



Comparing the growth of giant freshwater shrimp, *Macrobrachium rosenbergii*, in different culture ecosystems using biofloc, aquaponics and synbiotic bacteria technologies

Fadaei M.M.¹; Rafiee Gh.^{1*}; Rezaei Tavabe K.¹; Nematollahi M.A.¹; Frinsko M.²; Kiarsi Alikhani E.³

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Abstract

In aquaculture, the use of new technologies such as biofloc, aquaponic and synbiotics has increased the efficiency of aquatic animal production. In this study, five culture systems as experimental treatments, 1-biofloc (B), 2-biofloc and synbiotic (BS), 3-biofloc and aquaponics (BA), 4-biofloc, aquaponic and synbiotics (BAS), and 5-aquaponic (A), were designed for growth of giant shrimp, *Macrobrachium rosenbergii*, post larvae. The growth indices and water quality parameters were measured and compared among the experimental treatments. The highest total body length was observed in treatment BS (74.28±18.63 mm) and had a significant difference compared to other treatments ($p \leq 0.05$). The highest concentration of ammonia was related to treatment A (0.77±0.22 mg/L) and the lowest was recorded in treatment BAS with the rate of 0.61±0.16. Nitrite had the highest rate in treatment BS, (8.01±7.08) and the lowest recorded in treatment BA (6.47±6.55). The nitrate had the highest rate in treatment BAS, with the rate of 6.11±5.52 and the lowest recorded in treatment B with the rate of 4.29±4.09. The results of this study indicated that using biofloc, aquaponics and synbiotics, in a culture system, increase the efficiency of culture system in production of freshwater giant shrimp.

Keywords: Water quality, synbiotics, Phytoremediation, *Macrobrachium rosenbergii*

1- Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran.

2- Department of Fisheries, Faculty of Natural Resources, University of Karolina, United States

3- PHD candidate, Department of Fisheries, Faculty of Natural Resources, University of Tehran, Karaj, Iran

*Corresponding author's Email:ghrafiee@ut.ac.ir

Introduction

In recent years, shrimp production is one of the most important aquaculture activities in most tropical countries, and grown significantly in most parts of the world (FAO, 2020). On the other hand, the aquaculture sector has always faced problems, including water quality control, the spread of diseases, inappropriate food ratio, and in some cases growth reduction. The outbreak of diseases as the main problem of aquaculture has affected the economic expansion of this sector in many countries (Hoseinifar *et al.*, 2018). For example, in Iran, in recent decades, extensive practical measures have been taken into identify potential talents, especially in the southern coasts of the country, in order to develop the shrimp culture to reach shrimp industry, it is needed construction of thousands of hectares of shrimp ponds in the identified lands. The main motive of such expansion is to increase economic activities, create productive employment, increase shrimp production as a food item with high nutritional value, and increase the export of non-oil products. In the way of shrimp production, many researches have been conducted in connection with reducing water use and increasing the density of culture system all over the world, and led to the presentation of special methods to increase shrimp production with high density (Rafiee *and Saad.*, 2005). Although in many cases, the high density of shrimps in shrimp ponds has caused the transmission of more pathogens. At the same time, the demand

for sustainable and environmentally friendly aquaculture is increasing, and many researchers are looking for the use of materials to increase the growth and safety of aquatic animals in addition to being environmentally friendly (Adineh *et al.*, 2018; Minabi *et al.*, 2020). Probiotics increase the resistance of shrimp against diseases, prevent the growth of harmful bacteria, such as salmonella, clostridium and E-coli, and ultimately prevent the development of serious diseases in shrimp or its effect. It should be mentioned that prevention of disease occurs in a good microflora balance state. In this case, the beneficial bacteria in the right presence conditions and with a certain density prevent the establishment of harmful bacteria and prevent their reproduction, thus preventing the harmful bacteria from gaining strength. Therefore, the power of colony formation by beneficial bacteria is an important criterion for the performance of probiotics, and unstable bacteria can also be useful in high density if they are used continuously. Another useful way is bioflocs, in which the density and activity of the heterotrophic aerobic bacteria manipulated the ratio of carbon and nitrogen concentrations in the water for the benefit of the cultured organisms. The high ratio of carbon to nitrogen decreases the total ammonia nitrogen by bacterial biomass and increases the biomass of microorganisms. This absorption of nitrogen by heterotrophic bacteria, is much faster than nitrifying bacteria, and accelerate the reduce or remove of toxic nitrogen in water. This

biological mass includes a heterogeneous composition of the population of microorganisms and contains mass forms of filamentous bacteria, colloidal particles, suspended particles, organic polymers, cations and dead cells that flake on each other. Another creative system for intensive production of aquatic animals, to increase the productivity and growth of aquatic animals is named aquaponics system. In aquaponics technology, the cultivation of plants and aquatic animals are integrated, all of these technologies could not be extended to a large scale, however, saves water and reduces water use, especially in culture facilities, as a result, are friendly with the environment. It becomes compatible with the environment and prevents the pollution and destruction of the environment in dry and water-deficient areas, and the wastewater produced in aquaculture is used for the growth of the desired plant. Also, Economic efficiency increases. Unfortunately, due to hardware and software problems, this technology has not yet reached its nominal production capacity in some countries (Rafiee and Saad., 2005). Therefore, the role of heterotrophic bacteria, nitrifying bacteria, algae and plants and their performance according to the target species or the biological function of organisms, according to the type and quality of consumed food and in relation to the physical part of the structures, a balanced aquatic culture system with maximum water efficiency initiated (Rafiee and Saad., 2005). Therefore, in this research, the roles and functions of

biofloc, plants and the presence of synbiotic bacteria separately and in combination in increasing the efficiency of closed-loop system were compared for culture of *macrobrachium rosenbergii* larvae.

Materials and methods

Place of research and test plan

In order to running this research, the number of 1000 shrimp post-larvae with an average weight of 0.015 grs, transferred from breeding center of giant fresh water shrimp (*Macrobrachium rosenbergii*), farm of Qasr Shirin city, Kermanshah province, to the laboratory of water quality control and aquatic reproduction of the Faculty of Natural Resources, University of Tehran, located in Karaj city. The five experimental treatments were adjusted according to the presence of probiotics, regulation of the ratio of carbon to nitrogen (biofloc) and the presence of plants in the rearing systems (Table 1).

Table 1: Characteristics of the treatments.

Treatments	Type of treatment
B	Biofloc
BS	Biofloc and Synbiotic
BA	Biofloc and Aquaponics
BAS	Biofloc, Aquaponic and Synbiotics
A	Aquaponics

Preparation of shrimp tanks and base system

Before transferring the post-larvae to the test site, preparation of tanks and test units was done in the aquatic quality control laboratory. Then 36 peppermint plants seedlings were introduced to the

experimental unites. To adjust the ratio of carbon to nitrogen in tanks with biofloc, 8.575 grs of beet molasses was poured with 6.125 grs of food in eight litres of water and gently aerated for one week, due to the activity of microalgae and heterotrophic bacteria, while its colour changed from green to brown at the end of the week, biofloc formed. One litre of the solution was poured into the Imhof funnel and after settling the biofloc rate, it was introduced in treatments containing biofloc and

repeated on the 47th day of initiation of the experiment. The beet molasses was used to provide a carbon source for bacterial activity. After running the experimental system, pots were also placed in the shrimp tanks to create a favourable environment and prevent cannibalism, then the post-larvae introduced to the experimental units and post larvae fed on for 2 months (Figs 1 and 2).



Figure 1: A and B: Placement of peppermint plants on the culture tank.

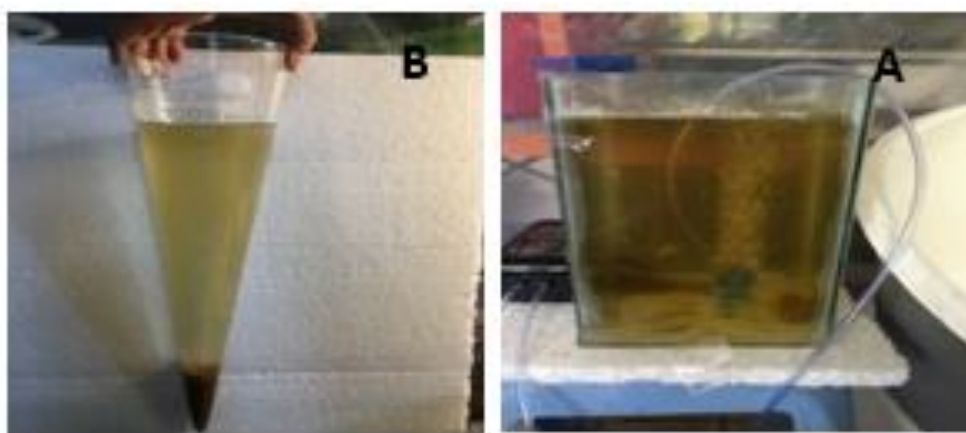


Figure 2: Biofloc. A: Produced biofloc; B: Pouring the biofloc into the Imhof funnel.

Feed and feeding

The post-larvae fed with salmon food S₀, brand of 21 Bayza production company, containing 50% protein, at the rate of

20% of the body weight daily, (0.075 grams), at 9, 15, 21, and 15 hrs, and in the middle of the experiment, feeding regime was reduced to two times, this

rate increased to 40% until the 17th day of initiation the experiment while sampling for biometry, counting and weighing of post larvae were done, nutritional calculations and the biofloc rates were taken into account, for each treatment and after that, feeding was increased by five percent compared to the previous day. On the 9th, 23rd and 44th days of the experiment, synbiotic with the brand of Tekgen company, D

Pro Plus model, special for aquatics, mannan oligosaccharide (MOS), inoculated in treatments containing synbiotic, seven grams solved in four liters of water, then aerated for 20 minutes and introduced into each tank. Molasses also added to the shrimp tanks based on nitrogen of food consumption at 10 am every day as calculation follows (Hosseini Far *et al.*, 2019):

$16\% \times 29\% \times \text{daily food amount} = \text{food nitrogen amount}$

$\text{The amount of nitrogen in food consumed daily} \times 70\% = \text{nitrogen input to the system}$

$50\% \times (\text{the amount of molasses} + \text{the amount of daily food}) = \text{carbon entering the system}$

In order to compensate the water evaporation through the systems, 32 litres of water added to each tank weekly. On the 17th day, 20 litres of water replaced with fresh water in each experimental treatment.

Water quality parameters measurement

In order to evaluate the physical and chemical quality of water in shrimp tanks, water sampled and total phosphorus, phosphate, total ammonia, nitrate, nitrite and total nitrogen were measured, the samples sent to the clean environment laboratory in Tehran province and were analysed. The physical indicators of water, including temperature, pH, electrical conductivity and TDS, were recorded daily by the HANNA four-index measuring device, model SN F8819867.

Recording the growth indices and biometry

Shrimp samples were done from the shrimp tanks and the weight, total length and carapace length of the shrimps were recorded using Image J software.

Results

Total length

The most significant increase in total length was observed in BS treatment with a rate of 74.28 mm ($p \leq 0.05$), this rates in BPA was 72.67 mm and in BA was 62.86 millimetre. There was no significant differences in the carapace length among the treatments at the 5% level. The final weight did not show a significant difference between the treatments ($p \leq 0.05$) at the end of experiment. The mortality rate was high due to cannibalism, among the treatments and the highest survival rate was seen in the biofloc treatment, but no significant difference was observed among the treatments ($p \leq 0.05$) (Table 2).

Table 2: Average changes in total length, carapace length, species weight, survival rate and final biomass in experimental treatments (mean±sd, n=5).

A	BAS	BA	BS	B	Treatment
ab 61.16 ±7.38	abc 72.67 ±7.21	bc 62.86 ±11.55	c 74.28 ±18.63	a 60.33 ±11.97	Total Body Length
a 12.69 ±1.72	a 13.75 ±1.47	a 12.98 ±2.26	a 13.67 ±2.34	a 12.36 ±1.48	Carapace Length
a 1.4 ±0.50	a 1.81 ±0.02	a 1.7 ±0.3	a 1.65 ±0.35	a 1.26 ±0.35	Final Individual weight
a 16.50 ±3.53	a 10.50 ±6.36	a 10.50 ±6.36	a 8.00 ±5.65	a 14.00 ±8.48	Secondary Number
a 66.00 ±14.14	a 42.00 ±25.45	a 42.00 ±25.45	a 32.00 ±22.62	a 56.00 ± 33.94	Survival rate
a 22.22 ±3.33	a 18.96 ±11.26	a 18.92 ±14.07	a 12.26 ±6.56	a 19.22 ±15.73	Final Biomass

The same superscript letters above the numbers in a row mean that there is no significant at the 0.05 level.

Water quality indicators

The Ec was not significantly different among the treatments until 3th week, an increasing trend was seen until the end of experiment ($p \leq 0.05$) (Fig. 3).

pH

The pH increased until 3 weeks of initiation the experiment then a high depletion occurred by 5th week, after that had an increase trend until the end of experiment, no significant difference was recorded among the treatments during the experimental period ($p \leq 0.05$). (Fig. 3).

Total dissolved solids concentration TDS

During the first to the ninth week, no significant difference was seen among the treatments ($p \leq 0.05$). But treatment B had a significant difference in the second week compared to other treatments ($p \geq 0.05$) (Fig. 3).

Water temperature

The water temperature did not show any significant difference among the treatments during the first to the ninth week ($p \geq 0.05$) (Fig. 3).

Total Ammonia concentration

Ammonia concentration showed a decreasing trend in all treatments from the first week to the third week, and then an increasing trend with an exponential slope from the fourth to the sixth week recorded, then showed a sharp decrease in the seventh week. From the third week onwards, the reduction of ammonia concentration was more noticeable among some treatment (Fig. 4).

Nitrite

The amount of nitrite in the first week was low in all treatments. In treatment B, there was a significant increase in the second week, and this value remained constant in the third week, and then in the fourth week, it increased again. After

that, in the fifth week, decreased sharply, and in the sixth and seventh weeks, the amount of nitrite decreased a lot. In BAS treatment, the highest amount of nitrite was seen in the second week, and after that, it decreased until the fifth week,

then it decreased to a negligible amount in the sixth and seventh weeks. In treatment A, a constant trend was recorded from the second to the fifth week, and it reached a negligible value in the sixth and seventh weeks (Fig. 4).

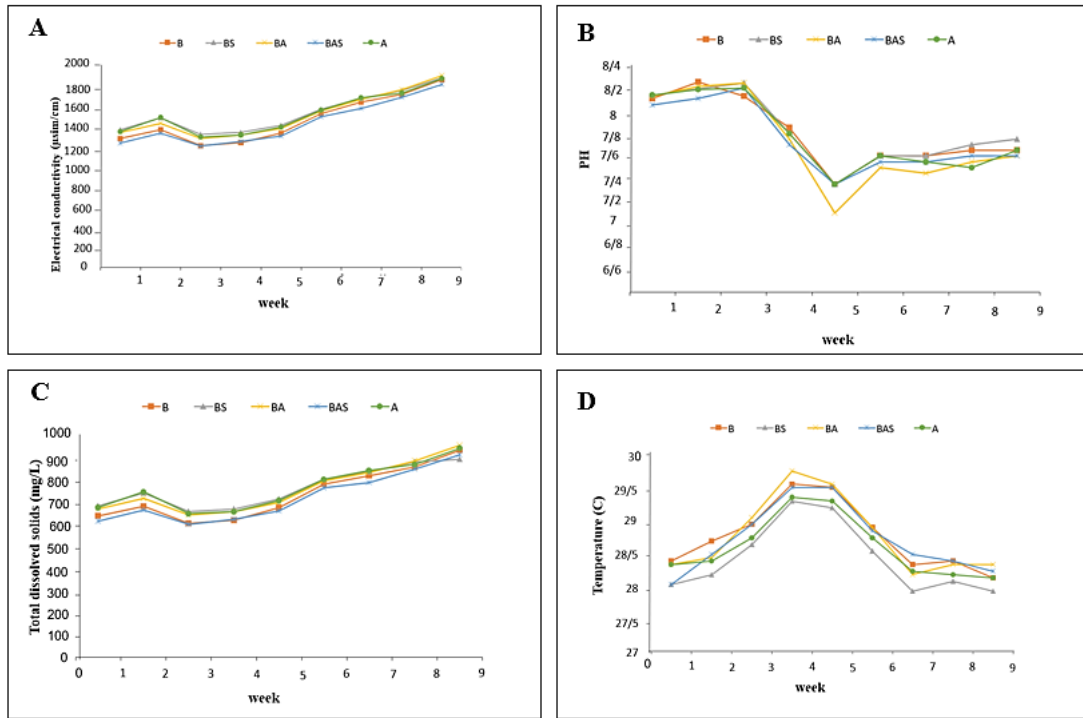


Figure 3: A. The mean (mean±sd) of Ec ($\mu\text{sim.cm}$) changes among the treatments during the experimental period. B. The mean (mean±sd) of pH changes among the treatments during the experimental period. C. The mean (mean±sd) of TDS changes among the treatments during the experimental period. D- The mean (mean±sd) of Temperature ($^{\circ}\text{C}$) changes among the treatments during the experimental period. D. Changes (mean±sd) of total water ammonia (mg.liter) among the treatments during the experimental period.

Nitrate

There was a slight increase in nitrate concentration in treatment B in the second week. Then in the third week, its amount decreased slightly. Finally, in the fourth, fifth, sixth and seventh weeks, it decreased to an insignificant amount. In BS treatment, there was a decrease in the trend in the second week and an increase in the third week and then a slight decrease in the fourth, fifth, sixth and seventh weeks. In BAS

treatment in the second week, there was a decreasing trend, then an increasing trend in the third week, and then it reached a negligible value in the fourth, fifth, sixth and seventh weeks. In treatment A, it remained constant in the first, second and third weeks and reached a negligible value in the fourth, fifth, sixth and seventh weeks (Fig. 4).

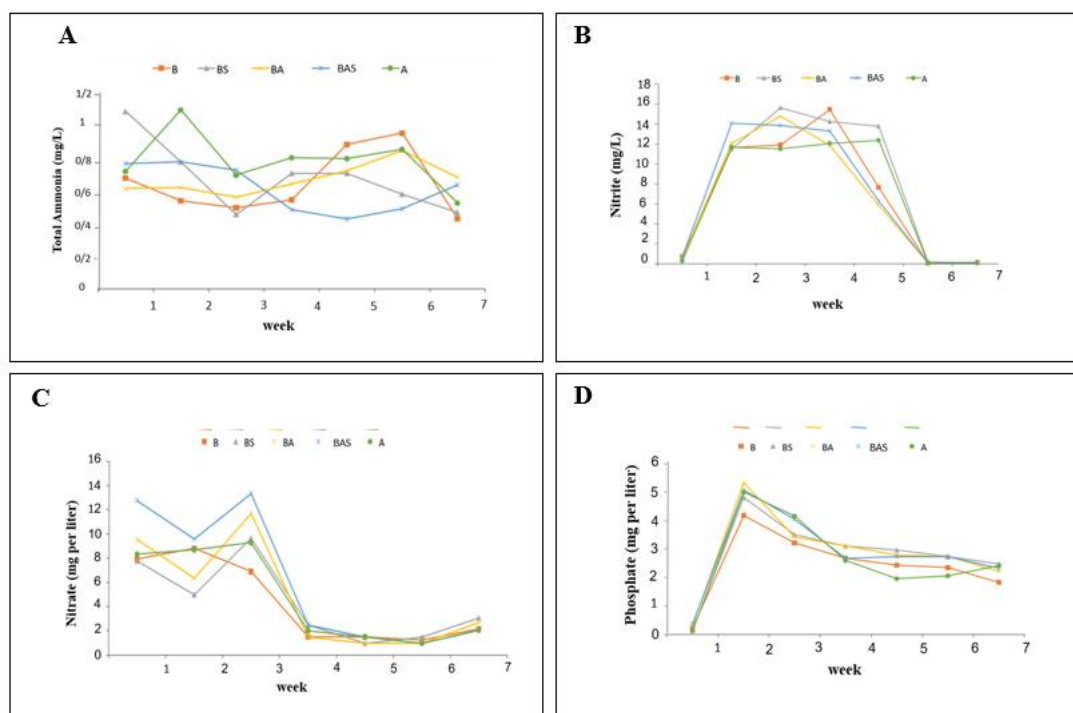


Figure 4: A. Changes (mean \pm sd) in total water ammonia (mg.liter) among the treatments during the experimental period. B. Average changes (mean \pm sd) of water nitrite (mg.liter) among the treatments during the experimental period. C. The mean changes (mean \pm sd) of water nitrate (mg.litre) among the treatments during the experimental period. D. Mean changes (mean \pm sd) in water phosphate (mg.liter) among the treatments during the experimental period.

Total Phosphate

The amount of phosphate in the first week was insignificant in different treatments due to the lack of biochemical reactions. Then, in the second week, its value increased in all treatments and then showed a decreasing trend in all treatments until the end of the experiment (Fig. 4).

Discussion

The first case of *M. rosenbergii* shrimp production recorded by FAO statistics in 1970 (FAO, 2008), and reported that 10 tons produced by Mexico and less than half a ton by Mauritius. By 1979, the annual world production had reached more than 1100 tons, produced by Brazil, Ecuador, French Polynesia,

Guam, Honduras, Malaysia, Mauritius, Mexico, Myanmar, Thailand and the United States. By 1990, global production had increased to more than 20,000 tons annually, and many new countries were listed as producer of giant shrimp.

Now a- days, giant freshwater shrimp (*M. rosenbergii*) is an important commercial aquaculture species in tropical and subtropical regions (New, 2002; Kim *et al.*, 2013). This type of shrimp is widely cultivated in many Asian countries such as Bangladesh, China, India, Myanmar, Malaysia, Taiwan, Thailand and Vietnam (Iketani *et al.*, 2011; Pérez - Fuentes *et al.*, 2013; Marques *et al.*, 2016). In order to ensure the balanced growth of aquaculture, new

technologies and management strategies to get sustainable aquaculture production has been suggested. These methods enable maximum production per volume unit with minimum negative environmental effects and less use of water resources. In this sense, in the last decade, more studies have been focused on closed recirculating aquaculture systems that require a small volume of water and minimize wastewater discharge. Examples of this type of facilities are closed aquaculture facilities and systems (RAS) and facilities that use for biofloc technology (BFT). RAS is an aquaculture facility based on biological and physical filters, where water is continuously circulated and recycled. For some reasons, such as high biological security, environmental advantages and better marketing, aquaculture in closed facilities is more popular than open and semi-dense facilities. In closed facilities, due to the return and reuse of water, the possibility of some risks such as some diseases, the exit of non-native species is restricted (Ray *et al.*, 2010). Also, several types of probiotics are used in production of marine crustaceans. Among these probiotics, we can mention *Lactobacillus*, *Enterococcus*, *Bacillus*, *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Microbacterium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodospiridium*, *Roseobacter*, *Streptomyces* and *Vibrio*. In this study, higher total length significantly observed in the BS treatment with a rate of 74.28 mm and

followed by BAS with a rate of 72.67 mm and BA with a rate of 62.86 mm, carapace length had not any significant differences between treatments. Comparing the final weight, the highest value for BAS treatment was 1.81 ± 0.001 grs and the lowest recorded in B treatment with the rate of 1.26 ± 0.001 grs, which can be related to good condition for growth of shrimp (Pérez Jesús *et al.*, 2022). Ammonia removal with higher efficiency reported by *Bacillus* supply around 91.73% to 90.57%, comparing the commercial photosynthetic bacteria (86.69%) (Thawinwan *et al.*, 2022). The released nitrogen through the culture system shifted to the production of microbial protein and this protein can be consumed as food. It is reported that consumption of microbial protein in biofloc systems is two times more than in normal condition. Considering that nitrogen is an essential element for the production of protein, nucleic acid and cell components, and phosphorus is an essential element for the cell wall and energy transfer (Mohammadi *et al.*, 2021).

It is shown that availability of biofloc significantly increase the activity of digestive enzymes, weight gain and average daily growth of fish (Mohammadi *et al.*, 2021).

The rate of nitrate was the most in BAS treatment, which was due to operation of nitrifying bacteria, and improvement of organisms by synbiotics. The results showed that between 39.3-24.1% of nitrogen and 19.4-14.8% of the total feed input can be

removed as a result of the integration of shrimp and plant production. It is important to mention that both nutrients usually accumulate in biofloc water (Pinho *et al.*, 2021).

In general, the results obtained from this research showed that the use of plant extracts did not have a significant effect on the growth and nutrition performance in the biofloc, while the consumption of yucca plant extract in shrimp diet increased hepatopancreas enzyme secretions and reduced ammonia in the breeding environment compared to other experimental treatments (Ahmed *et al.*, 2020).

According to the description above, the competition between plants and bacteria in absorbing the ammonia was higher in BAS treatment, and the absence of bacteria in treatments A caused the amount of increase, showing that integrating the Biofloc, aquaponics and symbiotic bacteria as an integrated system is more efficient in reduction of ammonia from the culture system (Thawinwan *et al.*, 2022). When bacteria are fed with organic materials containing high carbon and low nitrogen (sugar, starch and molasses), they need to absorb nitrogen from water in order to produce the protein needed for cell growth and reproduction. One of the important aspects of microbial control is the control of inorganic nitrogen in water and the production of microbial protein by adjusting the ratio of carbon to nitrogen in food. Instead of being toxic compounds, the uneaten nitrogen is used for production of microbial protein and this protein is used as food. The

consumption of microbial protein in biofloc systems is twice as much as in normal system, and in this system, there are signs of reducing the spread of microbial diseases due to the presence of a diverse population of heterotrophic bacteria (Mohammadi *et al.*, 2021).

In the examination of nitrite, in the BA treatment, nitrite was the least due to the low amount of ammonia and the presence of heterotrophic, autotrophic and biofloc bacteria, and the highest was recorded in the BS treatment, which was due to the consumption of oxygen by the bacteria. synbiotics and an increase in bacterial load as well as low conversion of nitrite to nitrate. In a study, the effect of potential synbiotics on single-strain (SSP; *Lactobacillus plantarum*) or multi-strain (MSP; *Bacillus subtilis*, *L. plantarum*, *L. rhamnosus*, *L. acidophilus*, *L. delbrueckii*) diets on Nile tilapia (*Oreochromis niloticus*) were investigated and resulted that bacterial supplements show significant effects on the levels of EC, TAN, NH₃, NO₂ and NO₃ (Mohammadi *et al.*, 2021).

The lowest amount of nitrate was recorded in treatment B, in fact, it indicates that nitrate was used to feed heterotrophic bacteria, and it was the highest in BAS treatment, and the reason for this was the increase in the activity of heterotrophic and autotrophic bacteria and the improvement of organisms was caused by synbiotic action (Fadhilah *et al.*, 2019). It has been shown that in shrimp culture tanks, adjusting the concentration of ammonia and nitrate is very important in maintaining the proper quality of water during the culture

period. The five-day biological oxygen demand will increase significantly with the increase in organic matter load in the rearing tanks (Khoda Bakhsh *et al.*, 2011). The total nitrogen in B treatment was the least due to its conversion into microbial protein in biofloc structures, and the highest amount was recorded in BS treatment. In examining the amount of phosphate and total phosphorus, it can be concluded that in treatment B, its amount was lower than other treatments and in treatment BA, it was the highest in BAS with a difference of 0.1. Biofloc was also shown to contribute to the needs of farmed freshwater shrimp, as shown by improved feed utilization, phosphorus retention, and growth performance. *Bacillus* spp., *Pseudomonas* spp., the dominant microbial community in a biofloc-based culture system have also been reported as efficient phosphate reducers. In fact, heterotrophic bacteria play a big role in absorbing a large part of orthophosphate in fresh water with an estimated rate of about 60%. Application of probiotics significantly reduced total phosphorus and total mineral phosphorus in sediments. The possible reason could be the use of phosphate by the bacterial consortium created in the biofloc structures, in which a wide variety of microorganisms participate. Phosphate concentration decreases significantly with increasing C/N ratio as well (Dash *et al.*, 2018). In aerobic conditions, the pH of the solution decreases after respiration and nitrification. Carbon dioxide as a final product of respiration helps to reduce pH, likewise, lowering

the pH below 6 in the bioreactor increases mineralization, nutrient mobility, so more phosphorus is recovered at low pH. Therefore, it is important to ensure that the pH is constantly maintained below six during the mineralization process (Da Silva *et al.*, 2016).

Ions, where the last two ions are the main forms of phosphorus, are taken up by plants. Therefore, when the pH is slightly acidic, the maximum amount of phosphorus is provided in a nutrient solution. The most suitable pH values of food solution for the development of crops are between 5.5-6.5 (Goddek *et al.*, 2015). Using plants as filters to remove nutrients from water is a capable approach to nutrient recycling that has been explored in floc-ponics research. In these studies, the focus has been on recycling nitrogen and phosphorus and converting them into plant biomass. For long-term rotations, the chemical composition must be well known and regularly monitored to avoid imbalances in nutrient supply. In aquaponic systems, nitric acid (HNO₃) and potassium hydroxide (KOH) are used to adjust the pH and at the same time providing the macronutrients in the systems. (Goddek *et al.*, 2015). During the cultivation of giant fresh water shrimp, with biofloc technology, survival of higher than 85% has been obtained, which is consistent with the results of the present study (Pérez *et al.*, 2022).

Ammonium conversion to microbial protein requires less dissolved oxygen compared to the oxygen needed for nitrification (Rafiee and Saad., 2005;

ME *et al.*, 2008). As a result, it can be concluded that shrimp effectively reduces the accumulation of settled solids without considering the mortality rate in the plant layer. The arrangement of flocconic structures should be designed in a way that provides the best possible conditions for the production of aquatic animals and plants and the maintenance of BFT microorganisms. The main issue identified in this study, is related to the maintenance of suspended solids in water at suitable concentrations for plant and fish production. As mentioned in the above sections, plant growth seems to be limited due to the presence of additional solids in flocconic structures. In examining the percentage of protein, it is pointed out that bacteria are very small and have a diameter of one micrometer. When the density of microbial biomass is high, they tend to gather and form flocs that are between 0.1 and several millimeters. The formation of flocs affects their shape and stability, and many organisms secrete extracellular polymers that are made of polysaccharides and proteins and act like glue and connect other particles together, it seems that the flocs attract bacteria and higher organisms and form a dynamic ecological system, and their establishment period is about ten hours. On the other hand, due to the destruction of organisms due to existing nutrition and microbial decomposition, production of new microbial cells occurs, and they change twice a day, and new flocs are formed (Rafiee *et al.*, 2021).

Conclusion

It was concluded that there are differences between the performance of organisms in biofloc technology, photosynthesizing plants and symbiotic bacteria while are used separately and together. Also, water consumption is less and the amount of mineral and organic substances increased. It can be said that the use of biofloc technology is significantly more practical and even the use of biofloc or the combined biofloc, symbiotic and aquaponic systems compared to traditional methods greatly improve the existing culture ecosystem conditions and it improves the nutritional conditions of shrimp and probably prevents the occurrence and spread of pathogenic agents. Increasing the resistance of aquatic animals against many diseases is also a new solution in the fishery industry, however it is needed gathering new data from the results of future aquaculture researches.

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