THE ROLE OF SULFUR-DRIVEN AUTOTROPHIC DENITRIFICATION IN SUSTAINABLE WASTEWATER TREATMENT

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ABSTRACT

Sulfur-driven autotrophic denitrification (SdAD) utilizes reduced sulfur compounds as electron donors for nitrate reduction. Compared to conventional heterotrophic denitrification, SdAD can treat wastewater with low organic carbon to nitrogen ratios, such as dairy factory cleaning wastewater and post-anoxic municipal wastewater. Using sulfur compounds as electron donors avoids the need for external carbon sources for denitrification and reduces the overall carbon footprint of the denitrification process. In addition, previous studies have reported that SdAD produced less N_2O emissions than the conventional heterotrophic denitrification processes. N_2O is a potent greenhouse gas (GHG) with a global warming potential 295 times greater than carbon dioxide. The low GHG-emissions highlights that SdAD can be a more sustainable and environmentally friendly approach for the wastewater treatment industry.

However, one of the major challenges of SdAD is the generation of a large quantity of protons, which can consume alkalinity, reduce pH, and inhibit denitrification. To address this challenge, this study evaluated insoluble pH buffering materials—$FeCO_3$ and sustainable $CaCO_3$ materials (e.g., mussel shells)—for their capabilities to provide alkalinity in the SdAD process. Batch cultures with elemental sulfur powder as the sole electron donor were set up. The control experiments contained baseline alkalinity using $NaHCO_3$. $FeCO_3$ (1.74 g/L) and ground green-lipped muscle shell (GLS) (2.13 g/L) were added to different batch cultures, respectively. The nitrate concentrations, pH, sulfate, and phosphorus concentrations were monitored during a time-course

experiment. The results showed that New Zealand green-lip mussel shells, among other tested materials, can act as an efficient pH buffer.

This study also investigated the prevalence of sulfur-utilizing denitrifiers in New Zealand Wastewater Treatment Plants (WWTPs). Return activated sludge samples were collected from three WWTPs in Auckland and used as seed sludge for batch experiments. Sodium nitrate and elemental sulfur powder were used as the sole electron acceptor and donor. The results showed that sulfur-utilizing denitrifies were ubiquitous in these WWTPs.

In conclusion, sulfur-driven autotrophic denitrification is a promising technology for sustainable wastewater treatment. This technology has the potential to significantly reduce the carbon footprint of the treatment process and provide a more cost-effective and sustainable alternative to conventional denitrification processes. Further research and development are needed to optimize and scale up the process for full-scale applications.

KEYWORDS

Autotrophic Denitrification, Elemental Sulfur, Sustainable Wastewater Treatment, Net-zero-emissions, Low Carbon Footprint

PRESENTER PROFILE

As a passionate and dedicated environmental engineering Ph.D. student, my research focuses on sulfur-driven autotrophic denitrification in net-zero-emissions, sustainable wastewater treatment.

INTRODUCTION

Wastewater treatment is a critical aspect of environmental engineering aimed at mitigating the impact of human activities on water bodies. Removing nitrogen compounds, such as nitrate, is essential in wastewater treatment to prevent eutrophication and potential harm to aquatic ecosystems. Nitrate and its metabolic by-product, nitrite, can be harmful to human health (Singh et al., 2022). Therefore, the World Health Organization (WHO) suggests the maximum acceptable level for nitrate in public drinking water shall be less than 11.3 mg/L NO_3–N (Edition, 2011; Organization, WHO., & Staff, 2004).

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Biological denitrification can be carried out by both heterotrophic and autotrophic bacteria. Conventional heterotrophic denitrification has been the prevailing method for nitrogen removal, utilizing organic carbon sources as electron donors. Due to organic carbon being oxidized by heterotrophic denitrifying bacteria, carbon dioxide is released from denitrification processes (Mohsenipour, Shahid, & Ebrahimi, 2014; Singh et al., 2022). Notably, in the post-anoxic denitrification processes, external organic carbon sources (e.g., methanol or acetate) may be added to boost denitrification efficiency, resulting in both higher CO_2 emissions and sludge production (Xiao et al., 2014). Furthermore, this process may face challenges when treating wastewater with low C:N ratios, such as wastewater from dairy processing plants or post-anoxic municipal wastewater.

Autotrophic denitrification is a metabolic process in which inorganic compounds such as elemental sulfur (S^0), reduced iron compounds (Fe^0 /Fe^{2+}), or hydrogen gas (H_2) are utilized as electron donors with an inorganic carbon source (Tong, Rodriguez-Gonzalez, Feng, & Ergas, 2017). Advantages of autotrophic denitrification include lower excess sludge production and less carry-over of organic substrate to the effluent than heterotrophic denitrification (Sengupta, Ergas, & Lopez-Luna, 2007). Notably, it can effectively treat wastewater with limited organic carbon content, reducing the need for external carbon sources and, consequently, the overall carbon footprint of the denitrification process. Among the different autotrophic denitrification processes, sulfur-driven autotrophic denitrification (SdAD) has emerged as an attractive alternative for heterotrophic denitrification due to its low cost and ease of use of elemental sulfur (S^0) as a packing medium and biofilm carrier in packed bed bioreactors (Li, Morrison, Collins, Li, & Zhan, 2016). (Di Capua, Papirio, Lens, & Esposito, 2015).

As shown in the SdAD stoichiometry below, the proton generation to nitrate reduction is almost 1:1 ratio (NO_3–N:H^+). Removing 1 mg N–NO^{3-}/L produces 7.83 mg SO_4^{2-} /L and uses 3.36 mg/L of $CaCO_3$ as alkalinity. These protons can consume alkalinity and reduce pH levels, leading to the inhibition of denitrification efficiency. It has been observed in our study and reported previously that SdAD inhabitation occurred when the pH was below 6 (Di Capua, Pirozzi, Lens, & Esposito, 2019). To overcome this challenge, innovative approaches such as the use of low-cost, low-carbon footprint pH buffering materials need to be explored.

Equation (1):

$$S + 0.876\, NO_3^- + 0.343\, H_2O + 0.023\, CO_2 + 0.08\, NH_4^+ + 0.379\, HCO_3^- \\ = 0.08\, C_5H_7O_2N + 0.44\, N_2 + SO_4^{2-} + 0.824\, H^+$$

The specific aim of this report was to assess the performance and efficiency of insoluble chemicals as pH buffers. The New Zealand green-lipped mussel shell emerged as a promising low-cost and sustainable insoluble alkalinity option among some other materials. In addition, this study focused on using insoluble S^0 to determine if denitrifying sulfur-oxidizing denitrifying bacteria existed in WWTPs and assess the impact of operating factors on the bioreactor performance.

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MATERIALS AND METHODS

SYNTHETIC WASTEWATER

Both trace element and mineral salts medium stock solutions were prepared using milli-Q water and chemicals of ACS grade and above (Zhuang et al., 2011). The ingredients of the two stock solutions are shown in **Error! Reference source not found.** and **Error! Reference source not found.**. Synthetic wastewater (1 L) was prepared using 1 mL trace element stock solution, 10 mL mineral salt stock solution, and 989 mL milli-Q water.

Table 1:Mineral salts stock solution (100×)

Chemical formula	Concentration (g/L)
NaCl	100
$MgCl_2 \cdot 6H_2O$	50
KH_2PO_4	20
NH_4Cl	30
KCl	30
$CaCl_2 \cdot 2H_2O$	1.5
Na_2SO_4	5

Table 2:Trace element stock solution (1,000×)

Chemical formula	Concentration (g/L)
HCl (33% solution)	7.5 mL
$FeCl_2 \cdot 4H_2O$	1.50
$CoCl_2 \cdot 6H_2O$	0.19
$MnCl_2 \cdot 4H_2O$	0.10
$ZnCl_2$	0.07
H_3BO_3	0.006
$Na_2MoO_4 \cdot 2H_2O$	0.036
$NiCl_2 \cdot 6H_2O$	0.024
$CuCl_2 \cdot 2H_2O$	0.002

BATCH TESTS

To investigate the existence of sulfur-oxidizing denitrifying bacteria, return activated sludge (RAS) samples were taken from three major WWTPs in Auckland, New Zealand, namely the Mangere (MG) WWTP, the Rosedale (RD) WWTP, and the Army Bay (AB) WWTP. These three plants play a pivotal role, collectively managing over 90% of Auckland's daily wastewater (WatercareLtd, 2015) making them highly suitable candidates for investigating the potential ubiquity of sulfur-oxidizing denitrifying bacteria in New Zealand's WWTPs. The collected activated sludge samples were stored in 20 L carboys in a 4°C refrigerator to preserve microbial activity.

A 200 mL of RAS was directly transferred into a 500 mL reagent bottle containing 200 mL of the culture medium (2× strength). For each WWTP RAS, three different pH buffers were used, soluble $NaHCO_3$ (as a control), mussels shell, and a sulfur composite granular packing media material (S^0-Composite). The total of nine reactors is summarized in Table 3.

Then the nine bottles were transferred onto an orbital shaker incubator set at 30°C and incubated in the dark during the experimental period. The bottles were replenished with S^0 and nitrate when they had been depleted. Additional buffering chemicals were replenished to increase pH if it fell below 6.

Table 1: Detailed information of the batch tests conducted in this study

NO.	WWTP Sludge	pH buffer	Note
RD 1	Rosedale	$NaHCO_3$	control reactor with a soluble alkalinity
RD 2	Rosedale	mussels shell	Sustainable alkalinity
RD 3	Rosedale	sulfur composite granular packing media material	(S^0-Composite)
MG 1	Mangere	$NaHCO_3$	control reactor with a soluble alkalinity
MG 2	Mangere	mussels shell	Sustainable alkalinity
MG 3	Mangere	sulfur composite granular packing media material	(S^0-Composite)
AB 1	Army Bay	$NaHCO_3$	control reactor with a soluble alkalinity
AB 2	Army Bay	mussels shell	Sustainable alkalinity
AB 3	Army Bay	sulfur composite granular packing media material	(S^0-Composite)

ANALYTICAL CHEMISTRY

Prior to taking the daily samples, pH measurements for each bottle were conducted using an Orion 3 stars pH meter to monitor any changes in pH over time.

Then, sample aliquots (1 mL) were taken periodically to analyze nitrate (NO^{3-}), nitrite (NO^{2-}), phosphate (PO_4^{3-}), and sulfate (SO_4^{2-}) concentrations using an Ion Chromatography (IC) Instrument (Thermo Scientific ICS-2100). All samples were filtered using 0.45 μm syringe filters and stored at 4°C if not measured immediately. At selected time intervals, a portion of 200 mL of spend medium was withdrawn from each reactor and was replenished with 200 mL of the fresh culture medium.

The samples were also measured using HACH kits for nitrate (HACH method number 836) and nitrite (HACH method number 840) concentrations as an alternative to the IC methods.

RESULTS AND DISCUSSION

UBIQUITY OF SULFUR-DRIVEN AUTOTROPHIC DENITRIFYING BACTERIA

Table 4 shows that sulfur-driven autotrophic denitrification happened in all three WWTPs' RAS samples without needing acclimatization. The results demonstrated that sulfur-oxidizing denitrifying bacteria are ubiquitous in all three major WWTPs in Auckland. However, the concentrations of sulfur-oxidizing denitrifying bacteria might be different in these WWTPs. Nevertheless, the results indicate that new SdAD treatment units installed in the future can be self-seeded using RAS from the local WWTP in New Zealand.

Table 4: Ubiquity of SdAD bacteria in three WWTPs' RAS samples.

	average NO_3-N removal rates (mg/L·d) within the first 24-hr incubation	pH on Day 1 ($NaHCO_3$)
MG	143	6.30
RD	1,073	6.63
AB	419	6.49

SUSTAINABLE INSOLUBLE BUFFER

Batch culture experiments were conducted to search for effective and sustainable insoluble pH buffer chemicals. RAS samples from the three WWTPs were used to inoculate the bioreactors with different buffering chemicals. The experiment lasted for six days, with measurements taken on Day 1, Day 2, Day 5, and Day 6.

For the RAS from Mangere WWTP, the control experiment using the soluble alkalinity ($NaHCO_3$) was seen a reduction of nitrate nitrogen concentrations decreased from 424 mg/L NO_3–N to 182 mg/L NO_3–N in six days. No nitrite was detected using IC (0.5 mg/L NO_2 detecting limit) during this period. The pH values of the water samples showed a gradual decline trend from 6.30 on Day 1 to 5.58 on Day 6. The results are shown in *Figure 1* below.

In the test using New Zealand Green-lipped mussels shell (GLS) as the pH buffer, the nitrate concentration is reduced from 440 mg/L NO_3–N to 24 mg/L NO_3–N in six days. Again, no nitrite was detected during this period. The pH values of the water samples showed a gradual decline trend from 6.22 on Day 1 to 5.88 on Day 6. Results can be seen in *Figure 2* below.

Based on the stoichiometric calculations *Equation (1)*, the pH would have been dropped to 3.22 ($NaHCO_3$ reactor) and 3.37 (GLS reactor) without these buffering chemicals. Notably, the GLS exhibited a nearly identical pH buffering capacity and performance when compared to the soluble alkalinity $NaHCO_3$. Furthermore, intriguingly, the GLS seemed to induce a slight enhancement in the denitrification process. Notably, both tests demonstrated a decreasing pH trend, indicating the acidification of the systems during the denitrification process. But their buffering capacity could keep the pH above 5.5 even with over 400 mg/L NO_3-N, an extremely high concentration used to get results quickly.

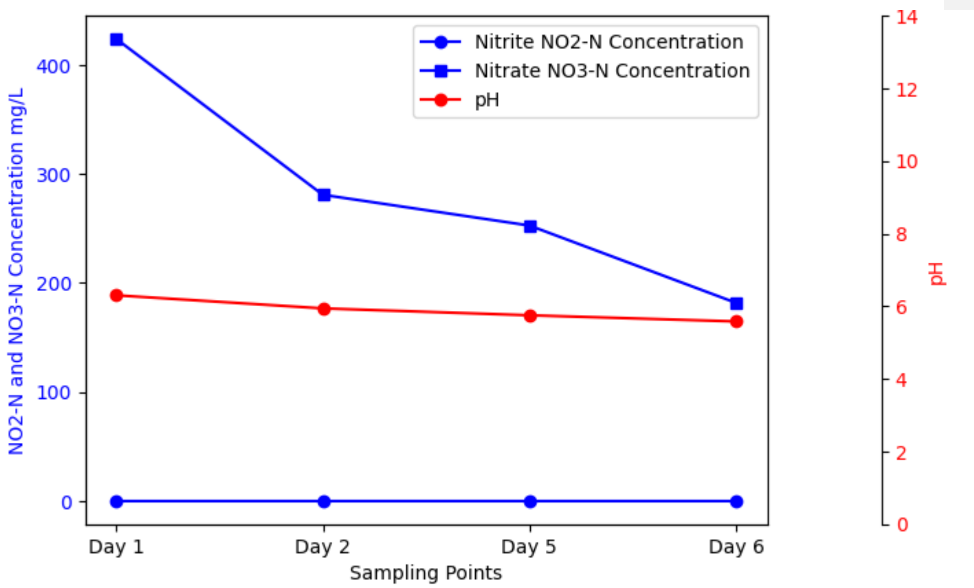

Figure 1: Denitrification test results using Mangere WWTP sludge and $NaHCO_3$ as pH buffer.

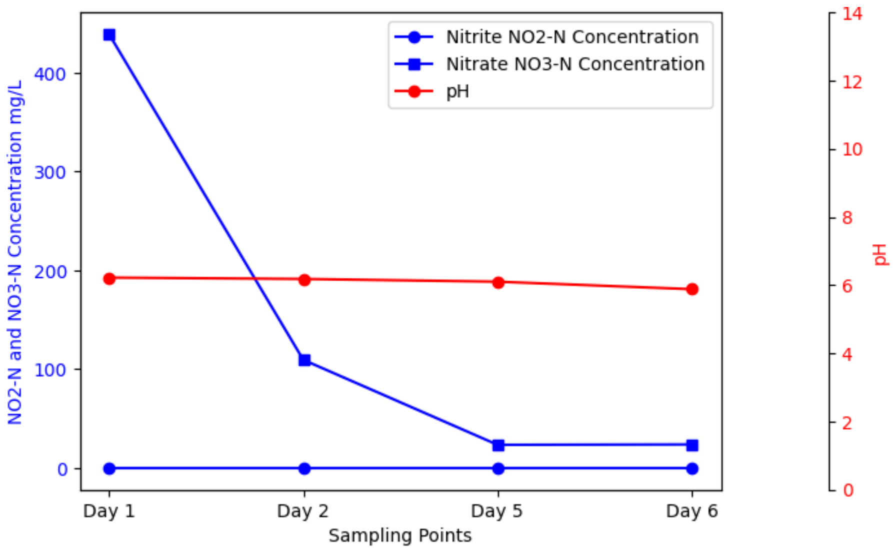

Figure 2: Denitrification test results using Mangere WWTP sludge and GLM as a pH buffer.

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GREEN-LIPPED MUSSEL SHELL AS A SUSTAINABLE BUFFER

Because GLS showed relatively better buffering capacity in the previous experiments, we conducted experiments using sludge samples from three different WWTPs to have a holistic view. The experiments stopped when the nitrate removal rate reached 95% or more. As shown in *Table 5 and Figure 3,* the GLS could buffer a stable pH which supported a consistently good nitrate removal performance across all three WWTPs sludge. These consistent patterns of nitrate reduction across different WWTPs underscore the robustness of sulfur-driven autotrophic denitrification processes when employing the mussel shell as a pH buffer.

Several studies (Liang, Chen, Tong, Liu, & Feng, 2018; Sengupta et al., 2007) have investigated the viability of using oyster shells as an alkalinity source for autotrophic denitrification. Adding crushed oyster shells also improved the microbial attachment as it increased the surface areas (Sengupta et al., 2007). Green-lipped mussels are indigenous to New Zealand. Farming green-lipped mussels is accountable for the annual production of 90,000 tones of shell waste, and approximately half of these shells are disposed of at landfills (O'Sullivan, Jul 26, 2021). Starting to look at using those shells as a buffer substrate in our SdAD processes could be a solution to reduce solid water and denitrification carbon footprint.

Table 5: End-of-cycle pH values in the bioreactors using GLS as an insoluble and sustainable pH buffer.

WWTP	MG	RD	AB
pH	6.22	6.25	6.49

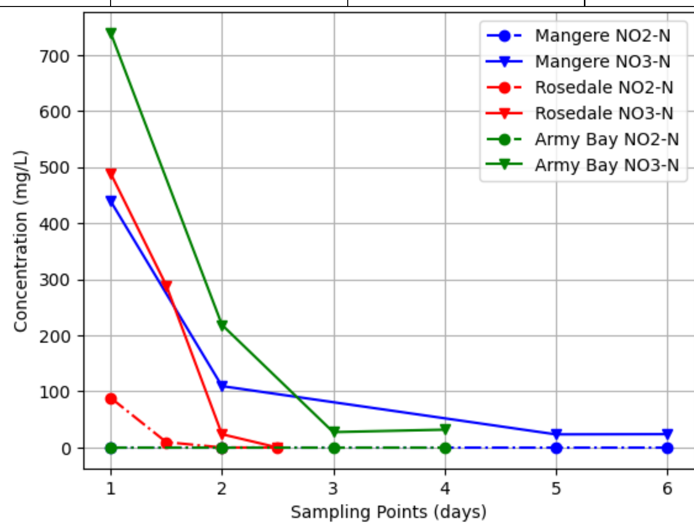

Figure 3: Denitrification performance with different WWTP sludges and GLS as the sustainable pH buffer. Extremely high NO_3–N concentrations were used in these batch tests to obtain results quickly.

SULFUR COMPOSITE GRANULAR PACKING MEDIA

Another study has been done to assess the sulfur composite granular packing media denitrification performance. Sodium bicarbonate and shell were also used to compare with the composite media for supporting the nitrate removal efficiency. The nitrate concentrations were measured periodically and compared for each buffer, as shown in and *Figure 4*.

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Overall, the data indicates that the sulfur composite granular packing media material consistently demonstrated the lowest nitrate concentrations across all sampling points, highlighting its efficient performance in nitrate removal during the denitrification process. The shell buffer showed competitive results with decreasing nitrate concentrations, suggesting its potential as a pH buffering material for denitrification. The $NaHCO_3$ buffer, although effective, exhibited comparatively higher nitrate concentrations at all sampling points,

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implying slightly less optimal nitrate removal efficiency compared to the other buffers.

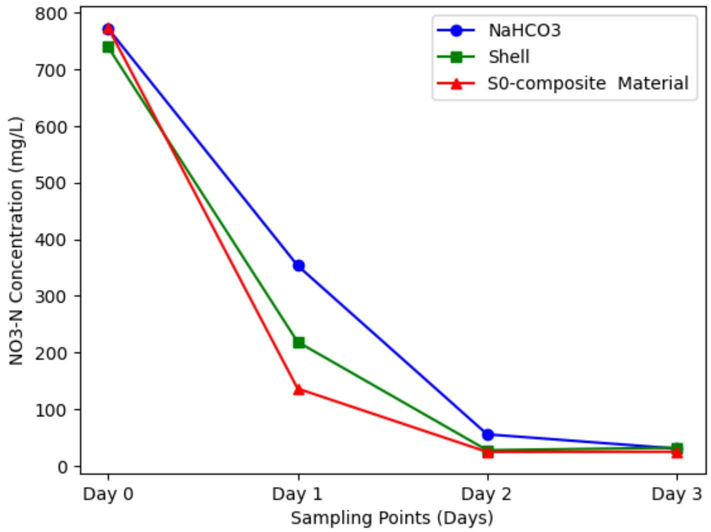

Figure 4: Nitrate concentrations using Army Bay WWTP sludge with buffer of NaHCO3, shell and the sulfur composite granular packing media material.

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CONCLUSIONS

The study investigated the role of sulfur-driven autotrophic denitrification (SdAD) in sustainable wastewater treatment, focusing on the utilization of different pH buffering materials to address the challenges associated with the process. Through a series of batch experiments using sludge from various WWTPs, the effectiveness of different pH buffers, including $NaHCO_3$, New Zealand green-lipped mussel shells, and a sulfur composite granular packing media material, were evaluated. The results underscore the buffering capacity of the GLS, which consistently exhibited its capability and even appeared to modestly enhance denitrification efficiency. This result suggested mussel shells as a sustainable alternative to conventional chemical alkalinities for SdAD systems.

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The study reveals that sulfur-driven autotrophic denitrifiers are widely present in various WWTPs, as evidenced by their successful utilization of S^0 as electron donors in denitrification. This finding has significant implications for the potential implementation of SdAD as an effective and sustainable approach in wastewater treatment processes.

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