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Quantification and characterization of nitrifying bacteria isolated from an aquaponic system

Cuantificación y caracterización de bacterias nitrificantes aisladas de un sistema acuapónico

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ABSTRACT

Nitrifying bacteria are essential in aquaponic systems because they transform nitrogenous waste into useful plant nutrients, preventing ammonium toxicity in fish. This study aimed to quantify and characterize cultivable nitrifying bacteria in an aquaponic system over time, using two replicated systems with watercress (*Nasturtium officinale*), and the fishes Nile tilapia (*Oreochromis niloticus*), red tilapia (*Oreochromis* sp.), and white pacu (*Piaractus orinoquensis*). Samples were collected at three moments (0, 3, and 6 months) from the fish tank, hydrocyclone, and biofilter. The highest bacterial abundance was detected in the fish tanks, likely due to higher oxygen levels and nutrient availability, with a consistent increase over time. Correlation analysis indicated that certain nutrients, such as potassium, phosphate, manganese, and nitrate, could favor the proliferation of nitrifying bacteria. Nine bacterial morphotypes were isolated and phenotypically characterized, with most displaying Gram-positive staining and negative urea hydrolysis. This study provides insight into the spatiotemporal dynamics of nitrifying bacteria in aquaponic systems, highlighting their role in nutrient cycling. The high bacterial abundance observed underscores the system's potential for efficient nutrient reuse. Molecular techniques such as 16S rRNA gene sequencing and metagenomics are recommended to confirm bacterial identity and better understand community structure. These findings reinforce the ecological importance of nitrifying bacteria in system performance and advancing sustainable agricultural practices.

Keywords: Hydroponics; Nitrification; Pisciculture; Recirculating nutrient systems; Sustainable agriculture.

RESUMEN

Las bacterias nitrificantes son esenciales en los sistemas acuapónicos porque transforman los desechos nitrogenados en nutrientes útiles para las plantas, evitando la toxicidad por amonio en los peces. Este estudio buscó cuantificar y caracterizar las bacterias nitrificantes cultivables en un sistema acuapónico a lo largo del tiempo, utilizando dos sistemas replicados con la planta berro (*Nasturtium officinale*), y los peces tilapia del Nilo (*Oreochromis niloticus*), tilapia roja (*Oreochromis* sp.) y cachama blanca (*Piaractus orinoquensis*). Se tomaron muestras en tres momentos (0, 3 y 6 meses) del tanque de peces, hidrociclón y biofiltro. La mayor abundancia bacteriana se detectó en los tanques de peces, probablemente debido a mayores niveles de oxígeno y disponibilidad de nutrientes, observándose un incremento a lo largo del tiempo. El análisis de correlación indicó que ciertos nutrientes como potasio, fosfato, manganeso y nitrato podrían favorecer la proliferación de bacterias nitrificantes. Se aislaron y caracterizaron fenotípicamente nueve morfotipos bacterianos, la mayoría de los cuales presentó tinción Gram positiva e hidrólisis de urea negativa. Se proporciona información sobre la dinámica espaciotemporal de las bacterias nitrificantes en sistemas acuapónicos, destacando su papel en el reciclaje de nutrientes. La elevada abundancia bacteriana registrada resalta el potencial del sistema para la reutilización eficiente de nutrientes. Se recomienda aplicar técnicas moleculares, como la secuenciación del gen 16S rRNA y el análisis metagenómico, para identificar los morfotipos y entender mejor la comunidad microbiana. Estos hallazgos refuerzan la importancia de las bacterias nitrificantes en el rendimiento del sistema y el avance de prácticas agrícolas sostenibles.

Palabras clave: Agricultura sostenible; Hidroponía; Nitrificación; Piscicultura; Sistemas de recirculación de nutrientes.

INTRODUCTION

The aquaponic system is an integrated and sustainable method that simultaneously cultivates plants and aquatic organisms by recirculating nutrients through water, combining the principles of conventional aquaculture with hydroponics (Love *et al.* 2015). This model is fundamentally based on the Recirculating Aquaculture System (RAS), which includes essential components such as a fish tank, mechanical and biological filtration units, aeration systems, and water circulation infrastructure. The three major biological communities: plants, fish, and nitrifying bacteria that interact within a closed-loop, nutrient-recirculating environment (Love *et al.* 2014).

Through a diverse microbial consortium of microorganisms with metabolic pathways involved in nitrogen transformations, the fish waste and uneaten fish feed can become plant-accessible nutrients (Goddek *et al.* 2016; Bartelme *et al.* 2019). This process enhances sustainability by reducing nutrient discharge and utilizing fish-derived waste as a nutrient source for plants, closing the loop in the nutrient cycling (Lal *et al*. 2024).

Nitrogen recycling in aquaponic systems begins when fish excrete nitrogen primarily in two forms: ammoniacal nitrogen (NH_4^+/NH_3), which is released mainly through the gills and to a lesser extent via urine, and organic nitrogen, which is expelled as solid feces (Canfield *et al.* 2010). Additionally, uneaten feed contributes to the system's nitrogen load by introducing both organic compounds, such as proteins, and trace amounts of inorganic nitrogen (Bartelme *et al.* 2018). Although ammonium (NH_4^+) is relatively non-toxic, un-ionized ammonia (NH_3) is highly toxic to aquatic organisms, with its toxicity increasing under higher pH and temperature conditions. Toxic effects in fish have been observed at concentrations as low as 0.05–0.2 mg/L of NH_3, a significant concern for species such as Nile tilapia (*Oreochromis niloticus*) and white pacu (*Piaractus orinoquensis*), both commonly farmed in tropical aquaponic systems (Gyamfi *et al.* 2024). From the plant perspective, both ammonium and nitrate are assimilable forms of nitrogen; however, nitrate is generally preferred in hydroponic and aquaponic setups due to its chemical stability, lower toxicity, and greater compatibility with nutrient uptake mechanisms (Britto & Kronzucker, 2013).

Studies demonstrate different environmental conditions and microbial abundance in each component of an aquaponic system, which can be related to specific functions (Schmautz *et al.* 2021). Therefore, identifying the microbial communities in each compartment is essential for understanding how the system operates holistically, particularly in terms of water quality, and plant and fish health (Munguia-Fragozo *et al.* 2015). Such insights can help address current gaps in aquaponic crop production and enhance system efficiency (Bartelme *et al.* 2018).

Central to this process are nitrifying bacteria, which play a vital role in converting ammonia first to nitrite (via ammonia-oxidizing bacteria, AOB) and then to nitrate (via nitrite-oxidizing bacteria, NOB), thereby supporting plant growth and preventing toxic ammonia buildup (Maier, 2000; Owen & Jones, 2001). Key bacterial genera involved in these nitrification steps include *Nitrosomonas*, *Nitrosospira*, and *Nitrobacter* (Wongkiew *et al.* 2017).

Despite the growing adoption of aquaponic systems in Latin America, data on the composition and activity of nitrifying bacterial communities in Colombian systems remain scarce. Therefore, the objective of this research was to describe and quantify the nitrifying bacteria present in an aquaponic system to contribute to the broader understanding of microbial nitrogen dynamics and support the development of more efficient and ecologically sustainable aquaponic practices.

MATERIALS AND METHODS

Experimental setup. The study was conducted in the Hydrobiology Laboratory and aquaponics system of greenhouse of Ictiology of Universidad Militar Nueva Granada located in Cajicá, Colombia (5°01′ N, 74°01′ W; 2,558 m a.s.l.). The study followed the methodology described by Aguirre (2023), with adaptations to a vertical NFT system. Two identical small-scale aquaponic systems (S1 and S2) were constructed and operated under identical environmental and operational conditions as parallel replicates for six months.

Each system was composed of the following main components: a fish rearing tank, a hydrocyclone (clarifier), a biological filtration unit (biofilter), and a vertical hydroponic system based on the Nutrient Film Technique (NFT). All components were connected through PVC piping in a closed-loop configuration. In each system, watercress (*Nasturtium officinale*) was planted in the hydroponic NFT vertical with 40 plants, and a polyculture of Nile tilapia (*Oreochromis niloticus*), red tilapia (*Oreochromis* sp.), and white Cachama (*Piaractus orinoquensis*) was arranged in a tank of 1,000 Liters with a stocking density of 3-8 kg/m^3 (Figure 1). The system was equipped with an airflow blower for air circulation and a water pump for water flow regulation, so the water was circulated continuously. Fish were manually fed twice daily with a commercial diet containing 32% crude protein. Feeding rates were adjusted biweekly to correspond to 2–4% of the total fish biomass, with average daily feed input ranging from 150 to 300 g per system. No automated feeding systems were used.

Aquaponics system description. The aquaponic system consisted of a 1,000-liter cylindrical polyethylene fish tank stocked at a density of approximately 100 fish/m^3, comprising a polyculture of *Oreochromis niloticus*, *Oreochromis* sp., and *Piaractus orinoquensis*, with an overall biomass ranging from 3 to 8 kg/m^3. Continuous aeration was provided by a 0.5 HP blower connected to fine bubble diffusers. Solid waste from the fish tank was directed to a 500-liter hydrocyclone clarification unit, which included an internal PVC structure and a Matala filter mat to facilitate sedimentation and solids removal. The clarified water then entered a 250-liter biofilter containing 10 kg of Kaldnes-type plastic media, which was aerated to promote the growth of nitrifying bacterial biofilms. The treated

water was subsequently delivered to a vertical NFT (nutrient film technique) hydroponic unit comprising 1.6-meter PVC towers, each supporting 40 watercress (*Nasturtium officinale*) plants. Nutrient-rich water was recirculated from the central reservoir to the top of each tower and flowed downward through the root zone. Water circulation throughout the system was maintained using submersible pumps with a flow rate of 2,000 L/h. Estimated hydraulic retention times were 24 h in the fish tank, 6–8 h in the biofilter, and 30–40 min in the hydroponic unit. The total operational volume of the system was approximately 1,200 liters.

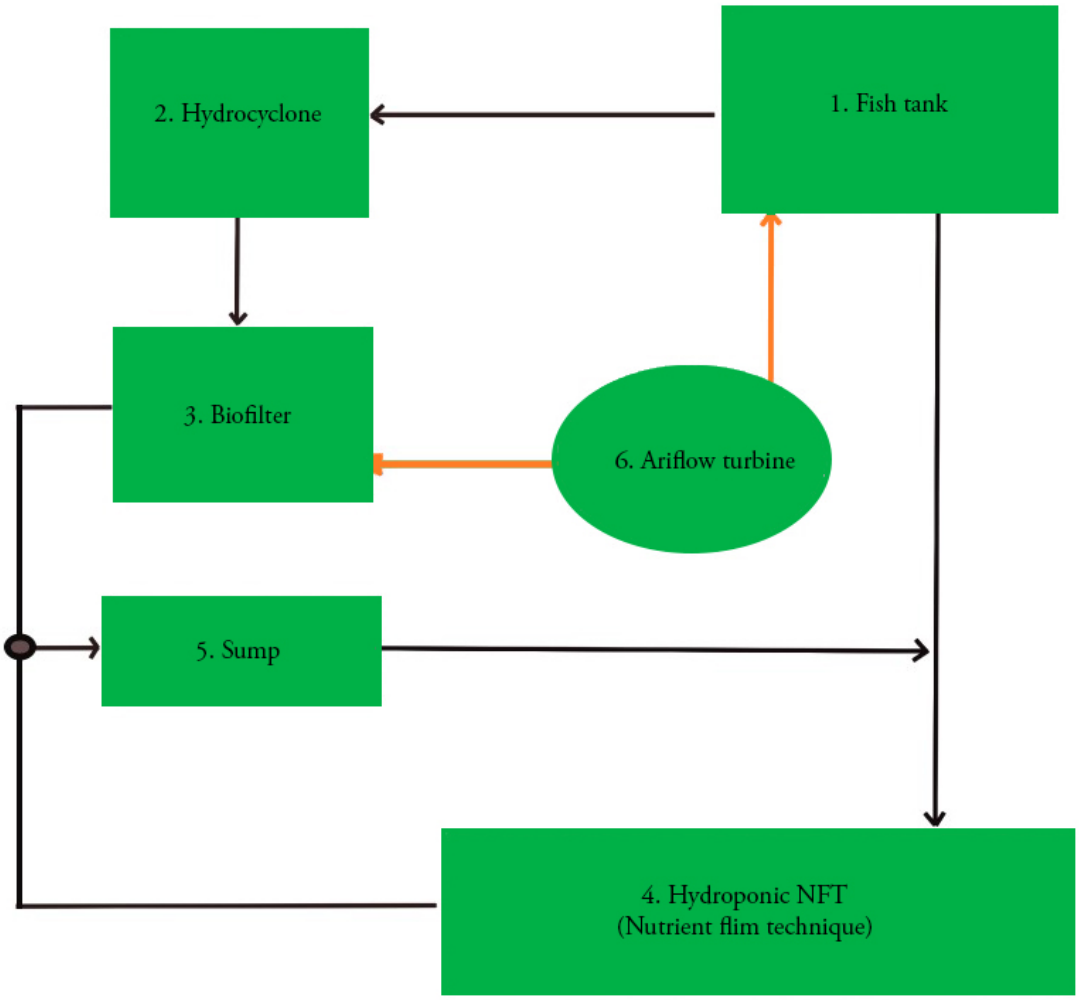


Figure 1. Schematic representation of the experimental aquaponic system. Components: 1. Fish tank, 2. Hydrocyclone (clarifier), 3. Biofilter, 4. Vertical hydroponic unit using the Nutrient Film Technique (NFT), 5. Water reservoir, and 6. Recirculation pump (turbine).

Water quality parameters. Water quality parameters were monitored to evaluate the physicochemical conditions of the aquaponic system and to correlate them with microbial activity. Temperature and pH were measured weekly using a multiparameter probe (HI 9829, Hanna® Instruments). Nutrient concentrations, including ammonium (NH_4^+), nitrite (NO_2^-), nitrate (NO_3^-), phosphate (PO_4^{3-}), calcium (Ca^{2+}), iron (Fe^{2+}/Fe^{3+}), potassium (K^+), and manganese (Mn^{2+}), were analyzed every 15 days via spectrophotometry using Spectroquant® kits (Merck).

Analytical procedures followed the manufacturer's specifications and were aligned with the Standard Methods for the Examination of Water and Wastewater (APHA, 2017), employing the following equivalent methods: 4500-NH_3 for ammonium, 4500-NO_2^- for nitrite, 4500-NO_3^- for nitrate, and 4500-P for phosphate. Also, it was performed weekly, replacing 10–15% of the total volume to maintain ionic balance and water quality. The pH levels were maintained between 6.5-7, and adjusted, when necessary, by adding calcium bicarbonate to increase alkalinity or hydrochloric acid (HCl) to lower the pH.

Isolate and bacteria quantification. Water samples for microbial analysis were collected from three components of each aquaponic system: (1) the fish tank, (2) the hydrocyclone (clarifier), and (3) the biofilter. In all cases, samples were obtained at a depth of 10 cm below the surface using sterilized containers of 10 mL. For

the biofilter, 50 g of water plastic biofilter media (biochips) was taken to obtain the surface-attached microbial communities. To detach bacteria from the biofilm, samples were transferred to sterile Erlenmeyer flasks and vortexed at 2,500 rpm for 10 minutes in 10 mL of water before inoculation.

A selective ammonium-based medium for nitrifying bacteria, as described by Verhagen & Laanbroek (1991), was used and adapted to the environmental conditions of the aquaponic system (Table 1) to promote bacterial growth under system-specific parameters. Serial dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were prepared in triplicate with the selective ammonium medium. These dilution levels were selected based on preliminary trials and methodologies reported in the literature to optimize the detection range for bacterial abundance under controlled conditions. From each dilution, 1 mL aliquots were inoculated into ammonium broth and incubated at 28 °C for 15 days. Following incubation, two drops of Griess reagent were added to detect nitrite formation (indicated by a red color), zinc powder was used to confirm nitrate presence (yellow coloration), and Nessler's reagent was added to detect residual ammonium (brown precipitate). Quantification of nitrifying bacteria was carried out using the Most Probable Number (MPN) method, following the protocol described by Chandrapati & Williams (2014).

Characterization of nitrifying bacteria. Triplicate isolation to obtain pure strains in a selective medium (Table 1). After 15 days of incubation at 21,5 °C, the pure strains were characterized macroscopically by size, color, and texture, while microscopically their morphology was identified by Gram staining. Biochemically, the strains were characterized by oxidase, catalase, motility, indole, urea, lysine, glucose, lactose, arginine, inositol, TSI, citrate, MacConkey, MRS, and the presence of CO_2 (Chauhan & Jindal, 2020).

Statistical analysis. The data were parametric, as the microbial abundance data were validated by the normality requirement Shapiro-Wilk test = 0.0001; $p < 0.05$. To assess the difference between MPN/L values per component, stage, and aquaponic system, a randomized block design factorial analysis was performed, followed by the Tukey-Kramer HSD Test. Likewise, the Pearson's correlation test was used to observe significant differences between nutrients related to the abundance of nitrifying bacteria. Statistical analyses were conducted using R Studio software v. 4.0.2 for Windows.

Table 1. Composition of the culture medium used for the selective growth of nitrifying bacteria.

Component	Concentration g/L
Ammonium sulfate	0.5g
Potassium phosphate	1g
Magnesium sulfate	0.3g
Sodium chloride	0.3g
Ferrous sulfate	0.03g
Calcium carbonate	1g
Water	1L
pH	6.6
Agar	18g

RESULTS AND DISCUSSION

Water quality parameters. On average, the aquaponic systems, based on water quality parameters such as concentrations of ammonium, nitrite, and nitrate, were within the recommended range for effective nitrification and optimal growth of plants and fish. Considering the species used, optimal growth temperatures range from 14 to 30 °C (Wongkiew *et al.* 2018) (Table 2).

Parameters such as pH, temperature, nitrite, phosphates, calcium, iron, potassium, and manganese showed no significant variation ($p>0.05$) within the aquaponics systems. This suggests that the aquaponics system maintained appropriate water quality throughout the experiment (Birolo *et al.* 2020; Torres-Mesa *et al.* 2023). The pH remained within the recommended range for the optimal development of the aquaponic system (pH 6-7), and the temperature (18-30°C) was within acceptable ranges for the plant growth (Somerville *et al.* 2014).

During the study, fish mortalities were minimal (< 5%), and no outbreaks of disease or major stress symptoms were observed. Watercress plants showed healthy vegetative growth throughout the cultivation period, with no significant signs of nutrient deficiency or wilting.

Nitrifying bacteria quantification. An abundance >1100 (MPN/L) was quantified (Figure 2), where F-test yielding a p-value >0.05, indicating that the data followed a normal distribution. The experiment was replicated under identical conditions, and no statistically significant differences were observed between the

replicate systems ($p < 0.50$). When analyzing grouped data, no significant differences were found among the means. Therefore, increasing the number of biological replicates is recommended to reduce variability and improve the statistical robustness of abundance measurements.

Table 2. Water quality parameters in the fish tank of aquaponic systems S1 and S2.

Parameters	Mean ± SD (S1)	Mean ± SD (S2)
pH	6,63 ± 0,63	6,53 ± 0,72
Temperature °C	21,45 ± 1,63	21,68 ± 1,57
Ammonium mg/L	0,22 ± 0,36	0,40 ± 0,49
Nitrite ug/L	29,0 ± 21,36	29,0 ± 19,1
Nitrate mg/L	68,60 ± 26,38	95,71 ± 48,04
Phosphate mg/L	10,31 ± 7,14	10,84 ± 5,40
Calcium mg/L	53,15 ± 26,89	46,65 ± 24,45
Iron mg/L	0,23 ± 0,31	0,16 ± 0,18
Potassium mg/L	11,46 ± 13,18	9,92 ± 6,55
Manganese mg/L	0,41 ± 0,38	0,21 ± 0,09

In the quantitative analysis of bacterial abundance across the components of the aquaponic system, the fish tank and biofilter exhibited the highest nitrifying bacteria abundances (Figure 2). This trend is likely influenced by the combination of nitrogenous waste from fish excretion and the continuous aeration typically present in these components, which together create favorable conditions for the proliferation of nitrifying bacteria. Although dissolved oxygen levels were not measured in this study, similar patterns have been reported by Schmautz *et al.* (2021), who demonstrated that higher oxygen availability promotes nitrifier activity and abundance. Furthermore, despite the biofilter representing only one-quarter of the volume of the fish tank, it incorporated a plastic substrate with a high specific surface area that likely enhanced bacterial colonization and biofilm formation (Aguirre *et al.* 2023).

In contrast, the hydrocyclone exhibited the lowest bacterial abundance. This component primarily functions to facilitate the sedimentation of suspended solids, such as feces and uneaten food, for subsequent removal from the system (Ramírez Sánchez *et al*. 2011). Notably, the hydrocyclone does not feature aeration, as it could resuspend the particles being settled. As a result, low oxygen concentrations in this component likely contributed to the observed reduction in bacterial abundance, particularly nitrifying bacteria. Anoxic conditions generated within the hydrocyclone likely hindered bacterial growth, as these environments are unfavorable for nitrification (Schmautz *et al*. 2021). This hypothesis is supported by Carrasquero Ferrer *et al.* (2014), who found a positive correlation between elevated oxygen levels and enhanced nitrification rates.

Additionally, the variability of bacterial abundance was examined over time, using the average abundance of each component. The results showed that bacterial abundance generally increased over time, as illustrated in Figure 2.

Assessing bacterial abundance over time revealed the application of the STR Species-time reactions for Bacteria model (Oliver *et al.* 2012). This model elucidates relationships within ecological communities by defining patterns that illustrate fluctuations in abundance, diversity, and richness over time in bacterial communities. These findings reinforce the results: as the system matures, a symbiotic relationship evolves between bacterial communities and the developing environment, leading to lower initial abundance and a subsequent increase in later stages (Torres-Mesa *et al.* 2023).

This study represents the first report of bacterial abundance in aquaponic systems using the MPN technique. Previous research, however, has documented bacterial abundance at the molecular level. For instance, Mehrani *et al.* (2020) identified the genus *Nitrospira* with a relative abundance of 3.8% through 16S rRNA gene sequencing. Similarly, Bartelme *et al.* (2019) highlighted *Nitrospira* as the predominant taxon across all aquaponic system components, emphasizing its role in nutrient recycling and nitrification. A study by Castro-González *et al.* (2023) also found *Nitrospira* to be the dominant genus in all compartments of an aquaponic system from the fourth month onwards. In contrast, *Nitrosomonas* exhibited minimal abundance in the biofilter (< 5%), while archaea oxidizers such as *Candidatus Nitrosopelagicus*, *Candidatus Nitrosopumilus*, and *Candidatus Nitrosotalea* were detected at low levels (< 10%) in the fish tank (Castro-González *et al*. 2023).

The bacterial community's abundance according to the nutrient system. Several interactions were established between the nutrients measured in the aquaponic system and the abundance of nitrifying bacteria (Figure 3).

The nutrients that showed a strong positive correlation were Potassium with a correlation of 0.957, Phosphate with 0.697, and Manganese with 0.669. In addition, a high negative relationship between nitrite and abundance of -0.767 was found (Figure 4). These results displayed notable correlations with bacterial abundance, likely due to their pivotal cellular roles.

On the other hand, ammonia did not show a clear trend or correlation with bacterial abundance (-0.123), despite its known role as a primary substrate for nitrifying bacteria (Figure 4). This weak correlation may be explained by the fact that nitrifying communities, such as *Nitrosomonas* and *Nitrobacter*, thrive within specific ammonia concentration ranges. Optimal ammonia levels for *Nitrosomonas* activity are typically reported between 1–5 mg/L, beyond which inhibition or substrate limitation may occur depending on system conditions (Prosser, 1990; Vadivelu *et al.* 2007). Similarly, *Nitrobacter* requires sufficient nitrite levels derived from ammonia oxidation but is also sensitive to environmental fluctuations such as pH and oxygen availability. In this study, it is possible that the ammonia concentrations were either below or above optimal thresholds, thus limiting a stronger association with bacterial abundance.

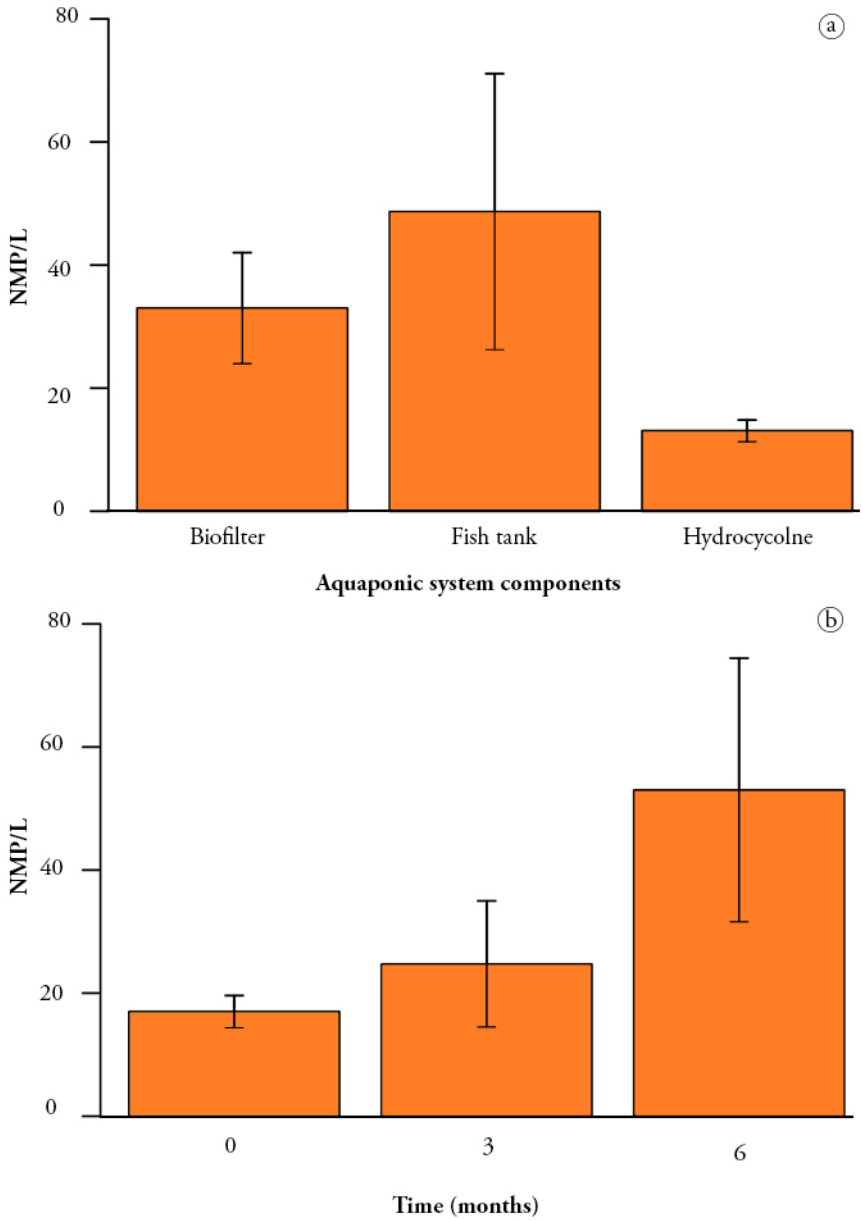


Figure 2. Variation in bacterial abundance (MPN/L). a) at the different aquaponic system components; b) at the different months of operation

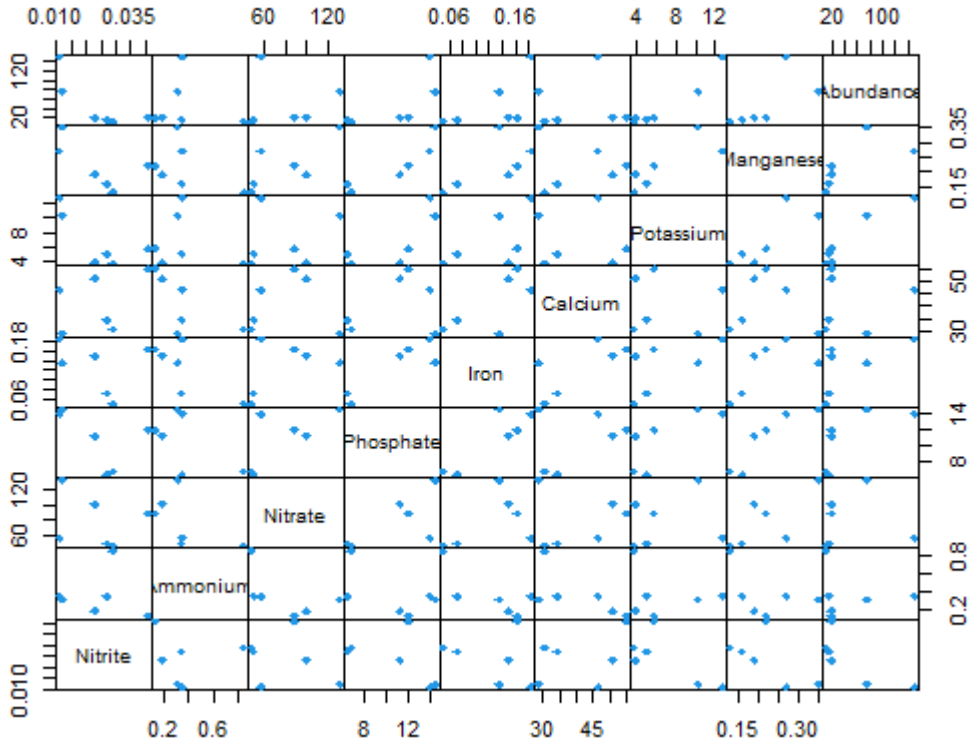


Figure 3. Pearson's correlation matrix, showing the relationships among nutrient concentrations and bacterial abundance in aquaponic systems S1 and S2.

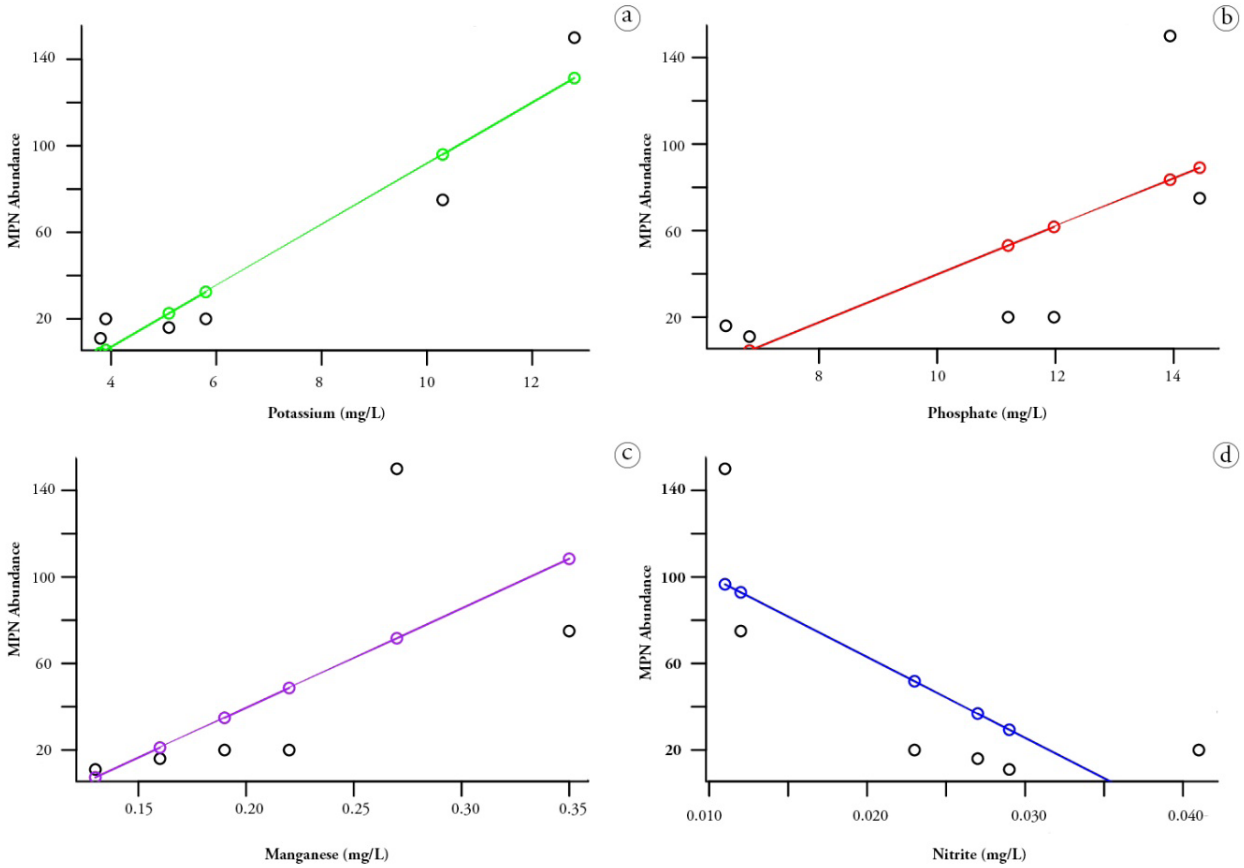


Figure 4. Pearson's correlation between bacterial abundance (MPN) and key physicochemical parameters in an aquaponic system. a. Potassium; b. Phosphate; c. Manganese; d. Nitrite.

The elevated correlation of potassium and the abundance of 66-99% reveal a crucial role in the growth and activity of nitrifying bacteria. Underhill & Prosser (1985) found that potassium ethyl xanthate can induce a prolonged lag in nitrification, while Welch & Scott (1959) established that low potassium levels can inhibit nitrification. Lees & Quastel (1945) further supported this, demonstrating the bacteriostatic effects of potassium chlorate on soil nitrification. Although aquaponic systems are deficient in potassium, perhaps potassium was maintained at relatively high concentrations when potassium hydroxide was added to stabilize the system's pH (Torres-Mesa *et al.* 2023). The potassium contributes to enzyme activation, protein synthesis, and forms a part of teichoic acids in Gram-positive cell walls (Caycedo Lozano *et al.* 2021).

Phosphate is also vital, as its deficiency can inhibit nitrifying bacteria and reduce nitrification efficiency (Nishio *et al.* 2008, Nishio *et al.* 2002). Furthermore, the addition of phosphate has been shown to increase nitrification activity, potentially promoting ammonification by heterotrophic bacteria (Nishio *et al.* 2002). This highlights the importance of phosphate for the growth and activity of nitrifying bacteria.

Manganese is essential for resistance to nitrosative stress, a key component of the innate immune response to bacterial pathogens (Yousuf *et al.* 2020). Manganese oxidation by bacteria is also important in biogeochemical cycles, particularly in the oxidative cycling of Mn in aquatic environments (Sujith & Bharathi 2011). In addition, biomineralization of manganese oxide, a process involving manganese oxidation, is a social trait that protects against nitrite toxicity in bacteria (Zerfaß *et al.* 2018). Manganese is involved in various biochemical processes, such as water splitting and oxygen production, and is a cofactor for enzymes involved in the detoxification of reactive oxygen species (Caycedo Lozano *et al.* 2021).

The negative correlation observed between nitrite and bacterial abundance may be attributed to nitrite accumulation, which is known to inhibit the growth of nitrifying bacteria, particularly *Nitrobacter* (Papp *et al.* 2016). Alternatively, this could reflect the rapid conversion of nitrite into nitrate by nitrite-oxidizing bacteria. Conversely, ammonium showed no clear trend or significant correlation with bacterial abundance (-0.123), despite being a primary substrate for ammonia-oxidizing bacteria such as *Nitrosomonas* (French *et al.* 2012). This lack of association may be explained by low ammonium concentrations in the system, potentially resulting from elevated dissolved oxygen levels in surface layers that favor rapid oxidation of ammonium (Hernández *et al.* 2013). *Nitrosomonas* typically thrives in environments with ammonium concentrations between 1–5 mg/L, and its activity may be limited when concentrations fall below this range (Vadivelu *et al.* 2007). Similarly, *Nitrobacter* prefers moderate nitrite availability but can be inhibited under conditions of nitrite accumulation or oxygen limitation. Given the continuous aeration and efficient nitrification processes in the aquaponic system studied, it is likely that substrate levels for both bacterial groups remained below threshold levels for significant variation in abundance, thus explaining the weak correlation.

Isolation and characterization of nitrifying bacteria. A protocol was standardized for the adequate growth and development of nitrifying bacteria isolated from aquaponic systems. The temperature and pH where the optimal results were obtained were 21.5°C, and pH 6.6. These results coincided with those observed *in situ* in the aquaponic systems evaluated.

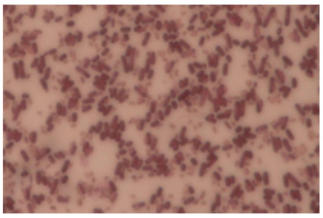
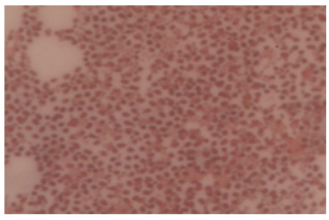
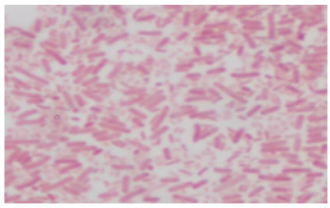
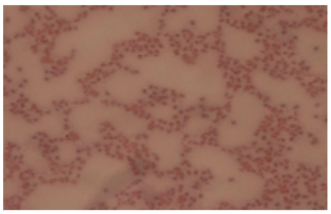
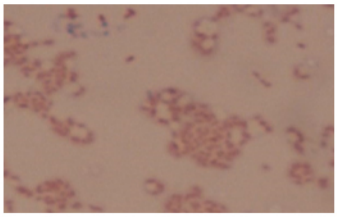
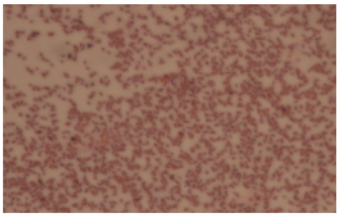
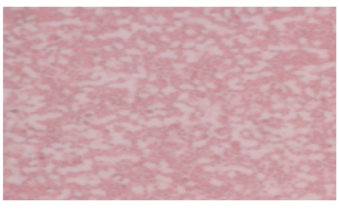
Despite their abundance in the environment, cultivation of nitrifying bacteria remains a challenge, as the nitrification process produces little energy, so the bacteria grow slowly (Farges *et al.* 2012). Cultivating these bacteria is hindered by slow and infrequent growth due to pH decreases and nitrite accumulation, impacting their metabolism (Papp *et al.* 2016). However, these challenges can shift when bacteria exist in a mixed environment where nitrite accumulation is rare, and pH changes are regulated (Farges *et al.* 2012).

Nine strains were isolated and characterized macroscopically, all of which were gram-negative, except for strain M9, which was gram-positive and exhibited a unique spiral morphology (Table 3). However, strain M9 was the only one that could not be purified; for this reason, enzymatic characterization was not performed (Table 3). Most morphotypes had a bright appearance (BA), smooth edges (SE), all were soft in consistency (SC), some exhibited flat elevation (FE), and others presented convex elevation (CE). Two gram-negative cocci morphotypes (M2 and M7) were isolated, along with four gram-negative coccobacillus morphotypes (M4, M5, M6, and M8), and two gram-negative bacillus (M1 and M3).

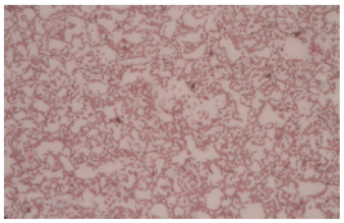
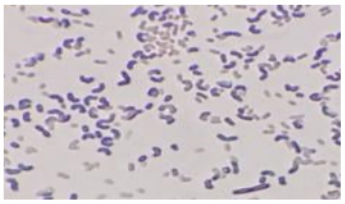
All isolated morphotypes were positive for oxidase, inositol, citrate, CO_2, and growth on MacConkey agar. However, the biochemical urea hydrolysis test was negative, which is of particular importance for the isolation of nitrifying bacteria. In the catalase test, most strains were negative except for morphotypes M1 and M7. Half of the morphotypes exhibited motility (M1, M6, M7, M8) while the other half did not. M1 was the only morphotype positive for indole and iron oxidation. Morphotypes like M5 and M6 can utilize arginine and lysine as their main amino acids for metabolism, unlike M2, M4, M7, and M8, which tested negative for both. M1 and M6 can use both glucose and lactose as a sugar source. On the other hand, M2 and M8 seem to use another sugar source as they tested negative for both (Table 3).

Regarding macro, micro, and biochemical characterizations performed on the isolates (Tables 2 and 3), it's plausible to assume that certain morphotypes such as M1, M3, and M7 could correspond to *Nitrobacter* sp., *Nitrospina* sp., and *Nitrosococcus* sp. based on their characteristics (Kim & Gadd, 2008; Kouki *et al.* 2011). However, precise identification necessitates molecular testing. Similarly, M9 could potentially represent *Nitrospira*, exhibiting a gram-positive, spirilloid morphology commonly found in freshwater (Kim & Gadd, 2008). *Nitrospira*, also known as Comammox (Complete ammonia oxidation), was detected in the same aquaponic system (Castro-González *et al.* 2023) and reported in other aquaponic systems (Bartelme *et al.* 2019; Mehrani *et al.* 2020).

Table 3. Morphological and biochemical characterization of bacterial morphotypes isolated from the aquaponic system.

Morphotype	Morphological traits	Biochemical profile	Image (1000×, Gram stain)
M1	BA, SE, SC, FE; Bacillus (Gram -)	OX+, CAT–, MOT+, IND+, UREA–, LYS–, GLU–, LAC+, ARG+, INO+, TSI+, CIT+, MAC+, MRS–, CO_2+	
M2	BA, SE, SC, CE; Coccus (Gram -)	OX+, CAT+, MOT–, IND–, UREA–, LYS–, GLU–, LAC–, ARG–, INO+, TSI–, CIT+, MAC+, MRS–, CO_2+	
M3	TA, SE, SC, FE; Bacillus (Gram -)	OX+, CAT+, MOT–, IND–, UREA–, LYS–, GLU+, LAC–, ARG+, INO+, TSI–, CIT+, MAC+, MRS–, CO_2+	
M4	BA, SE, SC, FE; Coccobacillus (Gram -)	OX+, CAT+, MOT–, IND–, UREA–, LYS–, GLU–, LAC+, ARG–, INO+, TSI–, CIT+, MAC+, MRS–, CO_2+	
M5	TA, SE, SC, FE; Coccobacillus (Gram -)	OX+, CAT+, MOT–, IND–, UREA–, LYS+, GLU+, LAC–, ARG+, INO+, TSI–, CIT+, MAC+, MRS–, CO_2+	
M6	TA, SE, SC, FE; Coccobacillus (Gram -)	OX+, CAT+, MOT+, IND–, UREA–, LYS+, GLU+, LAC+, ARG+, INO+, TSI–, CIT+, MAC+, MRS+, CO_2+	
M7	BA, SE, SC, FE; Coccus (Gram -)	OX+, CAT–, MOT+, IND–, UREA–, LYS–, GLU–, LAC+, ARG–, INO+, TSI–, CIT+, MAC+, MRS–, CO_2+	

Continuación tabla 3.

M8	BA, SE, SC, CE; Coccobacillus (Gram -)	OX+, CAT+, MOT+, IND–, UREA–, LYS–, GLU–, LAC–, ARG–, INO+, TSI–, CIT+, MAC+, MRS–, CO_2+	
M9	BA, SE, SC, CE; Spirillum (Gram +)	Not evaluated	

Macroscopic characteristics are described using the following codes: BA = Bright Appearance, TA = Translucent Appearance, SE = Smooth Edge, IE = Irregular Edge, SC = Soft Consistency, CE = Convex Elevation, FE = Flat Elevation. Microscopic morphology was determined by Gram staining at 1000× magnification using oil immersion. Biochemical tests included oxidase, catalase, motility, indole, urea hydrolysis, lysine decarboxylation, glucose/lactose/arginine/inositol fermentation, triple sugar iron (TSI) test, citrate utilization, MacConkey agar growth, MRS medium growth, and CO_2 production. Symbols: (+) positive result; (–) negative result.

No existing studies were found to compare morphotypes M2, M4, M5, M6, and M8 concerning their morphology and described characteristics. It's crucial to note that these comparisons are speculative, based on other studies characterizing nitrifying bacteria. Therefore, molecular tests are recommended to validate this information.

None of the isolated morphotypes demonstrated positive urease activity (Table 3). According to Castellanos-Rozo & Ramos-Parra (2015), urease activity can influence ammonium oxidation by inhibiting the process in certain microorganisms. Similar findings were reported by Masmela-Mendoza *et al.* (2019) in bacteria isolated from pisciculture environments. Furthermore, Koops *et al.* (2006) describe urea hydrolysis as a physiological response often associated with eutrophic conditions, particularly in systems with nutrient overload, such as stabilization ponds. In contrast, aquaponic systems are designed to maintain strict control over water quality parameters, minimizing nutrient accumulation and thus preventing eutrophication. Therefore, the absence of urease activity in the isolates analyzed may reflect the effective nutrient regulation within the studied aquaponic system, rather than being an indicator of eutrophication.

Additionally, the oxidase-catalase results indicated that the isolated microorganisms are primarily aerobic, except for M1 and M7, which lack the catalase enzyme, implying they could be facultative anaerobes (Fernández *et al.* 2010). M1 was the only strain demonstrating indole degradation via xindole, isatin, and anthranilic acid (Li *et al.* 2009). This morphotype was also the sole one capable of oxidizing Fe (II) into Fe (III), utilizing three types of sugars (dextrose, lactose, and sucrose) a trait found in certain nitrifying bacteria (Kim & Gadd, 2008). M6 exhibited growth in both MacConkey and MRS, indicating a potential weakening of bacteria upon isolation. It's essential to note that the system also harbors other microorganisms like archaea and other uncultivable bacteria (Schmautz *et al.* 2021).

The quantification of nitrifying bacteria using the MPN method revealed their high abundance throughout the entire aquaponic system. Among the system components, the fish tank stood out with the highest bacterial abundance, correlated with elevated nutrient levels and excellent oxygenation. The isolation of nine distinct morphotypes, including a noteworthy gram-positive spirillum, underscores the potential diversity within the system. Further complementary analyses, such as molecular, biochemical, and metagenomic techniques, are recommended to solidify the identity of these isolated bacterial morphotypes. These additional investigations will confirm the identity of the isolated bacteria and delve into their functional roles in enhancing nutrient recycling efficiency within aquaponic systems, thereby fostering environmental sustainability.

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REFERENCES

AGUIRRE, J.P.; TORRES-MESA, A.; PÉREZ-TRUJILLO, M.M.; RUBIO-CASTRO, S.A.; GÓMEZ-RAMÍREZ, E. 2023. Evaluation of *Fragaria* x *ananassa* in an aquaponic system with *Oncorhynchus mykiss* and substrate culture conditions. AACL Bioflux. 16(5):2589-2600.

AMERICAN PUBLIC HEALTH ASSOCIATION – APHA. 2017. Standard methods for the examination of water and wastewater. Volume 3. Andesite Press. 138p.

BARTELME, R.P.; OYSERMAN, B.O.; BLOM, J.E.; SEPULVEDA-VILLET, O.J.; NEWTON, R.J. 2018. Stripping away the soil: plant growth promoting microbiology opportunities in aquaponics. Frontiers in Microbiology. 9:8. https://doi.org/10.3389/fmicb.2018.00008

BARTELME, R.P.; SMITH, M.C.; SEPULVEDA-VILLET, O.J.; NEWTON, R.J. 2019. Component microenvironments and system biogeography structure microorganism distributions in recirculating aquaculture and aquaponic systems. mSphere. 3(4):143-19. https://doi.org/10.1128/msphere.00143-19

BIROLO, M.; BORDIGNON, F.; TROCINO, A.; FASOLATO, L.; PASCUAL, A.; GODOY, S.; NICOLETTI, C.; MAUCIERI, C.; XICCATO, G. 2020. Effects of stocking density on the growth and flesh quality of rainbow trout (*Oncorhynchus mykiss*) reared in a low-tech aquaponic system. Aquaculture. 529:735653. https://doi.org/10.1016/j.aquaculture.2020.735653

BRITTO, D.T.; KRONZUCKER, H.J. 2013. Ecological significance and complexity of N-source preference in plants. Annals of Botany. 112(6):957-963. https://doi.org/10.1093/aob/mct157

CANFIELD, D.E.; GLAZER, A.N.; FALKOWSKI, P.G. 2010. The evolution and future of earth's nitrogen cycle. Science. 84(330):192-196. https://doi.org/10.1126/science.1186120

CARRASQUERO FERRER, S.J.; PIRE SIERRA, M.C.; COLINA ANDRADE, G.; MAS Y RUBÍ, M.; MARTÍNEZ, D.; DÍAZ MONTIEL, A. 2014. Tasas de nitrificación y desnitrificación durante el tratamiento biológico de efluentes de tenerías en un reactor por carga secuencial. Boletín del Centro de Investigaciones Biológicas. 47(3):220-234.

CASTELLANOS-ROZO, J.; RAMOS-PARRA, Y.J. 2015. Caracterización de bacterias oxidadoras de amonio aisladas del humedal de la planta de tratamiento de aguas residuales de la Universidad de Boyacá. I3+. 2(1):82-95. https://doi.org/10.24267/23462329.78

CASTRO-GONZÁLEZ, M.; MOLINA-TRINCADO, V.; GÓMEZ-RAMÍREZ, E.; NARANJO-ROBAYO, N. 2023. Nitrous oxide production and microbial diversity in an aquaponic system. AACL Bioflux. 16(6):3032-3047.

CAYCEDO LOZANO, L.; CORRALES RAMÍREZ, L.C.; TRUJILLO SUÁREZ, D.M. 2021. Las bacterias, su nutrición y crecimiento: una mirada desde la química. Nova. 19(36):49-94. https://doi.org/10.22490/24629448.5293.

CHANDRAPATI, S.; WILLIAMS, M.G. 2014. Total viable counts | Most Probable Number (MPN). In: Batt, C.A.; Tortorello, M.L. (Eds.). Encyclopedia of Food Microbiology. Second Edition. Academic Press. p.621-624. https://doi.org/10.1016/B978-0-12-384730-0.00333-5

CHAUHAN, A.; JINDAL, T. 2020. Biochemical and molecular methods for bacterial identification. In: Chauhan, A.; Jindal, T. Microbiological methods for environment, food and pharmaceutical analysis. Springer. p.425-468. https://doi.org/10.1007/978-3-030-52024-3_10

FARGES, B.; POUGHON, L.; RORIZ, D.; CREULY, C.; DUSSAP, C.G.; LASSEUR, C. 2012. Axenic cultures of *Nitrosomonas europaea* and *Nitrobacter winogradskyi* in autotrophic conditions: a new protocol for kinetic studies. Applied Biochemistry and Biotechnology. 167(5):1076-1091. https://doi.org/10.1007/s12010-012-9651-6

FERNÁNDEZ, A.; GARCIA DE LA FUENTE, C.; SAEZ, J.; VALDEZATE, S. 2010. Métodos de identificación bacteriana en el laboratorio de microbiología. Seimc. 9(1):1-28.

FRENCH, E.; KOZLOWSKI, J.A.; MUKHERJEE, M.; BULLERJAHN, G.; BOLLMANN, A. 2012. Ecophysiological characterization of ammonia-oxidizing archaea and bacteria from freshwater. Applied and Environmental Microbiology. 78(16):5773-5780. https://doi.org/10.1128/AEM.00432-12

GODDEK, S.; ESPINAL, C.A.; DELAIDE, B.; JIJAKLI, M.H.; SCHMAUTZ, Z.; WUERTZ, S.; KEESMAN, K.J. 2016. Navigating towards decoupled aquaponic systems: A system dynamics design approach. Water. 8:303. https://doi.org/10.3390/w8070303

GYAMFI, S.; EDZIYIE, R.E.; OBIRIKORANG, K.A.; ADJEI-BOATENG, D.; SKOV, P.V. 2024. Nile tilapia (O*reochromis niloticus*) show high tolerance to acute ammonia exposure

but lose metabolic scope during prolonged exposure at low concentration. Aquatic Toxicology. 271:106932. https://doi.org/10.1016/j.aquatox.2024.106932

HERNÁNDEZ, E.; AGUIRRE, N.; PALACIO, J.; RAMÍREZ, J.J.; DUQUE, S.R.; GUISANDE, C.; ARANGUREN, N.; MOGOLLÓN, M. 2013. Evaluación comparativa de algunas características limnológicas de seis ambientes leníticos de Colombia. Revista Facultad de Ingeniería Universidad de Antioquia. 69:216-228. https://doi.org/10.17533/udea.redin.18151

KIM, B.H.; GADD, G.M. 2008. Bacterial physiology and metabolism. Cambridge University Press. United Kingdom. 512p. https://doi.org/10.1017/CBO9780511790461

KOOPS, H.P.; PURKHOLD, U.; POMMERENING-RÖSER, A.; TIMMERMANN, G.; WAGNER, M. 2006. The Lithoautotrophic Ammonia-Oxidizing Bacteria. In: Dworkin, M.; Falkow, S.; Rosenberg, E.; Schleifer, K.H.; Stackebrandt, E. (eds). The Prokaryotes: A Handbook on the Biology of Bacteria. 3rd ed., Volume 5, Proteobacteria: Alpha and Beta Subclasses. Springer. Germany. p.778-812.

KOUKI, S.; SAIDI, N.; M'HIRI, F.; NASR, H.; CHERIF, H.; OUZARI, H.; HASSEN, A. 2011. Isolation and characterization of facultative mixotrophic ammonia-oxidizing bacteria from constructed wetlands. Journal of Environmental Sciences. 23(10):1699-1708.

LAL, J.; VAISHNAV, A.; DEB, S.; KASHYAP, S.; DEBBARMA, P.; DEVATI; GAUTAM, P.; PAVANKALYAN, M.; KUMARI, K.; VERMA, D.K. 2024. Re-circulatory aquaculture systems: A pathway to sustainable fish farming. Archives of Current Research International. 24(5):799-810. https://doi.org/10.9734/acri/2024/v24i5756

LEES, H.; QUASTEL, J.H. 1945. Bacteriostatic effects of potassium chlorate on soil nitrification. Nature. 155(3931):276-278. https://doi.org/10.1038/155276A0

LI, P.; TONG, L.; LIU, K.; WANG, Y.; WANG, Y. 2009. Indole degrading of ammonia oxidizing bacteria isolated from swine wastewater treatment system. Water Science & Technology. 59(12):2405-2410. https://doi.org/10.2166/wst.2009.312

LOVE, D.C.; FRY, J.P.; GENELLO, L.; HILL, E.S.; FREDERICK, J.A.; LI, X.; SEMMENS, K. 2014. An international survey of aquaponics practitioners. PLoS One. 9:1-10. https://doi.org/10.1371/journal.pone.0102662

LOVE, D.C.; UHL, M.S.; GENELLO, L. 2015. Energy and water use of a small-scale raft aquaponics system in Baltimore, Maryland, United States. Aquacultural Engineering. 68:19-27. https://doi.org/10.1016/j.aquaeng.2015.07.003

MAIER, R. 2000. Biogeochemical cycling. In: Maier, R.; Pepper, I.; Gerba, C. (eds). Environmental Microbiology. Academic Press. San Diego, USA. 585p.

MASMELA-MENDOZA, J.E.; LIZARAZO-FORERO, L.M.; ARANGUREN RIAÑO, N.J. 2019. Bacterias nitrificantes cultivables de la zona limnética del lago de Tota, Boyacá. Revista U.D.C.A Actualidad & Divulgación Científica. 22(2):1378. https://doi.org/10.31910/rudca.v22.n2.2019.1378

MEHRANI, M.J.; SOBOTKA, D.; KOWAL, P.; CIESIELSKI, S.; MAKINIA, J. 2020. The occurrence and role of Nitrospira in nitrogen removal systems. Bioresource Technology. 3:13-21.

MUNGUIA-FRAGOZO, P.; ALATORRE-JACOME, O.; RICO-GARCIA, E.; TORRES-PACHECO, I.; CRUZ-HERNANDEZ, A.; OCAMPO-VELAZQUEZ, R.V.; GARCIA-TREJO, J.F.; GUEVARA-GONZALEZ, R.G. 2015. Perspective for Aquaponic Systems: "Omic" Technologies for Microbial Community Analysis. BioMed Research International. 2015:480386. https://doi.org/10.1155/2015/480386

NISHIO, T.; SHINYA, M.; FUJIWARA, Y.; YOSHIKURA, T.; FUKUYAMA, J. 2008. Phosphate deficiency and its effect on nitrification in the pond of a sea-based solid waste disposal site. Japanese Journal of Water Treatment Biology. 44(3):139-148. https://doi.org/10.2521/JSWTB.44.139

NISHIO, T.; SHINYA, Y.; YOSHIKURA, T.; FUKUYAMA, J. 2002. Effects of phosphate on nitrifying activity in a water treatment plant at low temperature. Japanese Journal of Water Treatment Biology. 38(1):21-28. https://doi.org/10.2521/JSWTB.38.21

OLIVER, A.; LILLEY, A.K.; VAN DER GAST, C.J. 2012. Species-time relationships for bacteria. In: Franklin, R.; Mills, A. (eds). Microbial Ecological Theory: Current Perspectives. ASM Press. Washington, DC, USA. p.71-86.

OWEN, A.; JONES, D. 2001. Competition for acids between wheat roots and rhizosphere microorganisms and the role of amino acids in plant N acquisition. Soil Biology and Biochemistry. 33(4-5):651-657. https://doi.org/10.1016/S0038-0717(00)00209-1

PAPP, B.; TÖRÖK, T.; SÁNDOR, E.; FEKETE, E.; FLIPPHI, M.; KARAFFA, L. 2016. High cell density cultivation of the chemolithoautotrophic bacterium *Nitrosomonas europaea*. Folia Microbiologica. 61(3):191-198. https://doi.org/10.1007/s12223-015-0425-8

PROSSER, J.I. 1990. Autotrophic nitrification in bacteria. Advances in Microbial Physiology. 30:125-181. https://doi.org/10.1016/S0065-2911(08)60112-5

RAMÍREZ SÁNCHEZ, L.M.; PÉREZ TRUJILLO, M.M.; JIMÉNEZ, P.; HURTADO GIRALDO, H.; GÓMEZ RAMÍREZ, E. 2011. Evaluación preliminar de sistemas acuapónicos e hidropónicos en cama flotante para el cultivo de orégano (*Origanum vulgare*: Lamiaceae). Revista Facultad de Ciencias Básicas. 7(2):242-259.

SCHMAUTZ, Z.; ESPINAL, C.A.; BOHNY, A.M.; REZZONICO, F.; JUNGE, R.; FROSSARD, E.; SMITS, T.H. 2021. Environmental parameters and microbial community profiles as indication towards microbial activities and diversity in aquaponic system compartments. BMC Microbiology. 21:12. https://doi.org/10.1186/s12866-020-02075-0

SOMERVILLE, C.; COHEN, M.; PANTANELLA, E.; STANKUS, A.; LOVATELLI, A. 2014. Small-scale aquaponic food production. Integrated fish and plant farming; FAO Fisheries and Aquaculture Technical Paper. Food and Agriculture Organization of the United Nations. Rome, Italy. 589p.

SUJITH, P.P.; BHARATHI, P.A. 2011. Manganese oxidation by bacteria: biogeochemical aspects. In: Müller, W. (eds). Molecular Biomineralization. Progress in Molecular and Subcellular Biology. Volume 52. Springer. Berlin, Heidelberg. p.49-76. https://doi.org/10.1007/978-3-642-21230-7_3

TORRES-MESA, A.; CIFUENTES-TORRES, L.; HURTADO-GIRALDO, H.; GOMÉZ-RAMIREZ, E. 2023. Evaluation of plant-fish ratio in an aquaponic system with different stocking densities of *Cyprinus carpio* and *Carassius auratus*. AACL Bioflux. 16(1):606-615.

UNDERHILL, S.E.; PROSSER, J.I. 1985. Inhibition of nitrification by potassium ethyl xanthate in soil and in liquid culture. Soil Biology and Biochemistry. 17(2):229-233. https://doi.org/10.1016/0038-0717(85)90119-1

VADIVELU, V.M.; KELLER, J.; YUAN, Z. 2007. Effect of free ammonia on the respiration and growth processes of an enriched *Nitrosomonas* culture. Water Research. 41(4):826-834. https://doi.org/10.1016/j.watres.2006.11.030

VERHAGEN, M.; LAANBROCK, J. 1991. Competition for limiting amounts of ammonium between nitrifying and heterotrophic bacteria in dual energy-limited chemostats. Applied and Environmental Microbiology. 57:3255-3263. https://doi.org/10.1128/AEM.57.11.3255-3263.1991

WELCH, L.F.; SCOTT, A.D. 1959. Nitrification in nutrient solutions with low levels of potassium. Canandian Journal of Microbiology. 5(5):425-430. https://doi.org/10.1139/M59-053

WONGKIEW, S.; POPP, B.N.; KHANAL, S.K. 2018. Nitrogen recovery and nitrous oxide (N_2O) emissions from aquaponic systems: influence of plant species and dissolved oxygen. International Biodeterioration & Biodegradation. 134:117-126. https://doi.org/10.1016/j.ibiod.2018.08.008

WONGKIEW, S.; POPP, B.N.; KIM, H.; KHANAL, S.K. 2017. Fate of nitrogen in floating-raft aquaponic systems using natural abundance nitrogen isotopic compositions. International Biodeterioration & Biodegradation. 125:24-32. https://doi.org/10.1016/j.ibiod.2017.08.006

YOUSUF, S.; KARLINSEY, J.E.; NEVILLE, S.L.; MCDEVITT, C.A.; LIBBY, S.J.; FANG, F.C.; FRAWLEY, E.R. 2020. Manganese import protects *Salmonella enterica* serovar Typhimurium against nitrosative stress. Metallomics (United Kingdom). 12(11):1791-1801. https://doi.org/10.1039/d0mt00178c

ZERFAß, C.; CHRISTIE-OLEZA, J.A.; SOYER, O.S. 2018. Manganese oxide biomineralization is a social trait protecting against nitrite toxicity. BioRxiv 294975. 85(92).26. https://doi.org/10.1128/AEM.02129-18