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Nitrogen Utilisation Efficiency in Aquaponics Circulation System Using Nitrobacter Treatment

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Abstract
Aquaponics is a sustainable system that combines aquaculture and hydroponics while relying on nitrifying bacteria to maintain its efficiency. However, the absence or low levels of these bacteria can hinder the system's performance, impeding its progress. Therefore, this study aimed to investigate the effect of Nitrobacter supplement used in the aquaponics system to enhance its nitrogen utilisation efficiency. The study utilised broadhead catfish (*Clarias macrocephalus*) and water spinach (*Ipomoea aquatica*) in the aquaponics setup. Two distinct treatments were applied, one with the addition of the Nitrobacter supplement and another without it. Water quality (pH, ammonia, nitrite, and nitrate levels), as well as plant and fish growth, were examined in both treated and untreated aquaponics systems. The results indicated that the treated aquaponics system maintained a pH level of 7.2 and reduced ammonia content to 10 ppm, creating a non-toxic environment for fish. Furthermore, the treated system exhibited significantly higher nitrate levels, with an increase of 80 ppm compared to the 10 ppm in the untreated system. Plant growth parameters, including length (21.60 ± 2.62 cm) and leaf count (7.67 ± 0.33), also show positive improvements in the treated aquaponics system. Fish in the treated system displayed a substantial enhancement in weight (41.76%) and length (28.15%) compared to those in the untreated system. Introduction of Nitrobacter into the aquaponics system had a positive impact on water quality and the well-being of both the fish and the plants, indicating its potential to improve overall system performance.
Keywords: nitrogen utilisation; aquaponics; nitrobacter

Introduction
Globally, there's a significant increase in the demand for food production, driven by the growing human population in the 21st century. Both aquaculture and agriculture are rapidly expanding sectors, but it's crucial to ensure their practices are sustainable to prevent long-term environmental harm. To address this challenge, one viable solution is the implementation of aquaponics systems, which can effectively support both aquaculture and agriculture in an environmentally friendly manner. Aquaponics is a holistic system that combines aquaculture (fish farming) and hydroponics (soil-less plant cultivation) within a single, interconnected cycle (Yep and Zheng, 2019). This system is characterised by its highly efficient water circulation, reducing the reliance on synthetic fertilisers, soil, and minimising wastewater production. Remarkably, aquaponics allows for the simultaneous cultivation of both plants and fish for food production, making it a sustainable and environmentally safer approach (Yep and Zheng, 2019)

Just like other living organisms, plants also require various types of elements and macronutrients that are essential for their growth and development which includes nitrogen (N). Nitrogen is one of the most essential elements that is needed to build up a biomolecule structure such as protein and nucleic acids in living organisms (Ueda et al., 2017). Nitrogen is an abundant element that can be found on earth where it makes up 78.1% of the atmosphere of the earth (Stevens, 2019).

Stevens (2019) also stated that most nitrogen exists in the form of gas (N_2) which is non-reactive and cannot be readily used by the living organisms. Thus, a portion of nitrogen undergoes a fixation process where it can happen naturally or through anthropogenic processes. Through the fixing process, nitrogen can be formed into a more reactive compound such as nitrogen oxides (NO_2), nitrous oxide (N_2O), nitric acids (HNO_3) and many more. In the aquaponics system, nitrogen can be produced by the fish in the form of ammonia (Sumeth et al., 2017). Several processes occur which make nitrogen in the form of ammonia turned into nitrate, which later can be used by the primary producer; the plants (Bernhard and Sara, 2010).

Due to the inability of plants to fully utilise nitrogen in the form of ammonia, the presence of nitrifying bacteria was required so that the ammonia can be converted into nitrate that can be utilised by the plant and act as fertiliser (Bernhard and Sara, 2010). There are a few examples of nitrite oxidising bacteria which includes Nitrobacter spp. and Nitrospira spp. These bacteria act as a filter that oxidises the nitrite and reduces the accumulation of waste product in the aquaponics system (Hu et al., 2015), and at the same time producing nitrate for plant growth and development. Those bacteria with nitrite oxidising ability will oxidise the nitrogen which is in the form of ammonia and nitrite to produce energy for its metabolism (Bernhard and Sara, 2010) and reduce the accumulation of fish waste which on the other way acts as water treatment.

Water treatment has become one of the problems in agriculture as it uses a lot of clean water source for the plantation and at the same time produces waste such as wastewater, excess fertiliser and pesticide to the environment. Without a proper solution to this problem, the amount of waste will increase and build up as time passes by in parallel with the production of products from the agriculture sector. Nonetheless, wastewater can be produced from many sectors, not only from agriculture. Another example that can contribute to wastewater production is aquaculture which also can have a bad impact to the environment such as water pollution. All of the events can be harmful to the environment if it is left untreated or not properly managed by the sustainable practices of the aquaculture system. Thus, to overcome this problem, a sustainable approach must be taken, which in this case is by practising aquaponics. However, the aquaponics system needs to be systematically operated so that the plant and the fish are able to produce maximum yield and reduce the waste produced from the farming activities simultaneously.

Hence, this study was conducted to investigate the effect of Nitrobacter, a nitrogen-fixing bacteria in an aquaponics system consisting of water spinach and catfish. The main objectives of the study were (1) To determine the water quality of the aquaponics system supplemented with Nitrobacter and without Nitrobacter. (2) To compare the growth of plants in Nitrobacter-treated and non-treated aquaponics systems. (3) To compare the fish growth in Nitrobacter-treated and non-treated aquaponics systems.

Materials and methods

Aquaponics system preparation

The aquaponics system consisted mainly of canvas with the size of 0.6m x 0.6m x 0.6m which is equal to 0.216m3, plant-grow bed (15cm x 10cm x 5cm) and electric water pump (Astro 300, China). According to the Malaysian Fisheries Institute, (2016), 1m3 can fit up to 129 fish with a length of 3 inches. Water volume in the fish tank was kept at 50 L for each pond and no water exchange was conducted throughout the 15 days period. The wastewater was pumped to the aquaponics beds at a water flow rate of 300 L per day. Aquaponics beds were filled with clay pebbles and placed on the position where the wastewater can recirculated back into the fish pond.

Fish and plants used in this study were broadhead catfish (*C. macroephalus*), and water spinach (*Ipomoea aquatica*). Three-month old broadhead catfish were reared in the system. The fish were fed twice a day (0900 hours and 1700 hours) with commercial fish starter feed (Leong Hup Aqua, Malaysia) which contained 34% of protein at 1.5% of fish weight. Water spinach seeds were germinated in a germination tray filled with peat moss for two weeks until three to four leaves of seedlings were produced. Then, healthy seedlings were selected and transplanted to the aquaponics beds with 5cm x 5cm spacing.

For this experiment, two separated aquaponic systems were constructed to differentiate between the Nitrobacter-treated aquaponics system with the non-treated aquaponics system. Each pond consisted of 25 catfishes and 6 water spinach. The Nitrobacter was purchased from Peeos Aquatic Company, Malaysia. Five g of Nitrobacter was applied on day one and followed by another 5g on day 10 following the recommendation of the company. Based on the study done by Degrange et al., (1997), the Nitrobacter population can grow in 15 days in a nitrite-enriched condition. Thus, this research was carried out within the 15-days period so that the necessity and efficiency of Nitrobacter treatment on the aquaponics can be investigated.

Water Quality Analysis

100 ml of water samples from treated and non-treated aquaponic systems were collected. The water quality analysis including pH level, ammonia, nitrite and nitrate content were identified using API Freshwater Master Test Kit. The result of the sample solution was compared to the pH Color Chart with white background. The water samples were collected and analysed at 3-days intervals.

Plant and fish growth experiment

The initial growth of both water spinach and the catfish were recorded on the first day of the experiment. At the end of the project (day 15), the final growth of both water spinach and catfish were recorded. The growth rate differences were examined by comparing the water spinach and catfish growth in the non-treated aquaponics system to the treated aquaponics system.

Plant growth

At the beginning of the experiment (day 1), plant height, number of leaves, leaf diameter and leaf length data were recorded. The data was then compared to the final reading at the end of the project (day 15) to measure the growth rate for both water spinach grown in the non-treated and treated aquaponics system. Plant height was measured starting from the surface of grow-beds media to the tip of the plant using measuring tape. Number of counted leaves, diameter and length of leaves were recorded for each plant.

Fish growth

At the start of the experiment (day 1), the data of the catfish were taken which includes fish length and fish weight. The data was then compared to the final reading at the end of the project (day 15) to measure the growth rate for both catfish cultured in the non-treated and treated aquaponics system.

Data Analysis

Water quality data were collected at 3-day intervals for 15 days, for both treated and non-treated aquaponics systems. Plant growth and fish growth performances data shown in the result were the average of 3 replications. The data were expressed in mean ± standard error. T-test for Equality of Means with significance value at $P \leq 0.05$ (2-tailed) was performed using Statistical Package of Social Science (SPSS) version 27 (IBM, USA). The 2-tailed test was used in this experiment because the difference tested is between two different groups. Formula for fish weight and fish length increase after 15 days of treatment was calculated as shown below (Olaniyi et al., 2013):

$$Percentage\ weight\ increase = \frac{mean\ weight\ gain\ (g)}{initial\ mean\ weight\ (g)} \times 100$$

$$Percentage\ length\ increase = \frac{mean\ weight\ length\ (cm)}{initial\ mean\ length\ (cm)} \times 100$$

Results and discussion

Water Quality Analysis

A total of four parameters were collected under water quality analysis which were pH value, ammonia content, nitrite content and nitrate content. Higher levels of ammonia were determined in non-treated water samples, contradicting the nitrate content which seems to be higher in treated water samples. On the other hand, the pH of the water sample for non-treated aquaponics system indicated higher overall reading.

pH level

The pH value of water in the aquaponics system was shown on Figure 1. The overall pH value for the aquaponics system that was not treated with *Nitrobacter* was higher and more acidic when compared to the water system treated with *Nitrobacter*. Li *et al.,* (2019) stated that the pH level in an aquaponics system needs to be around 7.0 or neutral to be favourable for fishes, plants and the *Nitrobacter* to live in the same system. However, the pH level of the water in the non-treated aquaponics system showed that it has a higher pH level. This can be caused by the amount of ammonia accumulation produced by the fish that cannot be transformed or oxidised into nitrite or nitrate due to the inexistence of the nitrifying bacteria in the non-treated aquaponics system. Eddy (2005) stated that ammonia can exist as ammonium ion (NH_4^+) and ammonia (NH_3) in water known as total ammonia nitrate, (TAN).

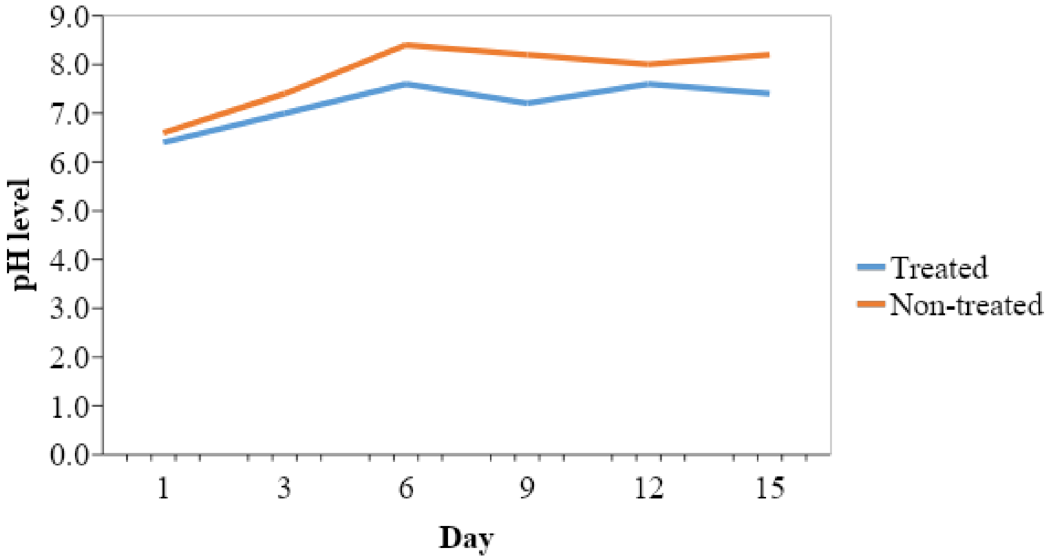

Figure 1 pH level in aquaponic water.

The pK (equilibrium constant) for the reaction of ammonia/ammonium was constant at 9.5 and it can be varying due to the temperature, where the concentration of unionised ammonia (NH_3) was stated to be higher in high pH and high temperature condition. It was reported by Tomasso *et al.*, (2011) that high pH level can increase the unionised ammonia where 600 mg/liter water containing TAN recorded high increment of unionised ammonia content from 2.5 to 24.6 mg/liter after the pH increased from 6.8 to 8.0. The unionised ammonia was toxic to the fish because most biological membranes are permeable towards the unionised ammonia (Eddy, 2005). According to Li *et al.*, (2019), fish can survive in water with a pH ranging from 6.5 to 9.0. Based on the research done on the ammonia toxicity to fish conducted by Thurston et al., (1981), it was stated that ammonia can be more toxic to the fish in higher pH. They stated that there was an increase of 10-fold of ammonia percentage in the water when the pH level was increased by one unit. On top of that, based on the result of water quality of tap water in Kuala Lumpur, the pH of the tap water tested can reached up to 7.99 (Ong *et al.*, 2007).

Ammonia content

From the result of the ammonia content, on the 15th day the graph shows a huge difference of ammonia content between the non-treated water sample and the treated water sample (Figure 2). The ammonia content differs by 4-folds where the ammonia content in the non-treated water sample is higher compared to the treated water sample with the result of 1.0 ppm and 4.0 ppm respectively. The lower amount of ammonia in the *Nitrobacter*-treated aquaponics system was due to the existence of the nitrifying bacteria where the ammonia undergoes nitrifying process to produce energy under aerobic condition (Zhu *et al.*, 2013). Apart from that, the ammonia accumulation occurs due to the excretion of waste by the fish in the aquaponics system and it can also be caused by the food given to the fish (Bernhard and Sara, 2010).

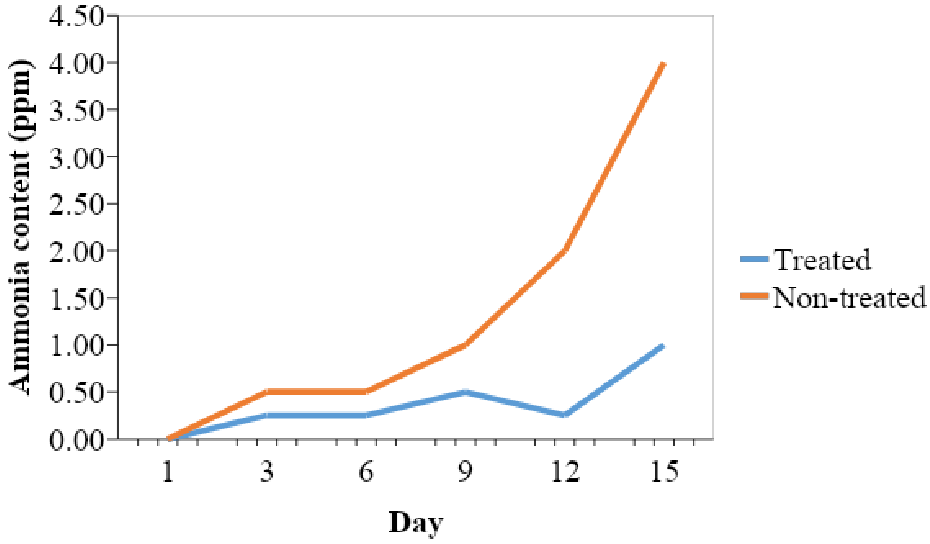

Figure 2 Ammonia content (ppm) in aquaponics water

In comparison with the result obtained on the pH of the water sample, the pH level for the non-treated aquaponics system showed higher pH level compared to the aquaponics system treated with the *Nitrobacter* treatment. Based on the research done by Tomasso *et al.*, (1980), the result showed that the ammonia present in the water was higher in the form of unionised nitrogen which was ammonia (NH_3). On top of that Park *et al* (2018) also stated that the concentration of unionised ammonia species increases as the pH of the water increases. For this reason, the content of ammonia is positively proportional to the pH level in the non-treated aquaponics system. Apart from that, the low reading of ammonia content showed that the *Nitrobacter* was able to undergo the nitrification process. Ruiz *et al.,* (2003) stated that the nitrifying bacteria can undergo the nitrification process in the pH ranging from 6.45 to 7.85. Based on the research done by Ruiz *et al.,* (2003), the ammonia nitrification is inhibited at pH 9.05.

Nitrite content

Just like ammonia, nitrite can also cause toxicity to the fish. From the data obtained in this research (Figure 3), the amount of nitrite content in the water was relatively low whereby the highest nitrate content recorded was 2.00 ppm. This indicated that only small amount of ammonia in the water was oxidised into nitrite for the aquaponics system that was not treated with *Nitrobacter* supplement, whereas in the *Nitrobacter* treated aquaponics system, more nitrite has been oxidised into nitrate. Based on the research done by Febriani *et al.*, (2018) on the application of aquaponic to the concentration of ammonia, nitrite and nitrate, she discovered that the amount of nitrite in the aquaponics system shows an increment and start to decrease on the 30th day of treatment which indicates that an adequate amount of nitrite was used by the plant for amino acids regulations. Between day 9 and day 12, there was a sudden drop in the nitrate content for non-treated *nitrobacter* systems due to the abiotic factor such as rain which had caused a drop in the readings due to excessive water.

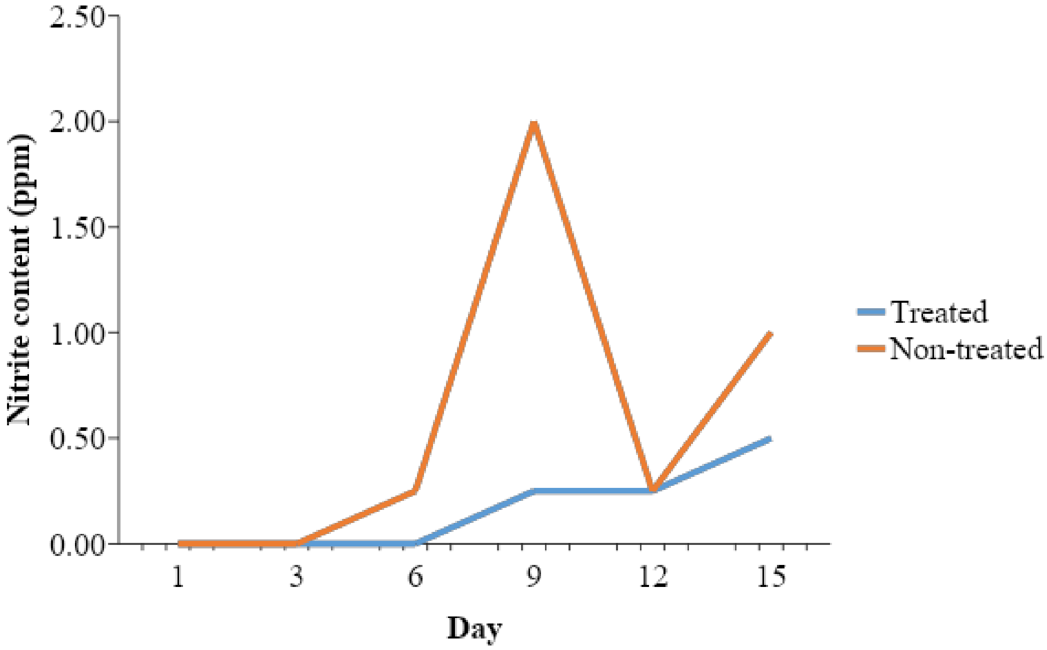

Figure 3 Nitrite content (ppm) in aquaponics water

From this research, a constant increment on the nitrite content was observed from the *Nitrobacter*-treated aquaponics system. Based on the research done by (Febriani *et al.*, (2018), the low amount of nitrite can be caused by the low population of ammonia oxidising bacteria. Thus, the conversion of ammonia into nitrite needs more time as the population of ammonia oxidising bacteria grows. Apart from that, the low amount of nitrite can also be caused by the successful conversion of ammonia into nitrate by the nitrifying bacteria (Manan *et al.,* 2017).

On the other hand, the process of ammonia oxidation into nitrite can take place due to the pH level of the aquaponics system that is not exceeding the pH level where the nitrification can take place. The range is not lower than 6.45 and not higher than 8.95 (Ruiz *et.*, 2003). Furthermore, research done by Kitidis *et al.*, (2011) reported that the low pH can decrease the ammonia oxidation rate. High content of nitrite can be harmful to the environment as it is an inorganic contaminant in aqueous environment (Zhang *et al.*, 2018).

Nitrate content

On the 15th day of the research, the amount of nitrate showed a significant difference between the water treated with *Nitrobacter* and non-treated water as shown in Figure 4. The result showed a difference by 8-folds where the nitrate content in the treated water was higher (80 ppm), whereas the nitrate content in the non-treated water showed a reading of 10 ppm. This indicated that the heterotrophic bacteria content in the water of the treated aquaponics system oxidises the ammonia nitrogen into nitrate. The heterotrophic bacteria such as *Nitrosomonas sp. Nitrospira sp.* and *Nitrobacter sp.* uses nitrate (NO_3^-) or nitrite (NO_2^-) as the alternate electron acceptor to oxygen (O_2) undergoes the process of heterotrophic denitrification (Zhu *et al.,* 2013). In the process of nitrification or the oxidation of ammonia, there were two steps involved (Bernhard and Sara , 2010).

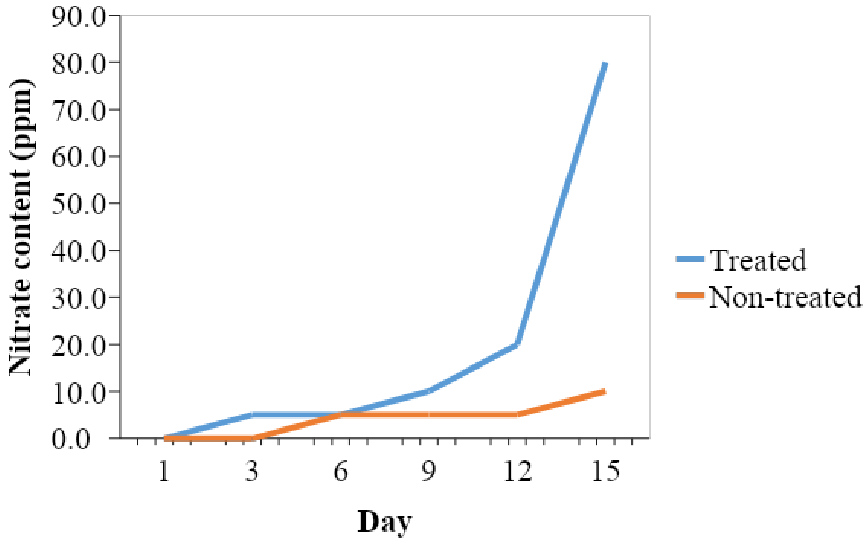

Figure 4 Nitrate content (ppm) in aquaponics water

The first step involved was the oxidation of the ammonia to nitrite by the ammonia-oxidising bacteria which are also known as ammonia-oxidizers. The next step involved was the nitrite later oxidised by the nitrifying bacteria into nitrate under aerobic condition. Bernhard and Sara, (2010) also stated that the process of ammonia oxidation required some enzymes for instance, ammonia monooxygenase and hydroxylamine oxidoreductase. The high nitrate content from the result obtained from the Nitrobacter-treated aquaponics system might indicate that the ammonia was successfully converted into nitrate by the ammonia oxidising bacteria. Aquaponics research done by da Silva Cerozi and Fitzsimmons, (2016) reported that the existence of nitrogen-fixing bacteria can accelerate the formation of nitrate compounds that was produced from the process of nitrite oxidation by the nitrogen-fixing bacteria. On top of that, plant require an efficient sensing systems in order to fully utilise the available nutrients (Gojon *et al.*, 2011).

Plant and fish growth analysis

For the plant growth analysis, three replications of data were measured with two repetitions for each replication. Meanwhile for the fish growth analysis, three replications of data were taken with two repetitions for each data. The overall growth of the plant showed a positive increment in the plant height, leaf diameter and leaf length and leaf number for both aquaponics systems. Similarly, fish growth also showed a positive increment on the fish weight and fish length for both treated and non-treated aquaponics systems.

Plant Growth

Plant growth performance was shown in Table 1. The result showed that there was a significant ($P \leq 0.05$) difference between the growth of the plant for both aquaponics system treated and non-treated with *Nitrobacter* supplement. Although the nutrient efficiency can be increased in an aquaponics system, the plant is still exposed to the plant diseases which can affect its growth (Stouvenakers *et al.*, 2019).

Based on the result obtained, the positive results were shown on the plant length and leaf number where high increment difference obtained in the aquaponics system that was treated with *Nitrobacter* supplement. The length recorded for the treated aquaponics system was 21.60 cm and the length of the plant for the non-treated aquaponics system was 12.27 cm. While, the leaf number for treated was 7.67 compared to 5.33 for non-treated (Table 1).

Table 1: Plant growth performances after 15 days in treated and non-treated aquaponics system

Treatment	Plant length (cm)	Leaf number	Leaf length (cm)	Leaf diameter (cm)
Non-treated	12.27 ± 0.55	5.33 ± 0.33	6.90 ± 0.10	1.00 ± 0.10

Treated	21.60 ± 2.62*	7.67 ± 0.33*	3.37 ± 0.18	0.40 ± 0.06

Note: Values were expressed in mean ± standard error (n=3), * indicated a significant difference at $P \leq 0.05$.

This finding indicated that the increment of nitrate content in the Nitrobacter treated aquaponics system was beneficial for the length and leaf number of water spinach. However, the result for the leaf length and leaf diameter of water spinach was not promising due to certain factors that need to be considered in the aquaponics system such as plant pathogen, nutrient uptake and others. Another factor affecting plant growth in the aquaponics system was the presence of plant pathogens such *Pythium* spp. and *Phytophthora* spp. probably because of the humid condition (Stouvenakers *et al.*, 2019). Apart from that, they also stated that in order to make a better production of the plant, some minerals such as phosphorus (P) and potassium (K) should also be included for a better plant yield. On the other hand, plant density is also important to make sure that the aquaponics system can produce maximum plant yield (Sunny *et al*., 2019).

In addition, light quality can also significantly affect the growth of the water spinach as it can affect the stem length and leaf number (Kitayama *et al.*, 2019). Based on a study by Guo *et al.*, (2020), a prolonged heat stress can also affect the development of water spinach and reduced the photosynthesis pigment content.

Fish Growth

Table 2 indicated the mean value for fish weight and fish length for non-treated and treated aquaponics systems after 15 days. Fish weight indicated no significant difference for both treated and non-treated aquaponics systems. On the other hand, fish length showed significantly different ($P \leq 0.05$) in the treated aquaponics system with 37.31 cm length.

Table 2: Fish weight and fish length after 15 days in treated and non-treated aquaponics system

Treatment	Fish weight (g)	Item 3
Non-treated	360.00 ± 13.47	34.47 ± 0.15
Treated	430.00 ± 34.80^{ns}	37.31 ± 0.98*

Note: Values were expressed in mean ± standard error (n=3), * indicated significant difference at $P \leq 0.05$, ns indicated not significant.

For the change in weight of the fish, there was a 41.76% increment for the fish in the treated aquaponics system, however the result was not significant compared to non-treated as shown in Table 3. Meanwhile, there was only 18.25% increment in weight of the catfish in the non-treated aquaponics system. This result showed that the fish grown in the treated aquaponics system showed better growth when compared to the non-treated aquaponics system. This may be indicated by a good water quality in the treated aquaponics system. Since low ammonia content is recorded in the treated aquaponics system, which is lower than 1 ppm, the catfish were not exposed to the ammonia toxicity where 4 ppm of ammonia content in water can harm the fish (Stathopoulou *et al.*, 2018).

Table 3: Changes in weight of the fish in treated and non-treated aquaponics system

Treatment	Day	Average Weight (g)	Change in weight (g)	Change in weight (%)
Treated	1	303.33	126.66	41.76
	15	430.00		
Non-treated	1	304.44	55.56	18.25
	15	360.00		

Similarly, the length of the catfish shown in Table 4 shows higher increment in the treated aquaponics system with 28.15% increment which indicates that the water treated with *Nitrobacter* can provide a better water quality for fish growth.

Table 4: Changes in length of the fish in treated and non-treated aquaponics system

Treatment	Day	Average length (cm)	Change in length (cm)	Change in length (%)
Treated	1	29.13	8.20	28.15
	15	37.33		
Non-treated	1	29.44	5.02	17.06
	15	34.47		

Conclusion

The treatment of *Nitrobacter* in an aquaponics system was observed to give a good impact towards the overall water quality parameters including the pH level, ammonia content, nitrite content and nitrate content as well as the wellbeing of the fish and the plant. The pH value of water in the aquaponics system treated with *Nitrobacter* shows that the *Nitrobacter* is able to maintain the optimum pH level for an aquaponics system which is 7.0. This value is suitable for the growth of the nitrifying bacteria, plant and also the fish. The treatment also shows a positive result for the ammonia content (10 ppm) in the *Nitrobacter*-treated aquaponics system where the ammonia content obtained from the water sample is relatively lower compared to the aquaponics system that is not treated with *Nitrobacter*. Apart from that, the *Nitrobacter* is shown to be able to oxidise the ammonia content in the aquaponics system which is indicated by the high nitrate compound detected in the water sample. From the result of the water spinach and catfish growth, the *Nitrobacter* treatment is shown to be able to increase the efficiency of the aquaponics system by increasing the growth rate for both water spinach (plant height: 21.60, leaf number: 1.67) and catfish (fish length: 37.33 cm). This also indicates that the plant is able to utilise nitrate in the water and the low ammonia content keeps the catfish from ammonia toxicity. In future, the research can be prolonged to better understand how the treatment of *Nitrobacter* in the aquaponics system can affect the water quality, plant growth and fish growth in a long term duration. Furthermore, the research shall be run with a larger scale of aquaponics system to study on the efficiency of the *Nitrobacter* treatment so that the product from the aquaponics system which is the plant and the fish can be harvested for commercialisation purpose.

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