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Oxidation of Ammonia in Fish Ponds to Nitrates Using Free and Immobilized Nitrifying Bacteria

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Abstract

In fish farming, ammonia stands out as a particularly detrimental toxicant, with the potential to significantly impede growth and even lead to fish mortality. The adverse effects of ammonia exposure in aquatic environments primarily stem from elevated concentrations of unionized ammonium (NH3), which can readily permeate gill membranes. This study aimed to mitigate ammonia levels in fish ponds by employing both free and immobilized nitrifying bacteria to convert ammonia to nitrates. Nitrifying bacteria were isolated from water samples, resulting in the identification of two Pseudomonas species and two Bacillus species. Bacillus spp., designated as B4, exhibited the ability to convert ammonia to both nitrite and nitrate while demonstrating resilience to high ammonia concentrations. When Pseudomonas spp. (C4) and Bacillus spp. (B4) were utilized, the total ammonia concentration was reduced from 2 mg/L to 1.4 mg/L and 1.3 mg/L, respectively. Moreover, the immobilized forms of C4 and B4 achieved a more substantial reduction, lowering the total ammonia concentration from 2 mg/L to 0.5 mg/L within a 5-day period. Both nitrifying bacteria not only exhibited ammonia removal capabilities but also demonstrated their proficiency in transforming ammonia into nitrite. Immobilization proved effective in enhancing microbial tolerance to high ammonia concentrations, ultimately leading to improved water quality and the preservation of aquatic animal health.

Keywords: fish pond, ammonia, nitrifying bacteria, consortium, immobilization

INTRODUCTION

Fish farming, or aquaculture, plays a pivotal role in meeting the global demand for protein. However, the sustainable growth of this industry is contingent upon maintaining optimal water quality within aquaculture systems. Among various water quality parameters, the concentration of ammonia (NH_3/NH_4^+) stands out as a critical factor that demands careful management (McKnight & Neufeld, 2021).

Ammonia, a natural byproduct of fish metabolism and organic matter decomposition within aquaculture systems (Yusoff *et al.*, 2011), presents a challenge. While aquatic organisms generally tolerate low levels of ammonia, the accumulation of toxic unionized ammonium (NH_3) can have deleterious effects on fish health and growth (Dauda *et al.*, 2019). High ammonia concentrations may result in reduced feed conversion, growth inhibition, and, in severe cases, fish mortality (Biswas *et al.*, 2006).

The presence of ammonia (NH_3/NH_4^+) is one of the most pernicious water quality issues in aquaculture. Its rapid accumulation in fish ponds poses a direct threat to the health and well-being of aquatic organisms. Elevated ammonia concentrations can lead to growth inhibition, compromised immune function, and, ultimately, increased mortality rates among fish populations (Guo *et al.*, 2022). Hence, effective ammonia management is essential for the sustainable operation of aquaculture systems.

Traditionally, strategies for ammonia removal have relied on physical and chemical treatments. However, these methods often come with environmental and economic drawbacks. As a sustainable and eco-friendly alternative, the utilization of nitrifying bacteria, either as single strains or in consortia, has garnered increasing attention (Dhanasiri *et al.*, 2011). These bacteria possess the remarkable ability to convert ammonia into less harmful nitrites (NO_2^-) and nitrates (NO_3^-), essential nutrients for aquatic plants and generally less toxic to fish (Yao & Peng, 2017). Nitrifying bacteria, whether as single strains or consortia, have shown promising potential for ammonia removal in aquaculture systems (Dhanasiri *et al.*, 2011).

This study focuses on harnessing the capabilities of nitrifying bacteria from the fish ponds, both in their free and immobilized forms, to efficiently oxidize ammonia in fish ponds. The use of immobilized cells offers the advantage of enhanced microbial tolerance to high ammonia concentrations, potentially improving the overall effectiveness of ammonia removal (Yusuf *et al.*, 2019). Immobilization, a technique where bacterial cells are confined within a matrix or support material, is crucial in this study for several reasons. It helps to prolong bacterial activity, stability, and protection from shear forces within the aquaculture system (Suvase, Annapure & Singhal, 2010; Ahmad, Shamaan & Arif, 2012). This technique also facilitates easier separation of bacteria from the treated water, allowing their reutilization and preventing the release of bacterial cells into the aquaculture environment, thereby reducing potential adverse effects (Li *et al.*, 2022).

The study aims to explore the performance of these bacterial systems, providing valuable insights into their application for maintaining water quality and ensuring the health and productivity of aquatic organisms within fish farming environments.

MATERIALS AND METHOD

Sample Collection

For this study, water samples were obtained from various sources within Kano Metropolis, including the Biological Sciences Aquarium at Bayero University Kano (BUK), Rumbun-Kifi Kano, and a resident's fish pond in Tarauni Metropolis labeled A, B, and C, respectively. One-liter water samples were collected using sterile and transparent water bottles. These samples were transported in a cooled container to the Microbiology Laboratory at Bayero University Kano and stored in a refrigerator for subsequent analysis.

Physiochemical Analysis of Water Samples

Various physicochemical analyses of the water samples were conducted. Temperature, pH, turbidity, ammonia, and nitrates were determined at the site of water sample collection, while dissolved oxygen and alkalinity levels were analyzed in the laboratory.

I. Temperature: Readings were obtained in the field using a mercury-in-glass thermometer calibrated in degrees Celsius. The thermometer was immersed in the water body for approximately 5 minutes to stabilize, after which readings were recorded.

II. pH: pH readings were taken using a pH meter (Hanna Instruments, Model 8621). The pH meter was submerged in the water body for about 2 minutes, and pH values were recorded.

III. Turbidity: Turbidity was measured using a Secchi disc with a 20cm diameter. The Secchi disc was lowered into the water body on a marked line until it disappeared, and the depth was recorded. It was then lowered slightly more and raised until it just reappeared, and the depth was recorded. The average of these two depths was calculated.

IV. Dissolved Oxygen: To analyze dissolved oxygen, fish pond water samples were collected in sterile brown bottles and analyzed using the Azide modification of Winkler's method (APHA, 2005). A 200 ml water sample was transferred into a 300 ml BOD bottle. 1 ml of manganese sulfate solution and 1 ml of alkaline azide reagent were added. The resulting mixture was titrated against 0.025 N sodium thiosulfate until the color change indicated the endpoint. The titre value was recorded as Dissolved Oxygen.

V. Alkalinity: Alkalinity was determined by titration methods (APHA, 2005). To 50 ml of the water samples in a clean 150 ml conical flask, 3 drops of phenolphthalein indicator were added. The samples were titrated with 0.05 M H2SO4 until the color disappeared. After that, 3 drops of methyl orange indicator were added, and titration continued until the color changed from yellow to a permanent reddish or orange-red color. The titre values were recorded and used to compute alkalinity.

VI. Nitrate and Ammonia: Ammonia and nitrates were measured using the Nessler method with the assistance of a Hanna Multiparameter. A small quantity of the sample was transferred into a cuvette, followed by the addition of 4 drops of Nessler reagent. The reaction between ammonia and the reagent caused a yellow tint in the sample, which was then measured colorimetrically, and the concentration was displayed in mg/L (ppm).

Isolation of Nitrifying Bacteria by Enrichment Method

The isolation of nitrifying bacteria was conducted using the basal inorganic medium by Brierley and Wood (Brierley & Wood, 2001). Four stages of enrichment were undertaken:

I. 1st Stage Enrichment

Water samples were enriched in 100ml of Brierley and Wood medium. The medium contained $(NH_4)2SO_4$ at 1g/L, along with other constituents and trace elements. After inoculation with samples A, B, and C, the media were incubated at 30°C and 140rpm. Growth assessment occurred after 4 days by measuring optical density at 610nm using a spectrophotometer.

II. 2nd Stage Enrichment

A similar medium was prepared and inoculated with previously enriched media. After 7 days of

incubation, growth assessment was conducted at 610nm.

III. 3rd Stage Enrichment

This stage involved enriching the mineral inorganic medium with an increased ammonium sulfate concentration of 2g/L for 4 days. Subsequent inoculation occurred into freshly prepared media with the same ammonium nitrogen concentration.

IV. 4th Stage Enrichment

The ammonium sulfate concentration was increased to 3g/L in the medium. After enrichment, cultures were transferred into freshly prepared media containing the same concentration of ammonium sulfate.

Purification of Nitrifying Bacteria

Purification of nitrifying bacteria was carried out using solidified Brierley and Woods medium containing 2 kg/m³ of ammonium sulfate and 1.5 g of purified agar. The agar plates were inoculated with 0.1 mL of the appropriate dilution of the water samples and then incubated at 30°C for one week. Distinct colonies obtained from isolation were sub-cultured onto freshly prepared Brierley and Woods media. The media was prepared aseptically and sterilized at 121°C. Colonies were inoculated using the streak plate method and incubated at 30°C for 5 days, following the procedure outlined by Grunditz and Dalhammer (Grunditz & Dalhammar, 2001).

Screening Tests for Nitrifying Bacteria

Ammonium Oxidation Test: Test tubes containing 5 mL of basal inorganic media with added ammonium sulfate (0.2% w/v) were prepared and sterilized. A loopful of each isolate was added to the respective test tubes, which were then incubated at 37°C for 5 days. After incubation, the presence of nitrite was determined using the Griess-Ilosvay reagent (a mixture of 30% sulfanilic acid and 15% alpha-naphthylamine in a 1:1 ratio). The appearance of a purplish-red color within 5 minutes was indicative of nitrite presence (Cheesbrough, 2006).

Nitrite Reduction Test: In this test, test tubes containing 3 mL of basal inorganic media with concentrated potassium nitrate (0.2% w/v) were sterilized. A loopful of each isolate was added to the respective test tubes, followed by incubation at 37°C for 5 days. After incubation, alpha-naphthylamine reagent and sulphanilamide reagent were added, and the test tubes were observed for the appearance of red coloration within 5 minutes (Bhusal, Muriana & Kerr, 2021).

Tolerance of Ammonia by Isolated Nitrifying Bacteria

Each isolated nitrifying bacterium (adjusted to an optical density of 0.5 nm) was cultured in 100 mL of basal inorganic media containing varying concentrations of ammonium sulfate (2, 4, 6, 8, and 10 mg/L). The medium was subsequently sterilized at 121°C for 15 minutes. All inoculated flasks were incubated at 30°C for 5 days using a shaking incubator, following the method described by Brierly and Wood (Brierley & Wood, 2001).

Identification of Confirmed Nitrifying Bacteria

Morphological characterization of the bacterial isolates was conducted according to Cheesbrough (Cheesbrough, 2006). The isolates' colonies were grown on nutrient agar and observed after 24 hours of incubation at 37°C. Characteristics such as color, shape, and appearance were recorded. Additional identification tests, including Gram staining, indole test, citrate test, urease test, oxidase test, and nitrate reductase test, were performed.

Formulation of Bacteria Consortium

The bacteria consortium is a combination of confirmed and identified bacterial groups, grown separately in nutrient broth as single cultures. They were incubated at 37°C with an agitation rate of 150 rpm for 24 hours. The cultures were adjusted to an absorbance reading of 0.5 at 600nm and then combined at a ratio of 1:1 to yield different combinations of nitrifying bacteria with an absorbance reading of 0.5 at 600nm as shown in Table 1.

Table 1: formulation of bacterial consortia

Code	Consortium composition
F1	*Pseudomonas species (C2)+ Bacillus species (B4)*
F2	*Pseudomonas species (C2) + Bacillus species (B2)*
F3	*Bacillus species (B4)+ Bacillus species (B2)*
F4	*Bacillus subtilis (B2) + Pseudomonas species (C2) + Bacillus species (B4)*

Screening for best bacteria consortium

To assess the performance of the bacteria consortium, both the nitrite reduction test and the ammonia oxidation test were employed. The nitrite reduction test determined the production of an enzyme that results in the reduction of

nitrate. It is based on the detection of nitrite and its ability to form a water-soluble azo dye, resulting in a red compound. The ammonia oxidation test used Griess Ilosvay reagent to detect the presence of nitrates. Both tests were carried out as previously described.

Tests for Tolerance of Ammonia by Nitrifying Bacteria Consortia

Each formulated bacteria consortium (0.5nm) was cultivated in 100ml of basal inorganic media with varying concentrations of ammonium sulfate (2, 4, 6, 8, and 10mg/L). For each media concentration, the pH of the medium was adjusted to 7, and the medium was sterilized at 121°C for 15 minutes while keeping all other chemical constituents constant. All the inoculated flasks were incubated at 30°C for 5 days in a shaking incubator (Brierly and Wood, 2001), after which AOB and NOB activities were determined as previously described.

Immobilization of Nitrifying Bacteria Cells

Immobilization of nitrifying bacteria cells was carried out using gellan gum following the method described by Yusuf et al. (Yusuf *et al.*, 2019; Yusuf & Sharu, 2022). Initially, gellan gum (0.75% w/v) was added to 100 ml of deionized water and heated to 75°C to dissolve the gum completely. Then, 0.06% (w/v) CaCl2 was added to the gum mixture, which was slowly cooled to 45°C. The pH of the solution was adjusted to pH 7.0 using 0.1M NaOH. The bacteria were grown in 2L of nutrient broth, centrifuged, and the resulting bacterial pellet was dispersed in the gum mixture and continuously stirred. Beads were formed by dropping the gum mixture into canola oil containing 0.1% Span 80 using a peristaltic pump. The beads were rinsed repeatedly with 0.1% (v/v) Tween 80 solution.

Reduction of Total Ammonia Nitrogen Concentration by Nitrifying Bacteria

Nitrifying bacteria and bacteria consortium F3 were grown in 100ml of basal inorganic media containing 2g of ammonium sulfate. They were incubated at 30°C in a shaking incubator for 5 days. The total ammonia nitrogen concentration was measured using the Nessler method with the aid of a Hanna Multiparameter (Jeong, Park & Kim, 2013). A small quantity of the sample was transferred into a cuvette, followed by the addition of 4 drops of the Nessler reagent. The reaction between ammonia and reagents caused a yellow tint in the sample, and the amount of ammonia was measured colorimetrically, with the concentration displayed in mg/L (Mustapha & Omotoso, 2008).

Reduction of Total Ammonia Nitrogen Concentration by Immobilized Nitrifying Bacteria

The same method as described for the reduction of total ammonia nitrogen concentration was applied to immobilized nitrifying bacteria. In each flask, 200 beads with diameters of 2-3 mm were used, and were incubated as previously described.

RESULTS

Physicochemical Analysis of the Water Samples

The results of the physicochemical analysis of the water samples (Table 2) are summarized as follows:

Water sample A exhibited the highest pH value at 7.35 ± 0.21, followed by water sample C with a pH of 7.32 ± 0.26. Water sample B had a pH of 7.20 ± 0.28. Temperature measurements for the water samples ranged between 18.28 ± 1.03°C and 23.15 ± 1.63°C. Turbidity levels varied among the samples, with values ranging from 34.00 ± 1.41 cm to 47.25 ± 1.06 cm.

The dissolved oxygen (DO) content of the water samples was 4.25 ± 0.35 mg/L for water sample A, 5.51 ± 0.44 mg/L for water sample B, and 4.61 ± 0.86 mg/L for water sample C. Alkalinity levels differed, with water sample B having the highest alkalinity at 121.17 ± 3.06 mg/L, followed by sample C with an alkalinity of 109.10 ± 2.69 mg/L. Sample A exhibited the lowest alkalinity at 95.00 ± 1.41 mg/L. The NH_3^+-N content in the samples followed the order: Sample C > Sample B > Sample A, with NH_3^+-N concentrations of 0.21 ± 0.03 mg/L, 0.17 ± 0.03 mg/L, and 0.06 ± 0.01 mg/L, respectively

Table 2: Physiochemical Characteristics of the Water Samples

Parameters (unit)	Sample A	Sample B	Sample C
pH	7.35±0.21	7.20±0.28	7.32±0.26
Temp (^{0}C)	21.00±1.41	23.15±1.63	18.28±1.03
Turbidity(cm)	34.00±1.41	47.25±1.06	38.55±2.05
DO (mg/L)	4.25±0.35	5.51±0.44	4.61±0.86
NO_2-N	0.03±0.01	0.12±0.02	0.06±0.01
Alkalinity(mg/L)	95.00±1.41	121.17±3.06	109.10±2.69
NH_3^+- N(mg/L)	0.06±0.01	0.21±0.03	0.17±0.03

Enrichment of Water Samples in Liquid Medium

Table 3 presents the results of the enrichment of water samples in a liquid medium, with the number of bacterial cells recorded (OD at 600nm). The results for each stage of water enrichment are as follows:

Table 3: Enrichment of Water Samples in Liquid Medium

Water Sample	1st Stage (OD 600nm)	2nd Stage (OD at 600nm)	3rd Stage (OD at 600nm)	4th Stage (OD at 600nm)
Sample A	0.54×10^{8} ± 0.01	1.06×10^{8} ± 0.68	1.30×10^{8} ± 0.21	1.67×10^{8} ± 0.06
Sample B	0.60×10^{8} ± 0.03	0.74×10^{8} ± 0.06	0.97×10^{8} ± 0.03	1.30×10^{8} ± 0.09
Sample C	0.43×10^{8} ± 0.03	0.64×10^{8} ± 1.20	0.85×10^{8} ± 2.30	0.95×10^{8} ± 0.00
Control	0.08×10^{7}± 1.00	1.08×10^{7}± 2.40	0.03×10^{8}± 3.40	0.08×10^{8}± 0.02

The recorded values represent the bacterial cell counts for each water sample at various stages of enrichment. The data reveal the progression of bacterial growth during the 1st, 2nd, 3rd, and 4th stages of water enrichment for each sample. However, the control remains constant at approximately 0.08×10^{7} CFU/ml throughout all stages. This suggests that the presence of ammonium sulfate in the liquid medium promotes the growth of the bacterial samples (Samples A, B, and C) compared to the control.

Screening Test for Nitrifying Bacteria Isolates

The results of the screening test for nitrifying bacteria isolates revealed that bacterial isolates B4, B2, C1, C2, and C4 were confirmed as ammonia-oxidizing bacteria. In contrast, bacterial isolates A1, B1, and B4 were confirmed as nitrite-reducing bacteria. Notably, bacterial isolate B4 exhibited the ability to oxidize ammonia and also reduce nitrite (Table 4). It is known that heterotrophic bacteria can utilize both organic and inorganic sources of carbon for growth.

Table 4: Screening Tests for Nitrifying Bacteria

Nitrifying Bacteria Isolates	AOB Test	NOB Test
A1	-	+
A2	-	-
B1	-	+
B2	+	-
B3	-	-
B4	+	+
C1	+	-
C2	+	-
C3	-	-
C4	+	-

Keys: AOB: Ammonia Oxidizing Bacteria; NOB: Nitrite Oxidizing Bacteria; - = no colour change, + = colour change

Tolerance of Ammonia by Free Nitrifying Bacterial Isolates

Table 5 shows the tolerance of ammonia by free cells of nitrifying bacterial isolates at five different concentrations 2, 4, 6, 8 and 10mg/L. The result revealed bacteria isolates A1, B2, B4, C1, C2, C4 and B1 had OD of 1.84 ±0.21, 2.00 ±0.17, 3.44 ±0.22, 2.08 ±0.15, 3.12 ±0.27, 4.08 ±0.21 and 2.40 ±0.10nm at 2mg/L while at 4mg/L, the OD_{600} of the isolates were 2.64 ±0.21, 4.80 ±0.23, 5.12 ±0.14, 2.80 ±0.12, 5.28 ±0.23, 5.60 ±0.31 and 2.80 ±0.11nm respectively. The result further showed that at 10mg/L, the bacteria isolates A1, B2, B4, C1, C2, C4 and B1 had the least ODs of 0.08±0.01, 1.68±0.29, 2.32 ±0.29, 1.04 ±0.12, 1.76 ±0.10, 3.20±0.21 and 0.08±0.03nm respectively.

Table 5: Tolerance of Ammonia by Nitrifying Bacteria Isolates

NH_3SO_4 Conc (mg/l)	Nitrifying Bacteria Isolates (Optical Density, 600nm)						
	A1	B2	B4	C1	C2	C4	B1
2	1.84±0.01	2.00 ±0.01	3.44 ±0.02	2.08 ±0.01	3.12 ±0.05	4.08 ±0.02	2.40 ±0.04
4	2.64 ±0.02	4.80 ±0.021	5.12 ±0.01	2.80 ±0.02	5.28 ±0.03	5.60 ±0.01	2.80 ±0.04
6	0.08 ±0.00	3.20 ±0.04	2.88 ±0.03	2.40 ±0.04	3.44 ±0.02	4.80 ±0.02	0.88 ±0.04
8	0.06 ±0.30	2.88 ±0.04	2.56 ±0.14	1.28 ±0.02	2.96 ±0.01	4.00 ±0.01	0.16 ±0.03
10	0.08 ±0.00	1.68 ±0.03	2.32 ±0.14	1.04 ±0.02	1.76 ±0.02	3.20 ±0.20	0.08 1±0.3

Values are Mean±SD of triplicate estimation

Morphological and biochemical characterization identified the isolates as *Bacillus* species (B4), *Bacillus* species (B2), and *Pseudomonas* species (C2).

Screening Tests for Nitrifying Bacteria Consortium

All the consortia were able to convert ammonia to nitrite and then to nitrate except F2, which comprised of *Pseudomonas species* (C2) + *Bacillus species* (B2) (Table 6)

Table 6: Screening consortium bacteria for Nitrifying bacteria

Consortium	AOB Test	NOB Test
F1	+	+
F2	+	-
F3	+	+
F4	+	+

Keys: AOB: Ammonia Oxidizing Bacteria; NOB: Nitrite Oxidizing Bacteria; - = no colour change, + = colour change

Tolerance of Ammonia by Nitrifying Bacteria Consortium

Figure 1 shows the ammonia tolerance of the four bacteria consortia at different ammonia concentrations 2, 4, 6, 8 and 10mg/L. From the result, it was observed that F1, F2, F3 and F4 had their optimum growth and tolerance of ammonia at 4mg/L. The least tolerance of ammonia by the consortia was observed at 10mg/L as F1, F2, F3, F4 and F5 had OD_{600} of 2.44, 1.48, 4.09, 2.12 and 2.41nm respectively. F3 had the highest level of tolerance to ammonia at varying concentrations of ammonium sulphate.

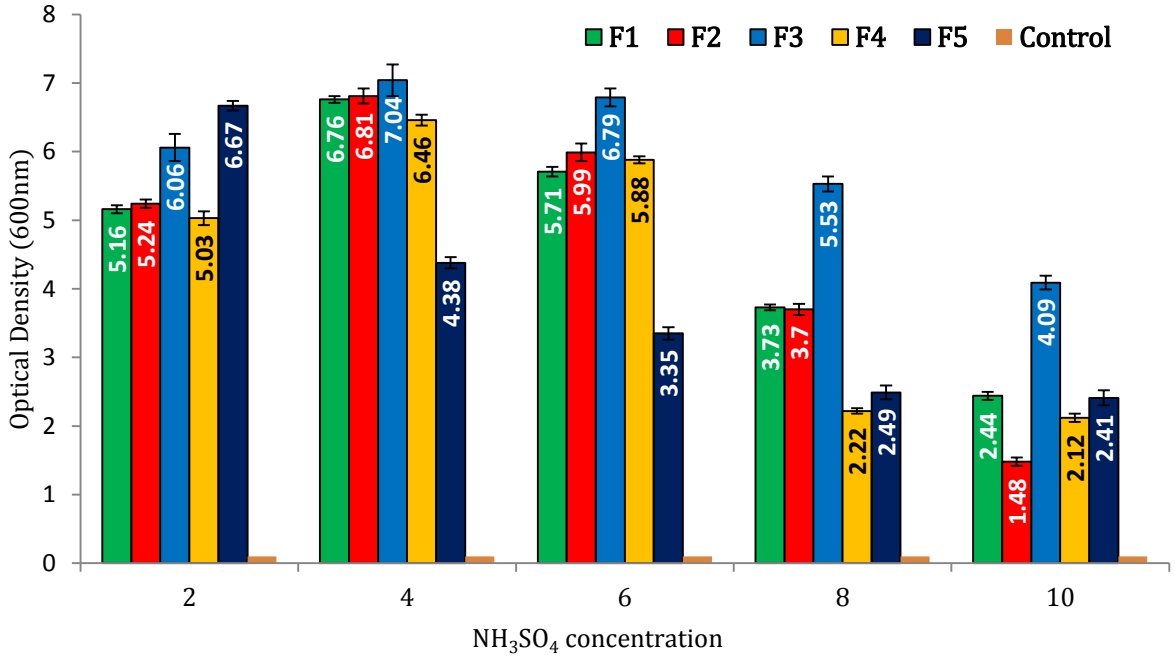

Figure 1: Tolerance of Ammonia by Nitrifying Bacteria consortium (F1, F2, F3, F4 and F5)

Reduction of Total Ammonia Nitrogen Concentration by Nitrifying Bacteria

Figure 2 shows the rate at which single nitrifying bacteria *Pseudomonas spp* (C4), *Bacillus spp* (B4) and nitrifying bacteria consortium F3 reduced the total ammonia concentration present in a period of 5 days. C4 and *Bacillus spp* (B4) were able to reduce total ammonia concentration from 2mg/L to 1.4mg/L and 1.3mg/L respectively. While F3 was able to reduce total ammonia from 2 to 0.7mg/L in 5 days.

Figure 2 illustrates the rate at which Pseudomonas spp. (C4) and Bacillus spp. (B4) reduced the total ammonia concentration over a period of 5 days. Both C4 and Bacillus spp. (B4) were able to reduce the total ammonia concentration from 2 mg/L to 1.4 mg/L and 1.3 mg/L, respectively.

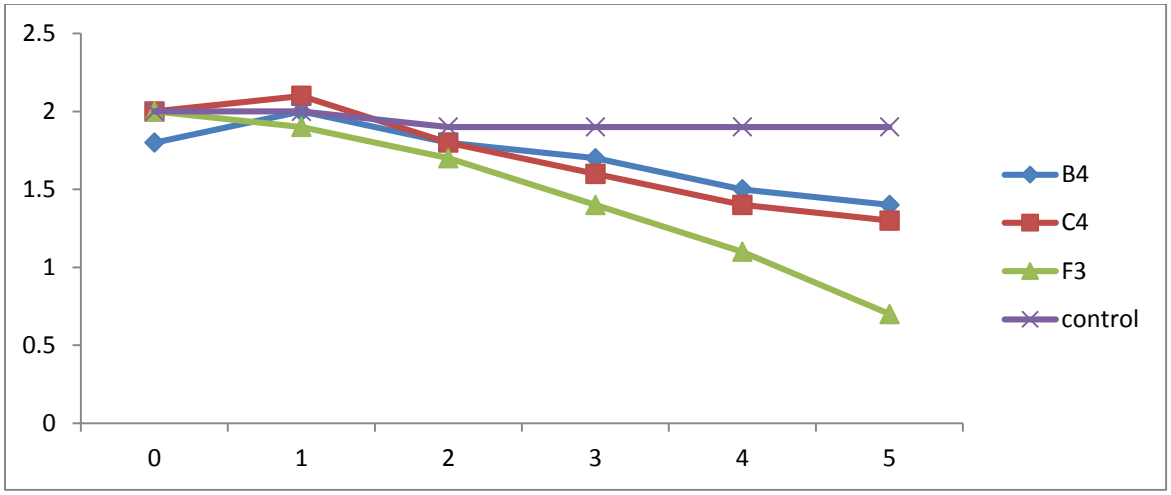

Figure 2: **Reduction of Total Ammonia Nitrogen Concentration by Nitrifying Bacteria**

Reduction of Total Ammonia Nitrogen Concentration by Immobilized Nitrifying Bacteria

Figure 3 shows the rate at which immobilized nitrifying bacteria *Pseudomonas spp* (C4), *Bacillus spp* (B4) and immobilized nitrifying bacteria consortium F3 reduced the total ammonia concentration present in a period of 5 days. *Pseudomonas spp* and *Bacillus spp* were able to reduce total ammonia concentration from 2mg/L to 0.5mg/L both. Meanwhile, F3 was able to reduce total ammonia from 2 to 0.3mg/L in 5 days.

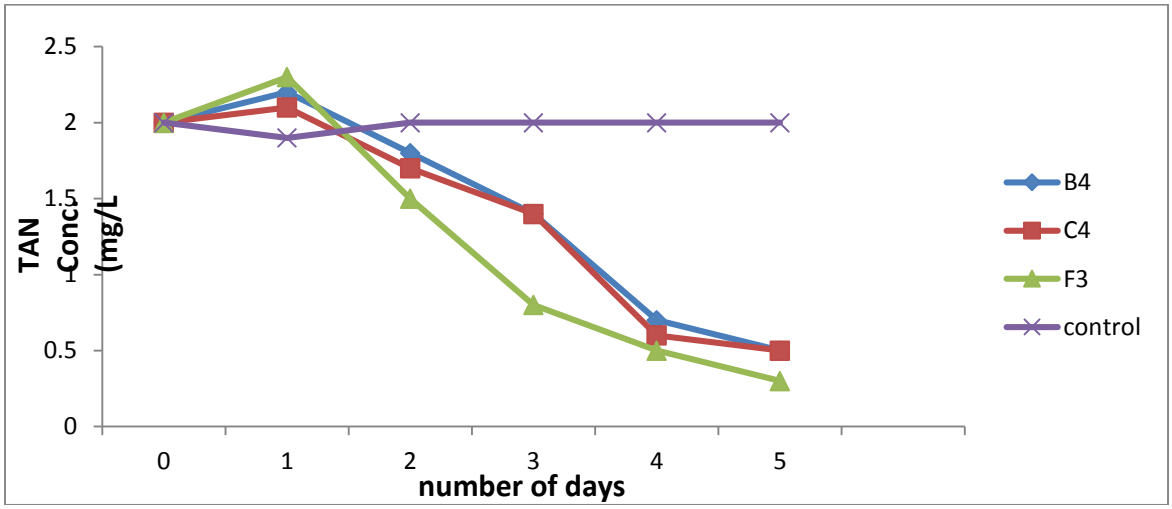

Figure 3: Reduction of ammonia by immobilized nitrifying bacteria

DISCUSSION

The comprehensive physicochemical analysis of water samples collected from distinct fish ponds within the Kano metropolis provided detailed insights into the environmental parameters crucial for sustaining aquatic life. The pH values, ranging from 7.00±0.00 to 7.47±0.05, were within the recommended optimal range for fish growth and productivity (Latap, Anyanwu & Ildefonso, 2015). These findings support the significance of pH in creating an environment conducive to aquatic life. The correlation between pH and its critical influence on fish health is consistent with previous studies, such as Pandey and Shukla (2005), who underscored the importance of neutral to slightly alkaline pH for fish survival.

Temperature readings observed in the range of 19.28±0.39°C to 23.00±1.41°C align with the ideal temperature range for fish pond ecosystems (Alabaster and Lloyd, 1980). The observed temperatures fall within the ranges suitable for fish health and activity, reinforcing the potential of these water bodies to support aquatic life.

Dissolved oxygen (DO) levels, vital for the respiration and metabolism of aquatic organisms, were recorded between 4.11±0.16 and 5.10±0.14mg/L.

These levels meet the requirements outlined by Nelson et al. (2015) and signify favorable conditions for sustaining aquatic life in the examined ponds. The adequate levels of DO are crucial to prevent hypoxia in water bodies, as lower concentrations of dissolved oxygen could result in fish mortality.

The alkalinity values, ranging between 95.67±0.47 and 120.17±0.23mg/L, conform to the guidelines that maintain a stable pH range and mineral concentration in the water, essential for maintaining suitable water quality conditions (Boyd and Tucker, 1992; APHA, 1998). In contrast, ammonia-N and nitrate concentrations were observed to be within the ranges of 0.06±0.01 to 0.15±0.00mg/L and 0.03±0.01 to 0.12±0.02mg/L, respectively. The recorded concentrations of ammonia-N fall below the recommended levels for optimal aquatic health, while the nitrate concentrations comply with values reported by Latap and co-workers (Latap, Anyanwu & Ildefonso, 2015). These findings indicate a relatively low ammonia concentration in the studied fish ponds, possibly due to lower fish population and regular water renewal.

Moving from the physicochemical analysis to the microbiological perspective, the study successfully enriched ammonia-oxidizing bacterial isolates and conducted a screening test to identify specific bacterial species capable of nitrogen cycling. The isolates identified, primarily *Bacillus* and *Pseudomonas* species, have been previously recognized for their nitrification and denitrification capabilities (Sheela, Beebi & Rao, 2015). Their identity align with a previous study by Sheela et al. (Sheela *et al.*, 2015), which identified *Bacillus* sp as bacteria capable of nitrifying and denitrifying ammonia in water. Efficient ammonia-degrading microorganisms, such as *Bacillus* spp. and *Ps. aeruginosa*, have been previously reported in marine environments (Zhou *et al.*, 2020; Li *et al.*, 2022; Wang *et al.*, 2022). *Bacillus* species have been recognized for their significant roles in the nitrogen cycle, including nitrification (Zhao *et al.*, 2020), denitrification (Li *et al.*, 2020), and nitrogen fixation (Yusoff *et al.*, 2011).

In this study, bacterial isolate C2 identified as *Pseudomonas* species possess the capacity to oxidize ammonia to nitrates. In a previous study, a novel heterotrophic nitrifying and aerobic denitrifying bacterium, *Pseudomonas stutzeri* SDU10 was isolated from piggery waste water (Chen *et al.*, 2020). This strain exhibited rapid growth, high nitrogen removal efficiency, tolerance to high dissolved oxygen levels, and excellent aggregation ability.

Moreover, the study accentuated the substantial influence of bacterial consortia, specifically emphasizing the efficiency of Bacillus species in ammonia nitrification. Previous research studies, such as Li et al. (Li, Zhao & Adam, 2016), have highlighted the significance of bacterial consortia in effectively managing high ammonia concentrations in aquatic environments.

The exploration of immobilized bacterial cells revealed their enhanced nitrification capacities compared to free cells. These findings signify the potential of immobilized cells in improving water quality, in agreement with the research conducted by Lau et al. (Lau, Tam & Wong, 1997).

Zhang *et al.*, 2014 demonstrated that immobilized nitrifiers have the potential to enhance water quality and sustain the well-being of aquatic organisms. The results of ammonia tolerance tests conducted with both free and immobilized bacterial cells indicated that immobilized bacterial cells exhibit a higher tolerance to elevated ammonia concentrations compared to their free counterparts. The reduction of ammonium in the aquaculture pond can be attributed to either nitrification or ammonia volatilization (Ramli *et al.*, 2017). Consequently, in this study, a control treatment devoid of the bacterial isolates was implemented to account for nitrification and volatilization in the pond water.

The intricate interplay between bacterial species, water quality, and environmental parameters is critical for understanding and managing aquatic ecosystems. The study findings underscore the importance of specific conditions and bacterial species in ensuring lower or reduced ammonia concentration in fish ponds, potentially applicable to aquaculture practices and water treatment processes.

CONCLUSIONS

The three nitrifying bacteria exhibited not only ammonia removal capabilities but also the capacity to convert ammonia to nitrite. Immobilization of these bacteria significantly enhanced their tolerance to high ammonia concentrations, thereby presenting a potential avenue for improving water quality and preserving the health of aquatic organisms.

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