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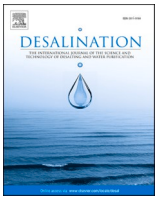

Valorization of reverse osmosis concentrate in the production of *Litopenaeus vannamei* and New Zealand spinach in an aquaponic system with biofloc

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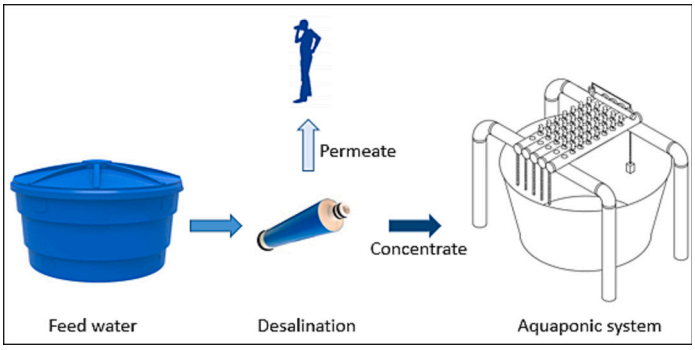
HIGHLIGHTS

- Desalination of water through RO system.
- Operation of an aquaponic system with RO concentrate.
- First study of an aquaponic system with L. *vannamei* and New Zealand spinach using BFT and NFT.
- The research studies possible valorization of the RO concentrate in northeastern Brazil.

GRAPHICAL ABSTRACT



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SUMMARY

The process of desalination through filtering membranes leads to the issue of generating a residue with a high concentration of salts, called concentrate. This residue is an environmental pollutant, especially when desalination is carried out far away from the sea (i.g., desalination in northeastern Brazil). In this context, the objective of the present research was to study the valorization of desalination concentrate through an aquaponic system. The feed water (brackish water) was desalinated, generating the concentrate, which was used for the operation of the experimental aquaponic pilot system in the cultivation of *Litopenaeus vannamei* (known as Pacific white shrimp) and New Zealand spinach (*Tetragonia tetragonioides*) in microbial biofloc. The initial average weight of the shrimp was 1.5 g and the stocking density used in the culture was 250 shrimp m^{-3}. The experiment was carried out for 74 days, and the final weight was 14.57 g and survival was 18 %. The low survival may be due to the high concentration of nitrite in the culture water 11.75 mg/L, which can be toxic to shrimp, and also due to the ionic balance of the system that was not close to seawater. Therefore, it is recommended to start the experiment when the biofloco system is in the chemoautotrophic stage, with the nitrification process established, or work with higher salinity. The spinach experiment was carried out for 49 days, with an initial biomass of 29.38 g and a final biomass of 519.41 g. Iron deficit was observed in the spinach leaves, thus, adding iron into the

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system is recommended. The results obtained could encourage the use of technology that is simple to apply and brings benefits to the community. Thus, the valorization of the waste produced in the desalination of water by reverse osmosis membranes reduces its inherent impact on the deposition of concentrate in natural environments.

1. Introduction

Along with an increasing global population the demand for fresh, clean water is also rising [1]. There are several measures to relieve the stress in water supply, such as water conservation, infrastructure repair, and better collection and distribution systems [2]. Also according to Elimelech and Phillip [2], although these measures are important, they can only improve the use of existing water resources, not increase them, thus, a way to increase the supply of fresh water beyond what is available in the hydrological cycle is desalination.

In Brazil, the northeast has the largest volume of dammed water in semi-arid regions in the world, and the discharges of its rivers provide water infiltration into aquifers of the order of 58 billion m^3/year [3]. According to Cirilo [4], with regard to groundwater, the northeastern territory is more than 80 % made up of crystalline rocks, there is a predominance of water with a high salt content captured in low-flow wells, on the order of 1 m^3 /h. The exception occurs in sedimentary formations, where the water is normally of better quality and higher flows can be extracted, in the order of tens to hundreds of m^3/h, continuously.

The regions of northeastern Brazil have underground water with hydrochemical composition: Magnesian Calcium Bicarbonate; sodium bicarbonate; mixed bicarbonate; Magnesian Calcium Chloride; sodium chloride; mixed chloride; mixed sodium sulfate; mixed sodium mixed [5].

To solve water shortages in northeastern Brazil, the Fresh Water Program (PAD) invests in small reverse osmosis desalinator systems to provide fresh water to low-income populations in semi-arid communities. According to the Ministry of Regional Development (MDR), there are currently 891 desalination systems in operation. The execution is thus distributed among the states: 252 systems in Ceará, 93 systems in Paraíba, 29 systems in Sergipe, 39 systems in Piauí, 98 systems in Rio Grande do Norte, 94 systems in Alagoas and 286 systems in Bahia. The desalination systems implemented so far have an installed capacity to produce about 3.5 million liters of drinking water per day and directly serve 214,000 people [6,7].

Desalination can be performed by thermal separation techniques or by membranes [1,8–10]. In recent years, big investments in the field of membranes, especially regarding reverse osmosis (RO), leveraged their use in the desalination process [9]. RO systems use high pressure water pumps to force the saline water through a semipermeable membrane, which allows water molecules to pass while retaining the salt molecules on the pressurised side of the membrane. The result of applying this technique is a liquid with a lower concentration of salts than the feed liquid, called permeate, and another one with a higher concentration of salts than the feed liquid, called concentrate [1,11].

When the desalination is carried out near the sea, usually the concentrate is released back into the sea, although there is some criticism about this, because of its impact on the marine organism [12]. However, when it occurs in places some distance away from the sea, as seen in several communities with a semi-arid climate in northeastern Brazil where there are small brackish water desalinators in order to make it suitable for domestic use, there is an issue regarding disposal of the concentrate.

The concentrate, when not treated and released directly into the soil, propitiates the accumulation of salts in the surface layers, which can be leached by the rainwater and reach aquifers, causing soil salinization and sodicity [13,14].

The residue produced, when applying the RO technique, has potential for reuse, mainly in relation to the agricultural production of species highly tolerant to large concentrations of salts [15–19]. Among the plant species with these characteristics, we can mention *Sarcocornia ambigua*, Saltbush (*Atriplex Nummularia*) and New Zealand spinach (*Tetragonia tetragonioides*).

In this context, seeking to enhance the value of the concentrate, Embrapa Semiarid has developed an integrated production system in northeastern Brazil, which aims to use the concentrate from the desalination system, minimizing environmental impacts and contributing to food security. This system uses the effluents from the desalination of brackish or saline groundwater in a combination of integrated actions in a sustainable way, in the search for the supply of good quality water. This system uses the concentrate in the fish farming tanks (tilapia) and in another moment this effluent of this breeding is used, enriched in organic matter, used for the irrigation of the saltbush (*Atriplex nummularia*) that, in turn, is used in the production of hay, with a protein content between 14 and 18 %, used for fattening goats, sheep and/or cattle in the region, thus closing the integrated production system [6].

The use of the concentrate in aquaponics in the production of fish and halophytic plants becomes attractive. Aquaponics integrates hydroponic plant and aquaculture fish production in a sustainable agricultural system that uses natural biological cycles to provide nitrogen and minimizes the use of non-renewable resources, providing economic benefits that can increase over time [20–22]. An alternative fish for cultivation in an aquaponic system is the *Litopenaeus vannamei* that can be produced by biofloc technology (BFT).

According to Kubitza [23], in several countries, BFT has enabled the production of *Litopenaeus vannamei* in areas a long way from the sea. Interest in cultivation in the interior of Brazil has been growing due to the cost of salinization and water's ion balance for shrimp cultivation. The vast majority of BFT systems have minimal discharge and water replacement, that is, they are cultivations with practically zero discharge.

In cultivations of *Litopenaeus vannamei* in inland waters, usually the water is salinized between 3 and 15 parts per thousand (ppt). Some producers use salinity of 25 ppt to reduce the risk of nitrite toxicity, a toxic metabolite, especially in waters with salinities below 15 ppt. In order to minimize problems with nitrite, salinity values below 5 ppt in BFT intensive cultivations are not recommended [23]. Salinities close to 25 ppt are considered ideal for the cultivation of this species because it is close to its isosmotic point [24]. Several studies like Carneiro [25], Pinheiro et al. [26], Pinheiro et al. [24], Silva [27], and Soares [28], evaluated the cultivation of the halophyte *Sarcocornia ambigua* and the shrimp *Litopenaeus vannamei* in aquaponics in nutrient film technique (NFT) with microbial biofloc, demonstrating its cultivation capacity.

An alternative plant for cultivation in an aquaponic system is the *Tetragonia tetragonoides* (Pall.), known as New Zealand spinach.

It belongs to the *Aizoaceae* family and produces herbaceous creeping plants, with dark green leaves [29]. New Zealand spinach is the most consumed type of spinach in Brazil, due to its adaptability to the tropical climate. The cultivation area in the state of São Paulo is 652 ha, with a production of 10.16 t, and productivity of 15.58 kg per hectare. Spinach is grown for the use of its leaves, which can be consumed "*raw*" or processed into canned or frozen products. It stands out among vegetables for its nutritional composition, with high levels of iron, excellent source of A and B2 vitamins, as well as calcium, phosphorus, potassium, and magnesium [30].

Yousif et al. [31], exposed the New Zealand spinach to saline stress by daily irrigation with NaCl solution, 0 mM (control), 50 mM (2.925 g

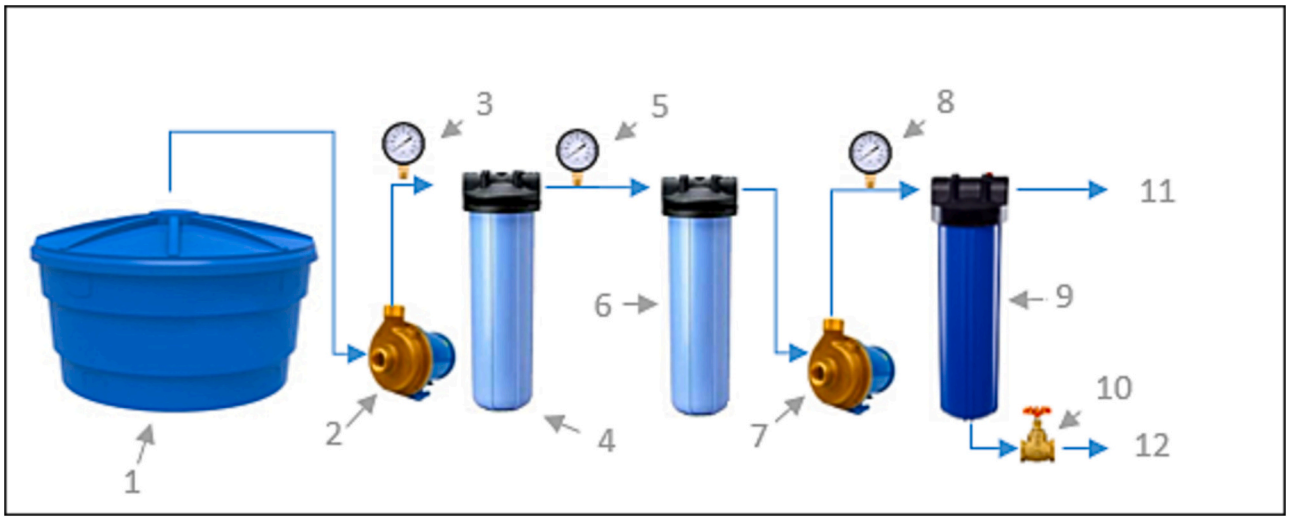

Fig. 1. Scheme of operation of the pilot system.
(Source: from the author.)
Legend: 1 – Feed water; 2 – Booster pump ¼ HP; 3 – Manometer; 4 – Cartridge filter 5 μm; 5 – Manometer; 6 – Activated carbon cartridge filter; 7 – Booster pump ¼ HP; 8 – Manometer; 9 – Reverse osmosis membrane; 10 – Gate valve; 11 – Permeate; 12 – Concentrate.

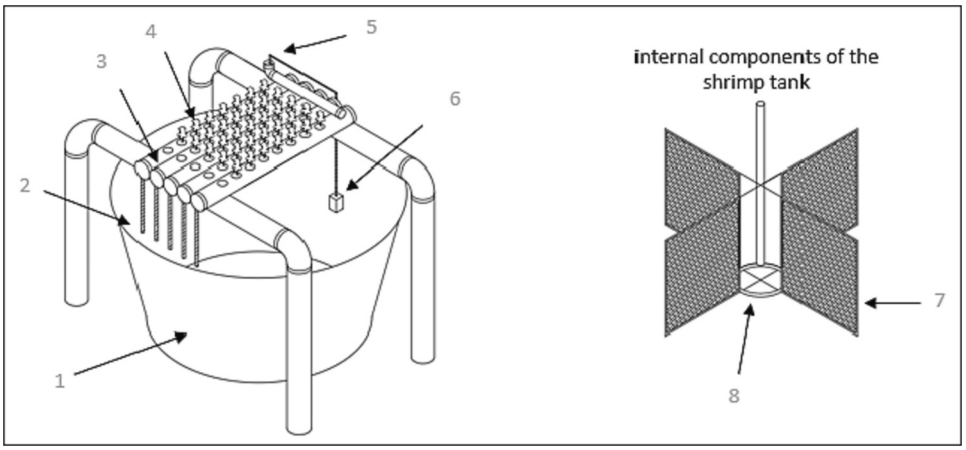

Fig. 2. Aquaponic pilot used in the study.
(Source: from the author.)
Legend: 1- shrimp tank; 2- water return pipes; 3- hydroponic countertop; 4- New Zealand spinach; 5- gutter irrigation; 6- irrigation pump; 7- artificial substrate; 8- aeration.

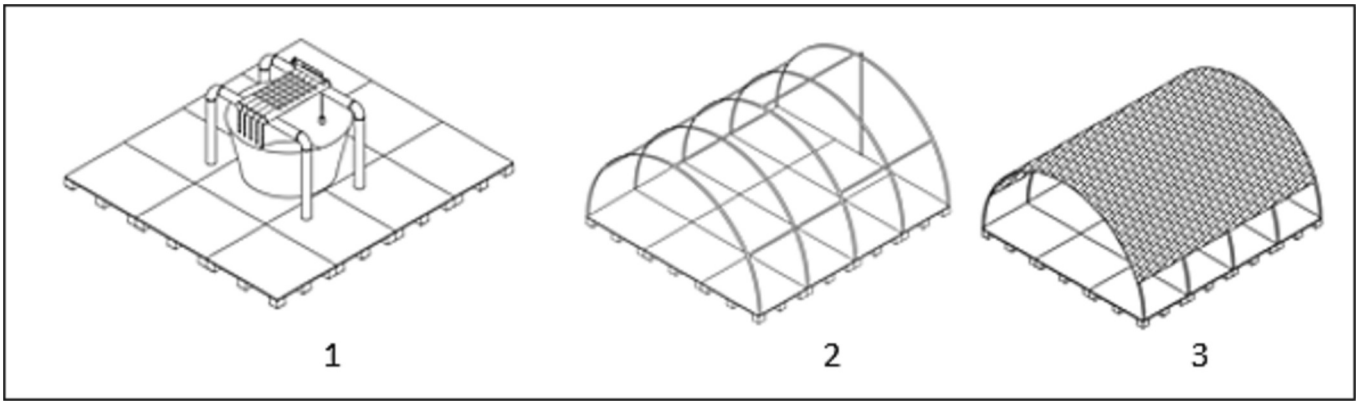

Fig. 3. Greenhouse: 1-shows the wooden pallet base with the aquaponic pilot system on top of it; 2-shows the PVC structure fixed on the pallet base; 3-shows the entire greenhouse with shade cloth and openings on the sides.
(Source: from the author.)

L^{-1} of NaCl), 100 mM (5.850 g L^{-1} of NaCl), and 200 mM (11.700 g L^{-1} of NaCl) for a period of 14 days, and observed that the growth increased with high salinity, indicating that the New Zealand Spinach is halophilic. Atzori et al. [32] also studied the growth of New Zealand spinach. They used 15 % and 30 % of seawater in the hydroponic solution, achieving electrical conductivity of 9.8 and 18 dSm^{-1},

Table 1
Characteristics of the feed water, concentrate and aquaponic effluent.

Parameter	Feed water	Concentrate	Aquaponic effluent
Temperature (°C)	25.78 ± 0.79	28.38 ± 0.85	27.86 ± 0.57
pH	7.42 ± 0.16	6.97 ± 0.27	8.00 ± 0.01
Apparent color (uH)	10.67 ± 1.41	1.33 ± 0.47	[a]
True color (uH)	1.00 ± 1.41	1.00 ± 0.00	73.00 ± 3.00
Turbidity (NTU)	1.38 ± 0.87	0.21 ± 0.03	110.60 ± 12.40
Absorbance 254 nm	0.02 ± 0.00	0.02 ± 0.00	0.40 ± 0.01
Salinity (ppt)	4.77 ± 0.12	9.01 ± 0.26	11.55 ± 0.15
Total dissolved solids (ppt)	4.52 ± 0.11	8.02 ± 0.22	10.08 ± 0.12
Conductivity (mS/cm)	9.04 ± 0.25	16.02 ± 0.43	20.16 ± 0.24
Dissolved oxygen (mg/L)	7.52 ± 0.33	6.89 ± 0.23	7.79 ± 0.17
Hardness (mg/L)	1000.00 ± 28.28	2000.00 ± 32.66	2660.00 ± 28.28
Calcium (mg/L)	173.68 ± 16.47	205.74 ± 29.51	408.82 ± 28.53
Magnesium (mg/L)	137.70 ± 16.52	361.26 ± 25.82	398.52 ± 24.14
Iron (mg/L)	0.51 ± 0.03	0.29 ± 0.03	0.43 ± 0.02
Zinc (mg/L)	0.30 ± 0.00	0.17 ± 0.02	0.00 ± 0.00
Copper (mg/L)	0.05 ± 0.00	0.08 ± 0.00	0.10 ± 0.01
Manganese (mg/L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Orthophosphate (mg/L)	0.00 ± 0.00	0.45 ± 0.11	1.50 ± 1.04
Sodium (mg/L)	1393.67 ± 21.69	2816.00 ± 7.79	3582.50 ± 110.64
Chloride (mg/L)	1638.90 ± 771.69	4006.66 ± 446.33	5636.34 ± 349.27
Potassium (mg/L)	96.83 ± 8.34	163.83 ± 0.24	211.17 ± 5.44
Sulfate (mg/L)	209.51 ± 95.49	574.58 ± 87.75	865.91 ± 88.50
Nitrite (mg/L)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Nitrate (mg/L)	1.47 ± 0.47	1.52 ± 0.47	86.55 ± 6.86
Total ammonia nitrogen (mg/L)	0.35 ± 0.02	0.16 ± 0.07	3.57 ± 1.51
Alkalinity (mg/L)	44.67 ± 5.25	63.33 ± 2.49	149.00 ± 11.00
Total suspended solids (mg/L)	72.00 ± 26.73	118.67 ± 1.89	697.00 ± 15.00

[a] Has not been analyzed/Data are a mean ± standard deviation.

respectively. New Zealand spinach has shown good cultivation results with these saline contents.

Thus, the objective of the present study was the valorization of RO concentrate in aquaponics, evaluating the cultivation of *Litopenaeus vannamei* and New Zealand spinach in an NFT aquaponic system with microbial biofloc.

2. Materials and method

2.1. Feed water

The backcountry regions of the states of Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, and Bahia commonly have brackish underground waters, with values ranging from 2 to 8 ppt [33]. In the present study, the saline content of the study water was considered as the average of these values, thus resulting in 5 ppt.

The preparation of the feed water (brackish water) was made from the dilution of seawater with the local supply water, until it reached salinity close to 5 ppt. Then it was filtered by RO to produce concentrate. To reach the salinity of the feed water, 17 % of seawater (salinity 30 ppt) was diluted with 83 % of tap water.

The sea water used in the dilution came from Moçambique beach (Florianópolis/Brazil), and was collected using the collection system from the Laboratory of Marine Shrimps (LCM) of the Federal University of Santa Catarina (UFSC).

2.2. Reverse osmosis pilot system

The RO pilot system was ordered and built especially for the Water Potabilization Laboratory (LAPOÁ), of the Department of Sanitary and Environmental Engineering (ENS) at UFSC.

The RO pilot system features feed water pretreatment with a 20″ × 2 1/2″ 5 μm Polypropylene Cartridge filter, and a 20″ × 2 1/2″ 5 μm Activated Carbon Cartridge filter that has the function of adapting the feed water to the RO membrane. RO membrane features: RO 3218 TMC HF membrane (spiral membrane). Maximum operating pressure 150 Psi. Manufacturer: R.O. Ultratec. TMC – trimesoyl chloride. Minimum 97 % NaCl rejection.

In general, following the flow of Fig. 1, first the feed water (1) was prepared and pumped (2) to the 5 μm cartridge filter (4) and activated carbon filter cartridge (6) in series, passing through the pressure gauges (3) and (5), and then pumped (7) to the RO membrane (9) passing through the pressure gauge (8), and thus resulting in two streams of water at the RO outlet, being a permeate stream (11) and the second stream which is the concentrate (12). The gate valve (10) was very important to control the outflow of the concentrate, thus being able to control the pressure of the RO manometer (8), keeping the pressure in the RO at 150 psi during all operations. During desalination, the permeate was discarded and the concentrate was recirculated twice in the system, thus simulating 3 serial filtrations, and resulting in a concentrate with a smaller volume and a higher salinity.

2.3. Biological material

The shrimp used in the present study were *Litopenaeus vannamei*, with an average weight of 1.53 g, provided by the LCM/UFSC. Post-larvae shrimp were cultivated in the nursery tank in the microbial biofloc system until they reached the weight necessary to start the experiment with salinity of 9 ppt, which was the salinity at the beginning of the aquaponic experiment.

New Zealand spinach seedlings were obtained from a local supplier. In the experiment, 35 seedlings were used and each of the seedlings had an average of seven spinaches with an average length of 5 cm (not including the length of the roots). Each spinach had 5 leaves on average.

2.4. Aquaponic pilot system, biofloc cultivation, and management

The aquaponic pilot system was installed in the ENS at UFSC. The experimental aquaponic pilot system used BFT and Nutrient Film Technique (NFT) cultivating New Zealand spinach and *Litopenaeus vannamei* shrimp. The pilot system consisted of a 1000 L tank, with 800 L of useful volume, with heating, aeration, artificial substrates, and a hydroponic bench for the plants. The water used in the system was the RO's concentrate. The pilot system contained 200 shrimp with an average weight of 1.53 g and initial density of 250 shrimp m^{-3}. The experiment began with salinity of 9 ppt, which was the concentrate salinity. A maximum salinity of 12 ppt was settled, which is the maximum salinity used in the study of Yousif et al. [31], with New Zealand spinach.

The pilot structure was adapted, assembled and operated according to Pinheiro et al. [26] (Fig. 2). On the hydroponic bench for plant growth the channels were formed by five PVC pipes of 75 mm in diameter and 1.10 m in length, arranged side by side, 0.5 m above the tank's water level, with 4 % of inclination, and in PVC supports 60 mm in diameter. The counter top had 0.4 m^2 of planting area. In the experiment, 35 seedlings were used and each seedling had on average seven New Zealand spinach. The spinaches were transplanted into the system in the fourth week of the experiment, which continued for 49 days until its completion. In the seventh week of the experiment, the amount of spinach in all cells of the hydroponic bench was reduced to three or four. When the spinaches were transplanted into the system, the shrimp had an average weight of 5.82 g.

The pilot structure had three thermostats of 300 W (300 W Thermostat Ocean Tech X5 220v), four artificial substrates of approximately 0.33 m^2, one air compressor (ACQ 003220 v) for system aeration, a

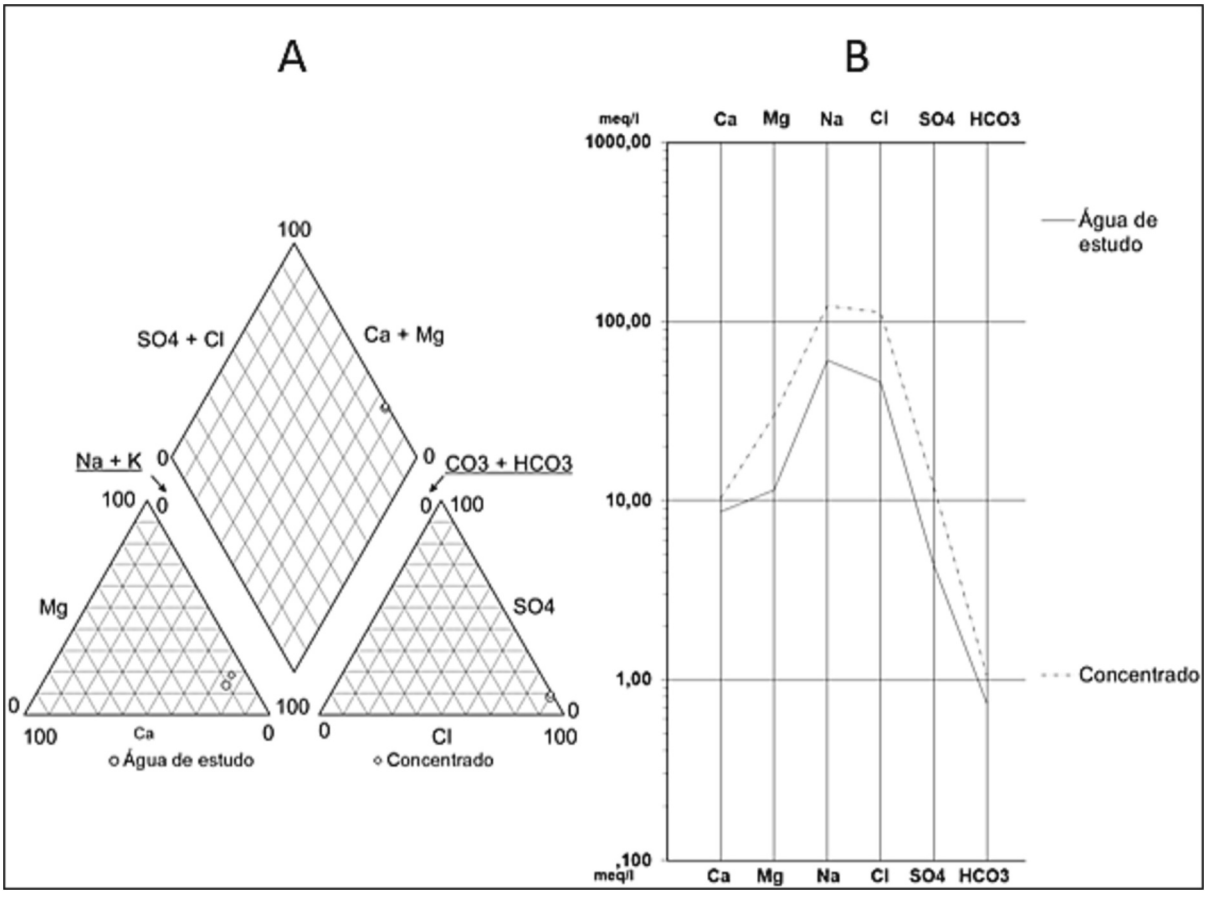

Fig. 4. Piper diagram (A) and Shoeller and Berkaloff diagram (B) of the feed water and concentrate.

Table 2
Water quality parameters of the aquaponic pilot system.

Parameters	Average ± standard deviation	Minimum	Maximum
Temperature (°C)	28.98 ± 1.08	26.60	31.50
pH	7.73 ± 0.30	7.02	8.27
True color (uH)	64.05 ± 17.51	25.00	97.00
Turbidity (NTU)	85.03 ± 30.17	5.30	136.00
Absorbance 254 nm	0.35 ± 0.10	0.15	0.52
Salinity (ppt)	10.72 ± 0.49	9.90	11.70
Total dissolved solids (ppt)	9.41 ± 0.42	8.69	10.20
Conductivity (mS/cm)	18.78 ± 0.79	17.53	20.40
Dissolved oxygen (mg/L)	7.32 ± 0.47	5.42	8.38
Orthophosphate (mg/L)	1.50 ± 0.94	0.34	4.74
Sulfate (mg/L)	935.09 ± 174.50	731.08	1559.08
Nitrite (mg/L)	11.75 ± 17.84	0.00	68.62
Nitrate (mg/L)	36.64 ± 36.07	11.66	110.52
Total ammonia nitrogen (mg/L)	2.32 ± 1.38	0.08	5.57
Alkalinity (mg/L)	129.00 ± 25.94	78.00	170.00
Total suspended solids (mg/L)	588.88 ± 107.71	410.00	792.00

nobreak (Nobreak Interactive SMS 27395 station II 600 va) that guaranteed continuous aeration of the system even with a power outage, and a submerged pump of 1000 L/h (Sarlo 1000 220 v), which continuously pumped water from the tank to the hydroponic bench, and distributed it into the channels. After irrigating the plants, the water returned to the tank by gravity. The tank was covered with shade cloth (50 % shading).

On day one the biofloc preparation was started. Therefore, 800 L of concentrate were placed in the pilot and fertilization was completed by adding 126 g of feed and 30.8 g of sugar (organic carbon) into the system in aeration. In order to start the experiment with a total suspended solids (TSS) of 350 mg/L and control the ammonia value, sugar was added for five days in a row. The instability in the ammonia results at the beginning of fertilization resulted in the sugar addition continuing beyond five days. The calculations of the amount of added sugar were made according to Avnmelech [34]. The system was stocked with shrimp on day 13, which is the day that the experiment weeks started to count. As the biofloc was not in the chemoautotrophic stage, the nitrification process was not established, so it was necessary to add sugar during the experiment in order to maintain water quality parameters [35,36].

Feeding was carried out three times a day at 09:00, 13:00, and 17:00 with a 38 % crude protein feed. The amount of feed provided weekly was calculated based on the feeding table proposed by Van wyk [37]. In order to adjust the amount of feed, 10 % of the shrimp were weighed weekly. After the post larvae shrimp were introduced to the system for cultivation, the addition of sugar was carried out between feeding intervals, as it could decrease the concentration of dissolved oxygen (DO) in the water and affect the shrimp [35]. The addition of organic carbon (sugar) was divided into two doses, at 11:00 AM and 03:00 PM.S

Whenever needed, alkalinity correction was carried out by adding calcium hydroxide. It was added at a rate of 20 % of the daily feed intake when the alkalinity was below 120 mg/L and 10 % of the daily feed intake when it was between 120 and 150 mg/L to maintain the alkalinity at optimal concentration. When alkalinity was above 150 mg/L, adding calcium hydroxide was not necessary. Sugar also helps to correct alkalinity, therefore, whenever sugar was added into the system, calcium hydroxide was not added.

During the experiment biofloc or solids were taken from the system. Concentrate and tap water were also added to replace the evaporating water, as well as biofloc and solids that were removed, always taking into account the maximum salinity value of 12 ppt settled for the system.

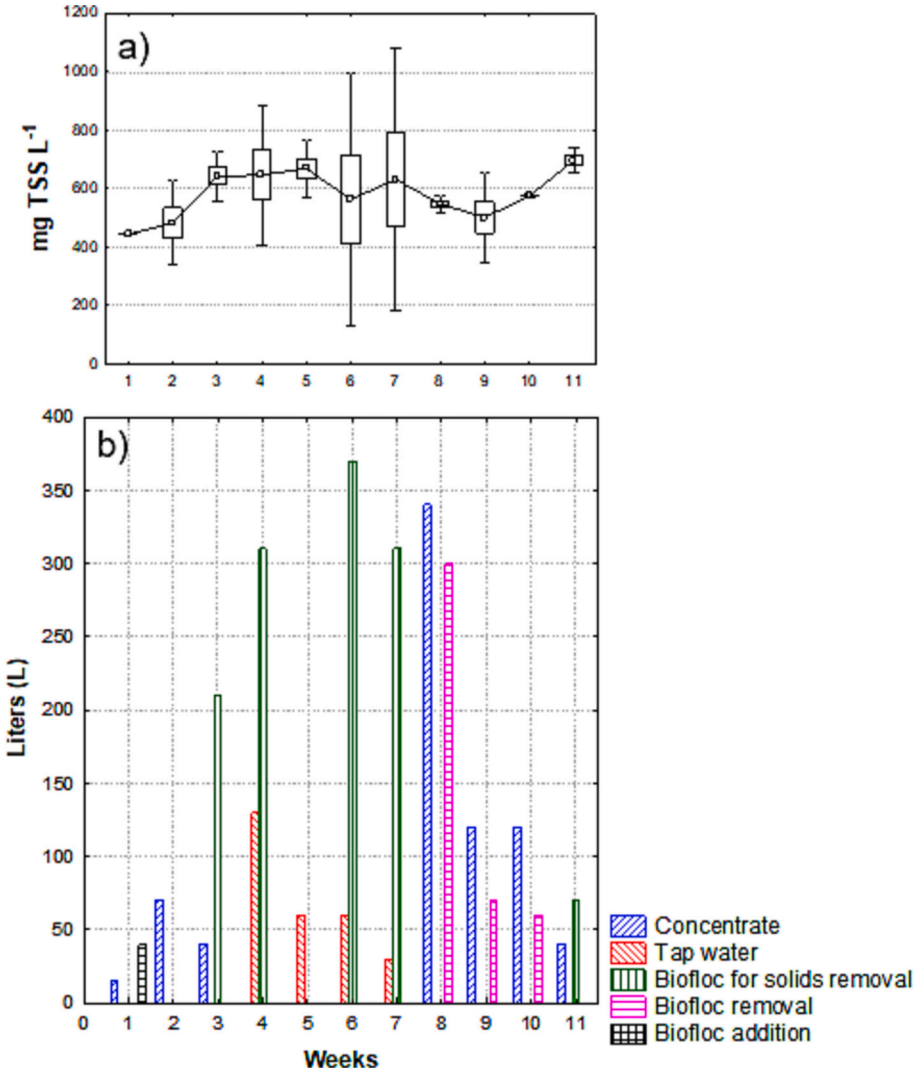

Fig. 5. (a) Total suspended solids and (b) shows the quantity (measured in liters) of input and output of water, concentrate, tap water, biofloc for solids removal, biofloc removal, and biofloc addition throughout the weeks of the experiment.

The aquaponic pilot system did not have a solids sedimenter, so to remove the solids from the system, a portion of water was removed and the solids sedimented, then the water, without the solids, was returned to the system. The pilot operated for 11 weeks after the post larvae were introduced to the system.

To accommodate the aquaponic pilot system, a greenhouse was designed and built (Fig. 3) with a base of wooden pallets, a structure of PVC pipes, 32 mm in diameter, and covered with transparent plastic tarpaulin. On top of the tarpaulin, a red shade cloth was placed (Red Chromatinet 35 % M2-polysack) that modifies the spectrum of sunlight by the greatest amount of red light, which stimulates the seedlings' growth [38].

2.5. Water quality parameters

Sulfate, nitrite, nitrate, and chloride analyses were undertaken by ion chromatography (Dionex Ion Chromatography). Measurements of total dissolved solids, electrical conductivity, salinity, and temperature were taken with a portable conductivity meter (brand: Akso; model: AK83). The other analyses were undertaken according to Apha [39].

2.5.1. Quality parameters of feed water, concentrate and aquaponic effluent

To evaluate the water quality parameters of the RO pilot, the feed water and the concentrate were analyzed. The temperature, pH, turbidity, salinity, total dissolved solids, electrical conductivity and DO were analyzed every hour of pilot operation, adding up to 32 analyzes of the feed water and 16 of the concentrate (for the analyzes of the concentrate it was considered from the second recirculation of filtration). They were also analyzed in triplicates apparent color, true color, absorbance 254 nm, hardness, calcium, magnesium, iron, zinc, copper, manganese, orthophosphate, sodium, chloride, potassium, sulfate, total ammonia nitrogen (TAN), nitrite, nitrate, alkalinity and TSS.

Analyzes of the aquaponic effluent were carried out in the last week of the aquaponic experiment. Analyzes of temperature, pH, turbidity, salinity, total dissolved solids, electrical conductivity, DO, absorbance 254 nm, orthophosphate, sulfate, TAN, nitrite, nitrate, alkalinity and TSS were carried out in two days in the last week. True color, hardness, calcium, magnesium, iron, zinc, copper, manganese, sodium, chloride and potassium were also analyzed once in triplicate.

2.5.2. Water quality parameters of aquaponic system

To evaluate the water quality of the aquaponic pilot system, two daily analyses (morning and afternoon) of temperature and DO were

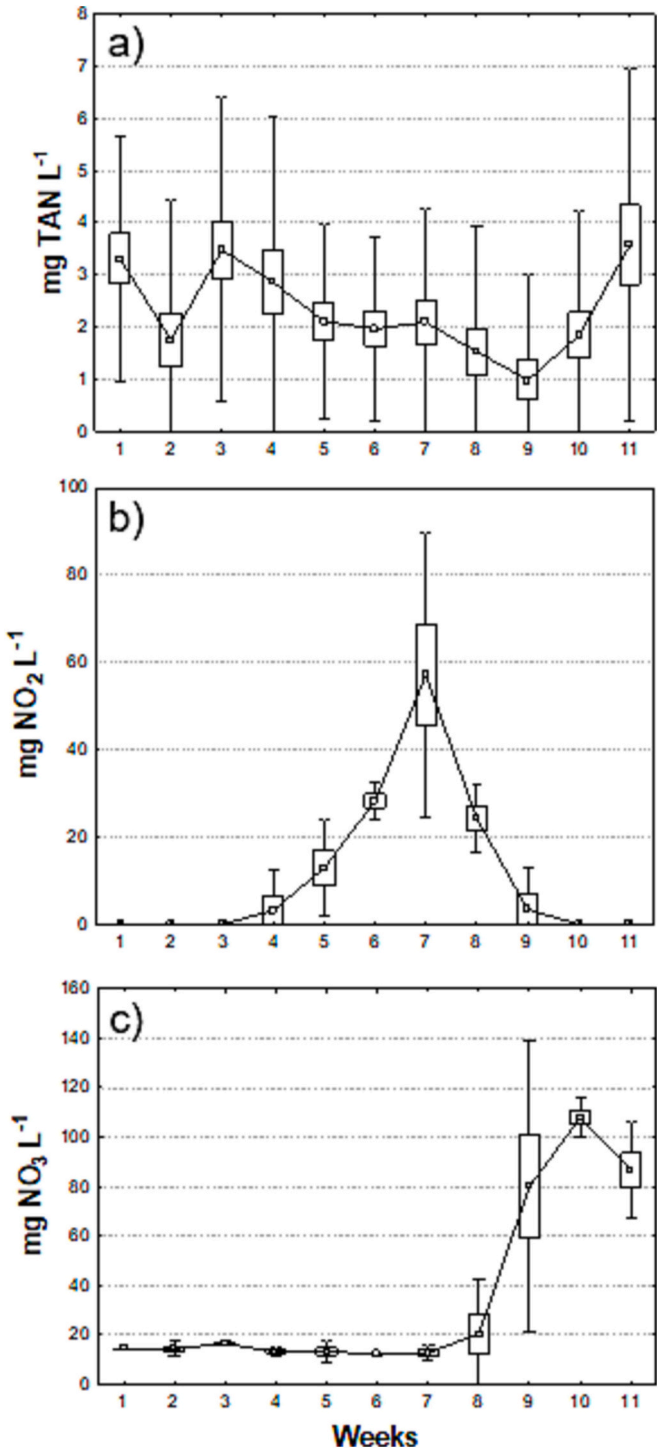

Fig. 6. (a) Total ammonia nitrogen (TAN), (b) nitrite and (c) nitrate in tank of *Litopenaeus vannamei* cultured in the aquaponic system.

performed. Turbidity, true color, pH, salinity, electrical conductivity, total dissolved solids, absorbance 254 nm, orthophosphate, sulfate, nitrite, nitrate, alkalinity, and TSS were also analyzed twice a week. Every morning the TAN was also analyzed.

2.6. Shrimp zootechnical performance indices and phyto technical performance of New Zealand spinach

Both the zootechnical performance of shrimp, weight, biomass, and survival, and phyto technical performance of New Zealand spinach, biomass weight, and length, were evaluated.

To calculate the initial average weight of the spinach, 20 spinach similar in size to those transplanted in the system were collected. The roots were cut and weighed and the value divided by 20, reaching an average value of 0.23 g. To calculate the initial biomass, the initial average weight was multiplied by the amount of spinach left in the system after the seventh week. In the calculations of weight, biomass, and length, the spinach roots were removed or disregarded.

Table 3
Zootechnical performance of *Litopenaeus vannamei* cultivated in an aquaponic system with biofloc for 74 days with a density of 250 shrimp m^{-3}.

Parameters	Average
Initial average weight (g)	1.53
Final average weight (g)	14.57
Weekly weight gain (g)	1.36
Final biomass (g)	524.49
Survival (%)	18

3. Results and discussion

3.1. Characteristics of the feed water, concentrate and aquaponic effluent

The results of the physical-chemical analyzes of the feed water, concentrate and aquaponic effluent can be seen in Table 1. As expected, it was observed that the water quality of the concentrate was better than that of the aquaponic effluent. Among the analyzed parameters, for example turbidity, we can observe that it was 0.21 ± 0.03 NTU and 110.60 ± 12.40 NTU, respectively, showing a great difference between the concentrate and the aquaponics effluent.

The RO pilot system operated with 150 psi of pressure and with 2 concentrate recirculations, thus simulating a series system of 3 filtration phases. It was possible to observe that the concentrate of the third filtration phase was approximately 40 % of the feed water. During the experiment, approximately 4500 L of study water was filtered and approximately 1800 L of concentrates were generated.

Fig. 4 presents the Shoeller and Berkaloff diagram (B), and through it we can see the amounts of cations and anions in equivalent amounts, and thus, being able to see the profile and compare the feed water and the concentrate. They have similar profiles, due to the fact that the concentrate is part of the feed water with the constituents in higher concentrations, due to the concentration that occurred in the filtration by RO. Fig. 4 also shows the Piper diagram (A) where it shows that both the feed water and the concentrate are classified as sodium chlorinated water, which can be compared with brackish groundwater in northeastern Brazil.

According to the classification of Richards [40], the feed water, the concentrate and the effluent of the aquaponic system are classified as C4S4 (Very high salinity and Very high sodium content), which characterize unfit for use in irrigation.

3.2. Water quality of the aquaponic pilot system

Table 2 shows the parameters of the water quality applied in the cultivation of *L. vannamei* and New Zealand spinach. The water temperature remained close to 30 °C due to the heater. Temperature, pH, turbidity, and DO alkalinity were within the limits for the cultivation of *L. vannamei* in biofloc. According to Emerenciano [35] and Hargreaves [36], the ideal temperature is between 28 and 30 °C, ideal pH between 6.8 and 8.0, turbidity between 75 and 150 NTU, DO above 4 mgL^{-1}, and alkalinity between 100 and 150 mgL^{-1}. Salinity, total dissolved solids, and conductivity increased over time, and on the day that the postlarvae shrimp were introduced in the system, the values were 9.9 ppt, 8.69 ppt, and 17.53 mS/cm, respectively, and on the last day of the experiment

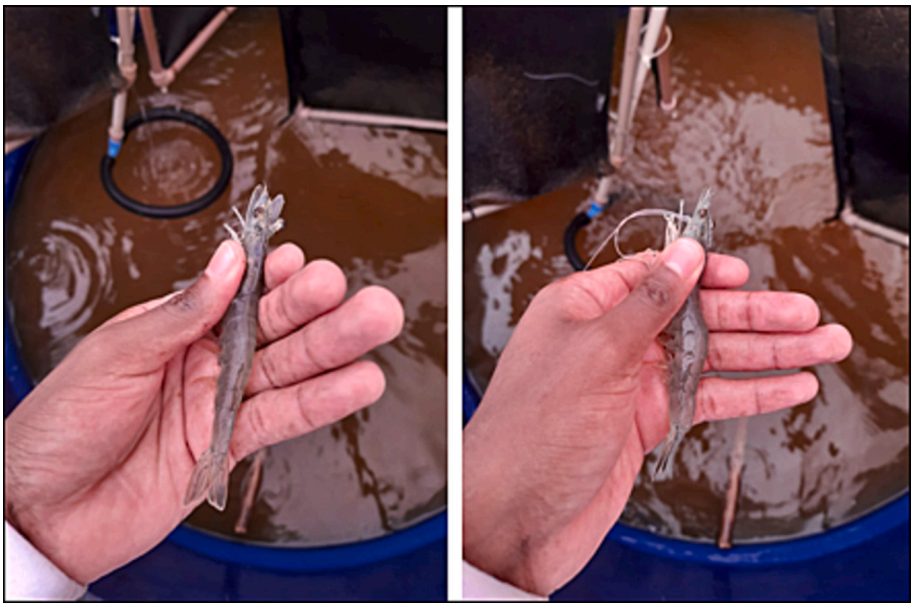

Fig. 7. *Litopenaeus vannamei* on the last day of the aquaponic experiment.

Table 4
Ionic concentrations of the ionic balance of the concentrate used in the experiment and of the aquaponic pilot system in week 7 (when shrimp deaths increased), week 8 (when exchange of part of the biofloc with concentrate was carried out), week 11 (last day of the experiment), and compared with the proportions of ions in seawater concentrations and an estimated proportion of 10 ppt (brackish water) according to Kubitza [23].

		Biofloc samples			Ideal concentrations	
Ions (mg/L)	Concentrate	Week 7	Week 8	Week 11	10 ppt	Sea 35 ppt
Chloride (Cl^-)	4006.66	5370.00	4914.82	5636.35	5529.00	19,350
Sodium (Na)	2816.00	3020.50	3036.67	3582.50	3074.00	10,760
Sulfate (SO_4^{2-})	574.58	722.84	665.86	865.91	774.00	2710
Magnesium (Mg)	361.26	403.38	379.08	398.52	369.00	1290
Calcium (Ca)	205.74	259.18	296.59	408.82	117.00	410
Potassium (K)	163.83	187.67	184.83	211.17	114.00	400
Hardness	2000.00	2306.67	2300.00	2660.00		
Na:K	17.19	16.10	16.43	16.97	26.96	26.90
Mg:Ca	1.76	1.56	1.28	0.97	3.15	3.15
Cl:Na	1.42	1.78	1.62	1.57	1.80	1.80
Cl:K	24.46	28.61	26.59	26.69	48.50	48.38

the values were 11.4 ppt, 9.96 ppt, and 19.91 mS/cm, respectively. This result occurs, probably, due to the addition of concentrate in the system to replace the evaporated water, increasing the salinity and total dissolved solids concentrations.

There was no accumulation of orthophosphate, which was an average value of 1.5 mg/L, which is different from the results in the study of Pinheiro et al. [24]. This low value could be because it was a new system and because of the removals that are made by the plants. Also, it may have sedimented with the solids in the system and in the gutters of the hydroponic bench, mainly because of the various removals of solids from the system and the water exchange that occurred. Buhmann et al. [41], state that 0.3 mg/L of PO_4-P is sufficient for the growth of several halophyte species, therefore the orthophosphate values measured in the system are acceptable.

The absorbance 254 nm on the day that the post larvae shrimp were introduced to the system was 0.151 and gradually increased until week 7, when the value was 0.520. At week eight, after changing part of the water in the system, it dropped to 0.350 and at the end of the experiment the value registered was 0.415. The increase in absorbance 254 nm indicates an increase in organic matter in the system [39]. True color and turbidity increased over time with a variation similar to Fig. 5(a), indicating that these increases were probably due to the increase in organic matter and/or TSS.

The concentration of TSS increased over time, reaching a maximum value of 792 mg/L. It is believed that this occurred, mainly, due to the addition of sugar in the system, which was carried out over the weeks to control the ammonia. According to Avnimelech [42] and Hargreaves [36], the addition of a carbon source into the system increases the concentration of TSS. Concentrations above 500 mg/L can influence both water quality and shrimp performance, being the recommended levels between 200 and 500 mg/L. The removal of solids when the level is above these values is recommended.

Fig. 5(a) shows TSS concentrations over the weeks. Fig. 5(b) shows the replacement of water in the system and the removal of solids. Analyzing the two figures, we can observe that the removal of solids in the system was carried out at weeks 3, 4, 6, 7, and 11 in an attempt to lower the concentration of TSS that was gradually increasing (as the system does not have a sedimenter, the removal of solids was carried out by removing amounts of biofloc from the system as presented in Fig. 5 (b), and after the solids sedimentation only the water was returned to the system). At weeks 8, 9, and 10 there was a decrease in TSS because the bioflocs were removed from the system and concentrate was added to control nitrite increase.

In the first week there was an addition of 40 L of biofloc, which came with the post larvae shrimp to be introduced to the system, Fig. 5(b). The replacement of water in the system was carried out when the water level,

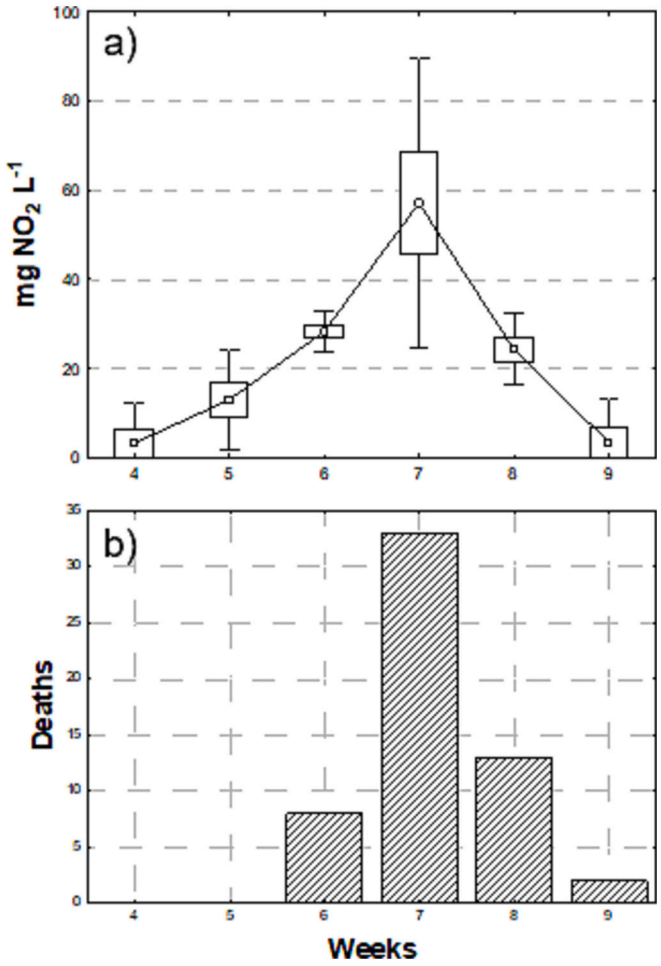

Fig. 8. (a) Nitrite and (b) number of deaths of *Litopenaeus vannamei* at weeks 4 to 9.

Table 5
Phytotechnical numbers for New Zealand spinach grown in the aquaponics system with microbial biofloc for 49 days.

Parameters	Average
Initial average weight (g)	0.23
Final average weight (g)	4.00
Initial biomass (g)	29.38
Final biomass (g)	519.41
Initial average length (cm)	4.94
Final average length (cm)	13.54

due to evaporation, was low. In the beginning, replacements were made with concentrate, but when the salinity in the system began to increase, the replacement was made with local supply water (tap water), therefore maintaining the system's salinity below 12 ppt, which was the maximum value settled for New Zealand spinach, based on the maximum value adopted in the study of Yousif et al. [31].

TAN (Fig. 6(a)) analyses were performed daily, as well as its control. Whenever the values were higher than 2 mg TAN/L, fertilization was done by placing sugar (a supplementary source of carbohydrates) in the system. Ammonia is highly toxic to shrimp, so for *L. vannamei* in water with salinity of 15 ppt, it should be maintained at concentrations less than 2.44 mg TAN/L [43]. The addition of a carbon source to the system stimulates the growth of heterotrophic bacteria, which creates a demand for nitrogen (such as ammonia), and organic carbon and inorganic nitrogen are usually incorporated in a fixed proportion in bacterial cells, decreasing the amount of ammonia in the system [35,36].

Weekly averages of ammonia gradually decreased from week 4 to week 9. Between weeks 8 and 10 little sugar was added into the system, since the ammonia values were stabilizing, which indicates nitrification by ammonia oxidizing bacteria (AOB), *Nitrosomonas*, *Nitrosococcus*, *Nitrospira*, *Nitrosolobus*, and *Nitrosovibrio* [44,45]. In the last week there was an increase in ammonia values. Instability in ammonia values is normal at the beginning of biofloc production, requiring a high C:N ratio to fertilize the water and ensure the growth of heterotrophic bacteria [45]. Thus, fertilization becomes necessary since the growth of heterotrophic bacteria is faster than that of nitrifying bacteria, which enables a faster control of ammonia. When sufficient carbon is available, control of ammonia by fertilization occurs, usually, within hours or days [36].

In the biofloc system there are three main processes that control ammonia: photoautotrophic assimilation by algae, assimilation by bacteria, and nitrification [36]. In aquaponic biofloc systems there is also removal of ammonia, which is done by plants.

The TAN results in the present experiment are higher than those in the studies of *L. vannamei* in aquaponics with *Sarcocornia ambigua*, and with biofloc [24–28], probably because these studies started when the biofloc was already in chemoautotrophic stage, with the nitrification process established.

Fig. 6(b) shows that nitrite was not detected in weeks 1, 2, and 3, and from week 4 to week 7, a large increase was observed, reaching the value of 68.62 mg/l. This is probably due to the decrease in the amount of sugar that was added to the system, which favored the development of chemoautotrophic bacteria. This includes nitrifying bacteria, and especially bacteria that oxidize ammonia into nitrite, usually developing before nitrite oxidizing bacteria (NOB) (*Nitrobacter*, *Nitrococcus*, *Nitrospira* and *Nitrospine*). Conditions were also favorable for bacteria that oxidize nitrite into nitrate, thus causing the accumulation of nitrite, which is one of the big problems when starting a new system [44,45]. Melo et al. [44], show that nitrite concentrations significantly influenced final weight, survival, and specific growth rate of *L. vannamei*. According to Sowers et al. [46] apud Kubitza [23], nitrite concentrations lower than 5 mg NO_2 L^{-1} are safe for *L. vannamei* for systems with 10 ppt salinity. However, from week 5 to week 8 the nitrite concentration was above 5 mg/L.

Fig. 5(b) shows that in weeks 8, 9, and 10 water was removed from the system, totaling 430 L, and concentrate was added to control the high nitrite values. In Fig. 6(b), nitrite decrease is observed, and at week 9 it reached satisfactory values (average value of 3.43 ± 3,43 mg NO_2 L^{-1}). At weeks 10 and 11 no nitrite was detected in the system, indicating the presence of nitrite oxidizing bacteria [44,45]. At week 8, to control the increase in nitrite, in addition to water replacement, shrimp feeding was stopped until nitrite levels were below 5 mg/L.

Based on the analyses of Fig. 6(b) and (c) it is noticeable that from week 8, nitrite started to lower (week 8–24.30 ± 2.80 mg NO_2 L^{-1}, week 9–3.43 ± 3.43 mg NO_2 L^{-1}, and weeks 10 and 11–0.00 ± 0.00 mg N-NO_2 L^{-1}) and nitrate started to increase, indicating nitrite nitrification into nitrate. In weeks 10 and 11 nitrites were not detected, also, the highest nitrate values were observed (week 10–107.78 ± 2.74 mg NO_3 L^{-1} and week 11–86.55 ± 6.86 mg NO_3 L^{-1}). According to Prangnell et al. [47], high concentrations of nitrites in new biofloc systems should drop to near zero after nitrite oxidizing bacteria are established, usually around six to eight weeks from the start of cultivation.

However, the chemoautotrophic bacteria community stabilization time can be shortened by avoiding peaks of ammonia and nitrite, using a mature biofloc inoculum with an established nitrification from previous productive cycles, or fertilizing the water with ammonium and nitrite salts at the beginning of the cycle to stimulate the growth of nitrifying bacteria [45].

The nitrite results in the present experiment are higher than those in the studies of *L. vannamei* in aquaponics with *Sarcocornia ambigua,* with biofloc [24–28], probably because these studies started when the biofloc was already in chemoautotrophic stage, with the nitrification process

Fig. 9. New Zealand spinach in the last week of the experiment.

established.

3.3. Zootechnical performance

Table 3 shows the zootechnical performance of *L. vannamei*. The weekly weight gain was 1.36 g, a value close to those obtained by Pinheiro et al. [24], which were 1.4 g for systems with salinity of 8 ppt and 1.2 g for systems with salinity of 16, 24, and 32 ppt for cultivation in a biofloc system. Survival rate was 18 %, which is lower than expected. Several studies of *L. vannamei* in biofloc in aquaponics systems with plants presented a survival rate between 50 % to 90 % such as Carneiro [25], Pinheiro et al. [26], Pinheiro et al. [24], Silva [27], and Soares [28]. In Fig. 7 we can see *L. vannamei* on the last day of the aquaponic experiment.

According to Kubitza [23], the initiatives of intensive cultivation of *L. vannamei* in BFT systems, with just a few exceptions, are not achieving the expected results. The main reason is high mortality rates (survival rates between 10 % and 30 % are common), due to illness or problems related to water quality, especially regarding nitrite in waters with salinities below 15 ppt.

In Table 4 we can see the values of the concentrate's ionic balance, and of the biofloc when an increase in shrimp deaths occurred (sample collected at the end of week 7); samples from one day after the water exchange, in which 310 L of biofloc were removed and 340 L of concentrate were added (at the beginning of week 8); samples from the last day of the experiment (week 11); and, a comparison with the proportion of ions recommended by Kubitza [23], where the author presents the amounts in seawater (35 ppt) and stipulates the quantities of 10 ppt that should be adopted for brackish water.

Therefore, it is noticeable that the ion balance of the aquaponic pilot system had ion proportion values similar to those recommended. Analyzing the values of concentrate and other samples from the system, we can observe an increase of these ions, which may be due to the water evaporation and replacement with concentrate that was carried out throughout the experiment, increasing their concentrations.

It is believed that both low salinity and high nitrite values are related to low shrimp survival. The low salinity of the system had a direct relationship with the mineral composition of the water. In the study of Pinheiro et al. [24], was observed that salinity affected shrimp survival, a direct relationship was found between the reduction in salinity and the increase in shrimp mortality, shrimp with salinity of 8, 16, 24 and 32 ppt were cultivated and had survivals of 56.3 ± 4.7, 83.3 ± 1.2, 82.6 ± 4.3 and 84.0 ± 4.0 respectively, Maicá et al. [48], observed the same behavior. Also according to Pinheiro et al. [24], although it is a euryhaline species, to ensure satisfactory survival and growth of *L. vannamei* in low salinity, the proportions of ions such as sodium, potassium and magnesium must be close to those found in seawater. According to Galkanda-Arachchige et al. [49], the ionic balance is important in the molting phase of shrimp, when the animal cannot change the shell properly, it is more susceptible to diseases and stress, especially the stress caused by nitrite spikes.

The low survival value was due to the high levels of nitrites recorded from week 5 to week 8 (Fig. 6(b)), which are toxic to shrimp in high amounts [35,42]. In Fig. 8, the direct link between the death of shrimp and nitrite increase is noticeable, since when the highest nitrite values (Fig. 8(a)) were observed, the highest numbers of deaths (Fig. 8(b)) were also recorded, as in week 7. The measures to contain the increase in nitrite were implemented behind schedule due to the delay in the production of concentrate in sufficient quantities to exchange part of the water in the system, which could have avoided some shrimp deaths. One way to avoid the death of shrimp due to nitrites in BFTs systems would be to work with higher salinity [23]. Pinheiro et al. [24], observed, through interpolation of the experiment data, the highest survival of *L. vannamei* in salinity of 25.7 ppt.

A high number of deaths was observed at the beginning of the experiment, after the post larvae shrimp were introduced to the system, probably due to the stress that the shrimp were subjected to in transportation (15.1 km), and the acclimatization period, where the most fragile shrimp ended up dying.

The death count of *L. vannamei* was made from what it was possible to observe and collect, therefore, there may be higher numbers of deaths. This count may have been influenced by the turbidity of the water in the system, which makes observation difficult, and also the practice of cannibalism by *L. vannamei* that feed on the dead, reducing the number of dead.

3.4. Phyto technical performance

New Zealand spinach was transplanted into the system at week 4, where it stayed for 49 days or until week 11. At the beginning of the experiment, seedlings with approximately seven spinaches were transplanted. In week 7, the amount of spinach in all cells of the hydroponic bench was reduced to three or four. This decrease in quantity was to avoid high plant density and shading, which could affect the growth of the spinach [50,51]. Table 5 lists the phytotechnical numbers, where the final biomass was 519.41 g and the final average length was 13.54 cm,

not taking the roots into account.

At the end of the experiment, some leaves showed indication of nutritional deficiencies, which could be a lack of iron. According to Veígas et al. [52], this is due to the presence of chlorosis in young leaves, veins with fine reticulation, initially green in color, and later pale green (Fig. 9). Iron deficit can be corrected by adding it to the system [53].

4. Conclusion

Valorization of reverse osmosis concentrate in the production of *Litopenaeus vannamei* and New Zealand spinach in an aquaponic system with biofloc was promising. We believe that by taking care of the system's ionic balance and keeping the nitrite level low, the experiment can be replicated with better survival rates for the largest shrimp. It is recommended to start the experiment when the biofloc system is in the chemoautotrophic stage, with the nitrification process established, or work with higher salinity. Working with a lower density of shrimp would also improve the result.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Ailton Borges Rodrigues reports financial support and equipment, drugs, or supplies were provided by National Council for Scientific and Technological Development.

Data availability

data can be given if requested

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