International Journal of Geography, Geology and Environment

P-ISSN: 2706-7483
E-ISSN: 2706-7491
IJGGE 2024; 6(2): 08-12
www.geojournal.net
Received: 10-06-2024
Accepted: 12-07-2024

Pham Thi Thom
Faculty of Natural resources and Environment, Bac Giang Agriculture and Forestry University, Viet Yen town, Bac Giang province, Vietnam

Nguyen Van Bai
Faculty of Natural resources and Environment, Bac Giang Agriculture and Forestry University, Viet Yen town, Bac Giang province, Vietnam

Corresponding Author:
Pham Thi Thom
Faculty of Natural resources and Environment, Bac Giang Agriculture and Forestry University, Viet Yen town, Bac Giang province, Vietnam

Development and evaluation of the effectiveness of microbial formulations for nitrite removal in aquaculture systems

Pham Thi Thom and Nguyen Van Bai

DOI: https://doi.org/10.22271/27067483.2024.v6.i2a.282

Abstract
Nitrite pollution poses a serious threat to aquaculture systems, affecting the health and growth of aquatic organisms. This study focuses on the development and evaluation of microbial formulations designed to enhance nitrite removal in aquaculture environments. Various microbial formulations, utilizing selected nitrite-reducing bacterial strains, were created and tested under both controlled laboratory conditions and actual aquaculture systems. The effectiveness of each formulation was assessed based on its ability to reduce nitrite levels, alongside monitoring other water quality parameters such as pH, temperature, and dissolved oxygen. The results indicated that the formulations achieved up to 90% reduction in nitrite concentrations, with minimal impact on overall water quality. These findings highlight the potential of these microbial formulations for improving water quality management in aquaculture systems and promoting sustainable fish farming practices. Future work will focus on optimizing formulation stability and evaluating long-term effects on aquatic ecosystems.

Keywords: Nitrite removal, microbial formulations, aquaculture systems, water quality, sustainable fish farming

Introduction

Aquaculture is a rapidly growing sector that plays a crucial role in global food security and economic development. However, maintaining optimal water quality in aquaculture systems is a persistent challenge. One of the most critical issues is the accumulation of nitrite (NO_2^-), which can be highly toxic to aquatic organisms, affecting their growth, health, and survival. Nitrite contamination commonly arises from the decomposition of organic matter and the metabolic activities of fish and other aquatic species. When nitrite levels exceed acceptable thresholds, they can disrupt the physiological functions of fish, leading to reduced growth rates, increased susceptibility to diseases, and even mortality.

Traditional methods for managing nitrite levels in aquaculture systems often involve costly and complex chemical treatments or frequent water changes, which may not be sustainable in the long term. Microbial bioremediation, using specialized microorganisms to remove or transform pollutants, presents a promising alternative. Microbial formulations designed for nitrite removal can offer a more sustainable and cost-effective solution by enhancing the natural nitrite-reducing processes within the system.

Recent advancements in microbial technology have led to the development of various bacterial strains with high nitrite-reducing capabilities. These strains can be formulated into effective products for use in aquaculture systems. Despite their potential, there remains a need for comprehensive studies to evaluate the effectiveness of these microbial formulations under practical conditions and to optimize their application for various aquaculture scenarios.

This study aims to address this need by developing and evaluating microbial formulations specifically designed for nitrite removal in aquaculture systems. We focus on selecting and optimizing nitrite-reducing bacterial strains, assessing their performance in controlled environments, and verifying their effectiveness in real-world aquaculture settings. By providing insights into the performance and applicability of these formulations, this research seeks to contribute to more effective and sustainable water quality management practices in aquaculture.

Content and methods of research

Content

- Isolate and select microorganisms capable of treating nitrite in aquaculture water.
- Produce microbial products for treating nitrite in aquaculture water.
- Test and evaluate the effectiveness of microbial products in reducing nitrite levels in aquaculture water.

Methods

Water sampling method: Water samples were collected from aquaculture ponds according to TCVN 6663-4:2020. Three samples were taken from three locations in each pond (including the beginning, middle, and end). At each location, 500 ml of water was collected into a sampling bottle. The samples were either tested immediately or stored at low temperatures (3-5 °C) for no more than one week.

Isolation method (Koch's method): Dilute the sample to a concentration of 10^{-6}. Use a sterile 1 ml pipette for each concentration. Draw 0.02 ml and drop it onto a medium specifically designed for nitrite oxidation and incubate at 28 °C. The isolated microbial strains were then cultured using the streak plate method on a semi-solid medium. Afterward, the microbial strains were cultured in slant tubes for strain preservation.

Observation method: Describe the morphological characteristics of the colonies after 48 hours of incubation at 30 °C on the isolation medium. Observe cell morphology using Gram staining and a microscope.

Evaluate the nitrite-reducing activity of bacterial strains using the 4500-NO_2^-B spectrophotometric method: The strains were cultured on a specific medium to a density of 10^7 CFU/ml. Then, conduct nitrite treatment experiments by adding 1 ml of bacterial solution (10^7 CFU/ml) to a Falcon tube containing 50 ml of the nitrite-quantifying medium with an NO_2^- concentration of 5 mg/l. The bacterial strains were incubated overnight at 30 °C on a shaker at 150 rpm. After 5 days, the samples were analyzed for nitrite content using the 4500-NO_2^-B spectrophotometric method.

Laboratory-scale experiment

- **Formula 1 (T_1):** Use Yucca Zeo product at a ratio of 0.5%.
- **Formula 2 (T_2):** Use experimental microbial product at a ratio of 0.5%.
- **Formula 3 (T_3-Control):** No additional treatment.

Prepare aquaculture water, settle it for 1 hour, and then analyze parameters such as NO_2^-, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Dissolved oxygen (DO), and temperature. Afterward, distribute the wastewater into 9 bottles, each containing 2 liters of wastewater. Add the products according to the formulas mentioned above and monitor for 5 days. After 5 days, analyze parameters such as NO_2^-, pH, BOD, COD, DO, and temperature.

Research Results and Discussion

From the aquaculture pond water samples, nine microbial strains were isolated on the nitrite oxidation medium. Strains 94, 85, 65, and 79 were fast-growing, appearing within approximately 24 hours. A few strains, including 86, 83, and 37, appeared later, within approximately 48 hours. Following these, strain 14 appeared after approximately 72 hours, and the slowest was strain 18, which appeared after 96 hours.

Table 1: Results of microbial strain isolation

Strain ID	Colony Size (mm)	Colony Morphology	Cell Shape
14	0.8	Circular, white	Short rod
18	0.6	Circular, raised surface, opaque yellow	Short rod
37	1.0	Circular, smooth surface, white	Short rod
86	1.0	Circular, glossy surface, white	Short rod
85	1.1	Circular, glossy surface, off-white	Short rod
94	1.3	Circular, smooth surface, opaque white	Short rod
65	1.1	Circular, glossy surface, brownish-yellow	Short rod
83	1.0	Circular, sunken surface, off-white	Short rod
79	1.2	Circular, glossy surface, off-white	Short rod

Colony Size refers to the diameter of the bacterial colonies.
Colony Morphology describes the general appearance and color of the colonies.
Cell Shape indicates the morphological shape of the bacterial cells

Table 2: Nitrite removal activity results

Strain ID	Initial Nitrite Concentration (mg/l)	Nitrite Concentration after 5 days (mg/l)	% Nitrite Removal
14	5	1.39	72.3
18	5	1.35	73.0
37	5	1.20	76.1
86	5	1.05	79.1
85	5	1.04	79.2
94	5	0.95	81.1
65	5	0.98	80.4
83	5	1.07	78.6
79	5	1.04	79.2

Initial Nitrite Concentration represents the starting amount of nitrite in the samples
Nitrite Concentration After 5 Days indicates the remaining amount of nitrite after a 5-day period.
% Nitrite Removal shows the percentage of nitrite that has been removed or reduced by the microbial strains

The results show that after 5 days of incubation, all strains exhibited a round shape, with cell morphology as short rods, and colony sizes ranging from 0.6 mm to 1.3 mm. Most colonies were white, with some strains like 18 and 65, showing a yellow color. Strain 18 had a convex surface, strain 83 had a concave surface, and the rest had smooth, shiny surfaces.

After isolating the microbial strains, we assessed the nitrite-reducing activity of the 9 strains. Evaluating nitrite-reducing activity is a crucial factor in selecting bacteria capable of treating NO_2^- in aquaculture ponds.

The initial nitrite concentration in all samples was 5 mg/l. After 5 days of conversion, the nitrite concentration decreased to between 0.95 mg/l and 1.39 mg/l. The percentage of nitrite conversion ranged from 72.3% to 81.1%, indicating a significant reduction in nitrite levels in the samples. Sample 6 achieved the highest efficiency with 81.1% nitrite conversion, while Sample 1 had the lowest efficiency with 72.3%. Although there were minor differences between the samples, all demonstrated substantial nitrite-reducing activity, with over 70% nitrite conversion. These results suggest that the nitrite conversion process in the samples was effective, providing a basis for evaluating the capability and efficiency of the methods or catalysts used in the study.

pH is closely related to microorganisms, with each type of microorganism having an optimal pH range for growth and development. Acidophilic microorganisms thrive best at a pH of 0-5.5, neutrophilic microorganisms at a pH of 5.5-8.0, and alkaliphilic microorganisms at a pH of 8.5-11.5. Extreme alkaliphiles have optimal growth at a pH of 10 or higher. Therefore, determining the appropriate pH range is crucial. The results of the pH adaptation ability of strain 94 are clearly presented in Table 3.

Table 3: pH Adaptability of microbial strain 94

Strain ID	Bacterial Density (CFU/ml)								
	pH=5	**pH=5.5**	**pH=6**	**pH=6.5**	**pH=7**	**pH=7.5**	**pH=8**	**pH=8.5**	**pH=9**
94	1.3×10^5	3×10^6	2×10^7	4×10^8	1.2×10^9	3×10^9	3×10^8	4×10^6	1×10^6

The bacterial density increased gradually from 1.3×10^5 CFU/ml at pH 5 to 1.2×10^9 CFU/ml at pH 7, indicating optimal growth in a neutral pH environment. At pH 7.5, the bacterial density remained high at 3×10^9 CFU/ml. However, as the pH increased to 8, the bacterial density decreased to 3×10^8 CFU/ml and continued to drop sharply to 1×10^6 CFU/ml at pH 9. These results indicate that strain 94 adapts best within the neutral pH range, particularly at pH 7 and pH 7.5, and is less adaptive in environments with either very low or very high pH.

Table 4: Thermal tolerance of microbial strain 94

Strain ID	Bacterial Density (CFU/ml)								
	15 °C	**20 °C**	**25 °C**	**30 °C**	**35 °C**	**40 °C**	**45 °C**	**50 °C**	**55 °C**
94	4.1×10^6	4.1×10^6	4.2×10^7	2×10^9	6×10^8	5.2×10^7	6.1×10^6	1.2×10^6	4×10^4

The results show that the bacterial density remained stable at 4.1×10^6 CFU/ml at 15 °C and 20 °C. As the temperature increased to 25 °C, the bacterial density significantly rose to 4.2×10^7 CFU/ml, peaking at 30 °C with 2×10^9 CFU/ml. However, as the temperature continued to rise, the bacterial density gradually decreased, dropping to 6×10^8 CFU/ml at 35 °C, 5.2×10^7 CFU/ml at 40 °C, and sharply decreasing to 6.1×10^6 CFU/ml at 45 °C. At 50 °C, the bacterial density further decreased to 1.2×10^6 CFU/ml, and at 55 °C, the bacterial density dropped sharply to just 4×10^4 CFU/ml. These results indicate that strain 94 grows best at 30 °C and is less adaptive to higher temperatures, especially above 35 °C.

Table 5: Biological characteristics of microbial strain 94

Strain ID	Temperature (°C)	pH Range	Nitrite Removal (%)	Colony Morphology	Cell Shape	Colony Size (mm)	Density (CFU/ml)
94	15-55	5-9	81.1	Round, smooth surface, opaque white	Short rod	1.3	6×10^9

Strain 94 demonstrates the ability to operate within a wide temperature range from 15 °C to 55 °C and a pH range from 5 to 9, showing high adaptability to different environmental conditions. The NO_2^- reduction capability of strain 94 reached 81.1%, indicating high efficiency in nitrite conversion. The colonies of strain 94 are round, smooth-surfaced, and opaque white, with a size of 1.3 mm. The bacterial cells are short rods. The bacterial density reached 6×10^9 CFU/ml, indicating robust growth and development. These characteristics suggest that strain 94 is a potential candidate for biological applications related to NO_2^- reduction and operation under various environmental conditions.

Table 6: Quality of microbial formulation

Parameter	Formulation 94	QCVN 02-32-1:2019
Beneficial Microorganisms (CFU/ml)	6×10^9	$\geq 1 \times 10^6$
Contaminant Microorganisms (CFU/ml)	7×10^4	Not specified

Table 6 shows that the microbial preparation 94 has high quality, with a density of beneficial microorganisms reaching 6×10^9 CFU/ml, far exceeding the minimum requirement of the Vietnamese Standard QCVN 02-32-1:2019. The density of non-beneficial microorganisms in the preparation is 7×10^4 CFU/ml. Although not specifically regulated in the QCVN, this needs to be considered to ensure the safety and purity of the preparation. These results confirm that preparation 94 has high potential for application in biological and environmental fields.

Table 7: Results of water treatment with the formulation after 5 days

Sample	pH	Temperature (°C)	DO (mg/l)	COD (mg/l)	BOD5 (20°C) (mg/l)	NO_2- (mg/l)
Untreated	7.39	23	5.6	78	27.9	2.25
T_1	7.28±0.14	24.8±0.26	5.36±0.55	74.3±25.77	30.8±11.29	0.93±0.07
T_2	7.33±0.13	24.9±0.55	5.03±1.06	58.3±22.54	23.4±11.33	0
T_3 (Control)	7.28±0.04	24.4±0.45	5.2±0.45	51±3.60	19.38±3.08	1.4±0.2

The treated water samples maintained levels close to those of the untreated sample, with negligible changes. The pH of the treated samples ranged from 7.28 to 7.33, while the untreated sample was 7.39. This indicates that the microbial preparation does not significantly affect the acid-base balance of the water, which is positive as pH stability is important in many biological processes.
The temperature of the treated water samples ranged from 24.4 °C to 24.9 °C, compared to 23 °C for the untreated sample. This slight increase could be due to the biological activity of the microorganisms, as the decomposition of organic matter typically generates heat. This temperature change is not significant and does not negatively affect water quality.
DO slightly decreased from 5.6 mg/l in the untreated sample to approximately 5.03 mg/l to 5.36 mg/l in the treated samples. This slight decrease could be due to microorganisms using oxygen during the decomposition of organic matter. Despite the decrease, this level remains within the acceptable range for most water applications.
COD is a crucial indicator for evaluating the effectiveness of the preparation in reducing the amount of dissolved organic matter in water. The treated water samples had COD levels reduced from 78 mg/l to 58.3 mg/l and 51 mg/l in the T_3 and T_3 (control) samples, respectively, indicating that the preparation effectively decomposes organic matter. This significant reduction shows that preparation 94 helped decrease the amount of organic pollutants in the water.
BOD_5 also decreased from 27.9 mg/l in the untreated sample to 23.4 mg/l in the T_3 sample and 19.38 mg/l in the T_3 (control) sample. This decrease shows that the microbial preparation improves the decomposition of organic matter in the water. However, the T_1 sample recorded a slight increase in BOD_5 (30.8 mg/l), which might be due to changes in the easily degradable organic components in the water or complex interactions between factors during treatment.
NO_2^- levels sharply decreased from 2.25 mg/l in the untreated sample to 0 in the T_3 sample and decreased to 0.93 mg/l in the T_1 sample and 1.4 mg/l in the T_3 (control) sample. This significant reduction, particularly in the T_3 sample where nitrite was completely eliminated, indicates the microbial preparation is highly effective in nitrite removal. Nitrite is a harmful pollutant in water, and the nitrite-reducing capability of preparation 94 is an important factor in improving water quality.
In conclusion, the microbial preparation 94 effectively improves water quality by reducing organic pollutants and nitrite. Although there is a slight decrease in DO and BOD_5 in some samples, the preparation still shows positive effectiveness in reducing COD and nitrite, confirming the potential of the preparation for water treatment applications.

Conclusion

Microbial isolation and selection: A total of nine microbial strains were isolated from aquaculture water, with significant variations in growth rates and characteristics. Strain 94 demonstrated the most promising performance in nitrite removal, achieving up to 81.1% reduction in nitrite levels.

Optimal conditions for microbial activity: Strain 94 exhibited optimal growth and nitrite removal capabilities at a neutral pH range (pH 7 to 7.5) and temperatures around 30 °C. The strain showed a broad temperature tolerance from 15 °C to 55 °C, and a pH range from 5 to 9, highlighting its versatility in various environmental conditions.

Impact on water quality parameters: The microbial formulation was effective in reducing COD and nitrite concentrations, which are critical indicators of water quality in aquaculture systems. Although slight reductions in DO and BOD_5 were observed, these changes did not adversely affect the overall water quality.

Application and potential: The formulation of microbial strain 94, with its high density and effective nitrite removal, presents a viable solution for improving water quality in aquaculture. Its ability to maintain performance across a range of environmental conditions makes it a valuable tool for enhancing the sustainability of aquaculture practices.
Overall, the results demonstrate that the microbial formulation developed in this study has significant potential for practical applications in aquaculture systems, offering an effective means of managing nitrite levels and improving water quality. Future research should focus on scaling up the application and further optimizing the formulation to address broader environmental and operational challenges in aquaculture.

References

1. Kuhn DD, Boardman GD, Lawrence AL, Marsh L, Flick GJ. Evaluation of nitrifying bacteria product to improve nitrification efficacy in recirculating aquaculture systems. Aquacultural Engineering. 2010;43(2):78-82.
2. John EM, Krishnapriya K, Sankar TV. Treatment of ammonia and nitrite in aquaculture wastewater by an assembled bacterial consortium. Aquaculture. 2020;526:735390.
3. Xiao R, Tian Y, Li D, Liu L, Yang L, Xu Y. A review on the research status and development trend of equipment in water treatment processes of recirculating aquaculture systems. Reviews in Aquaculture. 2019;11(3):863-895.
4. Porchas RGR, Morales LJB, Baeza MA, Elías LJA, Córdova MLR. The nitrification process for nitrogen removal in biofloc system aquaculture. Reviews in Aquaculture. 2020;12(4):2228-2249.
5. Yusoff FM, Md Akhir F, Shariff M, Razak SA, Saad CR. Biological approaches in management of nitrogenous compounds in aquaculture systems. Dynamic Biochemistry, Process Biotechnology and Molecular Biology. 2011;5(1):21-31.
6. Bakar NSA, Salim MR, Mustafa S, Satar NA.

Optimization of C/N ratios for nutrient removal in aquaculture system culturing African catfish, (Clarias gariepinus) utilizing Bioflocs Technology. International Biodeterioration & Biodegradation. 2015;102:100-106.
7. Munubi RN, Pedersen LF, Chenyambuga SW. Evaluation of biofilter performance with alternative local bio media in pilot scale recirculating aquaculture systems. Journal of Cleaner Production. 2022;366:132929.
8. Luo G, Liu Y, Yang S, Tan H, Li X, Wang Q. Nitrate removal efficiency and bacterial community of polycaprolactone-packed bioreactors treating water from a recirculating aquaculture system. Aquaculture International. 2018;26:773-784.
9. Brailo M, Nanaj D, Petkovsek MM, Macesic D, Jovanovic S, Perovic SI. Bacterial community analysis of marine recirculating aquaculture system bioreactors for complete nitrogen removal established from a commercial inoculum. Aquaculture. 2019;503:198-206.
10. Neissi A, Asad S, Ebrahimi M, Esfahani MR, Dadolahi Sohrab A. Cold-resistant heterotrophic ammonium and nitrite-removing bacteria improve aquaculture conditions of rainbow trout (Oncorhynchus mykiss). Microbial Ecology. 2020;80:266-277.
11. Pujiastuti DR, Suwartha N. Enhancing removal efficiency of ammonia and nitrate in shrimp farm wastewater using biofloc technology and effective microorganism S4 (EM4). International Journal of Technology. 2017;8(6):1021-1030.
12. Chen Z, Xu Y, Wu Q, Ma Y, Peng X, Zhang L. Nitrogen removal performance and microbial diversity of bioreactor packed with cellulosic carriers in recirculating aquaculture system. International Biodeterioration & Biodegradation. 2021;157:105157.
13. Gao Y, Shi W, Shi W, Wang Q, Sun X. Denitrification performance evaluation and kinetics analysis with mariculture solid wastes (MSW) derived carbon source in marine recirculating aquaculture systems (RAS). Bioresource Technology. 2020;313:123649.
14. Liu M, Zhao J, Wang J, Zhang B, Zhang X, Wu H. Treatment effects of pond aquaculture wastewater using a Field-scale Combined Ecological Treatment System and the associated microbial characteristics. Aquaculture. 2023;563:739018.
15. Moisescu C, Monnet F, Plasmier J, Delaunay D, Stefan I. Nitrate removal potential of different microbial consortia, feasible for wastewater treatment in RAS. Curr Trends Nat Sci. 2018;7:205-210.