# CHANGES IN WATER QUALITY DUE TO ANTHROPOGENIC ACTIVITIES AND FEASIBLE BIOLOGICAL NITROGEN REMOVAL METHODS FOR SALINE WATER– CASE STUDY IN NEW ZEALAND

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## ABSTRACT

New Zealand is an agricultural intensive country, surrounded by ocean. Therefore, it is regularly faced with water quality issues under the combined pressure of nitrogen pollution and seawater intrusion. As means of nitrogen treatment, biological methods are efficient and cost-effective. However, biological treatment in saline conditions possess difficulties or complexities, due to loss of cell activity or death of microbial species due to osmosis. To negate these effects for biological treatment, this study aimed to search indigenous nitrifiers and denitrifiers that can survive in saline conditions and function efficiently to remove inorganic nitrogen contaminants. For dentification, hydrogenotrophic denitrification was particularly investigated as an alternative method. Especially as, instead of requiring organic carbon, hydrogenotrophic denitrifiers synthesize organic carbon from carbon dioxide in the atmosphere, a characteristic which could help New Zealand achieve its recently set goal of being net zero carbon by 2050. The enriched communities oxidized ammonia from 170 mg $NH_4^+$-N $L^{-1}\cdot d^{-1}$ down to 27 mg $NH_4^+$-N $L^{-1}\cdot d^{-1}$ at peak removal rates of 50±5 mg $NH_4^+$-N $L^{-1}\cdot d^{-1}$, with nitrate and nitrite reduction (i.e. denitrification), achieving average removal rates of 25±5 mg $NO_3^-$-N $L^{-1}\cdot d^{-1}$ and 30±5 mg $NO_2^-$-N $L^{-1}\cdot d^{-1}$, respectively. In this regard, the removal achieved the maximum allowable nitrate and nitrite concentrations of 0.2 mg $NO_2^-$-N $L^{-1}$ and 11 mg $NO_3^-$-N $L^{-1}\cdot d^{-1}$, set by the World Health Organization. Therefore, it was found that by using this method the adverse impact of intensified agriculture on water resources can be mitigated, improving water quality of saline polluted water through sustainable methods.

*Keywords*: Nitrification, Hydrogenotrophic Denitrification, Saline water

## 1. INTRODUCTION

The nitrogen cycle (Figure 1) has important agricultural and environmental implications, especially being a major constituent for maintaining agricultural growth and the world's food requirements. However, increased agricultural growth results in increased nitrogen in the environment which inadvertently leads to pollution of our surface waters and groundwaters, with various nitrogen species. Consequently, wastewater treatment plants must also cope with increased nitrogen loading in their influents and meet stringent discharge regulations that prevent eutrophication in receiving water bodies.

Different countries maintain various regulatory standards for the discharge of nitrogen containing pollutants to the environment. The World Health Organization (WHO) recommends a maximum nitrite/nitrate concentration of 0.2 mg $NO_2^-$-N $L^{-1}$ and 11 mg $NO_3^-$-N $L^{-1}\cdot d^{-1}$ (World Health Organization, 2011). The New Zealand government however has stricter nitrite recommendations of 0.06 mg $NO_2^-$-N $L^{-1}$ (Ministry for the Environment and Statistics New Zealand, 2015). These recommendations are based on the adverse impacts nitrate and nitrite have. For example, nitrate or nitrite in drinking-water sources can be toxic to humans. More significantly, high concentrations of $NO_3$-N can be fatal to infants under the age of six months, causing a condition commonly known as blue baby syndrome or methemoglobinemia (Kapoor and Viraraghavan, 1997; Kim et al., 2002). However, it should be noted that aquatic ecosystems are more sensitive to the detrimental effects of elevated nitrate levels than humans, with water bodies classified as 'eutrophic' when exceeding 2 mg $NO_3^-$-N $L^{-1}$. Currently, over 50% of measured sources within the Organisation for Economic Co-operation and Development, (OECD) are enough to trigger algal blooms (OECD, 2019). This increased biomass load leads to oxygen depletion, decrease of quality and water clarity due to increased precipitation rates and is a threat to the biotic components of the ecosystem (Dorgham, 2011). Other effects of nitrogen in water are those caused by the toxicity of nitrite to aquatic animals, particularly fish and crayfish (Ansari et al., 2011).

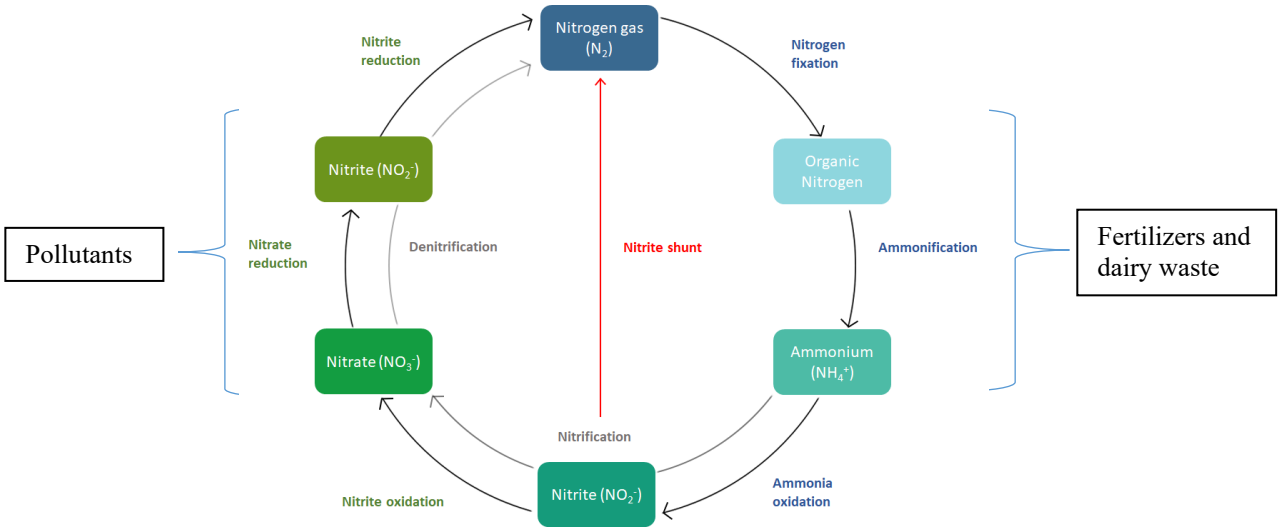


Figure 1: Overview of the nitrogen cycle. Red line represents a short cut nitrogen removal (nitrite shunt- mentioned in the discussion section of this paper). Briefly, nitrite shunt omits nitrite oxidation for the final step of Nitrification, and makes the first step of denitrification (nitrite reduction) redundant

Furthermore, nitrate contamination of groundwater has been a subject of concern since an association was made between the observation of elevated nitrate levels in well water, and the documentation of methemoglobinemia in 1945 (Comly, 1945). Increased agricultural production and the use of fertilizers have severely affected groundwater quality regarding nitrogen and phosphorus concentrations. Furthermore, due to the stability in oxygenated environments, nitrate can remain in groundwater for decades, accumulating to higher levels as farming continues (Ju, Kou, Zhang, & Christie, 2017).

More recently, climate change and rising sea levels have caused added stresses to water quality by seawater intrusion, which increases the salinity of many aquifers situated in coastal regions (as particularly seen in New Zealand). The treatment of saline water generally occurs through ion exchange (Ghafari et al., 2010), this creates a concentrated brine solution which needs further treatment before disposal. The brine to be treated typically exhibits elevated salinity (generally, 4%-26%) and concentrated nitrates/nitrite compounds. This study, therefore, investigated the use of indigenous microbes to perform nitrification and denitrification in saline conditions (4%). Hydrogenotrophic denitrification was investigated as an alternative method of denitrification. The process uses hydrogen gas as an electron donor to convert nitrate/nitrite to nitrogen gas. In this way, and compared to conventional wastewater treatment processes, this method requires no organic carbon and can be effective in treating low C/N waters such as agricultural run-off, tertiary wastewater effluent or nitrogen polluted groundwater (Grebliunas and Perry, 2016; Kim et al., 2002). Hydrogenotrophic denitrification also bears a few engineering advantages for water treatment. Mainly, hydrogen gas is benign to humans, results in a relatively low biomass yield (of denitrifying microbes compared to organic carbon electron donors) and has low costs per N-mass removed. Furthermore, and as can be seen in Equation 1, hydrogenotrophs utilize carbon dioxide for biosynthesis, such a method means carbon sequestration during nitrogen treatment will help achieve New Zealand's recently set goal of being net carbon zero by 2050 (Ministry for the Environment, 2019)

$$NO_3^- + 3.03\ H_2 + H^+ + 0.229\ CO_2 \rightarrow 0.0458\ C_2H_7O_2N + 0.477\ N_2 + 3.37\ H_2O \qquad (1)$$

Halophilic (salt loving) hydrogenotrophic denitrifiers and ammonia oxidizers were enriched over time in a saline environment (4%). We hypothesized that enrichment using indigenous strains will allow us to develop an efficient nitrogen removal treatment, capable of bioremediation in saline conditions. In this paper the issues of nitrogen pollution due to agricultural growth as well as sea water intrusion in New Zealand are highlighted. It then investigates removal of nitrogen in saline environments through biological means. This provides a solution to mitigate New Zealand's forthcoming issues of increased nitrogen pollution and seawater intrusion, while maintaining the outputs of its agricultural industries. To the best of our knowledge such a method has been overlooked in New Zealand, with many companies choosing to re-circulate organic carbon, or adding organic carbon to maintain sufficient nitrogen removal rates (Irvine et al., 2013)

## 2. NITROGEN-CENTRIC WATER QUALITY AND NITROGEN-POLLUTION IN NEW ZEALAND

### 2.1 Water pollution due to agriculture

Waterways around the world and in New Zealand are significantly affected by nitrogen contaminants due to agricultural non-point source pollution. Of blame is the ever growing and economically fruitful dairy industry, valued at around NZ$16.7 Billion in 2018 (NZIER, 2018). As can be seen in Figure 2, (a) represents the areas in New Zealand which has experienced the highest growth in dairy land since 2002. With the Canterbury (i) and Southland (ii) regions growing the most. Overall between the years 2007-2017, the country's dairy farming grew by around 20% (5.3 million livestock to 6.5 million) (NZIER, 2018). This coincides with a 25% increase in the use of nitrogen-based fertilizers per hectare of agricultural land since 2002 (Ministry for the Environment and Stats NZ, 2019). Unfortunately, this has also resulted in a 29% increase in agricultural nitrogen leaching and a 12% rise in total nitrogen levels in rivers (Figure 2 – (d)).

One of the dominant freshwater resources within New Zealand is groundwater. As of 2015, over 200 groundwater sources had been identified, covering 26.3% of the countries surface area (Ministry for the Environment and Stats NZ, 2019), of which, around 40% of the community drinking water supplies around New Zealand use groundwater as well as many individual rural households (Close and Humphries, 2019). As can be seen by Figure 2 (c) the expansion of the dairy industry from 2002-2016 strongly relates to an upward trajectory of $NO_3^-$-N concentrations in groundwater from 2005-2014. A problem that would impact many communities around New Zealand. Lakes Figure 2 (b) have been less effected, however, still polluted in Canterbury and in Northland. The linkage between agricultural growth and nitrogen pollution of waters is quite evident.

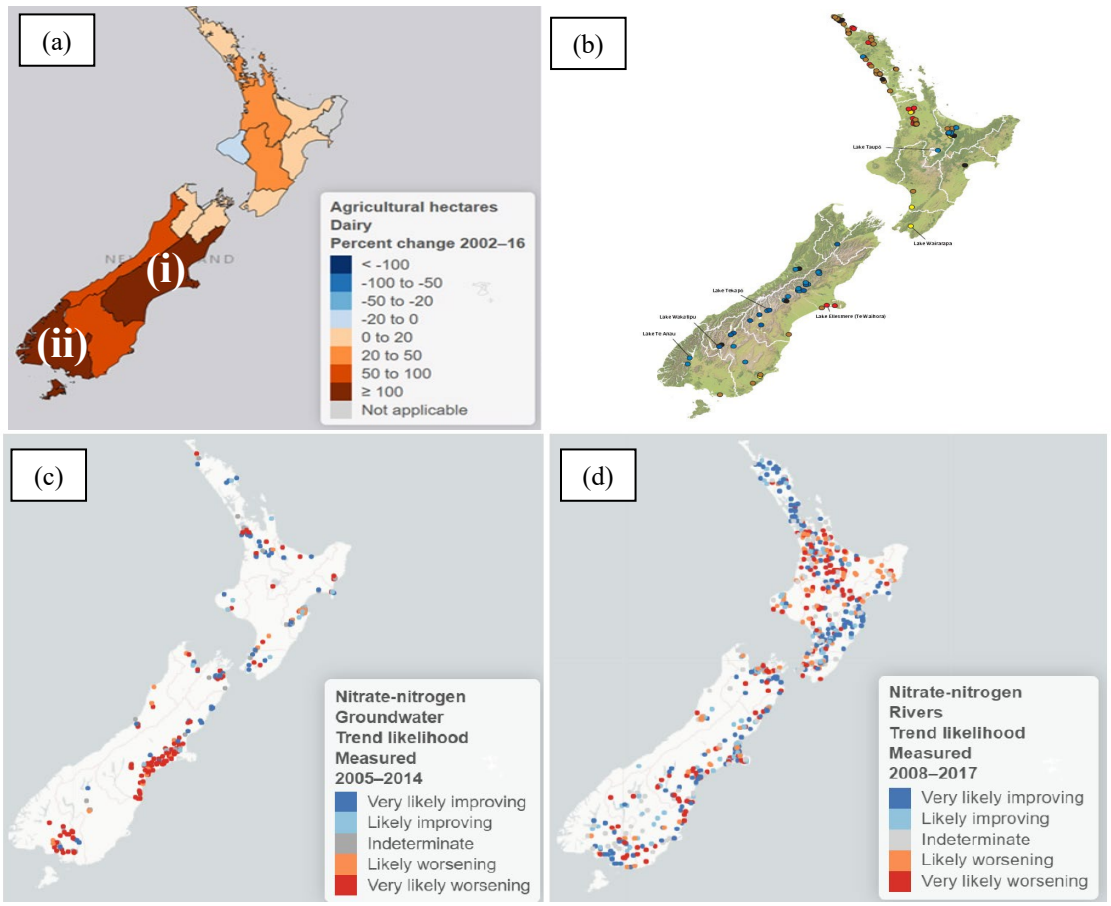


Figure 2: Agricultural growth and nitrogen pollution in New Zealand. (a) Agricultural growth (i) represents Canterbury region, (ii) represents Southland. (b) Nitrate-Nitrogen pollution of lakes in NZ, blue dots represent little/no pollution, red dots represent critical limits. (c) Nitrate-Nitrogen pollution of groundwater sources in NZ. (d) Nitrate-Nitrogen pollution of rivers in NZ (Ministry for the Environment and Stats NZ, 2019)

### 2.2 Seawater Intrusion

Seawater intrusion refers to the inflow of saltwater into freshwater aquifers due to the hydraulic connection of seawater and groundwater. Causes of intrusion are predominantly due to groundwater pumping, sea level rise,

or reduced recharge (Morgan et al., 2013). Throughout the world, coastal aquifers are a major source for water. Similarly, 150 of all identified groundwater sources in NZ are situated by the coast (NZ, 2015), supplying over 50% of all water sourced from groundwater (NZ, 2015). This can be attributed to coastal areas often having higher population densities with higher water demands, eventuating to increased pressure and often unsustainable use for water from nearby groundwater supplies (Michael et al., 2017). This was observed in Christchurch where groundwater abstraction for industrial and public supply exceeded recharge levels, causing groundwater levels to fall below high tide levels and catalyzing seawater intrusion (Aitchison-earl, 2003). Another study in Wellington also identified abstraction trends and its increased effect on saline intrusion (De Costa et al., 2004). Climate change and rising sea-levels have also become a topic of concern. It has been shown that in 2018 the global mean sea level was around 8.1 cm higher than the 1993 average (Lindsey and Lumpkin, 2018), similarly New Zealand has followed the same rate of increase as global trends (Intergovernmental Panel on Climate Change, 2014). Furthermore, around NZ the sea levels are predicted to rise by a further 0.6 m over the next 50 years (Ministry for the Environment and Stats NZ, 2019). Along with sea level rising, seawater intrusion in New Zealand will become increasingly prevalent, as was recognized by groundwater quality measurements in Christchurch (Aitchison-earl, 2003).

## 3. RESEARCH METHODS

### 3.1 Enrichment and growth conditions

Enrichment cultures were grown using a highly saline mineral salts medium; NaCl 40.0 g/L, $MgCl_2.6H_2O$ 0.5 g/L, $KH_2PO_4$ 0.2 g/L, $NH_4Cl$ 0.3 g/L, KCl 0.3 g/L, $CaCl_2.2H_2O$ 0.015 g/L, $NaSO_4$ 0.05 g/L and a trace elements solution (Zhuang et al., 2011). 1.1 g/L $NaHCO_3$ was added to the medium as a buffer and a sole inorganic carbon source. The final pH of the culture medium was adjusted to between 7 – 7.5 using HCl or NaOH solutions.

For enrichment of halophilic nitrifiers, $NH_4Cl$ was amended to the culture medium to provide the desired ammonia concentrations. Seed inoculum was sourced from biological filtration balls used by a local aquarium. Enrichment was conducted in 1 L bioreactors with a working volume of 700 mL (Figure 3) operating in batch mode. Aeration was provided by using air pumps connected to diffusors fixed on the bottom of the reactors as shown in Figure 3. Saturation dissolved oxygen was continuously maintained in the reactors. The temperature of each reactor was maintained between 20°C and 23°C using portable fan heaters. Each reactor was encased in aluminium foil, to prevent light from inhibiting the activity of ammonia-oxidising microorganisms (French et al., 2012). The salinity and temperature of each reactor was measured using a Water Quality 8371 Salinity Meter (AZ instruments, Taiwan). Technical triplicates were utilized for each analysis.

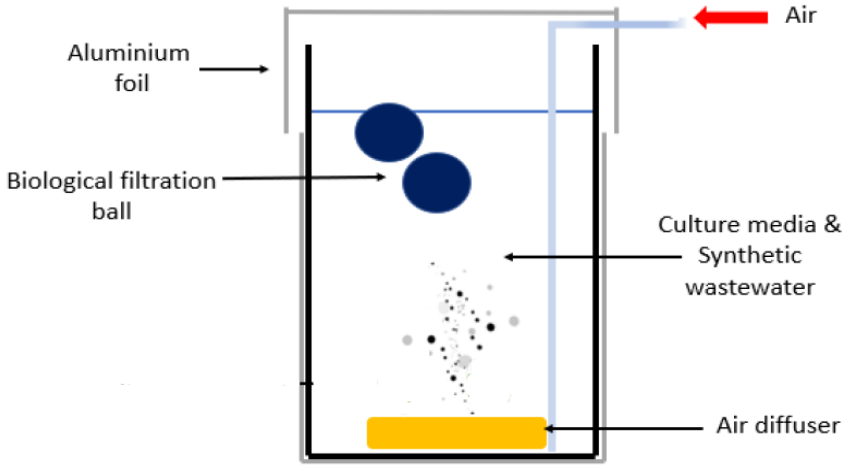


Figure 3: Schematic of Nitrifying reactor

The halophilic hydrogenotrophic denitrifiers were enriched in 160 mL glass serum bottles with a working volume of 100 mL. Marine sediment samples were collected from a local beach as inoculum. The bottles were prepared inside an anaerobic chamber with an $N_2$:$H_2$:$CO_2$ (90:5:5) atmosphere to minimize any oxygen contamination and kept for at least 24 hours before being sealed with butyl rubber stoppers. After inoculation, the bottles were further vacuumed for one minute to remove the headspace. Hydrogen gas (99.9%, BOC, New Zealand) was then injected into each bottle and pressurized to 10 psi under room temperature. The $H_2$ gas was replenished periodically depending on its utilization. For this experiment we desired to find the efficiency of both nitrite reduction and nitrate reduction, hence two concurrent experiments were conducted. For one experiment the culture media was amended with a substrate concentration of $NaNO_3$, while the second experiment was amended with $NaNO_2$ (both purities are ≥99%, Sigma, MO, USA).

### 3.2 Data analysis

Concentrations of ammonia, nitrite and nitrate were measured according to Standard Methods 22nd Edition (Rice et al., 2012), 4500-$NH_3$ Phenate Method, 4500-$NO_2^-$ Colorimetric Method and 4500-$NO_3^-$ Ultraviolet Spectrophotometric Screening Method. A UV-vis spectrophotometer (UV-2700 SHIMADZU, Japan) was used for the measurements. pH was measured using an HACH HQ40d portable meter (HACH). All the tests were performed in triplicate.

## 4. RESULTS AND DISCUSSION

### 4.1 Nitrification

Batch kinetic analysis was carried out in triplicates over a 120-hour time course experiment to determine the overall nitrification efficiency. This was computed through the rate of ammonia removal, proportion removed and the proportion of nitrogen accumulation. To do so, the $NH_4^+$, $NO_3^-$, and $NO_2^-$ were measured. As shown in Figure 4 The results show that ammonia oxidizing microorganisms were successfully enriched, achieving an 84% oxidation of ammonia at its current stage. This corresponds to a removal of 28±2 mg $NH_4^+$-N $L^{-1}{\cdot}d^{-1}$ loading per day and a peak removal rate of 50±5 mg $NH_4^+$-N $L^{-1}{\cdot}d^{-1}$. This experiment performed better than a study conducted by Ching and Redzwan (2017) who investigated ammonia oxidation in fish processing wastewater and observed a 60% $NH_4$-N removal at 4% salinity over 5 days. However, our enrichment performed slightly worse than another experiment which achieved 98% removal in batch experiments (Cui et al., 2014). This is a point which shows that our experiment was carried out at an early stage of the enrichment; hence it is envisioned that the performance will be improved as a stable microbial population is established. Another point observed in this study, and as be seen in Figure 4, is a high nitrite accumulation, indicating the inhibition of nitrite-oxidizing bacteria (NOB) compared to ammonia-oxidizing bacteria (AOB). Where in the case of complete nitrification the final product to be observed should be nitrate (Equation 2).

$$NH_4^+ + 1.86\ O_2 + 0.10\ CO_2 \rightarrow 0.021\ C_5H_7O_2N + 0.941\ H_2O + 0.98\ NO_3^- + 1.98\ H^+ \quad (2)$$

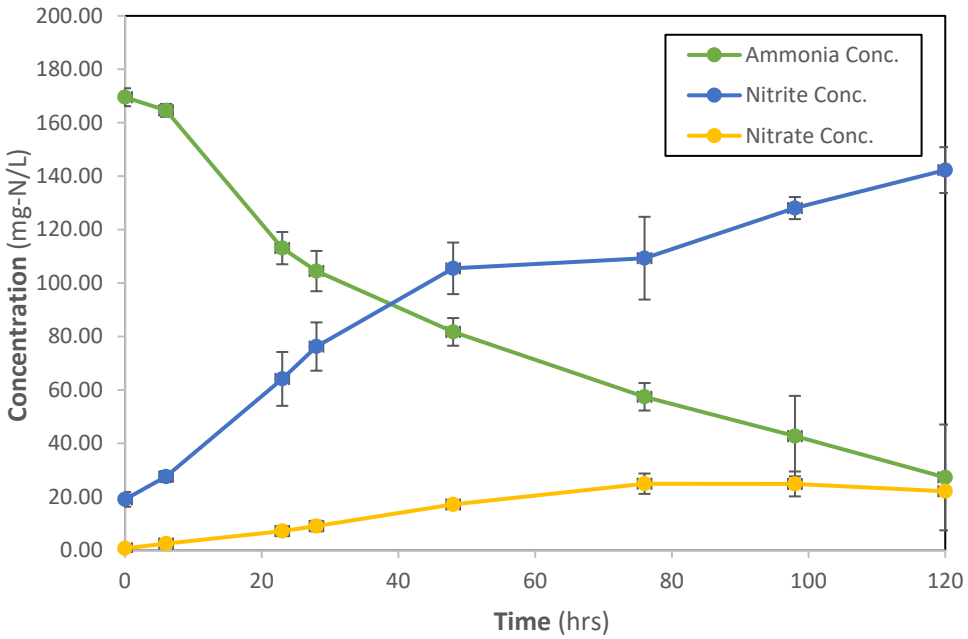


Figure 4: Ammonia Oxidation (4% salinity). Green line represents the oxidation of ammonia overtime. Blue line represents the accumulation of nitrite as ammonia is oxidized, whilst the yellow line represents the accumulation of nitrate.

Out selection of NOB is likely to have occurred due to the saline environment, as previous studies have indicated that NOB are more sensitive to salinity than ammonia oxidising microorganisms, (Ma et al., 2015; van den Berg et al., 2017; W. Cui et al., 2014). However, no studies, to our best knowledge have mentioned why NOB are more sensitive to salinity than AOB, a topic which could pose as an interesting research for future experiments. For this study, further experiments into the distribution and performance of the microbial population can confirm the inhibition of NOB over AOB due to the given conditions. Notwithstanding, such an instance of nitrite accumulation does not have to be considered as a negative, as in this case nitrite shunt denitrification techniques

can be utilized. A process that short-cuts the complete nitrogen removal process by cutting out the nitrite oxidation step during the final stage of nitrification (Figure 1). This means a reduction of energy requirements due to less aeration requirements, as well as a reduction of biomass (disposal costs) as the nitrite reduction in the first stage of denitrification also becomes redundant (Duan et al., 2019; Wang et al., 2019). Overall moving closer to the desired goal of having a highly efficient, low carbon footprint nitrogen removal system.

## 4.2 Denitrification

Mixed communities of $NO_3^-$ reducers and $NO_2^-$ reducers were each enriched in saline (4% NaCl) culture medium. Subsequently, these enrichment cultures were used in a batch kinetic analysis carried out in triplicate. The denitrification efficiency of these saline mixed communities was compared. Both $NO_3^-$ and $NO_2^-$ reducing mixed cultures were successfully enriched overtime. Figure 5 represents the progression of enrichment over 50-days where an increase in the Nitrogen Removal Rate (NRR, N-mg $L^{-1}\cdot d^{-1}$) was achieved. In all cases for the nitrate removal, the maximum allowable limit of 11 mg $NO_3^-$-N $L^{-1}\cdot d^{-1}$ for discharging in New Zealand was achieved (as depicted by the red dashed line). Similarly, for the nitrite reducer, apart from the first point, the maximum allowable discharge (0.2 mg $NO_2^-$-N $L^{-1}\cdot d^{-1}$) for nitrite was also achieved. Indicating a successful enrichment with improved removal rates.

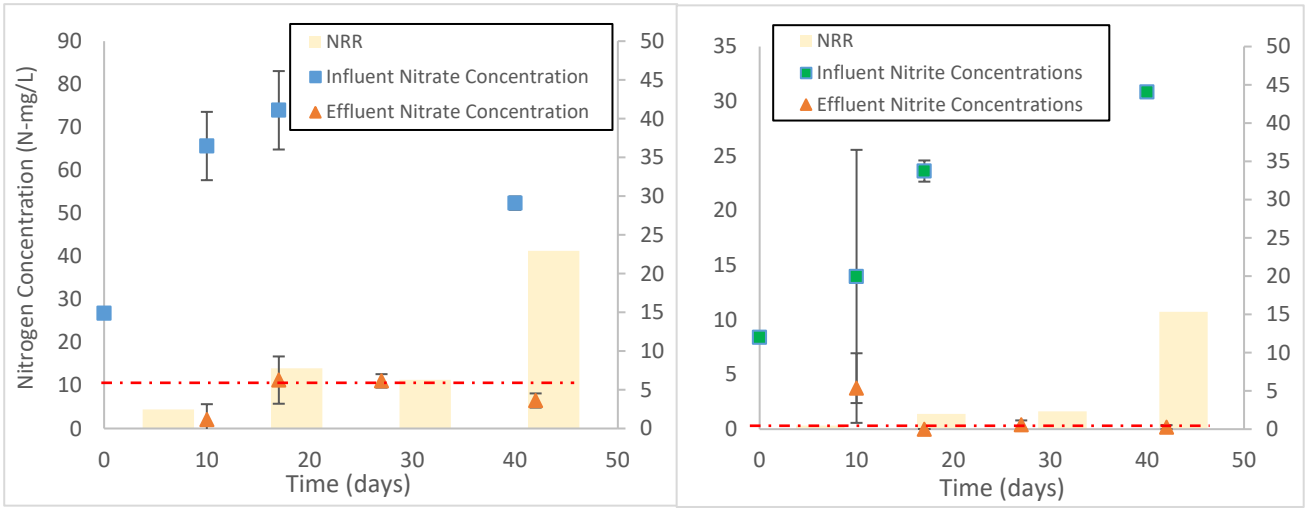


Figure 5: Enrichment and nitrate removal rates of hydrogenotrophic denitrifiers. Blue squares represent start-of-cycle nitrate, whilst green represents start-of-cycle nitrite concentrations. Orange triangles represent end-of-cycle concentrations for nitrate and nitrite. The yellow bars represent the improvement of nitrogen removal rates over the 50-day experiment.

Figure 6 represents the kinetic degradation of the enrichment cultures over a given time period, overall from a start-of-cycle concentration of 50 mg $NO_3^-$-N $L^{-1}\cdot d^{-1}$ and 30 mg $NO_2^-$-N $L^{-1}\cdot d^{-1}$ the average removal rates were 25±5 mg $NO_3^-$-N $L^{-1}\cdot d^{-1}$ and 30±5 mg $NO_2^-$-N $L^{-1}\cdot d^{-1}$, respectively. This corresponds to an 86% and 98% removal of nitrate and nitrite. If we compare this study to another study conducted on hydrogenotrophic denitrification by Visvanathan and colleagues (2008) at a 3% salinity, an average removal of 44 mg $NO_3^-$-N $L^{-1}\cdot d^{-1}$ in a hollow fibre membrane reactor was observed. The improved removal rates by Visvanathan et al.,'s (2008) was attributed to the use of hollow fibre membranes. Hollow fibre membranes are used to improve the solubility of hydrogen gas in the media and therefore improve removal rates. The results also indicate that hydrogen is likely the rate limiting factor. This is an area that we would look to progressing to in the future

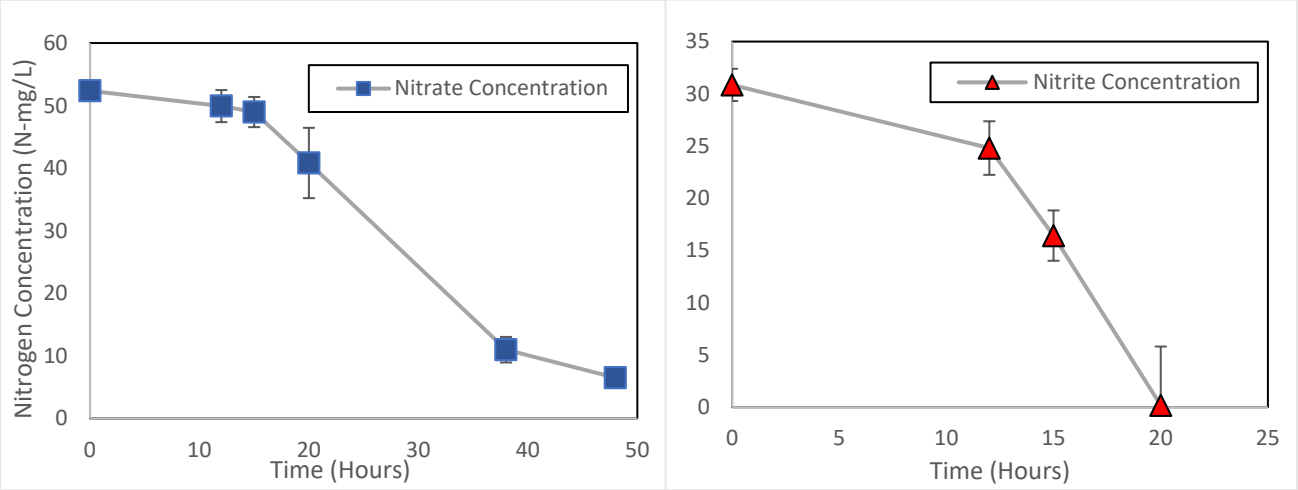


Figure 1: Nitrate and Nitrite degradation curves. Blue squares represent nitrate removal over time. Red triangles represent nitrite removal

## 5. CONCLUSIONS

This project was undertaken to investigate the enrichment of indigenous nitrifiers and denitrifiers in a saline environment. These findings could then be applied for further research as a combined system for wastewater treatment processes to improve the efficiency of treating nitrogen polluted high salinity wastewater. Over the course of this project halophilic hydrogenotrophic denitrifying microorganisms were successfully enriched and exhibited acceptable removal rates for potential industrial applications. Analysis determined that $NO_3^-$ and $NO_2^-$ removal rates were 25±5 mg $NO_3^-$-N $L^{-1}{\cdot}d^{-1}$ and 30±5 mg $NO_2^-$-N $L^{-1}{\cdot}d^{-1}$, respectively. More importantly, the denitrifiers achieved removal to the desired maximum allowable discharge limits as set by the WHO. It is envisioned that, with the use of hollow fibre membranes and continued enrichment, the overall removal would be further improved, which is a plan for a future with stricter discharge regulations. In contrast, the enrichment of nitrifying microorganisms achieved ammonia oxidation of 50±5 mg $NH_4^+$-N $L^{-1}{\cdot}d^{-1}$. Albeit with high inhibition of NOB, to a ratio of 4:1 $NO_2$-accumulation. The implications of these findings indicate the possibility for a nitrogen removal process in saline environments with nitrite shunt denitrification, to the benefit of achieving a cost effective, environmentally friendly process. The nitrite shunt process effectively bypasses the negative characteristics of $NO_2^-$ accumulation, with the added benefit of reducing operational costs. Future work will involve combining the saline nitrification and denitrification processes to assess the combined removal performance in a single reactor, with the use of hollow fibre membranes for the diffusion of hydrogen gas into the reactor. Furthermore, given the current direction of research, the possibility of nitrite shunt with various saline conditions can be further explored.

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