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EVALUATION OF WATER QUALITY PARAMETERS OF AN AQUAPONIC SYSTEM USING RED-BELLIED PACU (*Piaractus brachypomus*), STRAWBERRY (*Fragaria x ananassa*) AND BASIL (*Ocimum basilicum*)*

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Abstract: The physicochemical and microbiological parameters of water were evaluated in an aquaponic system for the production of red-bellied pacu ("*cachama blanca*"), strawberry and basil. Water samples were analyzed at the inlets and outlets of the system and in the three seeding methods employed - Nutrient Film Technique (NFT), floating root, and gravel bed - over three months. At the end of the productive process, nitrifying bacteria normal for this type of system were found. Chitinolytic bacteria, fundamental to aquatic cultures, were also growing, with colonies being found of bacteria of the *Bacillus* genus that assist in improving conditions in this environment, in particular *Bacillus subtilis*, important in modification of the aquaculture microbiota. Similarly, the presence was identified of phosphorus solubilizing fungi, which improve nodulation, nitrogen fixation, growth and crop yield. In the physicochemical parameters, variation in the final temperature was observed in each crop and bed. In terms of pH, variations were found in the final measurements. The ammonium variable showed decreases in the average final value, while nitrites remained stable at the beginning and at the end of each measurement in both cultures and in each bed. Finally, nitrate concentrations were seen to decrease in the floating beds for both cultures, by more than 20 mg/L.
Keywords: Aquaponic systems, physicochemical parameters, microbiological load of water, water quality.

INTRODUCTION

Aquaponics combines the cultivation of aquatic animals such as fish, molluscs, crustaceans and plants in controlled environments, where organic waste produced by some aquatic organism, generally fish, is sought to be converted through the action of bacteria into nitrates that serve as food source for plants. The plants then perform the function of cleaning the water by acting as a biological filter (Ramirez *et al*., 2008

Taking account of the operation and the important actors within an aquaponic system, Bernal (2017) mentions that the fish take advantage of approximately 30% of the concentrate, which is transformed into excrement and the remaining 70% ends up not being transformed, thus forming deposits at the bottom of the pond, where a high content of nutrients and organic material is generated. This fraction of nutrients can be used for plant growth, a process carried out by the bacteria incorporated in the system.

Nitrifying bacteria (*Nitrosomas* sp. and *Nitrobacter* sp.) that form an important part of the aquaponics process, live in a wide variety of habitats, including freshwater, drinking water, wastewater, seawater, brackish water, and in the soil. The main genera of nitrifying bacteria in activated sludge use carbon dioxide or inorganic carbon for the synthesis of cellular material (Avendaño, 2012). For each molecule of carbon dioxide assimilated, approximately 30 molecules of the ammonium ion or 100 molecules of nitrite must be oxidized (Zornoza *et al*., 2012). These bacteria fulfill the function of colonizing the substrates of the biofilter and of the seeding methods implemented, since when the fish are released into the tank the ammonia level increases due to the feces they produce. It is here that the *Nitrosomas* sp. bacteria colonize the system and are responsible for converting ammonium into nitrite, whereupon the ammonium concentration tends to decrease and nitrite levels increase (Somerville *et al*., 2022). *Nitrobacter* sp. bacteria similarly intervene, converting nitrites into nitrates, this being the assimilable form for plants (Paco Alanoca, 2020).

Moreover, the physicochemical parameters (temperature, pH, ammonium, nitrites, nitrates) play an important role, since they make it possible to identify whether or not it falls within the normal ranges, to evaluate the quality of the water. Quality control in an aquaponic system is essential to obtain performance in both fish and plant breeding, so the deterioration of this resource will alter the development, growth, reproduction and could even lead to the mortality of the cultivated species (Gomez *et al*., 2022).

In addition, the plant can be considered as a biofilter for fish in a mutually beneficial symbiotic relationship by absorbing nitrates that run through the pipes, optimizing the biomass growth therein (Somerville *et al*., 2022). Carbon, nitrogen and phosphates are likewise extracted from the aquaculture system, maintaining ion concentrations at adequate levels, which favors the development of fish (Gómez, 2019).

As such, culture of Red-bellied pacu (known locally as *cachama blanca*) was implemented to be used experimentally in the aquaponic system due to their rapid growth rate and resistance to poor water quality and diseases, in addition to their tolerance to a wide range of environmental conditions (Eslava, 2009), with the aim of evaluating the physicochemical and microbiological parameters in water of an aquaponic system for the production of red-bellied pacu (*P. brachypomus*), strawberry (*Fragaria* x ananassa) and basil (*O. basilicum*).

MATERIALS AND METHODS

Study area. The samples were taken in the aquaponic system built on the farm of the Fundación Universitaria de Popayán, in the *vereda* or hamlet of Los Robles, in the municipality of Timbío, in the department of Cauca, 8 km southwest of the city of Popayán, on the Pan-American highway. It is located on the western flank of the central mountain range with geographic coordinates of 20° 2' north latitude and 76° 40' west longitude, at a height of 1850 m.s.n.m.

To comply with the proposed objectives, samples of water were taken at the entrances and exits of the fish tank in which red-bellied pacu were being farmed and in the planting methods - NFT (Nutrient Film Technique), floating root, and gravel bed, in which the basil and strawberry crops were planted. It is worth mentioning that water sampling was conducted over 3 months every 15 days, to carry out analysis of the physical-chemical and microbiological quality parameters of the water.

MEASUREMENT OF PHYSICAL PARAMETERS

Temperature. It is important to highlight that the temperature, in enclosed spaces, becomes a variable with the highest stability, since this could affect the metabolic rate of the cultured organisms (National Authority of Aquaculture and Fishing, 2019). In addition, they mention that this physical parameter contributes to determining the solubility of oxygen, the rate of photosynthesis and the distribution between the ammonium and ammonia fractions. That is why this parameter was recorded at each water inlet and outlet of the aquaponic system, using a mercury thermometer that was submerged in the water for two minutes until the red line reached the scale marking the degrees Celsius corresponding to the sample.

MEASUREMENT OF CHEMICAL PARAMETERS

This was carried out with a master water testing kit at each of the inlets and outlets of the aquaponic system (fish tank, beds: NFT, floating, gravel) and was evaluated by colorimetric means.

pH. In the kit, the minimum possible pH reading was 6.0 and the maximum 7.6. The test was performed as follows:

- A clean test tube was filled with 5 ml of water from each inlet and outlet points of the aquaponic system. This test tube had a line indicating the level to which the water ought to be filled.

- Subsequently, 3 drops of pH Test Solution were added, keeping the dropper bottle in a vertical position so that the drops were uniform.

- Next, the stopper was placed on the test tube and shaken, with the purpose of mixing the solution.

- Finally, the result of the analysis was read by comparing the color of the solution with the pH Color Chart. The tube was located in an illuminated area on the white background of the color chart. The closest color indicated the pH of the water sample and the previous recording of the final data was made.

Ammonium. The salicylate-based ammonia test kit made it possible to identify the total ammonia level in parts per million (ppm), equivalent to milligrams per liter (mg/L) from 0 ppm to 8.0 ppm (mg/L). This test was carried out as follows: A clean test tube was used with 5 ml of water from each inlet and outlet of the aquaponic system. Consecutively, keeping the bottle vertical, 8 drops of Ammonia (NH_3/NH_4^+) Test Solution from bottle No. 1 were added. Similarly, 8 drops from bottle No. 2 of Ammonia (NH_3/NH_4^+) Test Solution were added. The stopper was then placed on the test tube and shaken vigorously for 5 seconds. 5 minutes were waited for the color to develop and, finally, the result of the analysis was read by comparing the color of the solution with the Ammonia Color Chart. The comparison was carried out in a well-lit place, selecting the most similar color from the color chart to register it.

Nitrites. The nitrite test kit read the total nitrite level in parts per million (ppm), equivalent to milligrams per liter (mg/L) from 0 ppm to 5.0 ppm (mg/L). This test was carried out as follows: With a clean test tube, 5 ml of water was filled from each inlet and outlet of the aquaponic system. Then, keeping the bottle upright, 5 drops of Nitrite (NO2 -) Test Solution were added, 5 minutes were waited in order for the color to develop and finally, in a well-lit place, the result obtained from the solution was compared with the Nitrate Color Chart. The closest color indicates the nitrite concentration in mg/L and it was proceeded to record the data obtained.

Nitrates. The nitrate test kit read the total nitrate level in parts per million (ppm), equivalent to milligrams per liter (mg/L) from 0 ppm to 160 ppm (mg/L). The procedure used for this test was as follows: A test tube was filled with 5 ml of water from each inlet and outlet of the aquaponic system, 10 drops of bottle No. 1 of Nitrate (NO_3^-) Test Solution were added. The dropper bottle again must be held upright to ensure uniform drops. The test tube was then stoppered and shaken several times, in order to mix the solution. Next, bottle No. 2 Nitrate (NO_3^-) Test Solution was shaken vigorously for 30 seconds, since this was a very important stage. Additionally, 10 drops from bottle No. 2 were added, keeping the dropper bottle in a vertical position so that the drops fall uniformly. The stopper was placed on the test tube and this was shaken vigorously for 1 minute, then waiting 5 minutes in order for the color to develop. Finally, the result of the solution was read using the Nitrate Color Chart. The tube was placed in an illuminated area, with the chart providing a white background. The closest color indicated the concentration in mg/L.

MEASUREMENT OF MICROBIOLOGICAL PARAMETERS

Disinfection protocol. All the materials were washed with sufficient soap and water and cleaned with absorbent towels. They were then washed again with distilled water and dried. Finally they were washed with 70% alcohol, wrapped in crack paper and placed in the autoclave of the laboratory.

Culture of bacteria. The tube was filled to 1040mm with distilled water, 3.92 gr of nutrient agar was applied and mixed with the distilled water in an Erlenmeyer, then the Erlenmeyer was placed on the hotplate with a speed of 300-350. The capsule of magneto was placed in the container and it was waited for it to boil three times, removed from the hotplate and placed on a flat surface with 2 burners on to preserve the heat while the water sample was taken at the inlets and outlets of the aquaponic system. Filling the Petri dish with the mixture with agar, the blade was heated until the tip turned red and it was then submerged in the water samples taken, passing the blade in a zigzag pattern in the Petri dishes. Finally, it was sealed with a lot of masking tape, marking each Petri dish with the date and sample point in order to put the dishes in the incubator. This was carried out in the laboratory 15 days after placing the cultures in the incubator, selected by date and place of sampling (fish tank, NFT bed, gravel bed, floating bed).

RESULTS AND DISCUSSION

PHYSICAL-CHEMICAL PARAMETERS

Temperature. According to Figure 1, the average initial temperatures of the two crops in each bed were found not to vary (21.8 °C). The final temperature in each crop and bed meanwhile did vary, showing negative values that indicate an increase in temperature. For Crop 1 (basil) the greatest variation was identified in Bed 3 (floating) with an average value of -0.5, while for Crop 2 (strawberry) the greatest variation was found in Bed 1 (gravel). with an average value of -1.

The variation of the system between the values of 21.8°C and 22.8°C on average over the days measured, indicates that, in terms of the requirements for fish and plants in the same system, the temperature is in the optimal range, which ensures adequate and sustainable growth of the system, which coincides with what is reported in the literature, (Caldas Quiñonez, 2019).

pH. Regarding the pH, the initial average values of both crops in each bed did not show variations (6.5). However, in the final measurements, differences were found, the pH values being slightly lower for the basil crop in the gravel bed (difference 0.5) and the NFT bed (difference 0.4), while for the strawberry crop the most marked difference was in the gravel bed (difference 0.4). This indicates that the variation by crop in each bed between the initial and final measurement was not substantial (Figure 2)

pH is of the utmost importance in aquaponic systems since it makes it possible to have a balance in the ecosystem wherein fish, plants and bacteria are integrated. For these types of aquaponic system it is recommended to maintain pH levels between 7 and 8 in order to increase nitrification. As regards the hydroponic component, however, slightly (6.1 - 6.5) and moderately acidic (5.6 to 6) pHs are recommended, which favor the solubility of the ionic forms absorbed by the plants (Reyes Flores *et al.*, 2020).

Ammonium. The average initial ammonium levels of both crops in each bed did not show variations (1.0). The average final value in each measurement showed decreases. The slightest was found in the NFT bed (variation of 0.9 for the strawberry crop and 0.8 for the basil crop), while the values

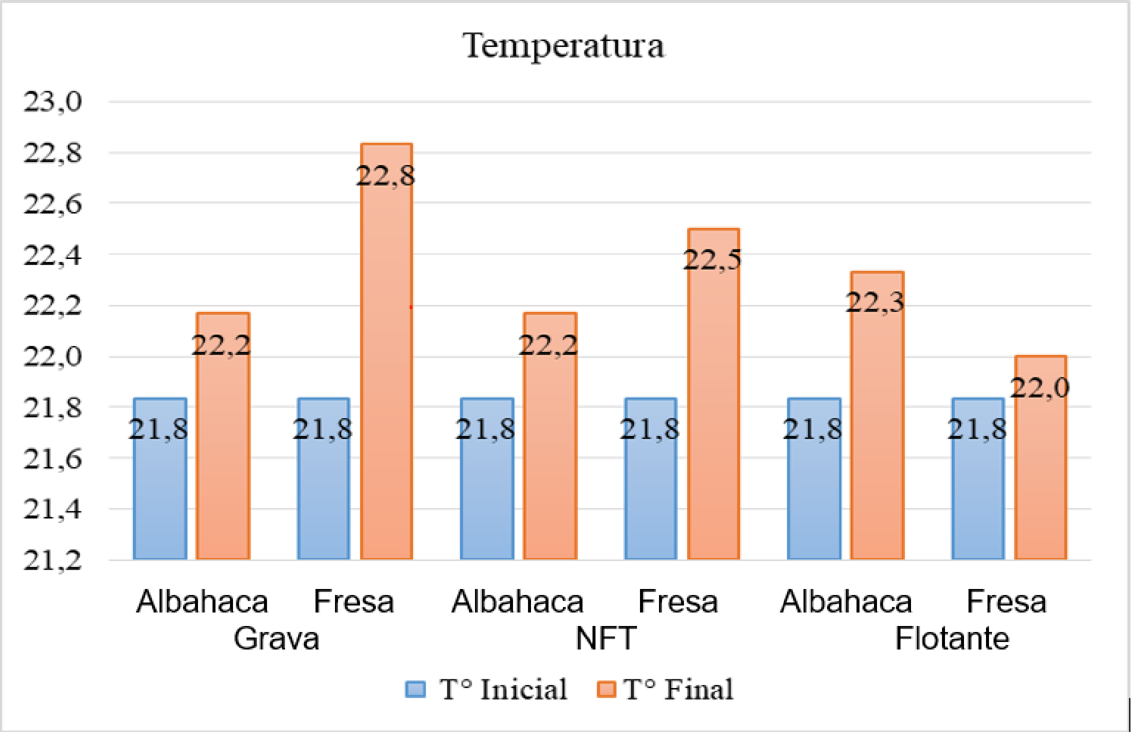

Figure 1. Mean variation of water temperature in each planting method.

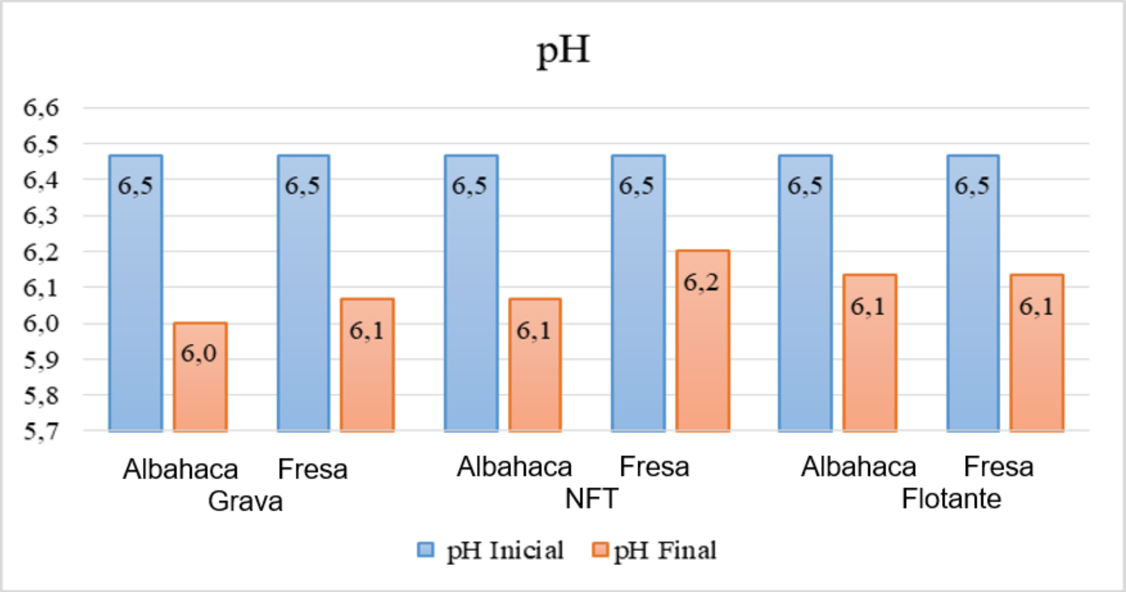

Figure 2. Mean variation of pH in the water in each planting method.

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were reduced by half with the strawberry crop in the gravel bed. (0.5), with a difference of 0.6 found for the floating bed for both crops (Figure 3).

According to the results obtained in the different measurements carried out, (Carranza Goicochea *et al*., 2021) argues that the range of 1.00 - 4.01 mg/L is tolerable in aquaponic production since its presence in these quantities does not produce mortality in the cultivated species. It should be noted that the levels are low because the biomass present at the beginning of the process was low. Its concentration tends to rise however when biomass increases and due to the food not consumed by the fish.

Nitrites. Nitrite concentrations stayed stable at the beginning and end of each measurement for both cultures and in each bed. These began at 0.4 mg/L and dropped to 0.2 mg/L (Figure 4).

It should be noted that in the period where there is a higher concentration of nitrosome bacteria, nitrite levels may show high variations, which indicates bacterial activity in the nitrification process. Moreover, when the concentrations are higher than 5mg/L they can cause slight stress or death of the cultivated species (Carranza Goicochea *et al*., 2021).

Nitrates. Finally, the initial concentrations of nitrates on average for both crops in each bed did not show variations (33.0 mg/L), with the exception of the strawberry crop in the floating bed, whose value was 25 mg/L. In the final measurement, meanwhile, they were reduced, especially in the floating bed for both crops by more than 20 mg/L. In the other measurements they remained the same or below 10 mg/L (Figure 5).

It is recommended to maintain levels between 5 and 150mg/L and to change the water when levels increase (Carranza Goicochea *et al*., 2021). In this research, results were obtained within the lower limits of the minimum range, with the highest

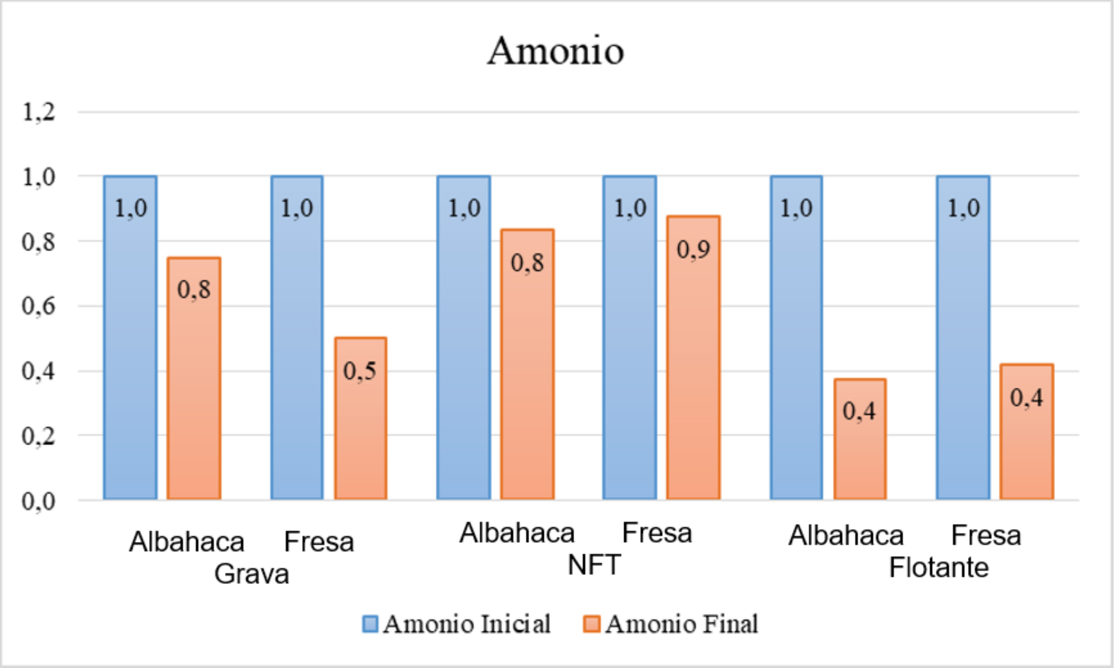

Figure 3. Mean variation of ammonium in the water in each planting method.

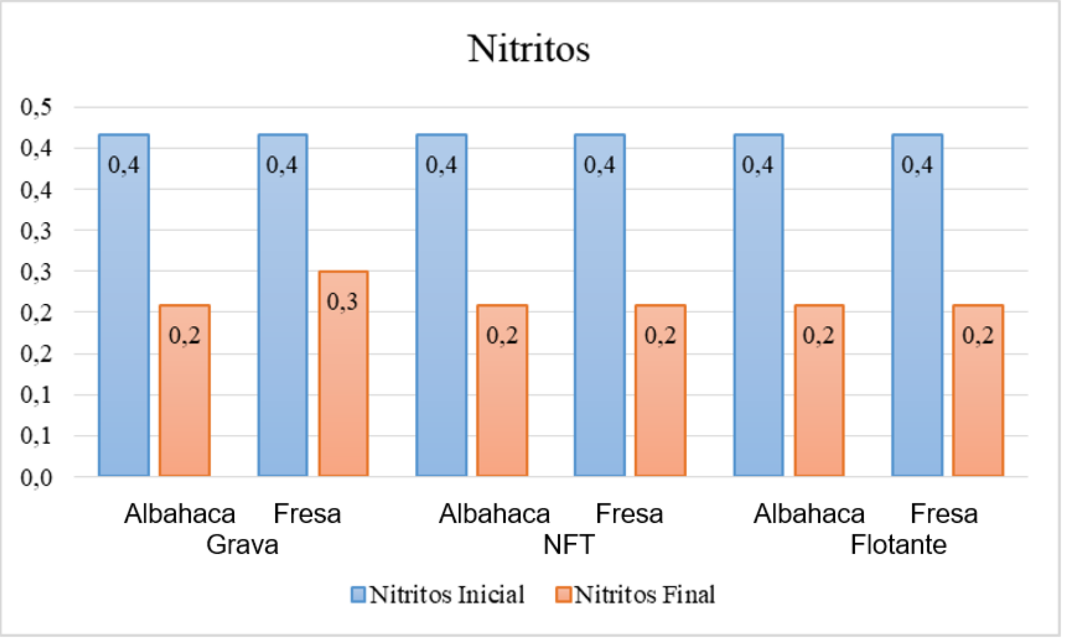

Figure 4. Mean variation of nitrites in the water in each planting method.

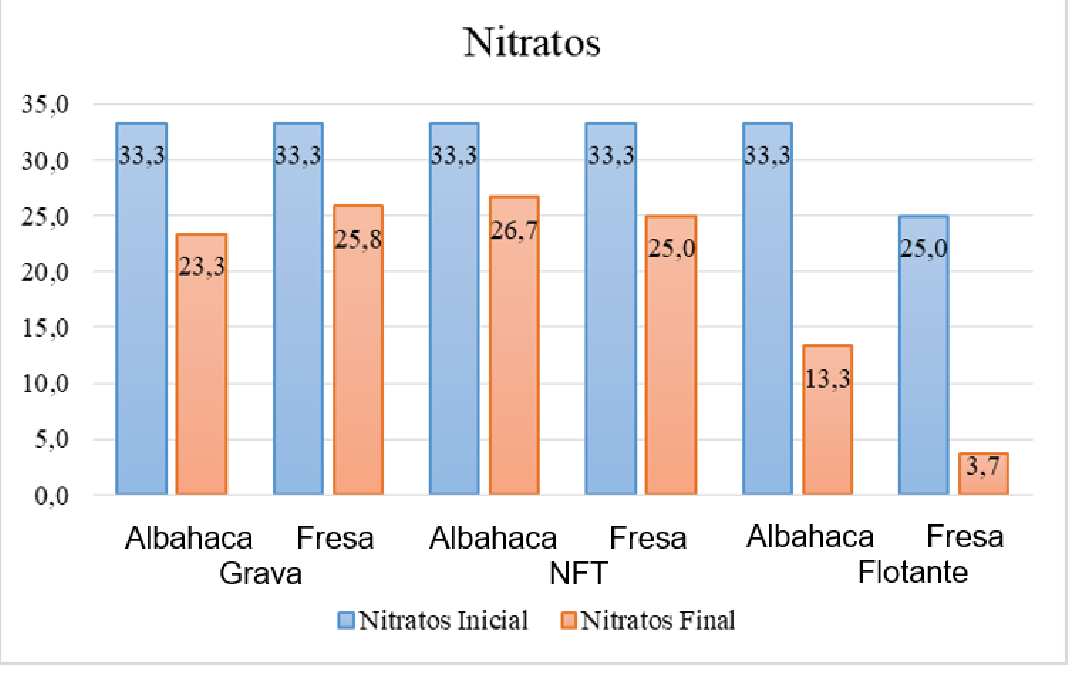

Figure 5. Mean variation of nitrates in the water in each planting method.

average value in the NFT bed for basil cultivation (26.7 mg/L) and 25.8 mg/L for strawberry cultivation on the gravel bed. It is worth noting that nitrates have a direct relationship with the amount of ammonia generated by the organic waste of the fish and the conversion carried out by beneficial bacteria in the biofilter, so that they can be used later by plants.

MICROBIOLOGICAL LOAD OF THE AQUAPONIC SYSTEM

Chitinolytic bacteria. These are a fundamental part of aquatic crops, since they allow the restoration of carbon and nitrogen levels through the degradation of chitin in the water column (Cardoso *et al*., 2012). In this group there is a great variety of microorganisms such as the genera *Pseudomonas*, *Bacillus*, *Alteromonas* and *Micrococcus*.

Studies such as the one carried out by (Figuerola *et al*., 2012) indicate that these genera are important for the solubilization of elements such as calcium phosphate, iron and aluminum, which makes them available in the environment for the production of various protozoa, rotifers, nematodes and a number of organisms that can be used as natural food on the site by cultivated species. That is why various researchers have suggested that the induction of heterotrophic microorganisms, combined with adequate aeration protocols, can provide benefits to aquaculture production, not only in profitability but also in sustainability (Martinez Cordoba *et al*., 2011).

Chitinolytic bacteria, despite not being nitrifying, are considered a source of nitrogen, playing an important role in protecting crops from pests, pathogens, and physiological disorders.

Bacteria of the genus *Bacillus*. *Bacillus* spp. have been described by (Stevens *et al*., 2002) as heterotrophic nitrifiers; it is evidenced that they can generate nitrate from ammonium directly. This activity, also reported by Stevens *et al*. (2002), is typical of heterotrophic nitrifiers and not of lithoautotrophs, which carry out only one stage of nitrification. Indeed, these types of isolate are of particular interest for optimizing the efficiency of biological nitrogen removal. However, further studies are necessary to understand their mechanism of action and their kinetic behavior, etc. On this aspect, the authors also argue through their research that *Bacillus* spp. are not only heterotrophic nitrifiers, but aerobic denitrifiers. In their study the experiments were carried out in an attempt to determine and quantify the contribution of heterotrophic nitrification and aerobic denitrification to total N removal. Taking the nitrogen balance in the culture condition of 41.1 mg/L of Initial NH_4^+-N at a C/N ratio of 15 in 96 h, 8.0% of the initial NH_4^+-N still remained in the medium in the forms of hydroxylamine, nitrite, nitrate, and organic N. 40.5% of NH_4^+-N was converted to biomass and it was estimated that 45.9% of NH_4^+-N was finally removed in the formation of N_2. This conversion of ammonium to N_2 with intermediate formation of N_2O under aerobic conditions was confirmed by gas chromatography. This allows us to conclude that with *Bacillus* sp., nitrogen removal occurs in a single step by simultaneous heterotrophic nitrification, while aerobic denitrification has great potential in wastewater treatment.

Bacillus subtilis. *B. subtilis* is a micro-organism widely used in aquaculture. It forms endospores in unfavorable conditions and its competitive qualities and exudation of enzymes against bacteria with gram-negative characteristics prevent the expansion of other cultivated genera. Its particularity of forming spores allows it to adhere to dry food without difficulty. In addition, this microorganism considerably improves the conditions of the environment in which it develops, which promotes and facilitates the decomposition of biological material and improves conditions and oxygenation in the water (Rodriguez, 2017).

Several studies have mentioned that proper management of microbial communities can make the general metabolism of these production systems more efficient. One of the most widely used technologies to modify the microbiota in aquaculture is the use of defined probiotics (live microorganisms applied in adequate amounts confer a benefit to hosted and cultured water). Several probiotics, such as *B. pumilus*, *B. clausii*, *B. subtilis*, *Lactobacillus plantarum* and *Saccharomyces cerevisiae*, have been widely used in recent years to improve the growth and survival of fish and crustaceans (Bentzon *et al*., 2016).

It should be noted that this technology was not applied in the present study. However, studies that have analyzed the microbiota or ecosystem of microorganisms from the gastrointestinal tract of the red-bellied pacu have identified microorganisms related to infectious alterations, as well as microorganisms from the group of sporulated bacilli and lactic acid bacteria, considered probiotics, among them, *B. subtilis*, *B. licheniformis*, *B. megaterium*, *Lysinibacillus sp*. and *Enterococcus faecalis* (Castañeda *et al*., 2019).

It is worth emphasizing that, although chemolithotrophic nitrification is the most recognized in N removal processes in aquaponic crops, the role of heterotrophic bacteria, which are detritivores par excellence, should not be underestimated. An example is the enzyme hydroxylamine oxido-reductase (participates in the oxidation of ammonia to nitrite), which is present in strains of *Bacillus* sp. highly efficient in nitrogen removal (Dominguez Mendoza *et al*., 2019).

***Fusarium* fungus**. Among the diversity of plant diseases that occur in aquaponics, soil-borne pathogens such as *Fusarium* spp., *Phytophthora* spp. and *Pythium* spp., are the most problematic due to their preference for wet/aquatic environmental conditions. However, it must be taken into account that not all the microorganisms detected are harmful or produce symptoms in the culture. Even species of the same genus can be harmful or beneficial (e.g. *Fusarium*, *Phoma*, *Pseudomonas*). The pathogens mentioned above are mainly pathogens linked to recirculating water, but can also be identified in greenhouses (Stouvenakers *et al*., 2019).

Elsewhere, (Sheema *et al*., 2017) point out that *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Mucor* and *Candida* fungi are efficient phosphorus solubilizers that improve nodulation, nitrogen fixation, crop growth and yield. They can thus be classified as beneficial microorganisms in aquaponic systems. In addition, (Manoharachary *et al*., 2005), reported on the importance of *Penicillium oxalicum*, *P. rubrum* and research on *Fusarium moniliforme* was carried out identifying their contribution to the energy flow and productivity of aquatic and semi-aquatic systems through degradation. of vegetable matter.

CONCLUSIONS

The physicochemical and microbiological parameters of the water for the aquaponic system did not show significant differences in any of the variables under study. It is worth noting the behavior of ammonium, which presented appropriate levels, which is attributed to the fact that the strawberry and basil crops functioned properly as biological filters in the system.

According to the microbiological load analysis carried out in the aquaponic system, the presence of chitinolytic bacteria was identified, as well as the genus *Bacillus* spp. which contribute considerably to environmental conditions since they promote and facilitate the decomposition of biological material. They likewise improve the conditions and oxygenation of the water, which leads to improved productivity and nutrient absorption.

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