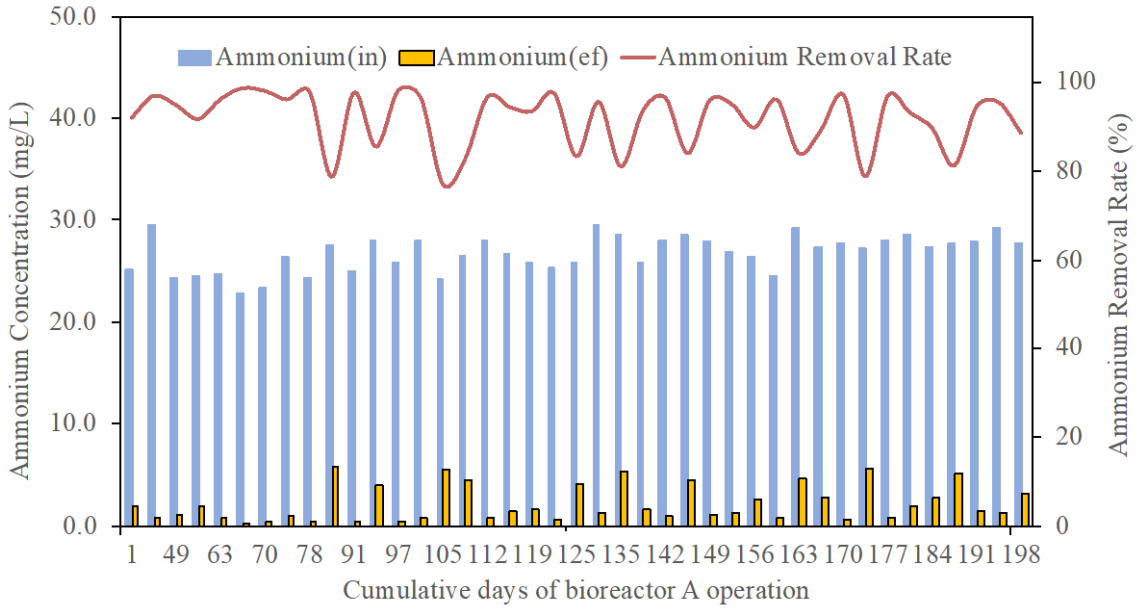

Figure 8. Influent and effluent concentration of ammonium nitrogen and ammonium removal rate from bioreactor A (without sea salt addition).

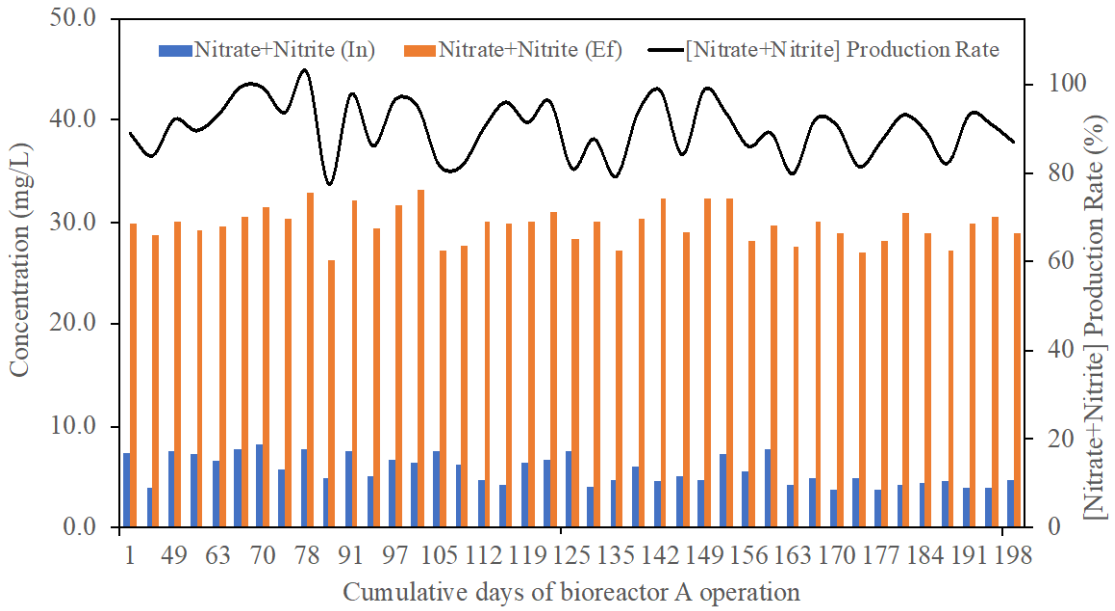

Figure 9. Influent and effluent concentration of nitrate + nitrite nitrogen and nitrate + nitrite production rate from bioreactor A (without sea salt addition).

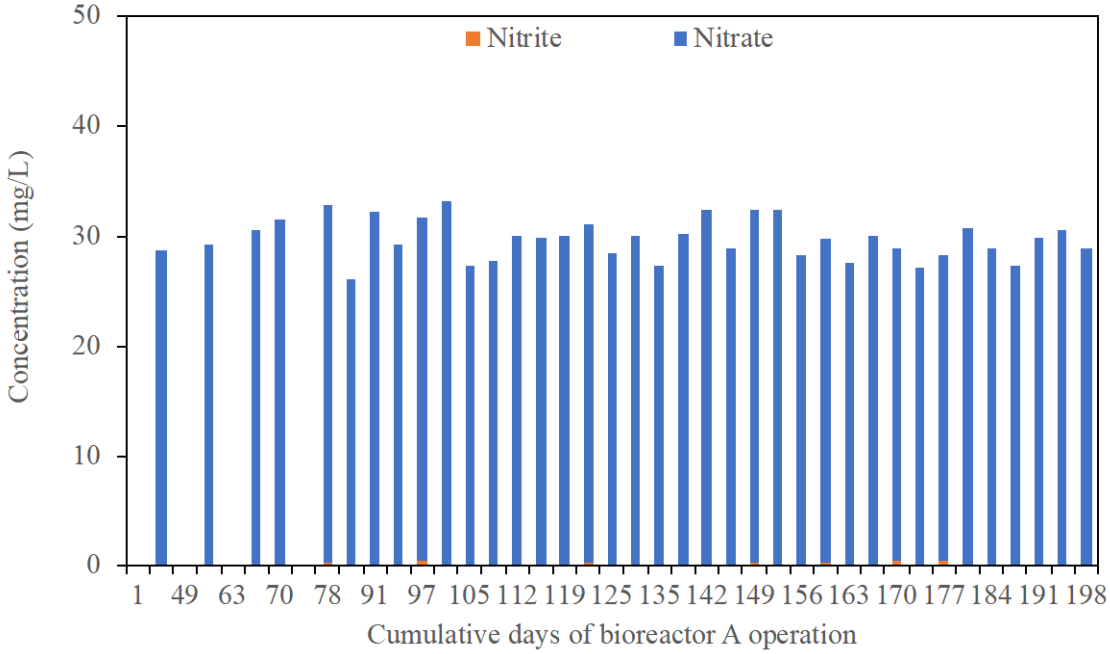

Figure 10. Effluent concentration of nitrate and nitrite nitrogen from bioreactor A (without sea salt addition).

3.4. Performances of Bioreactor B (with sea salt addition)

3.4.1. Ammonium

Experimental conditions were the same as bioreactor A except for the stepwise addition of sea salt (Table 1). The influent concentration of NH_4^+-N and NO_3^--N in bioreactor B was kept similar to bioreactor A, which was 26.3 ± 4 mg/L and 2.8 mg/L to 5.0 mg/L. Respectively, from 0% to 30% seawater, the effluent concentration of NH_4^+-N was less than 2.0 mg/L, which corresponded to an ammonium removal rate greater than 90%. Bioreactor B performed similarly as bioreactor A in a low level of seawater. There were not any significant effects of saltwater level on transformations of ammonium with seawater less than 30%. Following 30% to 80% sea salt addition, the NH_4^+-N levels started rising, and the nitrate levels started dropping (Figure 11). The effluent concentration of NH_4^+-N decreased with the increasing of seawater percentage and the ammonium removal rate dropped from 94.9% to 62.3%, demonstrating that nitrification was becoming increasingly inhibited under increasing levels of seawater. It is noticeable, however, at 100% saltwater NH_4^+-N levels were not stable and kept rising from 11.9 mg/L to 24.8 mg/L, and the ammonium removal rate dropped from 57.7% to 16.4%. The nitrification rate was low, which means the bacteria became inactive with the continuous input of salt.

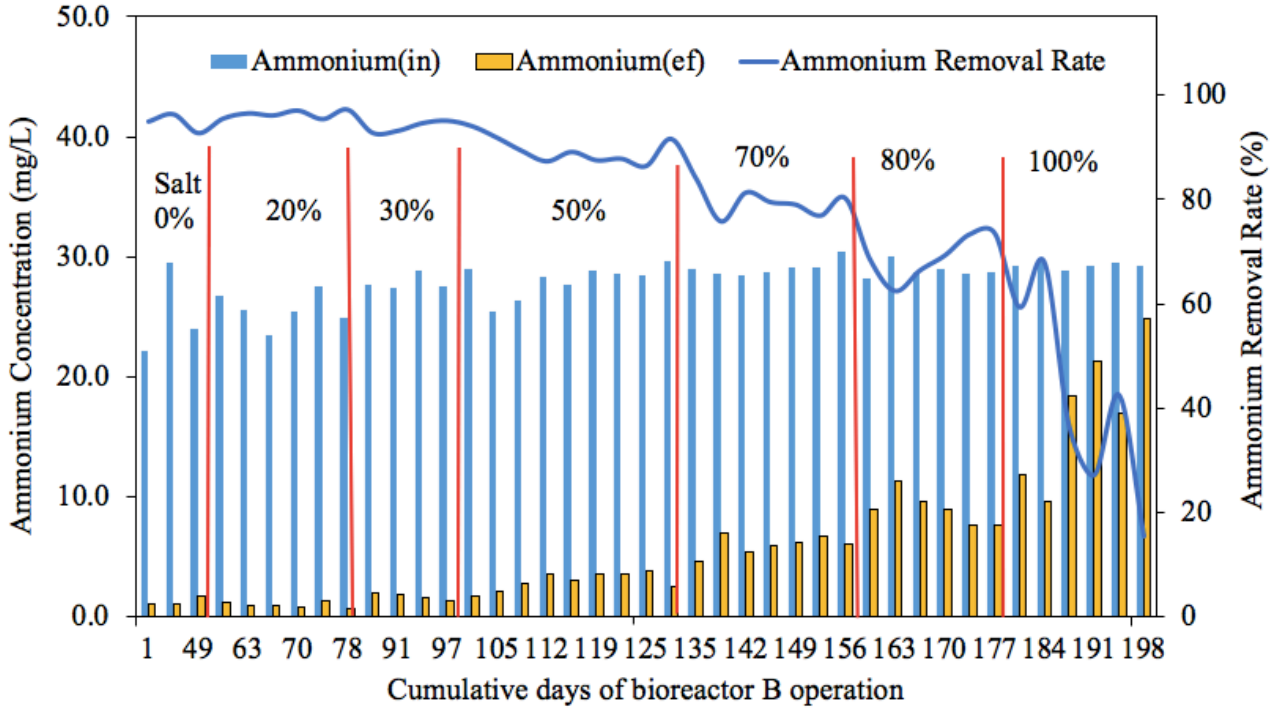

Figure 11. Influent and effluent concentration of ammonium nitrogen and ammonium removal rate from bioreactor B (with sea salt addition).

In order to analyze how salinity affects nitrification rate, linear regression methods in the ammonium removal rate were observed when salinity changed (Figure 12). From 0% to 30% seawater (fresh to light brackish water), as shown by the essentially zero-order function of ammonium removal rate versus saltwater level over this range of data, R^2 is 0.021. Light brackish water has no significant effects on ammonium removal rates. From 30% to 80% seawater (brackish water), the decrease in ammonium removal rate can be described by a first-order function with a strong correlation of $R^2 = 0.8523$. The ammonium removal rate was significantly inhibited by the level of salt content. From 80% to 100% seawater (dense brackish to saline water), the decrease in ammonium removal rate can be described by a first-order function with a weak correlation of $R^2 = 0.5282$.

From 80% to 100% seawater, the linear regression could not completely explain the relationship between nitrification rate and seawater. The ammonium removal rate changing with operation days was observed (Figure 13). At 80% seawater, the ammonium removal rate gradually increased with the operation day, which indicates bacteria were adapted to the salinity. At 100% seawater, the ammonium removal rate decreased with the operation day, which means the bacteria became inactive with the continuous input of salt. To test the activity of bacteria, the result of the recovery process was discussed later.

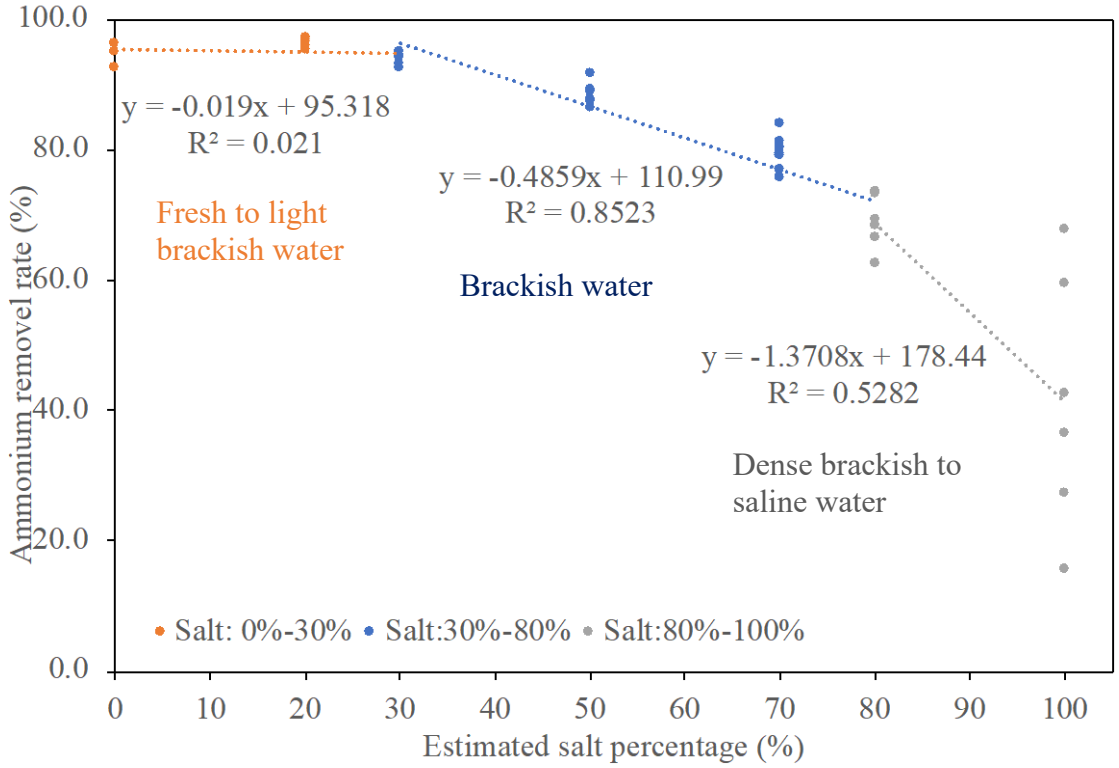

Figure 12. The mode fits with linear regression on the ammonium removal rate and salt percentage.

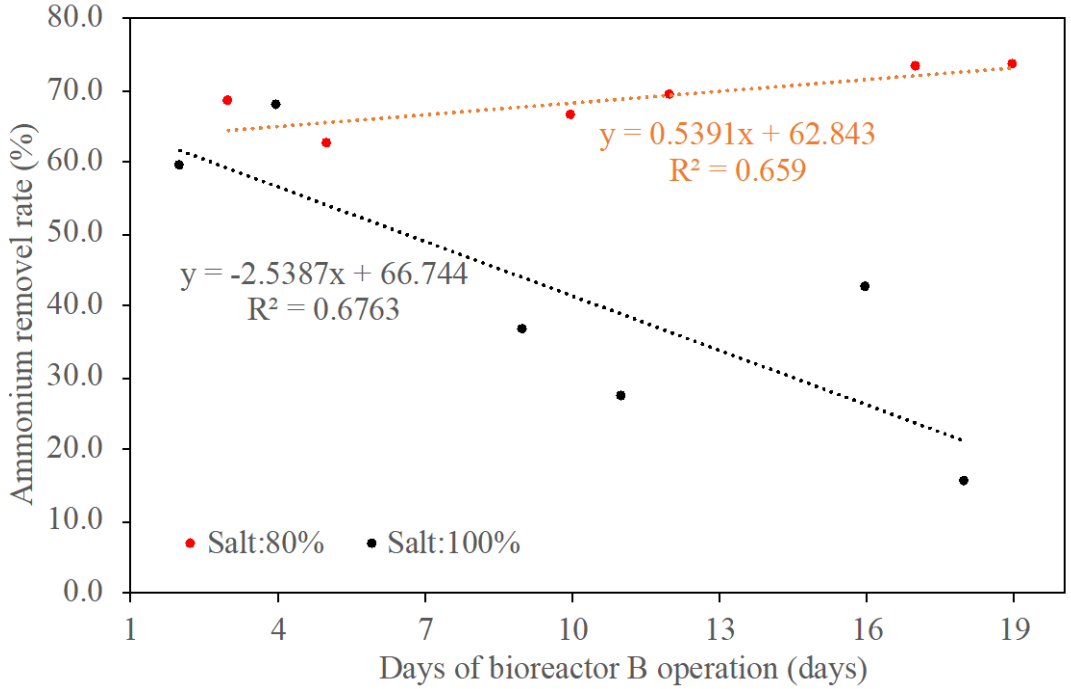

Figure 13. Ammonium removal rate at 80% and 100% seawater with operation days.

3.4.2. Nitrate and Nitrite

Nitrogen production was significantly affected by seawater. Salt can inhibit both nitrite and nitrate. The influent concentration of NH_4^+-N in bioreactor B was 26.3 ± 4.2 mg/L and influent concentration of NO_3^--N was between 3.3 mg/L to 5.1 mg/L which were similar to bioreactor A. The concentration of total NO_3^--N and NO_2^--N produced by the nitrification process was inversely related to the concentration of NH_4^+-N in bioreactor B (Figure 14). From 0% to 30% seawater, the production rate was greater than 86.6%. From 30% to 100% seawater, the production rate of nitrate and nitrite decreased with the increment of salinity due to the inhibition of nitrification.

During the nitrification process, nitrate was the main product. While the concentration of nitrate decreased with the increment of salinity, the concentration of nitrite increased with the increment of salinity in bioreactor B (Figure 15).

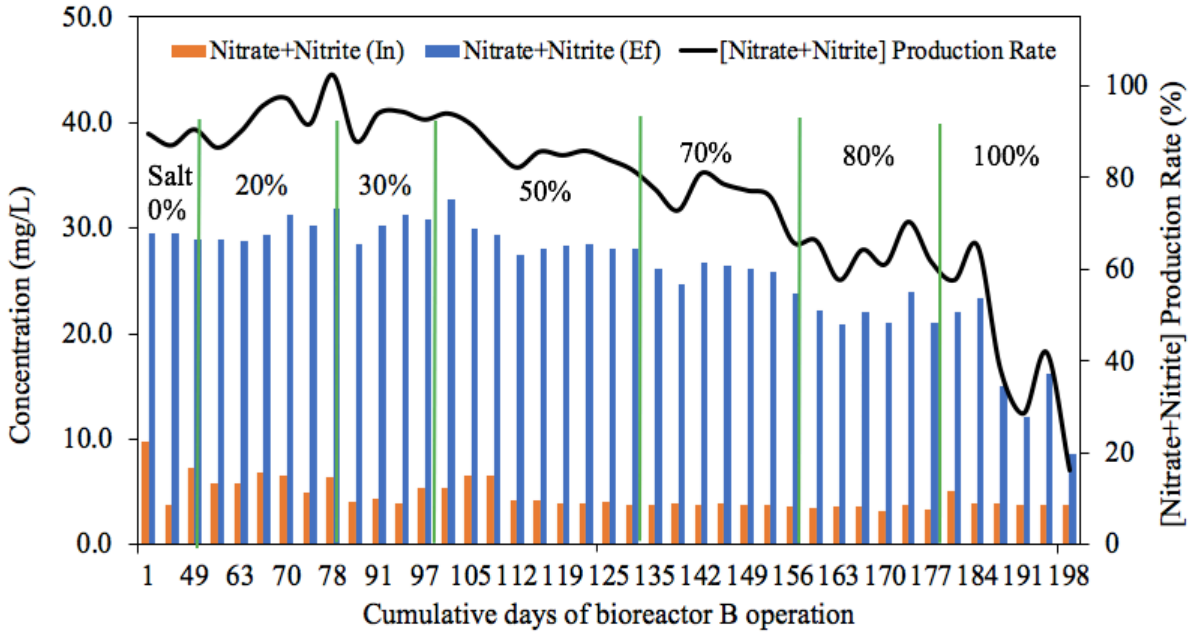

Figure 14. Influent and effluent concentration of nitrate and nitrate nitrogen, and nitrate + nitrite production rate from bioreactor B (with sea salt addition).

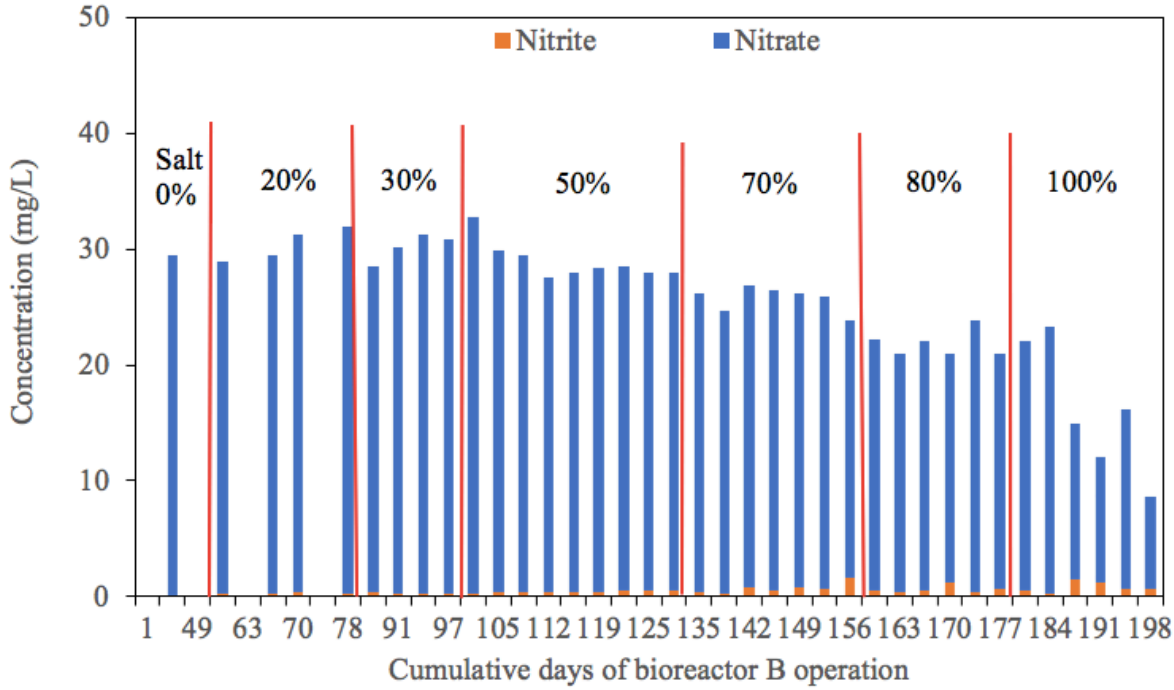

Figure 15. Effluent concentration of nitrate and nitrite nitrogen from bioreactor B (with sea salt addition).

In bioreactor A, the concentration of NO_2^--N was less than 0.5 mg/L. However, the concentration of NO_2^--N in bioreactor B was 0.2 mg/L to 1.7 mg/L. With seawater less than 30%, the influent concentration of NO_2^--N was from 0.3 mg/L to 2.8 mg/L and the effluent concentration of NO_2^--N was less than 0.6 mg/L, which was similar to bioreactor A (Figure 16). After 30% of seawater, the influent concentration of NO_2^--N was less than the detection limit (0.005 mg/L). However, in the bioreactor, incomplete nitrite oxidation was observed which corresponds to the concentration of NO_2^--N increase of up to 1.7 mg/L while the concentration of total nitrate and nitrite decreased. Ammonium in the bioreactor is converted to nitrate via nitrite during the whole nitrification process. After the experimental condition over 30% of sea salt compared to seawater, the production of nitrite in high salinity was more than lower salinity. On account of the increasing level of salinity, some nitrite accumulated in the bioreactor. At high salinity, the second step of nitrification which is converting nitrite to nitrate was inhibited. This showed that the NOB was more sensitive to salinity than AOB. Nitrite is toxic to eukaryotes and inhibits bacterial growth. NOB counteract nitrogen loss by converting nitrite to nitrate. NOB activity in WWTPs tends to be unstable, and breakdowns of nitrite oxidation can cause tremendous ecological damage if nitrite from WWTPs leaks into natural waters (Diams et al.,2016). Several studies found that high nitrite concentrations showed an inhibitory effect on the nitrifying activity, especially AOB cultures incubation (Castro-Barros et al., 2015).

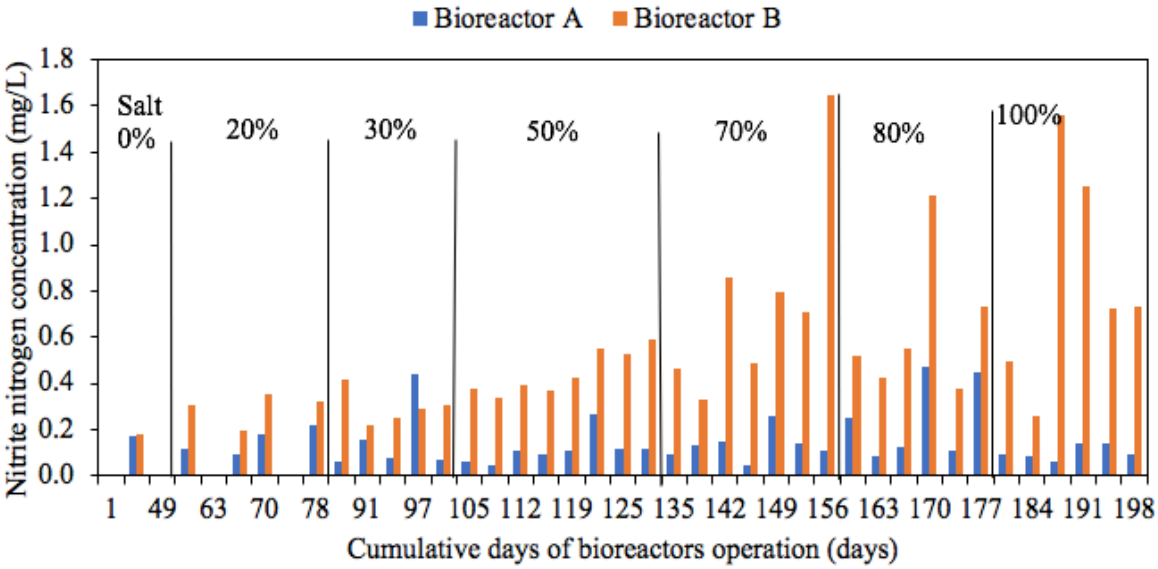

Figure 16. The concentration of nitrite nitrogen from bioreactor A (without sea salt addition) and bioreactor B (with sea salt addition).

3.4.3. Recovery Process

To determine the performance of the system and activity of nitrifiers after exposure to high salinity, bioreactor B was returned to the groundwater baseline condition of bioreactor A by removing sea salt. Figure 17 shows the ammonium removal rate in the bioreactors after the change back to free salt influent. At 100% of sea salt condition, which is same as seawater, the ammonium removal rate dropped from 59.2% to 15.4%; thus, all nitrifiers in the PVA-gel beads might have died. However, the ammonium removal rate in bioreactor B rebounded back to the original levels after five days. On day 5, the ammonium removal rate returned to 90.6%, and this suggests a fully reversible inhibition of salt effects on the nitrifying bacteria in the unit process used here with the PVA-gel bead biocarrier. The biomass of nitrifiers also affects the nitrification rate. Biofilm cultures in general offer protective niches to biomass against deleterious elements in the environment. The use of PVA-gel beads may also have assisted in protecting the biomass from the inhibitory effects of salt. In the case of a suspended biomass culture (i.e., conventional activated sludge), an inhibitory factor may have resulted from the washout of the biomass, thus potentially requiring a much longer recovery period, which would especially be true using very slowly growing nitrifying organisms, as in this case.

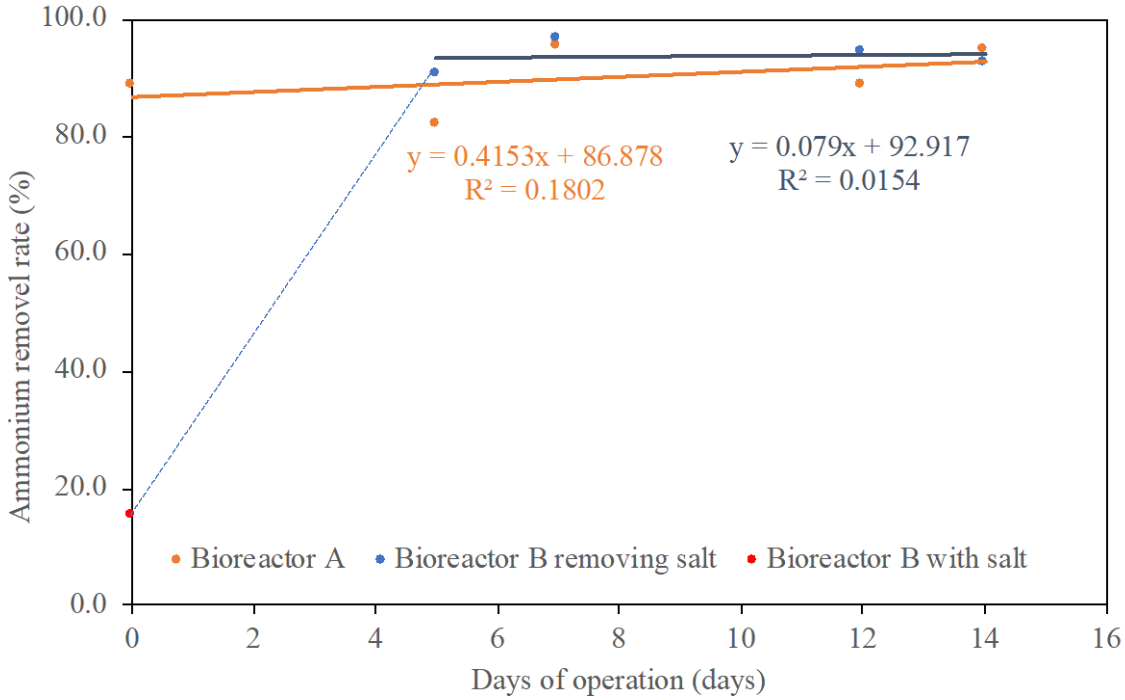

Figure 17. Ammonium removal rate in the bioreactors with influent free of salt addition.

4. Conclusions

In this study, nitrification was determined by adding sea salt to inhibit the oxidation of ammonium to nitrite and nitrate. It allowed the nitrification to be determined by measuring ammonium removal rate and nitrate/nitrite production rate. The results suggested that influent wastewater salinity had a direct influence on the nitrification rate in a biofilm treatment process. In light brackish water with seawater percentage less than 30%, the salinity had no significant effect on the nitrification rate. These results demonstrated that bacteria could survive and perform well in the low percentage saltwater. In brackish water with seawater percentage between 30% and 80%, the nitrification process was inhibited by the stepwise increasing salinity. Even at 80% seawater conditions, after the adaptive phase, the bacteria operated stably in the brackish water with low efficiency indicating that the system was still working. In saline water with seawater percentage greater than 80%, the transient decrease of nitrification was observed. Accordingly, it was crucial to control the seawater content in sewage in practice. Preventing high salinity is necessary for getting good nitrification performance in wastewater treatment.

Furthermore, nitrite is highly accumulated during nitrification processing with high salinity. This result agrees with the previous result found by Peng et al., (2004). At a salinity above 33g/L (saline water), the nitrite had a unique production in the nitrification (Vredenbregt et al., 1997). The nitrification rate might have relationships with the NOB and AOB ratio in the bioreactor. It is necessary to monitor the intermediate production to avoid nitrite accumulation.

According to the results, the short-term effects of salt on nitrification in the biofilm treatment process are revisable in short recovery time. Biofilm cultures (PVA-gel beads) can maintain high population density of nitrifying bacteria against the inhibitory effects of salt. When introducing

seawater to the wastewater treatment plant, an optimum ratio of salty wastewater and typical wastewater is important for the performance of the nitrification process, because high salinity might reduce the treatment efficiency of the system. However, the system still survives even at saline wastewater (100% of sea salt compared to seawater). The nitrification rate can recover back to the original level in a short time with the steady supply of freshwater which reduces the inhibitory effects of salt. Biofilm can reduce the HRT and increase system performance due to the high population density of biomass maintained in the reactor. Besides, biofilm treatment processes are simple to control and maintain in addition to having a low-energy requirement, and low-operation costs (Cortés-Lorenzo et al., 2006). Of course, with much longer exposure to high levels of saltwater, a different response might occur, which would be a venue for further study.

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Appendix

A. Determination of Optimum Hydraulic Retention Time.

HRT (hr)	Reactor					Duplicate				
	Influent NH4-N (mg/L)	Effluent NH4-N (mg/L)	Loading rate (mg/L/h)	Removal rate (mg/L/h)	efficiency (%)	Influent NH4-N (mg/L)	Effluent NH4-N (mg/L)	Loading rate (mg/L/h)	Removal rate (mg/L/h)	efficiency (%)
6	27.80	1.00	4.63	4.47	96.4	27.80	1.70	4.63	4.35	93.9
	33.91	0.58	5.65	5.56	98.3	33.91	0.89	5.65	5.50	97.4
	18.00	0.25	3.00	2.96	98.6	18.00	0.24	3.00	2.96	98.7
	27.62	0.33	4.60	4.55	98.8	27.62	0.31	4.60	4.55	98.9
	24.00	1.76	4.00	3.71	92.7	24.00	1.41	4.00	3.77	94.1
	21.30	1.11	3.55	3.37	94.8	21.30	0.94	3.55	3.39	95.6
	20.30	0.46	3.38	3.31	97.7	20.30	0.34	3.38	3.33	98.3
	21.20	0.58	3.53	3.44	97.3	21.20	0.31	3.53	3.48	98.5
	24.20	0.35	4.03	3.98	98.6	24.20	0.27	4.03	3.99	98.9
	20.00	0.29	3.33	3.29	98.6	20.00	0.24	3.33	3.29	98.8
	33.70	0.50	5.62	5.53	98.5	33.70	0.38	5.62	5.55	98.9
	26.00	2.29	4.33	3.95	91.2	26.00	1.91	4.33	4.02	92.7
	24.20	0.24	4.03	3.99	99.0	24.20	0.21	4.03	4.00	99.1
	21.50	0.27	3.58	3.54	98.7	21.50	0.23	3.58	3.55	98.9
	24.00	0.15	4.00	3.98	99.4	24.00	0.12	4.00	3.98	99.5
	25.50	1.73	4.25	3.96	93.2	25.50	1.53	4.25	4.00	94.0
	30.37	0.66	5.06	4.95	97.8	30.37	0.46	5.06	4.99	98.5
	34.00	2.20	5.67	5.30	93.5	34.00	2.05	5.67	5.33	94.0
	32.30	0.58	5.38	5.29	98.2	32.30	0.50	5.38	5.30	98.5
	39.15	3.04	6.53	6.02	92.2	39.15	2.85	6.53	6.05	92.7
	29.35	0.62	4.89	4.79	97.9	29.35	0.48	4.89	4.81	98.4
	23.76	0.66	3.96	3.85	97.2	23.76	0.55	3.96	3.87	97.7
	23.83	0.59	3.97	3.87	97.5	23.83	0.48	3.97	3.89	98.0
	30.40	0.58	5.07	4.97	98.1	30.40	0.50	5.07	4.98	98.4
5	29.85	0.78	5.97	5.81	97.4	29.85	0.59	5.97	5.85	98.0
	26.30	1.99	5.26	4.86	92.4	26.30	1.73	5.26	4.91	93.4
	30.60	1.20	6.12	5.88	96.1	30.60	1.05	6.12	5.91	96.6
	30.36	0.80	6.07	5.91	97.4	30.36	0.90	6.07	5.89	97.0
	31.07	1.73	6.21	5.87	94.4	31.07	1.07	6.21	6.00	96.6
	28.95	1.57	5.79	5.48	94.6	28.95	1.10	5.79	5.57	96.2
	30.06	0.97	6.01	5.82	96.8	30.06	0.73	6.01	5.87	97.6
	29.70	0.94	5.94	5.75	96.8	29.70	0.45	5.94	5.85	98.5
	28.60	0.77	5.72	5.57	97.3	28.60	0.45	5.72	5.63	98.4
	30.17	0.87	6.03	5.86	97.1	30.17	0.84	6.03	5.87	97.2
	29.23	1.65	5.85	5.52	94.4	29.23	1.51	5.85	5.54	94.8
4	30.70	1.04	7.68	7.42	96.6	30.70	0.99	7.68	7.43	96.8
	31.50	1.16	7.88	7.59	96.3	31.50	1.16	7.88	7.59	96.3
	34.00	1.09	8.50	8.23	96.8	34.00	1.10	8.50	8.23	96.8
	32.64	1.34	8.16	7.83	95.9	32.64	1.34	8.16	7.83	95.9
3	33.40	1.49	11.13	10.64	95.5	33.40	1.34	11.13	10.69	96.0
	33.40	1.32	11.13	10.69	96.0	33.40	1.87	11.13	10.51	94.4
	30.98	1.35	10.33	9.88	95.6	30.98	1.19	10.33	9.93	96.2
	34.70	1.15	11.57	11.18	96.7	34.70	1.18	11.57	11.17	96.6
2	34.50	8.90	17.25	12.80	74.2	34.50	2.96	17.25	15.77	91.4
	32.75	3.80	16.38	14.48	88.4	32.75	3.30	16.38	14.73	89.9
	33.65	2.70	16.83	15.48	92.0	33.65	2.44	16.83	15.61	92.7
	31.78	4.42	15.89	13.68	86.1	31.78	2.37	15.89	14.71	92.5
1	37.84	12.00	37.84	25.84	68.3	37.84	12.75	37.84	25.09	66.3
	34.35	20.05	34.35	14.30	41.6	34.35	22.10	34.35	12.25	35.7
	33.66	18.60	33.66	15.06	44.7	33.66	16.58	33.66	17.08	50.7
	35.03	21.00	35.03	14.03	40.1	35.03	23.60	35.03	11.43	32.6

B. Salinity, conductivity and chloride concentration during the experiments.

[1]Estimated salt concentration (%)	Measured value		Calculated value		Defeintiion of Water Type
	Conductivity (ms/cm)	Chloride ion (mg/L)	[2]Salinity (‰)	Salt content (%)	
0%	0.766	43.1	0.09	0%	Fresh Water
	0.790	48.8			
	0.787	48.8			
	0.800	54.3			
	0.754	49.2			
	0.826	48.4			
	0.717	41.9			
	0.781	48.9			
	0.805	48.6			
	0.755	55.6			
	0.821	48.4			
	0.776	49.0			
	0.780	45.6			
	0.748	49.3			
	0.828	48.4			
	0.818	48.5			
	0.798	48.9			
	0.758	49.2			
	0.867	47.9			
	0.796	48.7			
	0.786	48.9			
20%	11.020	3200.0	6.85	19%	Brackish Water
	11.630	4134.9			
	11.410	4048.0			
30%	19.740	6660.0	12.43	35%	
	19.210	7127.9			
	18.520	6855.4			
50%	27.340	9790.0	18.69	52%	
	26.890	10160.3			
	29.250	11092.2			
70%	35.860	13700.0	24.52	69%	
	35.860	13700.0			
	34.900	13323.1			
80%	41.490	15800.0	28.48	80%	
	40.860	15676.4			
	41.200	15810.6			
100%	50.260	19388.0	36.87	103%	Saline Water
	50.880	19100.0			
	53.590	20702.8			
	55.280	21370.1			
	53.810	20789.7			
	54.580	21093.7			

[1]Salt added (%) compared to salt amount in real seawater (36.0g/L)

[2]Salinity (‰) -= 1.80655×chlorinity (mg/L)/1,000; It is assumed that seawater contains 19,800 mg/L of chloride ion.

C. Chemical parameters in bioreactor A.

Sampling Date	Cumulative Days	Influent		Effluent		
		T	pH	T	pH	DO
Average		25.4	7.6	25.8	6.8	7.1
Maximum		25.9	8.3	26.5	7.4	8.4
Minimum		24.7	7.1	23.3	5.7	6.1
08/15/18	1	25.7	7.1	26.1	7.1	-
09/24/18	41	-	7.3	-	6.6	-
10/02/18	49	25.4	7.1	25.7	6.6	7.1
10/09/18	56	25.8	7.3	26.0	7.0	6.9
10/15/18	63	-	-	-	-	-
10/16/18	64	25.5	7.3	25.9	7.2	7.4
10/23/18	70	25.7	7.2	25.6	7.4	6.3
10/26/18	73	25.9	7.3	25.9	6.9	6.7
10/30/18	78	24.7	7.3	25.7	7.2	8.4
11/08/18	86	25.5	7.4	26.0	7.3	-
11/13/18	91	24.7	7.2	25.3	6.9	7.8
11/15/18	93	25.4	7.5	25.5	6.2	6.9
11/19/18	97	25.5	7.2	26.1	6.9	7.8
11/20/18	98	25.3	7.4	25.9	6.9	6.1
11/27/18	105	25.3	7.1	26.0	6.9	7.1
11/29/18	107	25.4	7.2	25.5	6.5	-
12/04/18	112	25.2	7.3	25.8	7.2	-
12/05/18	113	-	-	-	-	-
12/11/18	119	25.5	7.5	26.1	7.3	-
12/13/18	121	25.5	7.3	26.3	7.0	-
12/17/18	125	25.6	7.3	26.5	6.3	-
12/20/18	128	-	-	-	-	-
12/27/18	135	-	8.1	-	5.7	-
01/02/19	141	25.3	7.6	25.5	7.0	-
01/03/19	142	-	8.1	-	7.1	-
01/08/19	147	-	8.0	-	6.2	-
01/10/19	149	-	8.1	-	6.9	-
01/15/19	154	-	7.8	25.8	6.9	7.1
01/17/19	156	-	-	26.0	7.2	7.2
01/22/19	161	25.5	7.2	26.0	7.1	7.1
01/24/19	163	-	-	26.1	-	6.7
01/29/19	168	-	-	-	-	-
01/31/19	170	-	-	25.2		7.0
02/05/19	175	-	8.0	26.3	6.1	7.1
02/07/19	177	-	-	-	-	-
02/12/19	182	-	8.2	25.8	6.9	7.2
02/14/19	184	-	8.3	-	6.4	-
02/19/19	189	-	8.3	23.3	6.8	7.2
02/21/19	191	-	7.9	25.8	7.2	6.9
02/26/19	196	-	8.3	-	6.8	-
02/28/19	198	-	8.2	26.4	6.5	7.0

D. The influent and effluent nitrogen parameters in bioreactor A.

Sampling Date	Cumulative Days	Influent (mg/L)			Effluent (mg/L)		
		NH_4^+-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N
08/15/18	1	25.2	-	-	2.0	-	-
09/24/18	41	29.6	0.3	3.5	0.8	0.2	28.5
10/02/18	49	24.3	-	-	1.2	-	-
10/09/18	56	24.5	3.2	4.0	2.0	0.1	29.1
10/15/18	63	24.7	-	-	0.9	-	-
10/16/18	64	22.9	3.9	3.8	0.3	0.1	30.4
10/23/18	70	23.4	4.1	4.2	0.4	0.2	31.3
10/26/18	73	26.4	-	-	1.0	-	-
10/30/18	78	24.3	3.6	4.2	0.5	0.2	32.6
11/08/18	86	27.6	1.0	3.8	5.8	0.1	26.1
11/13/18	91	25.1	3.3	4.4	0.5	0.2	32.0
11/15/18	93	28.1	1.2	3.9	4.0	0.1	29.2
11/19/18	97	25.9	2.6	4.0	0.4	0.4	31.3
11/20/18	98	28.1	2.1	4.3	0.9	0.1	33.1
11/27/18	105	24.2	3.4	4.2	5.5	0.1	27.2
11/29/18	107	26.5	2.1	4.1	4.5	0.0	27.8
12/04/18	112	28.0	0.9	3.8	0.9	0.1	29.9
12/05/18	113	26.7	1.3	3.0	1.4	0.1	29.8
12/11/18	119	25.9	2.2	4.2	1.7	0.1	30.0
12/13/18	121	25.3	2.6	4.1	0.6	0.3	30.8
12/17/18	125	25.8	3.2	4.3	4.3	0.1	28.3
12/20/18	128	29.6	0.2	3.8	1.3	0.1	29.9
12/27/18	135	28.5	0.7	4.0	5.4	0.1	27.2
01/02/19	141	25.9	1.6	4.4	1.6	0.1	30.2
01/03/19	142	28.1	0.6	4.0	0.9	0.1	32.2
01/08/19	147	28.5	0.8	4.1	4.5	0.0	29.0
01/10/19	149	27.9	0.6	4.0	1.1	0.3	32.0
01/15/19	154	26.8	2.6	4.6	1.3	0.1	32.2
01/17/19	156	26.4	0.5	5.0	2.7	0.1	28.1
01/22/19	161	24.6	3.2	4.5	0.9	0.3	29.4
01/24/19	163	29.3	0.4	3.8	4.6	0.1	27.5
01/29/19	168	27.4	0.8	4.1	2.9	0.1	30.0
01/31/19	170	27.8	0.2	3.5	0.7	0.5	28.4
02/05/19	175	27.2	0.8	4.2	5.7	0.1	27.0
02/07/19	177	28.1	0.3	3.4	0.8	0.4	27.8
02/12/19	182	28.6	0.2	3.9	1.9	0.1	30.7
02/14/19	184	27.4	0.5	3.8	2.8	0.1	28.8
02/19/19	189	27.7	0.5	4.0	5.1	0.1	27.2
02/21/19	191	27.9	0.1	3.8	1.5	0.1	29.8
02/26/19	196	29.3	1.1	2.8	1.3	0.1	30.4
02/28/19	198	27.8	0.9	3.9	3.1	0.1	28.8

E. Ammonium removal and nitrate & nitrite production rate in bioreactor A.

Sampling Date	Cumulative Days	Removal/Production Rate (%)	
		[1]$NH4^+$ removal	[2](NO_2^- & NO_3^-) production
08/15/18	1	92.2	89.0
09/24/18	41	97.1	83.9
10/02/18	49	95.0	92.2
10/09/18	56	91.9	89.6
10/15/18	63	96.4	93.3
10/16/18	64	98.8	99.6
10/23/18	70	98.2	99.4
10/26/18	73	96.3	93.8
10/30/18	78	98.1	103.0
11/08/18	86	78.9	77.4
11/13/18	91	97.8	98.0
11/15/18	93	85.7	86.2
11/19/18	97	98.5	96.8
11/20/18	98	96.8	95.3
11/27/18	105	77.3	81.7
11/29/18	107	82.9	81.5
12/04/18	112	96.8	90.1
12/05/18	113	94.6	96.1
12/11/18	119	93.6	91.5
12/13/18	121	97.7	96.3
12/17/18	125	83.5	81.0
12/20/18	128	95.8	87.8
12/27/18	135	81.2	79.2
01/02/19	141	93.9	93.9
01/03/19	142	96.6	98.8
01/08/19	147	84.2	84.2
01/10/19	149	96.2	99.0
01/15/19	154	95.1	93.5
01/17/19	156	90.0	86.1
01/22/19	161	96.4	89.2
01/24/19	163	84.2	79.8
01/29/19	168	89.5	92.1
01/31/19	170	97.4	90.8
02/05/19	175	79.0	81.4
02/07/19	177	97.1	87.2
02/12/19	182	93.3	93.2
02/14/19	184	89.8	89.6
02/19/19	189	81.4	82.1
02/21/19	191	94.7	93.4
02/26/19	196	95.6	91.0
02/28/19	198	88.8	87.0

[1]NH_4^+ removal rate is equal to $\{[NH_4^+]_{in}-[NH_4^+]_{ef}\}/[NH_4^+]_{in} \times 100$

[2]NO_2^- & NO_3^- production rate is equal to $\{[NO_2^-+NO_3^-]_{ef} - [NO_2^-+NO_3^-]_{in}\}/[NH_4^+]_{in} \times 100$

F. Operation days in bioreactor B with salt.

Salt concentration (%)		sample No.	Sampling Date	Cumulative Days	Total operation days	Note
[1]Estimated	[2]Calculated					
0%	0%	1	08/15/18	1	49	Operation start
		2	09/24/18	41		
		3	10/02/18	49		
20%	20%	4	10/09/18	56	32	Salt added: 10/03/18
		5	10/15/18	63		
		6	10/16/18	64		
		7	10/23/18	70		
		8	10/26/18	73		
		9	10/30/18	78		
30%	35%	10	11/08/18	86	22	Salt added: 11/04/18
		11	11/13/18	91		
		12	11/15/18	93		
		13	11/19/18	97		
		14	11/20/18	98		
50%	53%	15	11/27/18	105	30	Salt added: 11/26/18
		16	11/29/18	107		
		17	12/04/18	112		
		18	12/05/18	113		
		19	12/11/18	119		
		20	12/13/18	121		
		21	12/17/18	125		
		22	12/20/18	128		
70%	70%	23	12/27/18	135	25	Salt added: 12/26/18
		24	01/02/19	141		
		25	01/03/19	142		
		26	01/08/19	147		
		27	01/10/19	149		
		28	01/15/19	154		
		29	01/17/19	156		
80%	81%	30	01/22/19	161	22	Salt added: 1/20/19
		31	01/24/19	163		
		32	01/29/19	168		
		33	01/31/19	170		
		34	02/05/19	175		
		35	02/07/19	177		
100%	105%	36	02/12/19	182	18	Salt added: 2/11/19
		37	02/14/19	184		
		38	02/19/19	189		
		39	02/21/19	191		
		40	02/26/19	196		
		41	02/28/19	198		Operation end

[1]Salt added (%) compared to salt amount in real seawater (36.0 g/L of salt is equal to 100%)

[1]Calculated Salt(%) is equal to {[1.80655×measured chlorinity (mg/L)]/36,000 mg/L}×100

G. Chemical parameters in bioreactor B with salt.

Estimated salt concentration	Sampling Date	Cumulative Days	Influent		Effluent		
			T	pH	T	pH	DO
	Average		25.4	7.6	25.7	7.1	7.3
	Maximum		26.0	8.6	26.5	8.3	8.2
	Minimum		24.2	7.0	23.4	6.4	6.0
0%	08/15/18	1	25.8	7.1	26.2	7.0	-
	09/24/18	41		7.3		6.7	-
	10/02/18	49	25.5	7.0	25.8	6.7	6.7
20%	10/09/18	56	25.4	7.2	25.7	6.8	7.1
	10/15/18	63	-	-	-	-	-
	10/16/18	64	25.5	7.3	25.8	7.0	7.3
	10/23/18	70	24.2	7.7	25.4	7.5	6.2
	10/26/18	73	26.0	7.3	25.5	6.7	6.9
	10/30/18	78	24.8	7.2	25.5	6.9	8.2
30%	11/08/18	86	25.7	7.4	25.9	6.9	-
	11/13/18	91	25.1	7.4	25.4	6.9	8.0
	11/15/18	93	25.6	7.3	25.6	6.8	7.0
	11/19/18	97	25.7	7.2	26.1	6.6	7.0
	11/20/18	98	25.5	7.3	25.7	6.8	6.0
50%	11/27/18	105	25.6	7.2	25.8	6.6	6.8
	11/29/18	107	25.7	7.2	25.6	6.4	-
	12/04/18	112	25.3	7.4	25.5	7.0	-
	12/05/18	113	-	-	-	-	-
	12/11/18	119	24.8	7.2	25.8	6.5	-
	12/13/18	121	25.6	7.3	26.2	6.8	-
	12/17/18	125	25.8	7.3	26.5	6.6	-
	12/20/18	128	-	-	-	-	-
70%	12/27/18	135	-	8.6	-	7.2	-
	01/02/19	141	25.4	7.3	25.4	6.5	-
	01/03/19	142	-	8.4	-	7.7	-
	01/08/19	147	-	8.4	-	7.5	-
	01/10/19	149	-	8.4	-	7.7	-
	01/15/19	154	-	8.3	25.7	7.7	7.6
	01/17/19	156	-	-	25.7	6.6	7.9
80%	01/22/19	161	25.6	7.1	25.9	6.8	7.8
	01/24/19	163	-	-	25.9	-	7.3
	01/29/19	168	-	-	-	-	-
	01/31/19	170	-	-	25.2		7.9
	02/05/19	175	-	8.1	26.3	-	7.5
	02/07/19	177	-	-	-	-	-
100%	02/12/19	182	-	8.0	25.6	7.6	7.9
	02/14/19	184	-	8.1	-	7.6	-
	02/19/19	189	-	8.0	23.4	8.3	7.9
	02/21/19	191	-	7.9	25.7	8.1	7.7
	02/26/19	196	-	8.0	-	8.1	-
	02/28/19	198	-	8.0	26.2	8.2	7.8

H. The influent and effluent nitrogen parameters in bioreactor B with salt.

Estimated salt concentration	Sampling Date	Cumulative Days	Influent (mg/L)				Effluent (mg/L)		
			NH_4^+-N	NOx-N	NO_2^--N	NO_3^--N	NH_4^+-N	NO_2^--N	NO_3^--N
0%	08/15/18	1	22.1	9.7	-	-	1.2	-	-
	09/24/18	41	29.5	3.8	0.3	3.6	1.1	0.2	29.3
	10/02/18	49	24.0	7.3	-	-	1.8	-	-
20%	10/09/18	56	26.7	5.8	1.9	3.9	1.2	0.3	28.6
	10/15/18	63	25.6	5.8	-	-	0.9	-	-
	10/16/18	64	23.5	6.9	2.8	4.1	1.0	0.2	29.2
	10/23/18	70	25.4	6.6	2.5	4.1	0.8	0.4	30.9
	10/26/18	73	27.6	5.0	-	-	1.3	-	-
	10/30/18	78	24.9	6.4	2.4	4.0	0.7	0.3	31.6
30%	11/08/18	86	27.7	4.1	0.4	3.7	2.0	0.4	28.0
	11/13/18	91	27.4	4.4	0.6	3.8	1.9	0.2	30.0
	11/15/18	93	28.9	3.9	0.3	3.7	1.6	0.2	31.0
	11/19/18	97	27.5	5.4	1.5	3.9	1.4	0.3	30.6
	11/20/18	98	29.0	5.5	1.4	4.1	1.8	0.3	32.4
50%	11/27/18	105	25.4	6.6	2.6	4.1	2.1	0.4	29.5
	11/29/18	107	26.4	6.6	2.4	4.2	2.9	0.3	29.1
	12/04/18	112	28.3	4.2	0.5	3.8	3.6	0.4	27.1
	12/05/18	113	27.7	4.3	0.4	3.8	3.1	0.4	27.6
	12/11/18	119	28.9	3.9	0.0	3.8	3.7	0.4	28.0
	12/13/18	121	28.6	4.0	0.0	3.9	3.5	0.6	27.9
	12/17/18	125	28.5	4.1	0.0	4.1	3.9	0.5	27.5
	12/20/18	128	29.7	3.7	0.0	3.7	2.5	0.6	27.4
70%	12/27/18	135	29.0	3.7	0.0	3.7	4.7	0.5	25.7
	01/02/19	141	28.6	3.9	0.0	3.9	7.0	0.3	24.4
	01/03/19	142	28.5	3.7	0.0	3.7	5.4	0.9	25.9
	01/08/19	147	28.7	4.0	0.0	4.0	5.9	0.5	26.0
	01/10/19	149	29.1	3.7	0.0	3.7	6.2	0.8	25.4
	01/15/19	154	29.1	3.8	0.0	3.8	6.8	0.7	25.2
	01/17/19	156	30.5	3.7	0.0	3.7	6.1	1.7	22.2
80%	01/22/19	161	28.2	3.5	0.0	3.5	8.9	0.5	21.7
	01/24/19	163	30.0	3.6	0.0	3.6	11.3	0.4	20.5
	01/29/19	168	28.7	3.7	0.0	3.7	9.7	0.6	21.6
	01/31/19	170	29.0	3.3	0.0	3.3	9.0	1.2	19.8
	02/05/19	175	28.6	3.8	0.0	3.8	7.7	0.4	23.5
	02/07/19	177	28.7	3.3	0.0	3.3	7.6	0.7	20.3
100%	02/12/19	182	29.2	5.1	0.0	5.1	11.9	0.5	21.5
	02/14/19	184	29.7	3.9	0.0	3.9	9.6	0.3	23.0
	02/19/19	189	28.9	4.0	0.0	4.0	18.4	1.6	13.4
	02/21/19	191	29.2	3.7	0.0	3.7	21.3	1.3	10.9
	02/26/19	196	29.5	3.8	0.0	3.8	17.0	0.7	15.5
	02/28/19	198	29.3	3.8	0.0	3.8	24.8	0.7	7.9

I. Ammonium removal and nitrate & nitrite production rate in bioreactor B.

Estimated salt concentration	Sampling Date	Cumulative Days	Removal/Production Rate (%)	
			[1]$NH4^+$ removal	[2](NO_2^- & NO_3^-) production
0%	08/15/18	1	94.8	89.5
	09/24/18	41	96.2	87.1
	10/02/18	49	92.6	90.5
20%	10/09/18	56	95.4	86.6
	10/15/18	63	96.4	90.0
	10/16/18	64	95.9	95.7
	10/23/18	70	96.9	97.3
	10/26/18	73	95.3	91.6
	10/30/18	78	97.1	102.4
30%	11/08/18	86	92.6	88.0
	11/13/18	91	93.0	94.1
	11/15/18	93	94.5	94.4
	11/19/18	97	94.9	92.7
	11/20/18	98	93.9	94.0
50%	11/27/18	105	91.7	91.7
	11/29/18	107	89.2	86.5
	12/04/18	112	87.2	82.2
	12/05/18	113	89.0	85.7
	12/11/18	119	87.4	84.9
	12/13/18	121	87.7	85.8
	12/17/18	125	86.3	83.9
	12/20/18	128	91.4	81.7
70%	12/27/18	135	83.9	77.5
	01/02/19	141	75.7	72.9
	01/03/19	142	81.2	80.9
	01/08/19	147	79.3	78.6
	01/10/19	149	78.9	77.2
	01/15/19	154	76.8	75.9
	01/17/19	156	80.1	66.0
80%	01/22/19	161	68.3	66.3
	01/24/19	163	62.3	57.8
	01/29/19	168	66.3	64.3
	01/31/19	170	69.1	61.2
	02/05/19	175	73.2	70.4
	02/07/19	177	73.4	61.6
100%	02/12/19	182	59.2	57.7
	02/14/19	184	67.8	65.2
	02/19/19	189	36.3	38.2
	02/21/19	191	27.1	28.7
	02/26/19	196	42.4	42.0
	02/28/19	198	15.4	16.4

[1]NH_4^+ removal rate is equal to $\{[NH_4^+]_{in}-[NH_4^+]_{ef}\}/[NH_4^+]_{in} \times 100$

[2]NO_2^- & NO_3^- production rate is equal to $\{[NO_2^-+NO_3^-]_{ef} - [NO_2^-+NO_3^-]_{in}\}/[NH_4^+]_{in} \times 100$

J. Ammonium removal rate and nitrate & nitrite production rate in bioreactors with influent free of salt addition.

Sampling Date	Cumulative Days*	[1]$NH4^+$ removal rate		[2](NO_2^- & NO_3^-) production rate	
		Bioreactor A	Bioreactor B	Bioreactor A	Bioreactor B
2/28/19	0	88.8	15.4	87	16.4
3/5/19	5	82.1	90.6	80.3	86.9
3/7/19	7	95.6	96.8	91.9	91.3
3/12/19	12	88.8	94.6	92.3	91.5
3/14/19	14	94.9	92.7	87.0	89.0

*3/1/2019 change to 0% seawater for seawater reactor

[1]NH_4^+ removal rate is equal to $\{[NH_4^+]_{in}-[NH_4^+]_{ef}\}/[NH_4^+]_{in} \times 100$

[2]NO_2^- & NO_3^- production rate is equal to $\{[NO_2^-+NO_3^-]_{ef} - [NO_2^-+NO_3^-]_{in}\}/[NH_4^+]_{in} \times 100$