HYDROGEN-DRIVEN AUTOTROPHIC DENITRIFICATION OF WASTEWATER USING A MEMBRANE BIOFILM PROCESS

Marc Russenberger[1], Kaleigh Biss[2], Hukerenui Bonnet[1], Jiabao Wendy Qi[1], Rob Fullerton[1, 3], Shan Yi[4], Wei-Qin Zhuang[1, ◦]

[1] Department of Civil and Environmental Engineering, University of Auckland, Auckland 1142, New Zealand

[2] Department of Civil and Environmental Engineering, University of South Florida, Tampa, FL33620, USA

[3] Beca, Auckland 1010, New Zealand

[4] Department of Chemical and Materials Engineering, University of Auckland, Auckland 1142, New Zealand

---

ABSTRACT

Nitrate contamination in water resources, stemming from agricultural intensification, excessive use of inorganic nitrogenous fertilizer, and inadequate municipal wastewater nitrate removal, poses serious environmental and health risks globally. Conventional heterotrophic denitrification has been used to remove nitrate from wastewater, but these processes often result in significant nitrous oxide emissions-a potent greenhouse gas (GHG) 298 times more potent than carbon dioxide. Moreover, some processes consume considerable energy by recycling nitrate-laden wastewater to mix with the primary effluent in pre-anoxic tanks to use its biochemical oxygen demand (BOD) for heterotrophic denitrification. Given the global urgency surrounding greenhouse gas emissions and New Zealand's commitment to the Paris 2050 emissions goal, a net-zero-emission nitrate removal process is essential.

Hydrogenotrophic denitrification is a promising bioprocess that uses hydrogen-oxidizing autotrophic bacteria to reduce nitrate to dinitrogen gas. Previous studies have shown that hydrogenotrophic denitrification can significantly reduce the production of direct and indirect greenhouse gas emissions. Due to no organic carbon being required by autotrophic microbes, there is no risk of organic carbon carryover or additional carbon dioxide production as in heterotrophic denitrification processes. Therefore, hydrogen can be provided in excess relative to the nitrate concentrations and stoichiometric needs of autotrophic denitrifiers, driving complete denitrification. However, technical challenges such as low water solubility and the explosive nature of hydrogen hinder the application of this novel wastewater treatment technology.

This research aims to develop a robust and efficient hydrogenotrophic denitrification process to minimize nitrous oxide emissions and energy consumption while maintaining wastewater discharge standards. Results from bench-scale testing using an innovative 20-liter membrane biofilm reactor (MBfR) highlighted that it could effectively and safely provide hydrogen through direct diffusion to hydrogen-oxidizing denitrifying bacteria (HODB) biofilm. Additionally, our results demonstrated that return-activated sludge from Rosedale Wastewater Treatment Plant (WWTP) could seed an MBfR effectively. The process

achieved a peak denitrification rate of 41 mg NO_3-N/L·d when operated in a batch mode with an applied hydrogen pressure of 5 psi. The denitrification rate could significantly increase when the MBfR was operated in continuous-flow mode. We noticed that pH control played an essential role in stabilizing performance and increasing denitrification rates. Overall, our MBfR-based hydrogenotrophic denitrification process can be a sustainable process to drop into existing wastewater treatment trains as a post-anoxic step.

KEYWORDS

Autotrophic denitrification, Hydrogenotrophic denitrification, Membrane Biofilm Reactor, Operating Parameters, pH control

PRESENTER PROFILE

Marc Russenberger is a second-year Ph.D. student at the University of Auckland's Civil and Environmental Engineering department. Marc completed his undergraduate study with first-class Honours at UoA, studying Chemical and Materials Engineering. His research focus is on Environmental Microbiology, specifically the biological removal of nutrients from wastewater with the aim of reducing greenhouse gas emissions occurring from traditional wastewater denitrification.

1.0 INTRODUCTION

Nitrate pollution has become a major environmental and public health concern globally (Bouchard et al., 1992; Wu et al., 2021). Elevated nitrate concentrations in water resources can result in eutrophication (Ashok & Hait, 2015), methemoglobinemia in infants, as well as gastric cancer (Lockhart et al., 2013). Thus, the current standard of nitrates in New Zealand drinking water is 11.3 mg NO_3-N/L and 0.91 mg NO_2-N/L, in line with the standards set by the World Health Organization. Intensifying farming practices, increased use of inorganic nitrogenous fertilizers, and inadequate nitrate removal from wastewater are major contributors to the elevating nitrate levels in ground and surface water (Baskaran et al., 2009; Lockhart et al., 2013; MfE, 2020). This issue is particularly prevalent in New Zealand, where agriculture is our primary industry, with a 629% increase in nitrogenous fertilizer use from 1991 to 2019 (Stats, 2021) and worsening nitrate-nitrogen groundwater quality (MfE, 2019). Furthermore, the nitrate concentration in the effluent of secondary domestic wastewater treatment plants (WWTP) can be over 40 mg N/L (Cao et al., 2019), making its remediation before discharge critical. Traditional heterotrophic nitrate reduction methods release carbon dioxide (Chen et al., 2016) and nitrous oxide (Andalib et al., 2018; Chung & Chung, 2000), two potent greenhouse gases that drive climate change.

Wastewater treatment is accountable for 3.2-10% of total anthropogenic nitrous oxide emissions (Law et al., 2012). Nitrous oxide is a powerful greenhouse gas with a carbon dioxide equivalent of 298 kg and a half-life of 114 years (EPA, 2023). It accounts for 10% of total global greenhouse gas emissions and the atmospheric concentration is 324 ppbv, increasing at a rate of 0.3% per year (Adouani et al., 2015). Nitrous oxide production occurs primarily during the biological removal of nitrogen via nitrification and denitrification (Richardson et al., 2009; Tallec et al., 2006). Nitrification and denitrification are the two main redox reactions in the nitrogen cycle (Fig 1.).

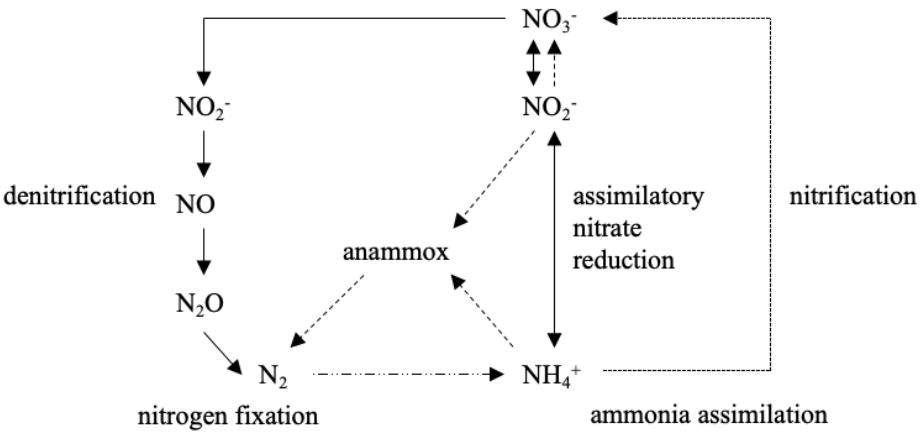


Figure 1: Schematic representation of the nitrogen cycle.

Conventional wastewater denitrification uses organic compounds (e.g., BOD) to drive the reduction of nitrate (Aslan, 2005) (Wąsik et al., 2001). In post-anoxic heterotrophic denitrification, carbon carryover is a concern. To prevent it, organic carbon is often inadequately supplied, resulting in incomplete denitrification and the production of nitrous oxide (Andalib et al., 2018; Chung & Chung, 2000). Therefore, heterotrophic denitrification is often carried out using a pre-anoxic approach using influent organic carbon to drive denitrification. To achieve this, a large volume of nitrate-laden wastewater from the back of the treatment train is returned to the front with an energy intensive recycle.

Autotrophic denitrification is an alternative process. Autotrophic denitrifiers use carbon dioxide as their carbon sources and reduced inorganic compounds, such as hydrogen, as electron donors (Vidal et al., 2002; Zehr & Kudela, 2011). Because no organic carbon is required in autotrophic denitrification, there is no resulting carbon carryover or additional carbon dioxide production. In particular, hydrogen, due to its low solubility and gaseous nature, can be provided in excess relative to the nitrate concentrations and stoichiometric needs of autotrophic denitrifiers to drive complete denitrification, reducing nitrous oxide production. It has been reported that hydrogen-driven denitrification can reduce nitrous oxide production in the order of magnitudes(He et al., 2023; Wu et al., 2021).

However, the low water solubility of hydrogen (1.6 mg/L) impacts the denitrification performance causing low mass transfer efficiency and hydrogen usage rates (Park & Yoo, 2009). Furthermore, hydrogen bubbling can result in the accumulation of hydrogen, creating an explosive environment (Mansell & Schroeder, 2002). The hydrogen-based membrane biofilm reactor (MBfR) is an emerging technology in biological denitrification for wastewater treatment (Jang et al., 2023). An MBfR supplies hydrogen via direct diffusion through the walls of a micro or non-porous gas transfer membrane to a denitrifying biofilm that accumulates on the exterior wall of the membrane. MBfRs are advantageous due to low energy consumptions, high specific surface area for biomass growth, safe and on-demand hydrogen delivery, and the need for no organic compound dosing (Martin & Nerenberg, 2012; Rittmann, 2007). Therefore, this study investigated the performance of indigenous (hydrogen oxidizing denitrifying bacteria) HODB from New Zealand wastewater in a MBfR. Investigating the performance of indigenous HODB is important to New Zealand as it has strict biosecurity restricting the import of foreign strains. More importantly the study investigates a sustainable denitrification method, which reduces greenhouse gas emissions in wastewater treatment.

2.0 MATERIALS AND METHODS

2.1 SYNTHETIC WASTEWATER

Table 1 presents the chemical composition of the trace element and mineral salts medium stock solutions used to prepare the synthetic wastewater. All chemicals used in this research are ACS grade or above from Sigma (Sigma, MO, USA). The synthetic wastewater consists of 1 mL/L of the trace element stock solution (Wagner et al., 2019), 10 mL/L of the mineral salts medium stock solution as described previously (Zhuang et al., 2014), and 1.1 g/L $NaHCO_3$. Whereas the concentration of $NaNO_3$ amended into the synthetic wastewater was varied from 394.1 to 607.2 mg/L, representing 65 to 100 mg N/L. This represented a respective loading rate of 36.1 to 55.6 mg N/L·d. This study's degree of nitrate contamination was representative of domestic wastewater characteristics. The pH of the prepared influent synthetic wastewater was adjusted to 7.4. Return-activated sludge taken from Rosedale WWTP in Auckland, New Zealand was used as the inoculum. Before inoculating the MBfR, the inoculum was diluted to 1,900 mg/L suspended solids (SS) using the synthetic wastewater.

Table 1: Chemical composition of trace element and mineral salts medium stock solutions.

Trace element stock solution [1000 ×]	Concentration (mg/L)	Mineral salts medium stock solution [100 ×]	Concentration (g/L)
HCl (33% solution)	7.5 (mL)	NaCl	100
$FeCl_2·4H_2O$	1500	$MgCl_2·6H_2O$	50
$CoCl_2·6H_2O$	190	KH_2PO_4	20
$MnCl_2·4H_2O$	100	NH_4Cl	30
$ZnCl_2$	70	KCl	30
H_3BO_3	6	$CaCl_2·2H_2O$	1.5
$Na_2MoO_4·2H_2O$	36	Na_2SO_4	5
$NiCl_2·6H_2O$	24		
$CuCl_2·2H_2O$	2		

2.2 LABORATORY-SCALE MBFR SET-UP

Figure 2 shows a schematic representation of the hydrogen-driven MBfR with respective batch and continuous-flow operational modes. Two lab-scale membrane modules (ZeeLung LS-1, VEOLIA) were housed inside a 20 L working volume acrylic column (175 cm diameter × 100 cm height). One module's nominal membrane surface area is 1.5 m^2 with a maximum process air pressure of 12 psi.

Synthetic wastewater was fed at a specified flow rate using a peristaltic pump (Cole-Parmer Masterflex, Illinois, United States) to give an HRT of 24 to 43 hours, and the effluent was collected for chemical analysis. The MBfR contents were stirred using a magnetic stirrer (SB301, Stuart) and magnetic stir bar at 150 rpm. The reactor was baffled with four equispaced baffles (1.5 cm length × 0.6 cm width × 75 cm height). Additional pure nitrogen mixing gas could be bubbled through the MBfR using the mixing air connection provided by the membrane modules at an adjustable flow rate of 0.5 to 5 L/min using a Kofloc in flow gas meter (RK-1350V, Kofloc). The applied pressure of pure hydrogen gas to the membranes was maintained at a specified pressure using a hydrogen gas regulator (HiQ, BOC) and monitored by a pressure gauge at the inlet.

The inoculation of biofilm onto the outer membrane surface was conducted in batch operation, whereas long-term performance analyses would be performed through a continuous-flow process.

2.3 MBFR OPERATING CONDITIONS

2.3.1 INOCULATION PHASE (BATCH-MODE

We also operate the MBfR in batch mode to enhance the growth and accumulation of a biofilm on the membrane module. Nitrogen gas sparging (1.5 L/min) and magnetic stirring was used during the batch mode to provide adequate mixing. In this well-mixed condition, the bulk biomass was kept suspended in the reactor with no sedimentation. Hydrogen gas (3 psi) was supplied to the membrane lumen to achieve bubbleless aeration. The applied hydrogen pressure was determined in preliminary testing in which the visible formation of bubbles occurred at higher pressures. During the inoculation phase, nitrate concentrations were maintained at 50 – 150 mg N/L through the direct supplementation of sodium nitrate.

The process was switched to continuous-flow operation after an eight-day inoculation period.

2.3.2 OPERATIONAL PHASE (CONTINUOUS-FLOW MODE)

After the inoculation phase, the MBfR performance went through an acclimatization period for approximately 30 days where the performance stabilized. During this period the MBfR was operated under a mixture of batch and continuous-flow modes. The applied hydrogen pressure during this period was 5 psi. During this period, the MBfR was fed with 65 mg NO_3-N/L with a 24-hr HRT (Day 9 to 18) and a 43-hr HRT (Day 19 to 46). Nitrogen sparging was stopped on Day 19 to enable the further accumulation of biofilm on the membranes. The pH of the MBfR was maintained between 6.7 to 8.1 through HCl dosing due to the increase in pH from denitrification. Once the process had acclimatized, the denitrification efficiency was in excess of 80% and the influent nitrate concentration was increased to 100 mg-N/L throughout the operational phase (Day 40 to 46).

2.4 BATCH BIOREACTOR SETUP (UBIQUITY STUDY)

The enrichment of HODB from Māngere, Rosedale, and Army Bay WWTPs was carried out in batch experiments, in glass serum bottle bioreactors (1 L) with a final working volume of 500 mL. 250 mL of Return activated sludge (RAS) from Māngere and Rosedale WWTPs and waste activated sludge (WAS) from Army Bay WWTP was diluted to the final working

volume using the prepared synthetic wastewater. During the enrichment 5 mL of a concentrated nitrate stock solution was intermittently dosed to provide 100 mg NO_3-N/L final nitrate concentration in each bioreactor. The bioreactors were capped and sealed with a butyl rubber stoppers and aluminium seals and flushed using pure nitrogen gas for 5 minutes to remove residual oxygen. The serum bottles were then pressurized to 10 psi using industrial grade hydrogen with a purity of 99.9%. The hydrogen gas was replenished periodically. The bottles were incubated at 30°C (Polar 1000C, Contherm) and shaken at 150 rpm using an orbital shaker (SHLD0415DG, Ohaus).

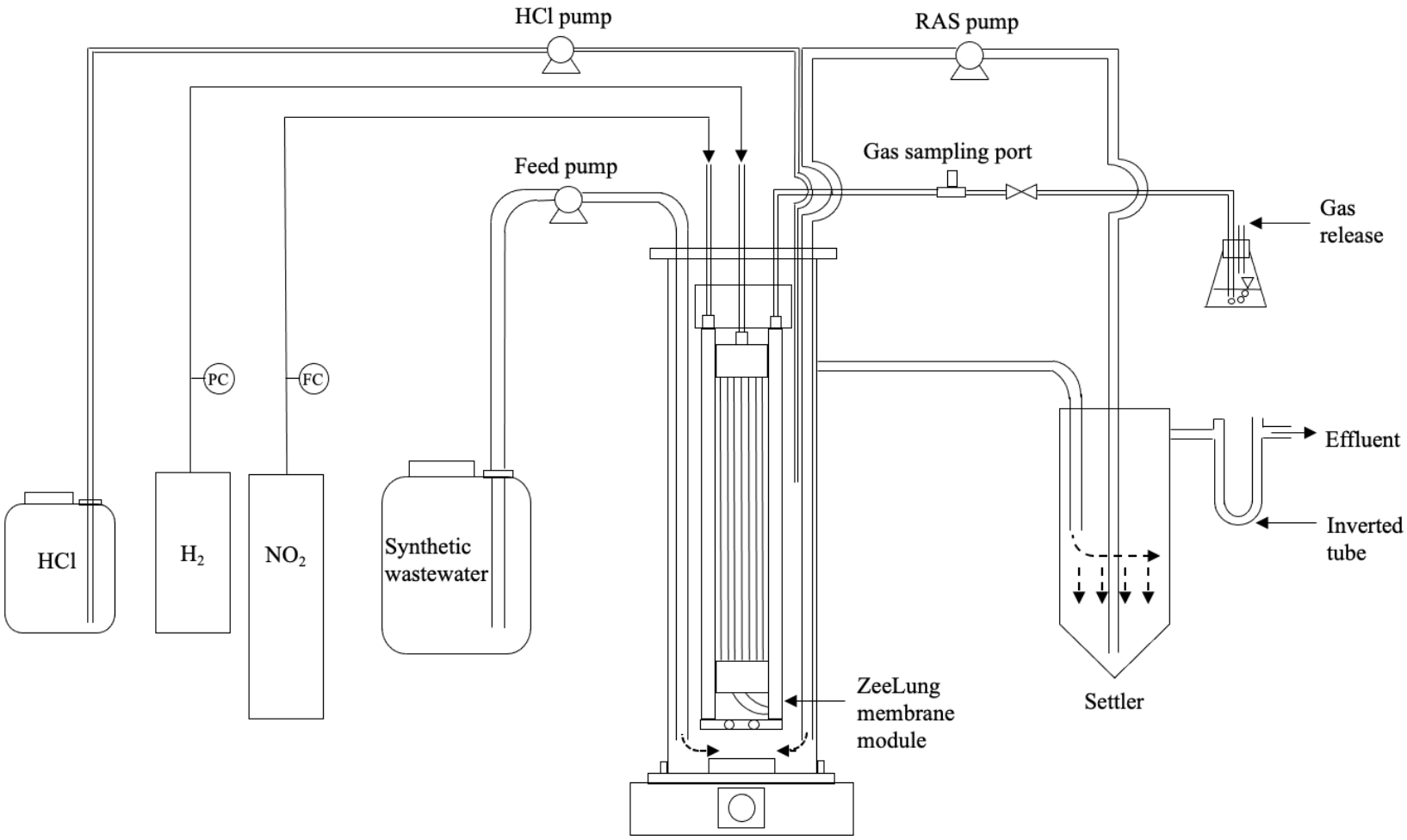


Figure 2: schematic representation of the hydrogen-driven denitrification MBfR set-up with ability to operate under batch (feed pump off) and continuous modes of operation.

2.5 SUSPENDED SOLIDS ANALYSES

Suspended solids analyses for the inoculation of the MBfR were completed in accordance with the Standard Methods for the Examination of Water and Wastewater 22nd edition (Rice & Bridgewater, 2012).

2.6 CHEMICAL ANALYSES

The reactor and effluent samples for analysis were centrifuged at 15,000 × g for 5 min to separate the supernatant for nitrate analyses via spectrophotometric and chromatographic methods. Nitrate concentrations during the start-up in the inoculation and acclimatization period were monitored directly using Hach Nitrate and Nitrite Test Strips (Hach, 2745425). Test strip results help determine the best dilution rates for more accurate quantification using Hach Nitrate TNTplus Vial tests (Hach, TNT 836). The vials were used following the manufactures protocol with a Hach DR1900 spectrophotometer.

NO_3-N and NO_2-N were quantified by IC (Dionex ICS-2100, Thermo Scientific) equipped with an AG18 column (Dionex IonPac). A 23 mM KOH solution was used as the eluent at a 1.0 mL/min flow rate.

3.0 RESULTS AND DISCUSSION

3.1 INOCULATION PERFORMANCE (DAY 0 TO 8)

The nitrate removal performance of the MBfR during the initial start-up phase (batch mode) is shown below in figure 3. (Day 0 to 8). The figure shows the bulk-liquid nitrate-nitrogen concentration during the batch inoculation phase. The nitrate reduction happened immediately when the MBfR was inoculated with sludge from Rosedale WWTP, indicating that hydrogenotrophic denitrifiers were already presented in the sludge. Sodium nitrate was replenished periodically to maintain nitrate nitrogen concentrations at above 50 mg-N/L to promote the selective growth of HODB.

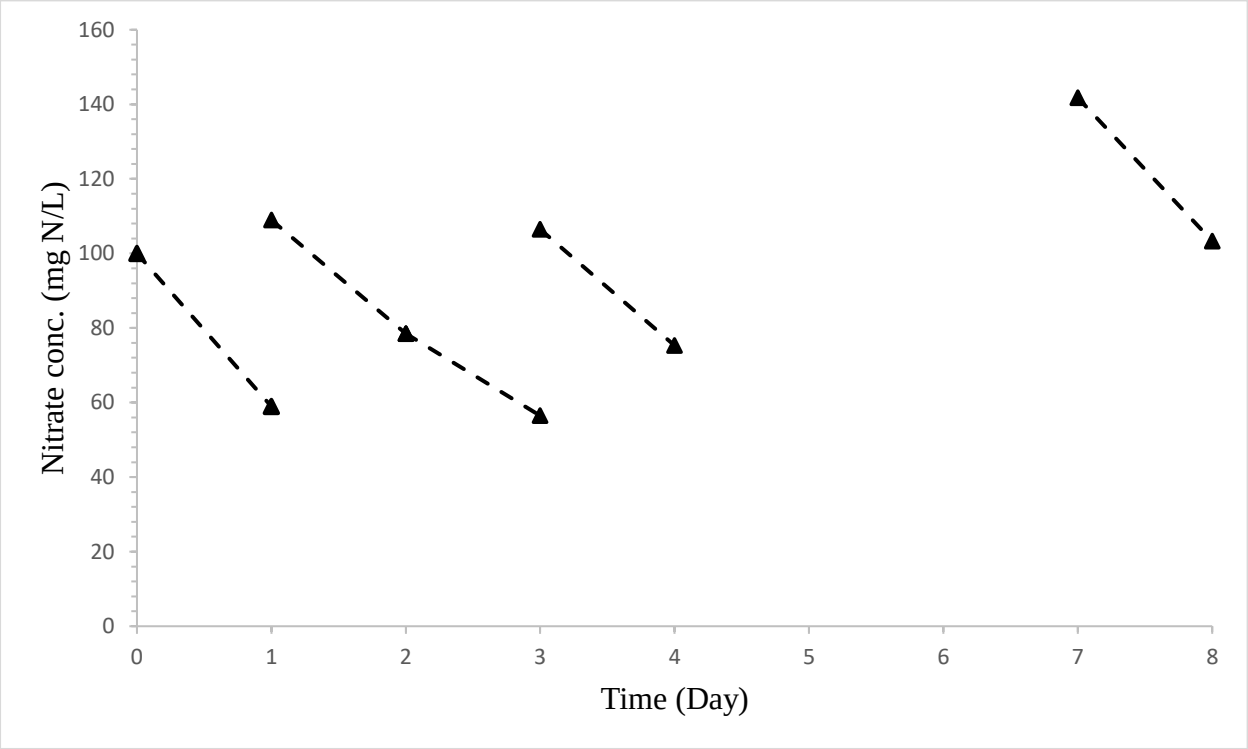


Figure 3: In the initial phase (Day 0 to 8), a batch operation mode in conjunction with high nitrate nitrogen concentrations in the MBfR was used to promote the growth of hydrogenotrophic denitrifiers using return activated sludge from Rosedale WWTP.

The peak nitrate removal rate achieved during the initial start-up phase was 41.0 mg N/L·d and the average removal rate was 34.2 mg N/L·d across the eight-day period. Given that the nitrate-nitrogen concentration in the post nitrification effluent is typically 40-50 mg-N/L, our process has the potential to be quickly started up using unacclimated return activated sludge and the effluent from the aerobic tank.

3.2 ACCLIMATIZATION PERFORMANCE (DAY 9 TO 39)

On day 9, the MBfR was changed to continuous mode after observing significant biofilm formation on the membrane modules. Figure 4. shows the nitrate-nitrogen removal efficiency across the acclimatization period between Day 9 to 39. Over the 30-day period, the nitrate-nitrogen removal efficiency improved from 14.8% (Day 9) to 68.6% (Day 39).

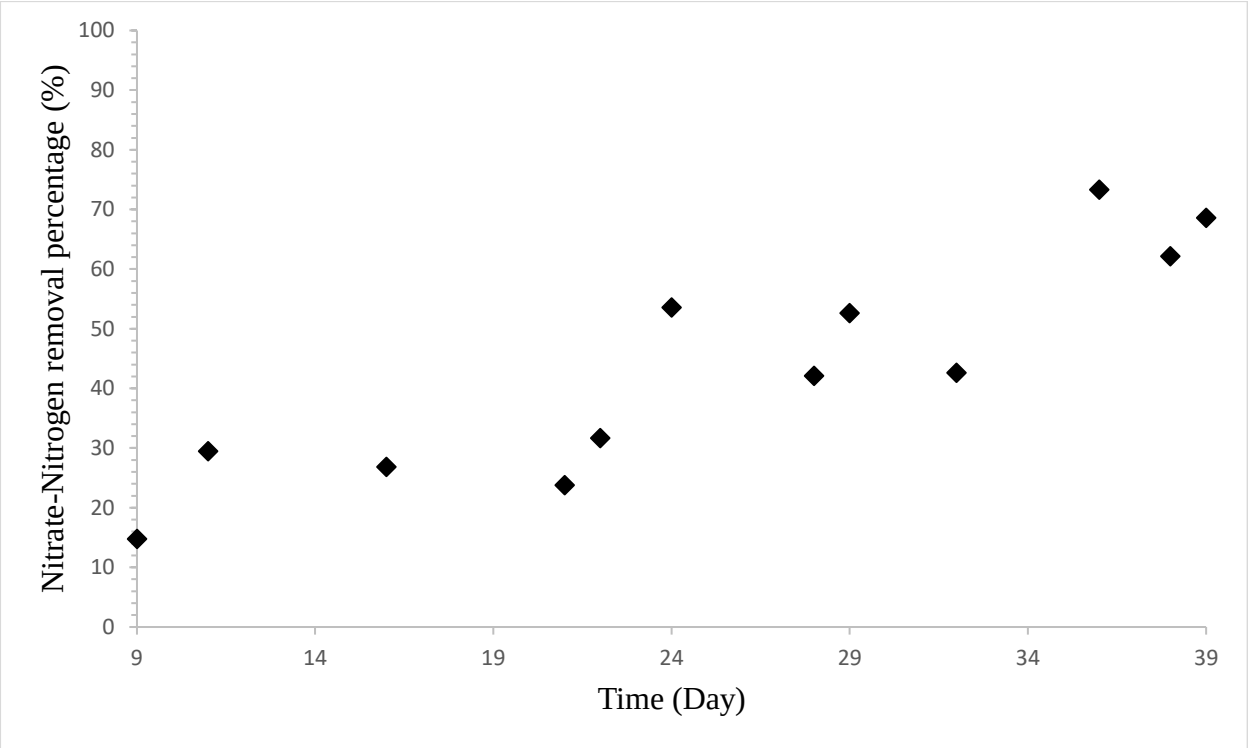


Figure 4: The nitrate nitrogen removal percentage during the stabilization phase (Day 9 to 39).

During Day 11 to 21, the performance of the MBfR fluctuated between 29.5 to 23.8%. The relatively low nitrate removal efficiency indicated losing HODB in the reactor from flush out or a decrease in HODB biofilm biomass quantity that could use hydrogen to conduct denitrification. Although we sparged the MBfR using nitrogen provide mixing, nitrogen sparging could prevent the accumulation of a biofilm on the exterior surfaces of the membrane module, reducing the amount of available HODB that could access hydrogen. The 24-hr HRT might also be shorter than the growth rate of the biofilm-forming HODB.

On Day 21, the continuous sparging of nitrogen gas was stopped, and the HRT was increased to 43-hr. These changes reduced the shearing of biomass off the membrane, enabled more HODB to accumulate on the membrane threads, and reduced the flush out of HODB suspended in the bulk liquid. The changes had positive results as the increase in denitrification efficiency from 23.8 to 68.6% (Day 21 to 39).

3.3 OPERATIONAL PERFORMANCE (DAY 40 TO 46)

Figure 5. shows the nitrate-nitrogen removal percentage achieved by the MBfR between Days 40 to 46. The system achieved a peak nitrogen removal percentage of 99.3%. The nitrate removal efficiency kept on improving to almost 100% on day 46. The peak specific removal rate under the 43-hr HRT was 55.2 mg N/L·d. Accounting for the membrane surface area (3 m^2) and reactor volume (20 L), the effective removal rate of nitrogen by the MBfR was 367.7 mg N/m^3·d (specific dentification rate × reactor volume ÷ membrane surface area). At these operating conditions, with a nitrogen removal efficiency ranging from 86.4 to 99.3%, we are confident that the MBfR can denitrify the effluent of secondary domestic wastewater effectively.

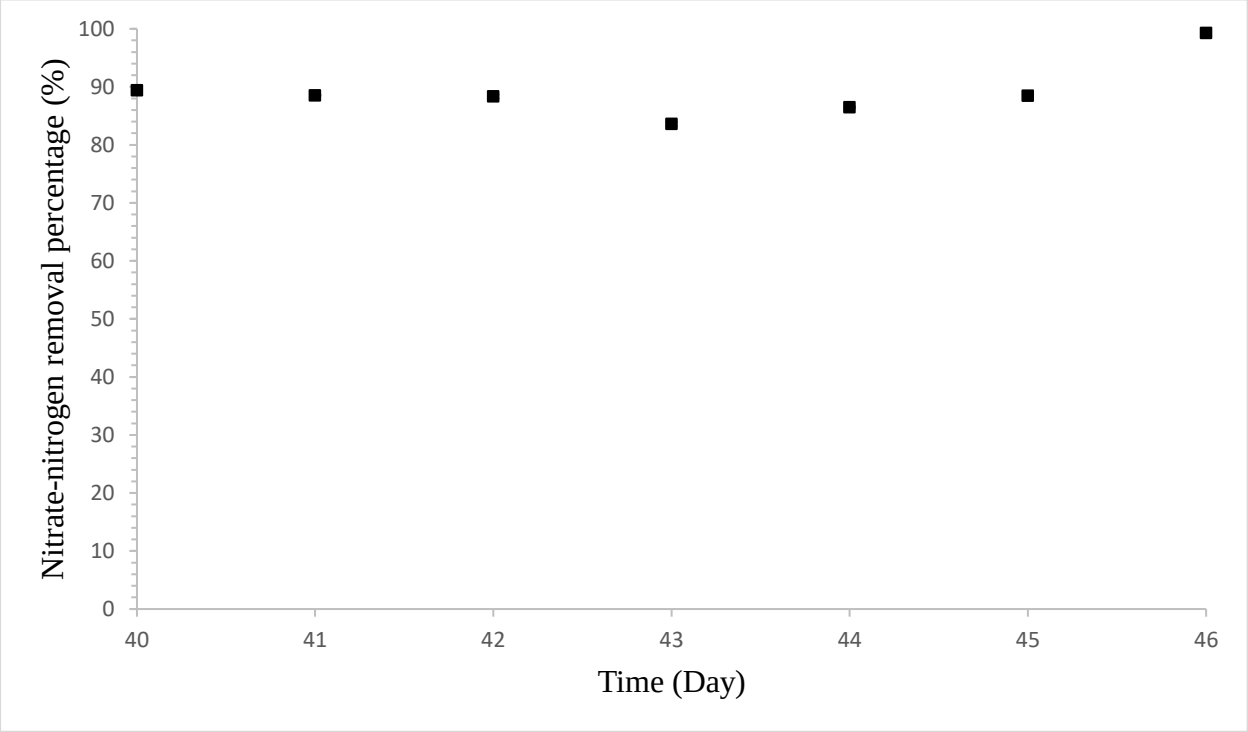


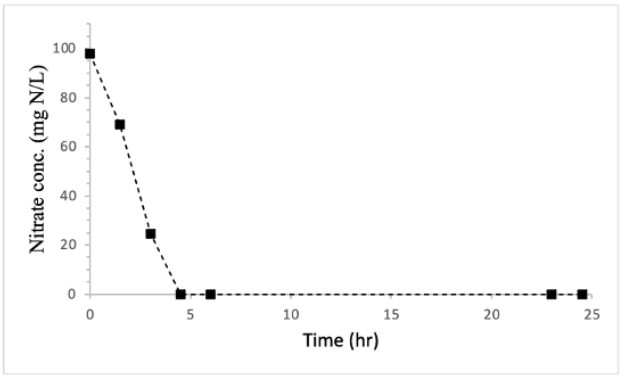
Figure 5: The nitrate nitrogen removal percentage during the operational period (Day 40 to 46).

The removal rates achieved by this process, considering the reactor volume and membrane surface area, compared favorably against results obtained in similar studies by, (Lee & Rittmann, 2000) and (Ergas & Reuss, 2001). In a study by (Lee & Rittmann, 2000), they achieved a specific nitrogen removal rate of 127.7 mg N/m^3·d. Another similar study (Ergas & Reuss, 2001) achieved a peak-specific removal rate of 245.6 mg N/m^3·d. The membrane surface area to reactor volume ratio is a key operational parameter, as the relationship determines the surface area provided for biofilm to accumulate with respect to the overall nitrate loading. The larger the comparative membrane surface area is, with respect to operating volume, the greater the corresponding concentration of HODB in the system. Increasing the surface area provides more space for the growth of HODB and improves the overall supply of hydrogen (electron donor) into the system relative to the nitrate concentration (electron acceptor). This indicates that the surface

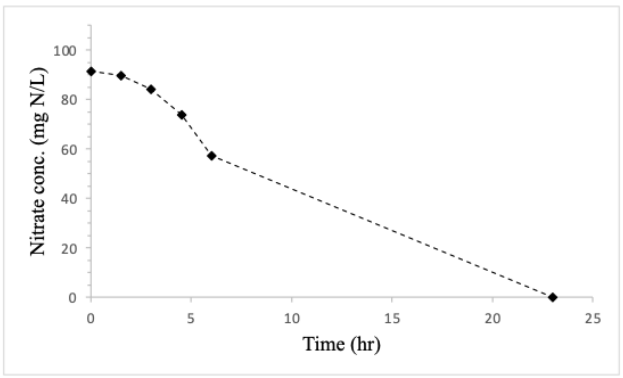
area comparative to the reactor volume can be increased to further improve denitrification performance.

3.4 UBIQUITY OF HODB IN NEW ZEALAND WWTPS (MĀNGERE, ROSEDALE, ARMY BAY)

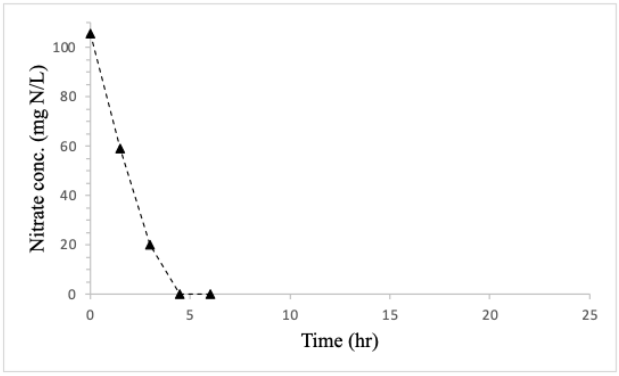
Figure 6. shows the nitrate-nitrogen concentrations in one hydrogen driven reduction cycle by batch enrichments using seed sludge from three main WWTPs in Auckland (Māngere, Rosedale, Army Bay). The results show that all three WWTPs could seed a HODB community capable of 100% nitrate removal within a 24-hr period. Māngere, Rosedale and Army Bay achieved an average nitrate removal rate of 522.1, 95.5, and 562.7 mg N/L·d, respectively. Rosedale's nitrate removal rate maybe in the same range as Māngere and Army Bay as 100% removal may have been achieved much earlier than the final sampling point (23-hrs).



(a) Māngere



(b) Rosedale



(c) Army Bay

Figure 6: The nitrate concentration over 25 hours in batch enrichments seeded by different wastewater treatment plants in Auckland.

The results demonstrate the ubiquity of HODB in Māngere, Rosedale and Army Bay WWTPs capable of being enriched and self-seeding a denitrification reactor. The enriched HODB in all three WWTPs are shown to have the ability to denitrify secondary domestic wastewater to below resource regulations.

4.0 CHALLENGES AND FUTURE DEVELOPMENT

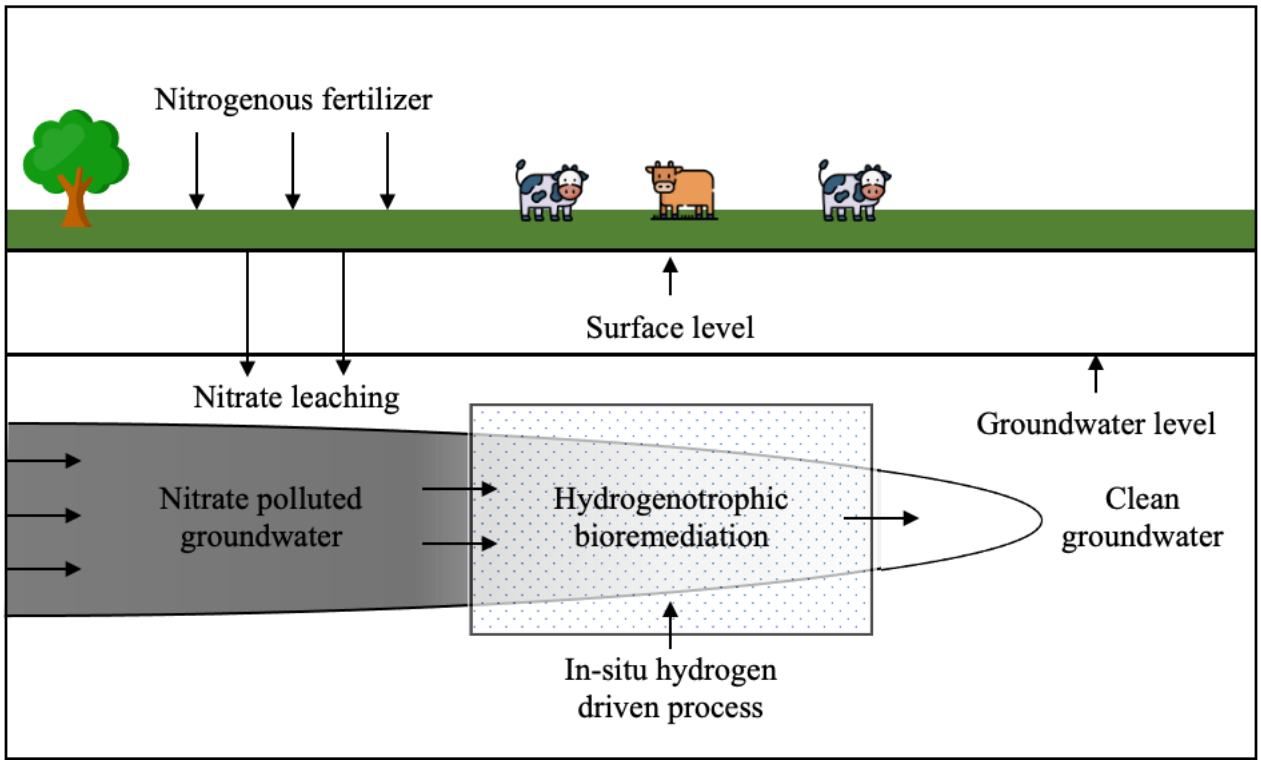


Figure 1: In-situ hydrogen driven denitrification model.

The following stages in this research will focus on two aspects; 1) Understanding the fundamental changes, interactions, and competition in the microbial community during the MBfR seeding and operating stages. This will focus on analyzing the sequenced DNA from key stages of the start-up and operating phases, and 2) Using the knowledge gained to shorten the inoculation and acclimatization periods to achieve quick start up hydrogenotrophic denitrification MBfRs.

We will also develop this technology to treat nitrate polluted groundwater using in-situ or pump-and-treatment strategies (EPA, 2013). New Zealand's most substantial source of nitrate pollution occurs from using nitrogenous fertilizers in the dairy industry and the subsequent leaching and pollution of groundwater. Applying an in-situ flow through hydrogen-driven denitrification process offers a clean and sustainable method of remediating nitrate-polluted groundwater. The principle of such a process is similar to that of the continuous flow MBfR developed in this study, applied in a non-point source configuration. The hydrogen-supplying modules are positioned downstream of nitrate-polluted ground waters, which flow through the process and are remediated (Fig 8.).

5.0 CONCLUSION

A continuous flow MBfR was successfully started up and achieved a nitrate-nitrogen removal efficiency of 99.3%. The specific denitrification rate of the process was 369.3 mg $N/m^3 \cdot d$ when the membrane surface area and the volume of the reactor were accounted for. This result compared favorably to similar studies done on hydrogen-driven MBfRs. Most importantly, the MBfR achieved a nitrate-nitrogen concentration well below that of water discharge regulations (10 mg NO_3-N/L).

The research carried out in this study proves the presence and feasibility of using indigenous HODB in a continuous hydrogen-driven autotrophic denitrification process for treating New Zealand wastewater. Denitrification performance analysis highlighted the ability to seed a bioreactor using indigenous HODB and denitrify a synthetic wastewater influent containing 100 mg NO_3-N/L below New Zealand discharge regulations. The study provides insight into the ability of this process as a drop-in denitrification system capable of mitigating denitrification-related greenhouse gas emissions. An exciting direction of further development in this area of research is the bioremediation of nitrate polluted groundwater.

ACKNOWLEDGEMENTS

M.R. thanks Callaghan Innovation for the Ph.D. scholarship to undertake the project. W.Q.-Z thanks Beca and the Warwick and Judy Smith Engineering Endowment Fund (ENG-2022-01W-Zhuang) for their generous support. The authors would also like to thank Ms. Kimberly Murphy, Michaela Strong, Emma Tyson and Mr. Kobe Daniel for their contribution to the project.

REFERENCES

Adouani, N., Limousy, L., Lendormi, T., & Sire, O. (2015). N2O and NO emissions during wastewater denitrification step: Influence of temperature on the biological process. *Comptes Rendus Chimie*, *18*(1), 15-22. https://doi.org/https://doi.org/10.1016/j.crci.2014.11.005

Andalib, M., Taher, E., Donohue, J., Ledwell, S., Andersen, M. H., & Sangrey, K. (2018). Correlation between nitrous oxide (N(2)O) emission and carbon to nitrogen (COD/N) ratio in denitrification process: a mitigation strategy to decrease greenhouse gas emission and cost of operation. *Water Sci Technol*, *77*(1-2), 426-438. https://doi.org/10.2166/wst.2017.558

Ashok, V., & Hait, S. (2015). Remediation of nitrate-contaminated water by solid-phase denitrification process-a review. *Environ Sci Pollut Res Int*, *22*(11), 8075-8093. https://doi.org/10.1007/s11356-015-4334-9

Aslan, Ş. (2005). Combined removal of pesticides and nitrates in drinking waters using biodenitrification and sand filter system. *Process Biochemistry*, *40*(1), 417-424. https://doi.org/https://doi.org/10.1016/j.procbio.2004.01.030

Baskaran, R., Cullen, R., & Colombo, S. (2009). Estimating values of environmental impacts of dairy farming in New Zealand. *New Zealand Journal of Agricultural Research*, *52*(4), 377-389.

Bouchard, D. C., Williams, M. K., & Surampalli, R. Y. (1992). Nitrate contamination of groundwater: sources and potential health effects. *Journal-American Water Works Association*, *84*(9), 85-90.

Cao, S., Du, R., Peng, Y., Li, B., & Wang, S. (2019). Novel two stage partial denitrification (PD)-Anammox process for tertiary nitrogen removal from low carbon/nitrogen (C/N) municipal sewage. *Chemical Engineering Journal*, *362*, 107-115. https://doi.org/https://doi.org/10.1016/j.cej.2018.12.160

Chen, D., Dai, T., Wang, H., & Yang, K. (2016). Nitrate removal by a combined bioelectrochemical and sulfur autotrophic denitrification (CBSAD) system at low temperatures. *Desalination and Water Treatment*, *57*(41), 19411-19417. https://doi.org/10.1080/19443994.2015.1101024

Chung, Y.-C., & Chung, M.-S. (2000). BNP test to evaluate the influence of C/N ratio on N2O production in biological denitrification. *Water science and technology*, *42*(3-4), 23-27. https://doi.org/10.2166/wst.2000.0354

EPA, U. S. E. P. A. (2013). *Introduction to in situ bioremediation of groundwater*

EPA, U. S. E. P. A. (2023). *Overview of Greenouse Gases*. United States Environmental Protection Agency. https://www.epa.gov/ghgemissions/overview-greenhouse-gases

Ergas, S. J., & Reuss, A. F. (2001). Hydrogenotrophic denitrification of drinking water using a hollow fibre membrane bioreactor. *Journal of Water Supply: Research and Technology-Aqua*, *50*(3), 161-171. https://doi.org/10.2166/aqua.2001.0015

He, Y., Li, Y., Li, X., Liu, Y., Wang, Y., Guo, H., Hou, J., Zhu, T., & Liu, Y. (2023). Net-zero greenhouse gas emission from wastewater treatment: Mechanisms, opportunities and perspectives. *Renewable and Sustainable Energy Reviews*, *184*, 113547. https://doi.org/https://doi.org/10.1016/j.rser.2023.113547

Jang, Y., Lee, S.-H., Kim, N.-K., Ahn, C. H., Rittmann, B. E., & Park, H.-D. (2023). Biofilm characteristics for providing resilient denitrification in a hydrogen-based membrane biofilm reactor. *Water Research*, *231*, 119654. https://doi.org/https://doi.org/10.1016/j.watres.2023.119654

Law, Y., Ye, L., Pan, Y., & Yuan, Z. (2012). Nitrous oxide emissions from wastewater treatment processes. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *367*(1593), 1265-1277. https://doi.org/doi:10.1098/rstb.2011.0317

Lee, K. C., & Rittmann, B. E. (2000). A novel hollow-fibre membrane biofilm reactor for autohydrogenotrophic denitrification of drinking water. *Water science and technology*, *41*(4-5), 219-226. https://doi.org/10.2166/wst.2000.0448

Lockhart, K., King, A., & Harter, T. (2013). Identifying sources of groundwater nitrate contamination in a large alluvial groundwater basin with highly diversified intensive agricultural production. *Journal of contaminant hydrology*, *151*, 140-154.

Mansell, B. O., & Schroeder, E. D. (2002). Hydrogenotrophic denitrification in a microporous membrane bioreactor. *Water research (Oxford)*, *36*(19), 4683-4690. https://doi.org/10.1016/S0043-1354(02)00197-5

Martin, K. J., & Nerenberg, R. (2012). The membrane biofilm reactor (MBfR) for water and wastewater treatment: Principles, applications, and recent developments. *Bioresource Technology*, *122*, 83-94. https://doi.org/10.1016/j.biortech.2012.02.110

MfE. (2019). *Pollution from our activities*. Ministery for the environment. https://environment.govt.nz/publications/environment-aotearoa-2019/theme-3-pollution-from-our-activities/

MfE. (2020). *Our freshwater 2020*. Ministry for the Enviroment. https://environment.govt.nz/assets/Publications/Files/our-freshwater-2020.pdf

Park, J. Y., & Yoo, Y. J. (2009). Biological nitrate removal in industrial wastewater treatment: which electron donor we can choose. *Applied Microbiology and Biotechnology*, *82*(3), 415-429. https://doi.org/10.1007/s00253-008-1799-1

Rice, E. W., & Bridgewater, L. (2012). *Standard methods for the examination of water and wastewater* (22nd 2012 / prepared and published jointly by American Public Health Association, American Water Works Association, Water Environment Federation ; joint editorial board, Eugene W. Rice [and others] ; managing editor, Laura Bridgewater. ed.). American Public Health Association.

Richardson, D., Felgate, H., Watmough, N., Thomson, A., & Baggs, E. (2009). Mitigating release of the potent greenhouse gas N2O from the nitrogen cycle – could enzymic regulation hold the key? *Trends in Biotechnology*, *27*(7), 388-397. https://doi.org/https://doi.org/10.1016/j.tibtech.2009.03.009

Rittmann, B. E. (2007). THE MEMBRANE BIOFILM REACTOR IS A VERSATILE PLATFORM FOR WATER AND WASTEWATER TREATMENT. *Environmental Engineering Research*, *12*(4), 157-175. https://doi.org/10.4491/eer.2007.12.4.157

Stats, N. (2021). *Fertilisers – nitrogen and phosphorus*. Stats NZ. https://www.stats.govt.nz/indicators/fertilisers-nitrogen-and-phosphorus#:~:text=Between%201991%20and%202019%2C%20estimates,in%20nitrogen%20applied%20from%20fertiliser.

Tallec, G., Garnier, J., Billen, G., & Gousailles, M. (2006). Nitrous oxide emissions from secondary activated sludge in nitrifying conditions of urban wastewater treatment plants: Effect of oxygenation level. *Water Research*, *40*(15), 2972-2980. https://doi.org/https://doi.org/10.1016/j.watres.2006.05.037

Vidal, S., Rocha, C., & Galvão, H. (2002). A comparison of organic and inorganic carbon controls over biological denitrification in aquaria. *Chemosphere*, *48*(4), 445-451. https://doi.org/https://doi.org/10.1016/S0045-6535(02)00073-5

Wagner, A. O., Markt, R., Mutschlechner, M., Lackner, N., Prem, E. M., Praeg, N., & Illmer, P. (2019). Medium Preparation for the Cultivation of Microorganisms under Strictly Anaerobic/Anoxic Conditions. *J Vis Exp*(150). https://doi.org/10.3791/60155

Wąsik, E., Bohdziewicz, J., & Błaszczyk, M. (2001). Removal of nitrates from ground water by a hybrid process of biological denitrification and microfiltration membrane. *Process Biochemistry*, *37*(1), 57-64. https://doi.org/https://doi.org/10.1016/S0032-9592(01)00177-7

Wu, L., Wei, W., Xu, J., Chen, X., Liu, Y., Peng, L., Wang, D., & Ni, B.-J. (2021). Denitrifying biofilm processes for wastewater treatment: developments and perspectives. *Environmental Science: Water Research & Technology*, *7*(1), 40-67.

Zehr, J. P., & Kudela, R. M. (2011). Nitrogen Cycle of the Open Ocean: From Genes to Ecosystems. *Annual Review of Marine Science*, *3*(1), 197-225. https://doi.org/10.1146/annurev-marine-120709-142819

Zhuang, W. Q., Yi, S., Bill, M., Brisson, V. L., Feng, X., Men, Y., Conrad, M. E., Tang, Y. J., & Alvarez-Cohen, L. (2014). Incomplete Wood-Ljungdahl pathway facilitates one-carbon metabolism in organohalide-respiring Dehalococcoides mccartyi. *Proc Natl Acad Sci U S A*, *111*(17), 6419-6424. https://doi.org/10.1073/pnas.1321542111