water

MDPI

Communication

Study on the Treatment of Simulated Recirculating Mariculture Wastewater by Thiosulfate-Based Autotrophic Denitrification

Fan Gao [1,2], Ting Yu [1], Zhongtai Chen [1,3], Junbo Zhang [1,3], Huchun Xu [1,3], Guangjing Xu [1,3,*] and Cuiya Zhang [4,*]

[1] College of Marine Technology and Environment, Dalian Ocean University, Dalian 116023, China; gaofan@nmemc.org.cn (F.G.); zxcqwe000@foxmail.com (J.Z.)
[2] National Marine Environmental Monitoring Center, Dalian 116023, China
[3] Key Laboratory of Nearshore Marine Environmental Science and Technology in Liaoning Province, Dalian Ocean University, Dalian 116023, China
[4] College of Ocean and Civil Engineering, Dalian Ocean University, Dalian 116023, China
* Correspondence: xuguangjing@dlou.edu.cn (G.X.); zhangcuiya@dlou.edu.cn (C.Z.); Tel.: +86-0411-84763287 (G.X.); +86-0411-84763497 (C.Z.)

Abstract: In this study, a sulfur-based autotrophic denitrifying filter (SADF) was developed for the purpose of removing nitrate from simulated recirculating mariculture wastewater. Results showed that over 90% of the nitrate could be effectively eliminated by utilizing thiosulfate as the electron donor, with a molar ratio of thiosulfate-S to nitrate molar ratio of 2:1 or greater. Additional batch tests confirmed that thiosulfate was a suitable sulfur source for nitrate removal even without prior accumulation of the biomass to nitrite. Excess thiosulfate had a minor impact on N-removal efficiency, so an external sulfur source was not required for nitrate removal, however, it could still help to reduce nitrate accumulation and water replacement to some extent. High-throughput sequencing results illustrated that *Thiomicrospira* and *Thioalkalivibrio* were the dominant autotrophic denitrifying genera in the SADF, while *Thiomicrospira* was more significantly affected in the case of insufficient sulfur sources. As the issue of nitrate accumulation in the mariculture recirculating system has been resolved, only a small amount of water needs to be added to the system daily. Therefore, the thiosulfate-based SADF process has the potential to be implemented for nitrate removal in mariculture systems, which could present a promising sustainable solution to the nitrate pollution issue.

Keywords: denitrifying filter; external sulfur source; S/N molar ratio; thiosulfate

Citation: Gao, F.; Yu, T.; Chen, Z.; Zhang, J.; Xu, H.; Xu, G.; Zhang, C. Study on the Treatment of Simulated Recirculating Mariculture Wastewater by Thiosulfate-Based Autotrophic Denitrification. *Water* **2023**, *15*, 2076. https://doi.org/10.3390/w15112076

Academic Editors: Catherine N. Mulligan, Daniel Mamais and Keke Xiao

Received: 21 April 2023
Revised: 21 May 2023
Accepted: 29 May 2023
Published: 30 May 2023

1. Introduction

In recent years, the mariculture industry has experienced rapid growth, resulting in the discharge of significant amounts of mariculture wastewater into the ocean. These wastewaters often contain residual feed and animal excreta, which introduce potential pollutants such as organic carbon, nitrogen, and phosphorus into the water. These pollutants can cause harm to marine life and degrade water quality, resulting in adverse environmental impacts [1,2]. To address these concerns, sustainable recirculating mariculture systems (RMAS) are being developed to replace traditional aquaculture practices. RMAS employs physical filtering technology to remove most of the solid organic matter, while the remaining pollutants are converted to components such as carbon dioxide, nitrate, and activated phosphorus [3]. Although recirculating mariculture can save up to 90% of the fresh seawater that would otherwise be used in traditional aquaculture, chemical pollutants such as nitrate can be accumulated over time [4]. The discharged nitrate can lead to issues, including blue-green algae and harmful algal blooms, making the removal of these pollutants necessary for compliance with mariculture wastewater discharge [5]. during mariculture and to further minimize the environmental impact of the discharged RMAS wastewater.

Biological denitrification is a widely used approach for removing nitrates from mariculture wastewater [6–9]. Nevertheless, high levels of salt and low availability of electron donors can hinder the efficiency of this process [10]. In the case of recirculating mariculture wastewater with high salt concentrations, the activities of denitrifying bacteria can be directly reduced. To facilitate an optimal environment for denitrifying bacteria to grow and replicate, it is recommended to apply anaerobic denitrifying filters, fixed/moved bed biofilm reactors, or other biofilm-based processes [11]. The low level of electron donors can also limit the activity of denitrifying bacteria, which can be addressed by adding external electron sources, such as organic carbon and reduced sulfur, etc. [12]. However, the organic carbon in seawater may combine with high levels of sulfate to produce toxic sulfides [13], making it necessary to consider non-toxic sulfur for autotrophic denitrification to reduce nitrate levels in mariculture wastewater.

Sulfur-based autotrophic denitrification (SAD) is a process that involves the use of reduced sulfur as an electron donor by sulfur-oxidizing bacteria (SOB) to reduce nitrate to nitrogen gas (as shown in Equation (1)) [14]. The process requires a source of reduced sulfur, which includes elemental sulfur (S^0), sulfide (S^{2-}), or thiosulfate ($S_2O_3^{2-}$). Elemental sulfur has the disadvantage of being insoluble in water and having a slow decomposition rate when exposed to microorganisms, leading to the accumulation of nitrite, which is toxic to the environment [15]. Sulfide is unstable in wastewater and can be easily converted to bio-refractory elemental sulfur or hydrogen sulfide, a gas readily evaporates from water [16,17]. Therefore, nitrite and sulfide are both byproducts that can be produced during the SAD process using sulfur or sulfide as electron donors, and both can be toxic to the environment if they are present in high concentrations [18,19]. Thiosulfate is a sulfur-containing compound that is non-toxic and biodegradable, making it an environmentally friendly option for removing nitrate from wastewater [15,20,21]. Commonly, the optimum molar ratio of thiosulfate to nitrate-N was about 0.89:1 for nitrate reduction to nitrogen gas according to the stoichiometric equation (Equation (2)) [22]. In the ecological floating beds, an S/N molar ratio of 0.7:1–1:1 was also demonstrated to be sufficient for effective nitrate removal without the formation of nitrite [23]. If the S/N ratio is not optimal (i.e., if there is excessive N or S), the process can be hindered.

$$1.1S^0 + NO_3^- + 0.76H_2O + 0.4CO_2 + 0.08NH_4^+ \rightarrow 0.08C_5H_7ON + 1.1SO_4^{2-} + 0.5N_2 + 1.28H^+ \quad (1)$$

$$1.25S_2O_3^{2-} + 1.41NO_3^- + 0.64CO_2 + H_2O\ 0.129C_5H_7O_2N + 0.64N_2 + 2.5SO_4^{2-} + 1.09H^+ \quad (2)$$

The study aims to develop a denitrifying filter to investigate the feasibility of using thiosulfate as an electron donor to remove nitrate from recirculating mariculture wastewater. The effects of S/N molar ratio and hydraulic retention time (HRT) on sulfur-based nitrate removal were also examined. Based on batch tests and DNA sequencing, the pathways and mechanisms of nutrient removal were analyzed. Furthermore, the study discussed the application of the thiosulfate-based SAD process in RMAS, emphasizing its ability to effectively remove nitrate and save seawater. This insight could play a crucial role in the development of sustainable mariculture practices.

2. Materials and Methods

2.1. Experimental System and Operation

Figure 1 displays a denitrifying filter that has a working volume of 3 L. The filter utilizes bio-carriers in the form of ceramic particles that have a diameter of 5 mm, which account for two-thirds of the total volume. To facilitate batch experiments and microbial DNA sequencing, several small sponges were placed on top of the ceramic particles to enhance biomass attachment. The temperature was controlled between 20 and 25 °C. Additionally, the reactor was completely shielded from light to impede any algae growth on the surface of the bio-carriers and the walls.

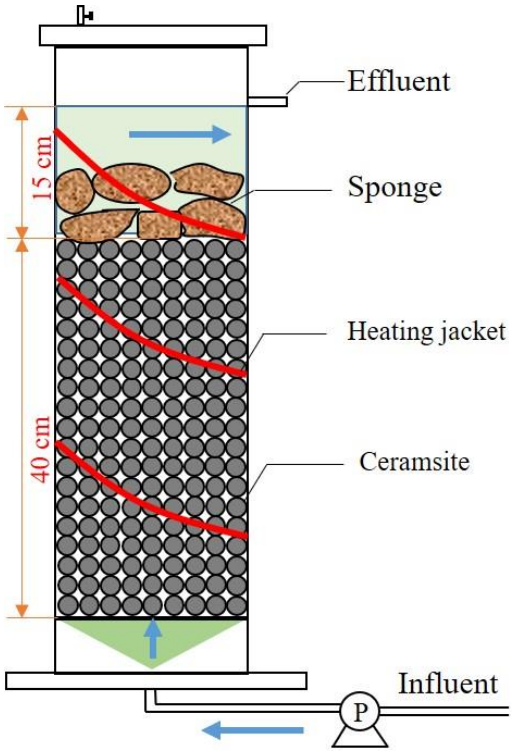

Figure 1. Flowchart of the denitrifying filter.

The whole experiment was conducted in three stages, which involved different $S_2O_3^{2-}$-S/NO_3^--N molar ratios (S/N). During the initial stage, which was conducted from day 1 to day 45 and named Stage I, the HRT was set to 6.7 h, and the S/N molar ratio was 3:1. This ratio provided more sulfur sources than required for SAD. To study the impact of S/N molar ratios on autotrophic denitrification, the molar ratio was lowered to 2:1 in Stage II, which was conducted from day 46 to 80. The molar ratio was further decreased to 1:1 in Stage III, which was conducted from day 81 to 123. Both Stage II and III had a decreased HRT of 4.8 h in order to increase the nitrogen removal load of the system.

The mariculture tail wastewater was prepared using offshore seawater with a salinity of 2.8%. Sodium nitrate and sodium thiosulfate were added as nitrogen and sulfur sources, respectively. The wastewater contained approximately 14 mg/L of NO_3^--N and 0.5 mg/L of PO_4^{3-}-P, while the thiosulfate concentration varied at different stages. Additionally, potassium bicarbonate was used as an inorganic carbon source (final concentration of 0.5 g/L) and pH buffering agent (maintained at 7.8).

The inoculated sludge was obtained from a terrigenous outfall near the coast. This outfall may release a large amount of organic matter, causing anaerobic fermentation of the sediments in the area and leading to the growth of a significant number of anaerobic bacteria, including sulfur-cycle and nitrogen-cycle anaerobic bacteria. Prior to use, the seeding sludge was pre-cultivated in a 2-L container with weekly doses of nitrate and thiosulfate for a period of about four weeks.

2.2. Batch Test of Autotrophic Denitrification at Different S/N Molar Ratios

Batch experiments were carried out to identify the effect of S/N molar ratios on nitrate removal. The nitrate concentration was about 20 mg/L, while the thiosulfate concentrations varied according to S/N molar ratios, i.e., 3:1, 2:1, and 1:1 (Table 1). Activated sludge was taken from the sponge bio-carriers. After three times of cleaning, the activated sludge was evenly divided into three airtight bottles. Nitrogen gas was used for agitation and to provide an anaerobic environment. After 5 min of batch testing, wastewater samples were taken at different time intervals for the analyses of NH_4^+-N, NO_2^--N, and NO_3^--N according to standard methods [24]. Before determination, the wastewater

samples were filtered with 0.22 μm filters. The nitrogen was detected in an ultraviolet/visible light spectrophotometer (MAPADA, Shanghai, China). The nitrate removal efficiency was calculated based on nitrate concentration in the influent and effluent, i.e., (NO_3^--N_{in}–NO_3^--N_{out})/NO_3^--N_{in} × 100%.

Table 1. The main components of batch experimental wastewater.

Stage	S/N Molar Ratio	$S_2O_3^{2-}$-S mg/L	NO_3^--N mg/L	$NaHCO_3$ g/L
I	3:1	144	21	0.5
II	2:1	96	21	0.5
III	1:1	48	21	0.5

2.3. Microbial Analysis

On the 46th day, a high-throughput sequencing technique was employed to analyze the microbial community. This involved collecting sludge samples on the sponge carrier for sequencing. Two primer pairs 341F/534R and 515F/805R were used to amplify the V3-V4 regions of the universal 16S rRNA gene. The 16S rRNA gene is a highly conserved gene in bacteria, and its variable regions can be used to identify bacterial taxa. The high-throughput sequencing was performed on an Illumina MiSeq platform by Sangon Biotech Co., Ltd. (Shanghai, China). High-throughput sequencing allows for the generation of millions of reads, providing a comprehensive view of the microbial community. These reads were then analyzed using bioinformatics tools to identify the taxa present in the sample, as well as their relative abundance.

3. Results and Discussion

3.1. Performance of Thiosulfate-Based Autotrophic Denitrifying Filter

Throughout the entire experiment, the concentration of ammonium and nitrite in the effluent were measured (Figure 2). The effluent pH was always stabilized between 8.1 and 8.2. However, it was found that both of these two inorganic nitrogen compounds were always below the detection limit, indicating that nitrate was the dominant nitrogen in the effluent. Especially, insufficient thiosulfate did not result in an obvious accumulation of nitrite in stage III. The reason might be that the rate constant of nitrate to nitrite (0.02–0.1/$gVSS^{-1}$ min^{-1}) was lower than that of nitrite to nitrogen gas (0.14–0.91/$gVSS^{-1}$ min^{-1}) [22].

Stage I was the initial stage of the experiment, mainly aimed at enriching sulfur-based nitrate denitrifiers. Suspended sludge was intercepted by sponges at the end of the SADF outlet (Figure 1), and then manually collected and added to the bottom of the SADF on a daily basis. This operation effectively promoted the attachment and enrichment of microorganisms on the surface of the bio-carrier. Over the first three weeks of stage I, there was a fluctuation in the effluent nitrate levels, which increased along with the influent nitrogen loading (Figure 1). Specifically, the nitrate concentration increased gradually from 10 to 14 mg/L, while the HRT decreased from 12 to 6.7 h. In fact, as the nitrate removal efficiency surpassed 80%, the influent nitrogen loading could gradually be increased. During days 21–45 with the same HRT of 4.8 h, the average nitrate removal efficiency reached 94.1% ± 2.2%, indicating that the thiosulfate-based autotrophic denitrification process was successfully established within the bio-filter system.

During Stage II, the nitrate concentration was maintained at about 14 mg/L, while the thiosulfate concentration gradually decreased, leading to the molar ratio of thiosulfate-S to nitrate-N decreased from 3:1 to 2:1. Figure 1 shows that the nitrate removal efficiency remained remarkably high at 94.5 ± 5.6%. This demonstrated that the sulfur-based nitrate denitrifiers had adequate sulfur sources to facilitate nitrate reduction, as indicated by the thiosulfate-S to nitrate-N ratio being maintained within the range of 2:1 to 3:1.

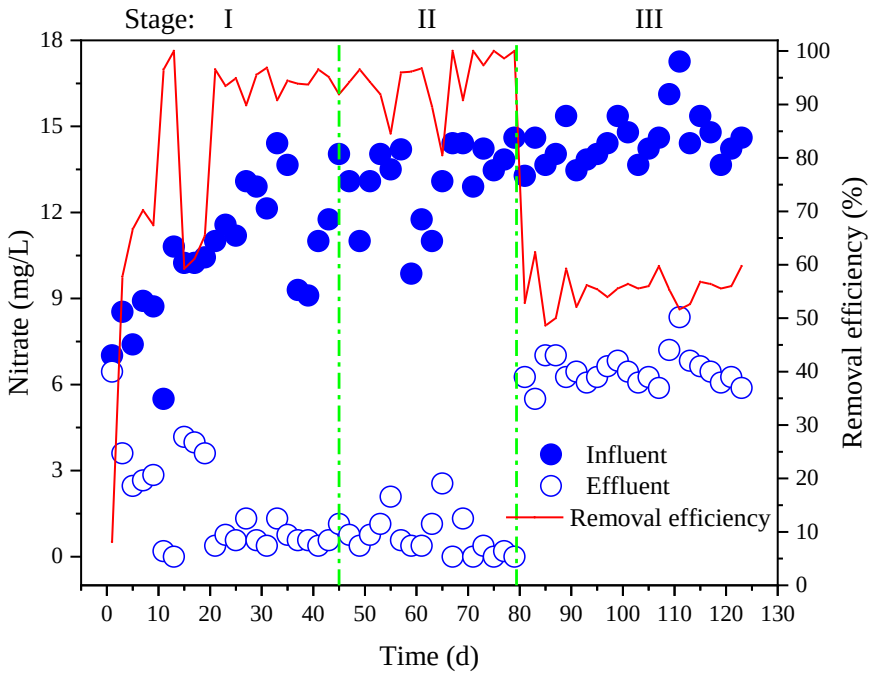

Figure 2. Nitrate removal efficiency in the SADF at different stages.

During Stage III, the molar ratio of thiosulfate-S to nitrate-N ratio was further reduced to 1:1. Consequently, there was a significant decrease in the nitrate removal efficiency. The average nitrate removal efficiency recorded was only 55.4 ± 3.3%, which indicated that such an S/N ratio was inadequate to sustain a thiosulfate-based autotrophic denitrification process. Meanwhile, the concentration of nitrite and ammonia in the effluent were always below the detection limits, but occasionally the nitrite concentration reached 0.02 mg/L. This small amount of nitrite nitrogen could be easily oxidized in the aeration biological filter of the RMAS system.

3.2. Batch Tests of Autotrophic Denitrification at Different S/N Molar Ratios

In order to determine the optimal conditions for the growth of SOB, a batch experiment was conducted to test their activity under different S/N molar ratios. Figure 3 shows that, in the first 4 h, the highest activity, at approximately 3.2 mgN/(L·h), was observed at an S/N molar ratio of 3:1. This was slightly higher than the activity observed at an S/N molar ratio of 2:1, which was approximately 2.9 mgN/(L·h). In the last 2 h, the nitrate removal rate (3.3 mgN/(L·h)) at an S/N molar ratio of 3:1 was still a little higher than that (2.8 mgN/(L·h)) at an S/N molar ratio of 2:1. In another group with a low S/N molar ratio of 1:1, the denitrification rate was only maintained at 2.1–2.5 mgN/(L·h). This result clearly showed that S/N molar ratios had a significant impact on the nitrate removal rate, and it was also anticipated that a low nitrogen removal efficiency would be observed, as seen in the continuous experiment (Figure 2).

Moreover, the nitrate removal rate remained similar as the S/N molar ratios increased from 2:1 to 3:1, which was in line with the results obtained from the continuous experiment. Thus, the optimal molar ratio for nitrate removal from RMAS wastewater was no more than 2:1. A higher sulfur ratio would result in an excessive amount of sulfur in the wastewater, while little nitrate accumulation could still reduce daily fresh seawater replacement.

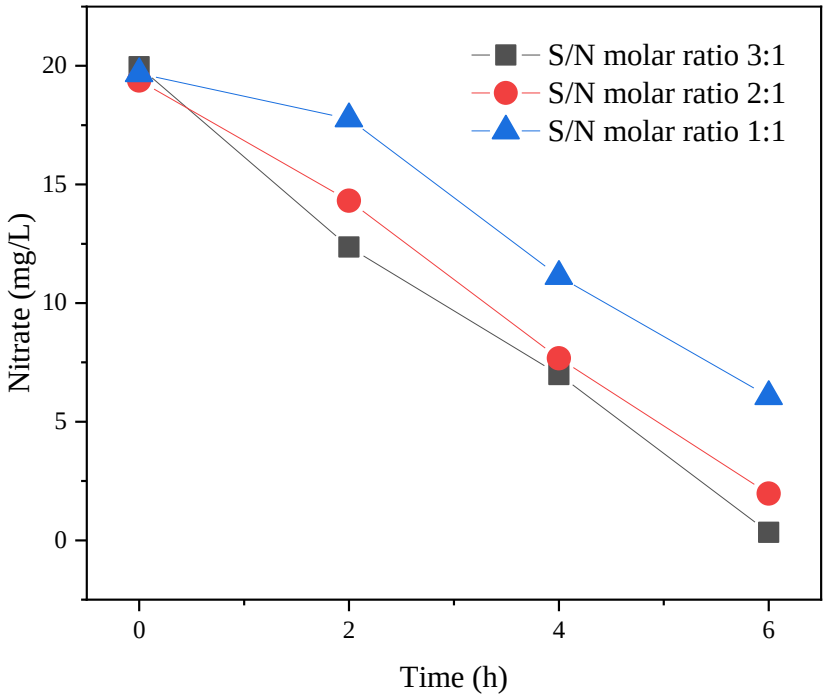

Figure 3. Nitrate concentration profiles at different thiosulfate-S/nitrate-N ratios.

3.3. Microbial Composition in Stages II and III with Different S/N Ratios

The bacterial abundance during the 68th day of stage II and the 99th day of stage III was determined using high-throughput sequencing methods (Figure 4). The results revealed a slight shift in the dominant community with a decreasing signal-to-noise ratio from stage II to stage III. However, a Venn diagram analysis showed that there were 138 common species, while the number of unique species in the 68th sample was much lower than that in the 99th. These findings suggested that the bacterial species became more diverse without sufficient sulfur sources.

In stage II, *Proteobacteria* and *Bacteroidetes* were the dominant phyla, accounting for 87.3% of the entire community. In stage III, the abundance of these two phyla decreased to 77.7%. The abundance of *Deinococcus-Thermus* (also known as *Deinococcota*), a phylum that possessed the function of DNRA (Dissimilatory Nitrate Reduction to Ammonium) that were highly resistant to environmental hazards, increased from 1.6% to 5.6% [25].

At the family level, the study found that *Rhodocyclaceae* and *Gammaproteobacteria* were the most dominant families in both stages II and III. As the S/N ratio decreased, the abundance of *Chromatiales* and *Bradymonadaceae* decreased. Genera and species affiliated with these two families are SOB known to utilize the accumulated sulfides, or thiosulfates using nitrate as an electron acceptor [26]. On the other hand, *Trueperaceae*, *Alphaproteobacteria*, *Desulfobacteraceae*, and Vibrionaceae showed significant increases. The most notable changes were observed in *Trueperaceae* and *Desulfobacteraceae* which experienced a decrease of over 230%. Furthermore, the abundance of *Bradymonadaceae* almost disappeared, declining from 0.93% to 0.06%.

At the genus level, *Thiomicrospira* and *Thioalkalivibrio* were found to be the dominant autotrophic denitrifiers in the SADF. As the S/N molar ratios decreased from stage II to stage III, their abundance also decreased from 2.1% to 1.6% and from 1.8% to 1.7%, respectively. This suggests that *Thiomicrospira* species with a higher growth rate were more affected in case of insufficient sulfur sources [27,28].

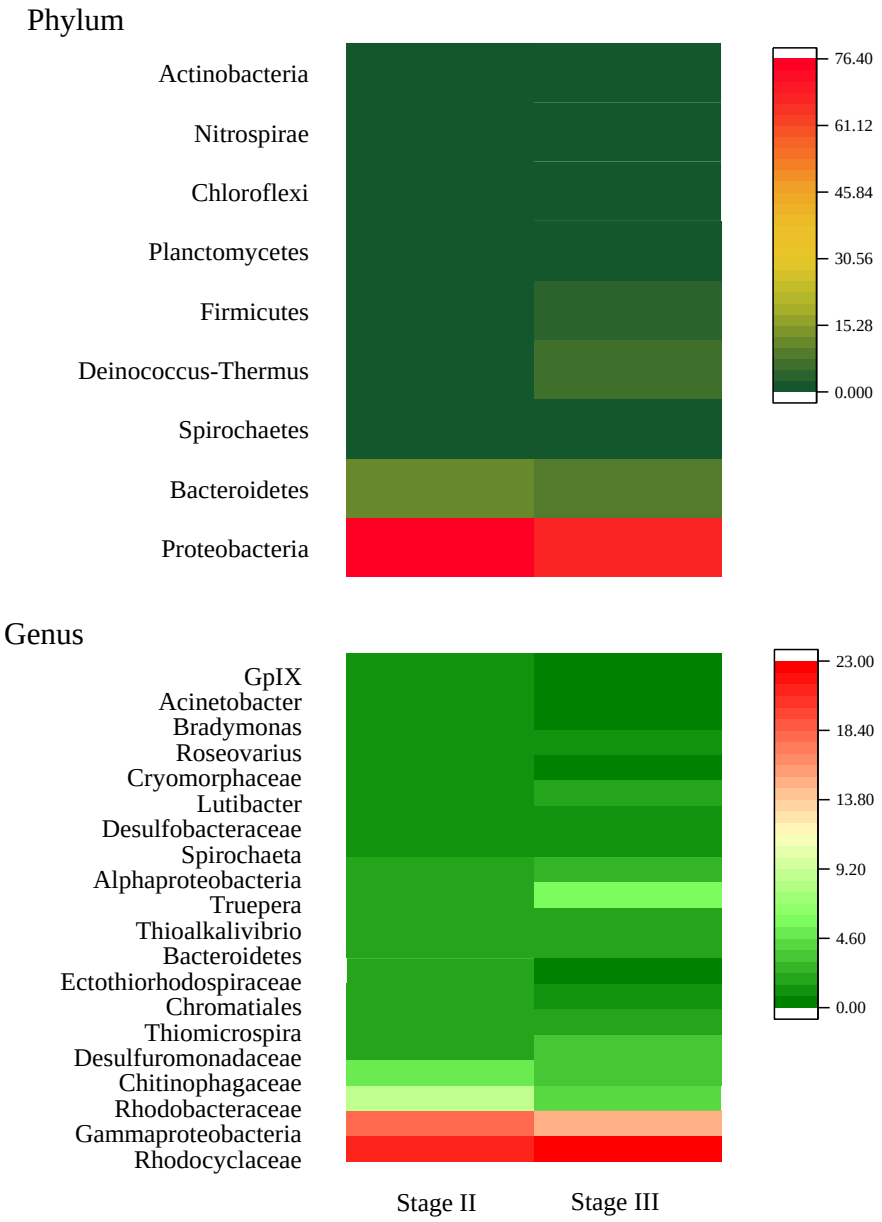

Figure 4. Heatmap of dominant bacterial phylum and genus at stages II and III.

3.4. A Proposed Recirculating Mariculture Model with SAD

Recirculating aquaculture, including mariculture technology currently faces challenges in managing high levels of nitrate produced by mariculture operations. To address this issue, ammonium should be oxidized to nitrate, without potential toxicity to animals, in the aerobic bio-filter system that treats RMAS wastewater, resulting in nitrate accumulation. According to the results of this study and the results of the publications [29,30], thiosulfate-based denitrification is a good option to efficiently remove nitrate and prevent the production of nitrite and hydrogen sulfide (Figure 5). For instance, if the total nitrogen released from residual feed and excreta is about 30 mg/L, the thiosulfate-based denitrifying filter needs to remove about 8 mg/L nitrate while producing approximately 96 mg/L of sulfate as a byproduct [31], which is about 1/28 of that of the seawater. Excessive sulphate accumulation can be reduced by daily adding a small amount of fresh seawater. Alternatively, the sulfate produced by SAD can be continuously removed or absorbed by a calcium-carbonate-based shell [32,33].

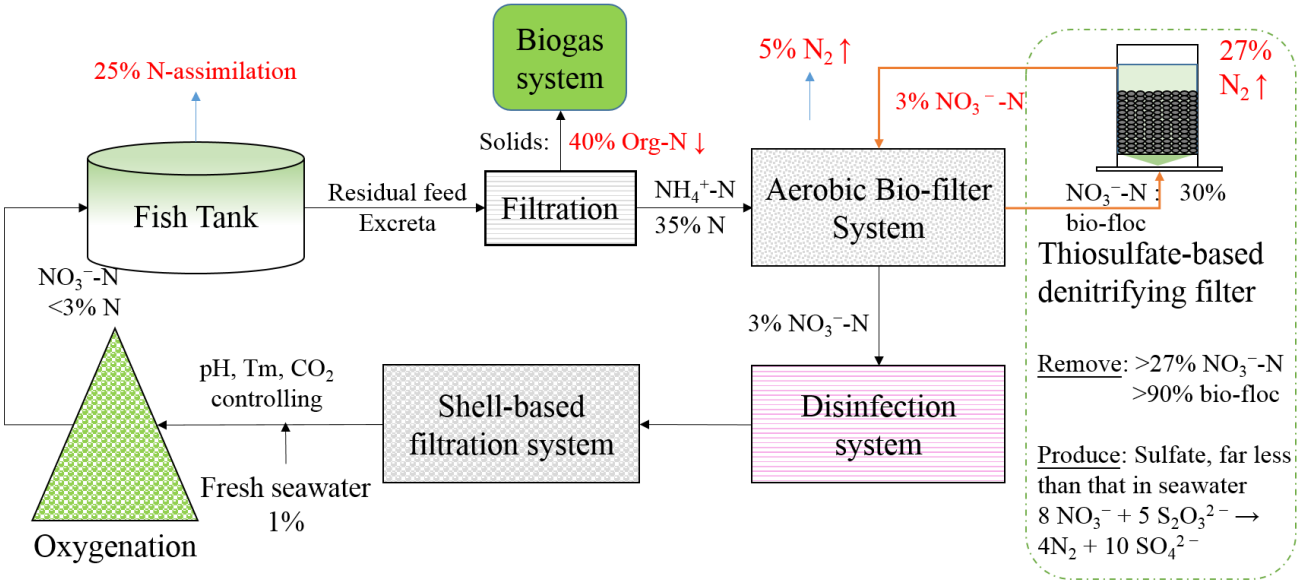

Figure 5. A new RMAS design that incorporates a thiosulfate-based denitrifying filter.

Figure 5 illustrates that the assimilation rate of feed for mariculture animals is typically ranging from 20% to 30% [34], and the remaining feed and fish secretion enter the wastewater. The mariculture wastewater first undergoes physical filtration, removing about 40% of pollutants, and the remaining pollutants are decomposed and transformed into nitrate and phosphorus in the aerobic bio-filter system, where a small amount of nitrogen is converted into nitrogen gas through simultaneous nitrification-denitrification. Then, over 90% of the residual nitrate nitrogen in the water can be removed by a thiosulfate-based autotrophic denitrification filter, which also functions to filter microbial flocs. Thiosulfate-based autotrophic denitrification generates a small amount of sulfate, which needs to be removed by adsorption with alkaline shells and chemical precipitation to prevent excessive accumulation of sulfates. Thus, the problem of nitrate nitrogen accumulation in the system is solved, and the marine aquaculture circulating water system only needs to supplement a small amount of uncontrollable loss of water every day.

4. Conclusions

This study aims to evaluate the efficiency of a thiosulfate-based SADF system for removing nitrate from RMAS wastewater, as well as to demonstrate its practicality. The research findings suggest that by optimizing the S/N molar ratio, the SADF system can effectively lower the levels of nitrogen in wastewater while reducing the frequency of daily seawater replacement. As nitrate is daily removed via SADF from the mariculture recirculating system, only a small amount of freshwater needs to be daily added to the system. These findings have important implications for the RMAS wastewater treatment field and provide alternative methods for mariculture industries to adopt more sustainable and environmentally friendly wastewater management practices.

Author Contributions: Methodology, F.G.; Software, T.Y.; Validation, Z.C.; Formal analysis, J.Z. and H.X.; Writing—original draft, G.X.; Writing—review & editing, C.Z.; Supervision, G.X.; Funding acquisition, F.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Major Science and Technology Projects of Liaoning Province (No. 2020JH1/10200002) and Scientific Research Project of Department of Education of Liaoning province (LJKMZ20221122).

Data Availability Statement: Data sharing is not applicable to this article.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Chen, W.; Gao, S. Current status of industrialized aquaculture in China: A review. *Environ. Sci. Pollut. Res.* **2023**, *30*, 32278–32287. [CrossRef] [PubMed]
2. Zhou, X.; Fang, J.; Shi, S.; Yang, L.; Xie, B.; Huang, L. Ecosystem Services of Ecosystem Approach to Mariculture: Providing an Unprecedented Opportunity for the Reform of China's Sustainable Aquaculture. *Front. Mar. Sci.* **2022**, *9*, 909231. [CrossRef]
3. Félix-Cuencas, L.; García-Trejo, J.F.; López-Tejeida, S.; de León-Ramírez, J.J.; Gutiérrez-Antonio, C.; Feregrino-Pérez, A.A. Nitrogen and phosphorus flux in wastewater from three productive stages in a hyperintensive tilapia culture. *Water Reuse* **2021**, *11*, 520–530. [CrossRef]
4. Feng, Y.; Wang, L.; Yin, Z.; Cui, Z.; Qu, K.; Wang, D.; Wang, Z.; Zhu, S.; Cui, H. Comparative investigation on heterotrophic denitrification driven by different biodegradable polymers for nitrate removal in mariculture wastewater: Organic carbon release, denitrification performance, and microbial community. *Front. Microbiol.* **2023**, *14*, 1141362. [CrossRef]
5. Zheng, L.; Liu, Q.; Liu, J.; Xiao, J.; Xu, G. Pollution Control of Industrial Mariculture Wastewater: A Mini-Review. *Water* **2022**, *14*, 1390. [CrossRef]
6. Gao, Y.; Guo, L.; Shao, M.; Hu, F.; Wang, G.; Zhao, Y.; Gao, M.; Jin, C.; She, Z. Heterotrophic denitrification strategy for marine recirculating aquaculture wastewater treatment using mariculture solid wastes fermentation liquid as carbon source: Optimization of COD/NO3−-N ratio and hydraulic retention time. *Bioresour. Technol.* **2020**, *304*, 122982. [CrossRef] [PubMed]
7. Zhang, T.; Wang, B.; Li, X.; Zhang, Q.; Wu, L.; He, Y.; Peng, Y. Achieving partial nitrification in a continuous post-denitrification reactor treating low C/N sewage. *Chem. Eng. J.* **2018**, *335*, 330–337. [CrossRef]
8. Wang, X.; Zhao, J.; Yu, D.; Du, S.; Yuan, M.; Zhen, J. Evaluating the potential for sustaining mainstream anammox by endogenous partial denitrification and phosphorus removal for energy-efficient wastewater treatment. *Bioresour. Technol.* **2019**, *284*, 302–314. [CrossRef]
9. Baeten, J.E.; Batstone, D.J.; Schraa, O.J.; van Loosdrecht, M.C.M.; Volcke, E.I.P. Modelling anaerobic, aerobic and partial nitritation-anammox granular sludge reactors-A review. *Water Res.* **2019**, *149*, 322–341. [CrossRef]
10. Zhao, Y.; Zhuang, X.; Ahmad, S.; Sung, S.; Ni, S.-Q. Biotreatment of high-salinity wastewater: Current methods and future directions. *World J. Microbiol. Biotechnol.* **2020**, *36*, 37. [CrossRef]
11. Wang, Z.; Gao, M.; Wei, J.; Ma, K.; Pei, J.; Zhang, J.; Zhou, Y.; Yang, Y.; Yu, S. Long-term effects of salinity on extracellular polymeric substances, microbial activity and microbial community from biofilm and suspended sludge in an anoxic-aerobic sequencing batch biofilm reactor. *J. Taiwan Inst. Chem. Eng.* **2016**, *68*, 275–280. [CrossRef]
12. Pang, Y.; Wang, J. Various electron donors for biological nitrate removal: A review. *Sci. Total. Environ.* **2021**, *794*, 148699. [CrossRef]
13. Fan, K.; Wang, W.; Xu, X.; Yuan, Y.; Ren, N.; Lee, D.-J.; Chen, C. Recent Advances in Biotechnologies for the Treatment of Environmental Pollutants Based on Reactive Sulfur Species. *Antioxidants* **2023**, *12*, 767. [CrossRef] [PubMed]
14. Chang, M.; Fan, F.; Zhang, K.; Wu, Z.; Zhu, T.; Wang, Y. Denitrification performance and mechanism of a novel sulfur-based fiber carrier fixed-bed reactor: Co-existence of sulfur-based autotrophic denitrification and endogenous denitrification. *J. Water Process Eng.* **2023**, *53*, 103618. [CrossRef]
15. Bao, H.-X.; Li, Z.-R.; Song, Z.-B.; Wang, A.-J.; Zhang, X.-N.; Qian, Z.-M.; Sun, Y.-L.; Cheng, H.-Y. Mitigating nitrite accumulation during S0-based autotrophic denitrification: Balancing nitrate-nitrite reduction rate with thiosulfate as external electron donor. *Environ. Res.* **2022**, *204*, 112016. [CrossRef]
16. Zwain, H.M.; Nile, B.K.; Faris, A.M.; Vakili, M.; Dahlan, I. Modelling of hydrogen sulfide fate and emissions in extended aeration sewage treatment plant using TOXCHEM simulations. *Sci. Rep.* **2020**, *10*, 22209. [CrossRef]
17. Feilberg, A.; Hansen, M.J.; Liu, D.; Nyord, T. Contribution of livestock H2S to total sulfur emissions in a region with intensive animal production. *Nat. Commun.* **2017**, *8*, 1069. [CrossRef]
18. Kroupová, H.K.; Valentová, O.; Svobodová, Z.; Šauer, P.; Máchová, J. Toxic effects of nitrite on freshwater organisms: A review. *Rev. Aquac.* **2018**, *10*, 525–542. [CrossRef]
19. Kuhn, D.D.; Drahos, D.D.; Marsh, L.; Flick, G.J., Jr. Evaluation of nitrifying bacteria product to improve nitrification efficacy in recir- culating aquaculture systems. *Aquac. Eng.* **2010**, *43*, 78–82. [CrossRef]
20. Deng, Y.-F.; Ekama, G.A.; Cui, Y.-X.; Tang, C.-J.; van Loosdrecht, M.C.M.; Chen, G.-H.; Wu, D. Coupling of sulfur(thiosulfate)-driven denitratation and anammox process to treat nitrate and ammonium contained wastewater. *Water Res.* **2019**, *163*, 114854. [CrossRef]
21. Fan, C.; Zhou, W.; He, S.; Huang, J. Sulfur transformation in sulfur autotrophic denitrification using thiosulfate as electron donor. *Environ. Pollut.* **2021**, *268*, 115708. [CrossRef]
22. Matsui, S.; Yamamoto, R. A New Method of Sulphur Denitrification for Sewage Treatment by a Fluidized Bed Reactor. *Water Sci. Technol.* **1986**, *18*, 355–362. [CrossRef]
23. Sun, S.; Liu, J.; Zhang, M.; He, S. Thiosulfate-driven autotrophic and mixotrophic denitrification processes for secondary effluent treatment: Reducing sulfate production and nitrous oxide emission. *Bioresour. Technol.* **2020**, *300*, 122651. [CrossRef] [PubMed]

24. Eaton, A.D.; Franson, M.A.H. *Standard Methods for the Examination of Water and WasteWater*; American Public Health Association: Washington, DC, USA, 2005.
25. Li, Q.; Bu, C.; Ahmad, H.A.; Guimbaud, C.; Gao, B.; Qiao, Z.; Ding, S.; Ni, S.-Q. The distribution of dissimilatory nitrate reduction to ammonium bacteria in multistage constructed wetland of Jining, Shandong, China. *Environ. Sci. Pollut. Res.* **2021**, *28*, 4749–4761. [CrossRef]
26. Mara, P.; Edgcomb, V.P.; Sehein, T.R.; Beaudoin, D.; Martinsen, C.; Lovely, C.; Belcher, B.; Cox, R.; Curran, M.; Farnan, C.; et al. Comparison of Oyster Aquaculture Methods and Their Potential to Enhance Microbial Nitrogen Removal from Coastal Ecosystems. *Front. Mar. Sci.* **2021**, *8*, 633314. [CrossRef]
27. Sorokin, D.Y.; Gorlenko, V.M.; Tourova, T.P.; Tsapin, A.I.; Nealson, K.H.; Kuenen, G.J. Thioalkalimicrobium cyclicum sp. nov. and Thioalkalivibrio jannaschii sp. nov., novel species of haloalkaliphilic, obligately chemolithoautotrophic sulfur-oxidizing bacteria from hypersaline alkaline Mono Lake (California). *Int. J. Syst. Evol. Microbiol.* **2002**, *52 Pt 3*, 913–920. [CrossRef] [PubMed]
28. Wang, H.-C.; Liu, Y.; Yang, Y.-M.; Fang, Y.-K.; Luo, S.; Cheng, H.-Y.; Wang, A.-J. Element sulfur-based autotrophic denitrification constructed wetland as an efficient approach for nitrogen removal from low C/N wastewater. *Water Res.* **2022**, *226*, 119258. [CrossRef] [PubMed]
29. He, Q.; Cheng, Z.; Zhang, D.; Main, K.; Feng, C.; Ergas, S.J. A sulfur-based cyclic denitrification filter for marine recirculating aquaculture systems. *Bioresour. Technol.* **2020**, *310*, 123465. [CrossRef]
30. Yogev, U.; Sowers, K.R.; Mozes, N.; Gross, A. Nitrogen and carbon balance in a novel near-zero water exchange saline recirculating aquaculture system. *Aquaculture* **2017**, *467*, 118–126. [CrossRef]
31. Chung, J.; Amin, K.; Kim, S.; Yoon, S.; Kwon, K.; Bae, W. Autotrophic denitrification of nitrate and nitrite using thiosulfate as an electron donor. *Water Res.* **2014**, *58*, 169–178. [CrossRef]
32. de Luna, M.D.G.; Rance, D.P.M.; Bellotindos, L.M.; Lu, M.-C. Removal of sulfate by fluidized bed crystallization process. *J. Environ. Chem. Eng.* **2017**, *5*, 2431–2439. [CrossRef]
33. Silva, A.M.; Lima, R.M.F.; Leão, V.A. Mine water treatment with limestone for sulfate removal. *J. Hazard. Mater.* **2012**, *221–222*, 45–55. [CrossRef] [PubMed]
34. Duarte, C.M.; Holmer, M.; Olsen, Y.; Soto, D.; Marbà, N.; Guiu, J.; Black, K.; Karakassis, I. Will the Oceans Help Feed Humanity? *Bioscience* **2009**, *59*, 967–976. [CrossRef]