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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2021 18(SI-2): 255-264. **OPEN ACCESS**

Effect of *Nitrosomonas sp. (*B43) on growth of *Brassica chinensis* and *Oreochromis sp* in Aquaponics system

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Aquaponics is a combination system consist of hydroponic production system and the aquaculture production into a sustainable agriculture system which depends on natural biological cycles in supplying nitrogen. Supplies of nitrogen in the aquaponic system depends on biological formation of amommium oxidation which produces nitrite (no^{-}_{2}) by *nitrosomonas* and nitrate (no^{-}_{3}) production by *nitrobacter* class of bacteria. Because the system depends on the two class of bacteria which occurs naturally in the system, the operator cannot control the conversion rate of ammonia released by the fish to nitrate needed by the plant in the system. In this study, *nitrosomonas* sp. (b43) was added into the aquaponic system. The purpose was to determine the effect of *nitrosomonas sp.* (b43) which were cultured into aquaponics system to the growth performance of the red tilapia (*oreochromis sp*.) And pak choy (*brassica chinensis*). Six aquaponics sets were set up, three for control (without the addition of *nitrosomonas sp.* (b43) and another three for bacteria treatment the plant seedlings of pak choy were transplanted into the aquaponics system after one week where the fish were released into the tank. The experiment started by culturing the *nitrosomonas sp.* (b43) at 8x105cfu/ml into 200 liters aquaponics system of cultivating red tilapia (30 tails) and pak choy (12 plant). The parameter for fish such as total length and body weight were recorded weekly. For plant growth parameters, the data were collected on plant height, root length, and chlorophyll content on weekly basis whereas water quality parameter such as ammomium content, nitrate content, nitrite content, and dissolved oxygen (do) were also recorded weekly.. The fresh and dry weight of the plant were recorded at the end of the experiment. In parameter of total length and body weight of fish, there were a significant difference between the bacteria treatment and control where control showed a significantly higher total length and fish bodyweight beginning on week 3rd of the experiment. Parameters of plant height, root length, chlorophyll content, fresh weight and dry weight of pak choy (b*rassica chinensis*) control showed significantly higher in parameters compared to treatment with *nitrosomonas sp* (b43). In water quality parameters, all control showed a significantly higher content of ammomium and nitrate content compared to treatment beginning on first week of experiment whereas nitrite (no^{-}_{2}) was observed to be significantly higher in treatment with *nitrossomonas sp* (b43) compared to control for all weeks during the experiment. Dissolved oxygen (do) parameter showed no significant differences between treatment and control except in week 4th of the experiment where treatment showed a significantly low do compared to control. As a conclusion, the addition of *nitrosomonas sp.* (b43) into aquaponics system in cultivation red tilapia and pak choy (*brassica* *chinensis*) showed a significant higher content of nitrite (no^{-}_{2}) in treatment which indicates higher conversion of ammomium to nitrites by *nitrosomonas sp*. (b43) added into the tank. However, high content of nitrites (no^{-}_{2}) and low content of nitrates (no^{-}_{3}) in the treatment resulted in significantly lower growth of the fish in the along with significantly low growth parameters of pak choy observed in 5 consecutive week..

Keywords: Aquaponics, *Nitrosomonas sp.*, Pak Choy, red tilapia, nitrate, nitrite

INTRODUCTION

Aquaponics refers to combination of hydroponics which is growing of plants without soil with aquaculture which is growing fish in a recirculating system (Okimoto, 2004). Plants in aquaponic system grows rapidly with uptake from dissolved nutrients which is excreted directly by fish or from microbial breakdown of fish wastes. Aquaponics possess a number of benefits over other recirculating aquaculture systems (RAS) while hydroponic systems uses inorganic nutrient solutions. Hydroponic system in aquaponics function as a biofilter which makes a separate biofilter is not needed as normally practice in other recirculating systems. Aquaponics systems depends on hydroponic system as its biofilter such as vegetables, herbs, and flowers as the biofilter system that generates profits and income (Rakocy & Hargreaves, 1993).

Plant growth in this aquaponics depends on nutrients from the fish feed. Many fish species utilize nitrogen in its metabolism between 20–30% of nitrogen (N) supplied thru fish diet (Penczak et al. 1982; Hall et al. 1992; Shpigel et al. 1993; Piedrahita,2003; Schneider et al. 2005). This can be interpret that 70–80% of the N supplied to fish thru feeding are being released into water in the form of waste (Krom et al. 1995).

Aquaponics can be separated to three components such as animals, plants and bacteria. The main components in the aquaponics is the aquatic animal which produce ammonia such as freshwater fish, crayfish and/or prawns (Diver & Rinehart, 2000). Low to medium nutrient requirements of green and leafy vegetables such as lettuce, basil, spinach Chinese cabbage, chives, herbs, and watercress are well adapted in aquaponics systems (Joel, 2007). Nitrification which involves ammonia oxidation into nitrates plays the most important process in an aquaponic system by decreasing the toxicity in the water which later result in nitrate compounds absorbed by the plants for its nourishment resulting in suitable environment for the fish (FAO, 2001). Ammonia are released by the product from proteins breakdown in fish into the water. Total ammonia content excreted by fish increased if the rates of feeding are also increased (Robert et al. 1997). To reduce the toxicity of the water for fish, plants can absorb nitrate which is a product from the conversion of ammonia. Ammonia oxidation which produces nitrite that are toxic by *Nitrosomonas* are then converted to nitrate that is relatively non-toxic by *Nitrobacter* (Rakocy et al. 2006).

Red Tilapia (*Oreochromis sp*.) were chosen because it is one of the commonly cultivated freshwater fish in the aquaponics system due to its characteristic in which the fish are fast growing and efficient at converting food into body mass (James, 2014). Tilapia eat plants, insects and algae. It also can hardly adapt to varying conditions (Sulaiman, 2018). In Jamaica and South American countries such as Colombia, red tilapia has a higher commercial price compared to the Nile tilapia (Lovshin, 2000). Pak Choy (*Brassica chinensis*) were chosen to be cultured in the aquaponics system because it is the leafy plants that need most nitrogen for its growth. Larger supply of nitrogen enhances more leaves formation per plant over a period of time. (Vos and Biemond, 1992). Pak Choy can be described as light green leaf cabbage under the *Brassicaceae* family with high nutrients such as Vitamin A, C, protein, and calcium (Myers, 1998). Pak Choy desires consistent watering, particularly within the fall but the drought will cause it to bolt to seed (Iannotti, 2018).

MATERIALS AND METHODS

Aquaponics System

In this study, six (6) aquaponics system were set up before the experiment was carried out. Each aquaponics system was prepared with the 300L fish tank, 15 L plastic container, pipe system, wooden block, 30 of red tilapia, plant seed, water pump air pump, aeration tube, and plant media consist of Lightweight expanded clay aggregate (LECA). All the tanks were filled with water and the water pump are initiated in order to increase its dissolved oxygen (DO). The fish were then transferred into each tank containing 30 red tilapia as the nutrient source for the plant. The experiment was carried out to determine the suitability and effect of *Nitrosomonas sp.* (B43) for the Pak Choy's growth and the fish in the aquaponics system. The *Nitrosomonas sp.* (B43) were inoculated at 8x105 cfu/ml into 200 liters per tank for bacteria treatment with 3 replicates while for control, three tanks consist of 3 replicates were filled only with water without any addition of bacteria.

Plant Materials

Pak Choy has been used in the study as as the experimental plant in the aquaponic system. There were 12 replicate of Pak Choy for each of the aquaponics system. Peatmoss was used as the germination media for Pak Choy seeds. The germination process takes around seven days and takes another seven days to wait for the

sprout of its third leaf before the plants were transferred into the aquaponic media.

Red Tilapia

Red tilapia or *Oreochromis* sp. were obtained in Kuala Berang, Terengganu from red tilapia breeder with a size between 3 cm – 6 cm.

Experimental Methodology

The experiment was conducted to determine the effect of the *Nitrosomonas sp.* (B43) on the growth of Pak Choy, red tilapia and water quality in the aquaponic system. All the tanks were filled with water and the system were run in order to increase the dissolved oxygen (DO) in the water. Then 30 red tilapia were transferred into each tank and seedlings of Pak Choy started to germinate. After one week, the plant seedling were transferred from seedling media to the all aquaponics system. After another week, the *Nitrosomonas* sp. (B43) were cultured into three of the tank for bacteria treatment, while the control tank was filled with water without any addition of bacteria for control. This study was conducted for 5 weeks and within this time the fish were fed three (3) times per day. In addition, the weight for the fish pellet that fed the fish were recorded daily.

Study on Water Quality

In an aquaponics system, water quality plays an important role where it determined the growth of the fish and plant in the system. If the ammonia or nitrite level is too high, then it will lead to disease to the fish and plant death. Therefore, the content of ammomium (NH_4^+) content, nitrate (NO_3^-) content, nitrite (NO_2^-) content and dissolved oxygen (DO) were observed and recorded weekly to make sure that the fish and plant to grow in an optimum condition. The YSI Multiparameter Water Quality instruments were used to obtain DO, NH_3, and NH_4^+ of every tank. LAQUAtwin HORIBA compact NO_3^- was used to obtain the nitrate content, and, API NO_2^- was used to obtain the nitrite content. For the content of nitrite, this study used color discs which apply a visual comparison method (color matching) in determining a finer resolution of nitrite concentration.

Pak Choy (*Brassica chinensis*) growth and development

This study was conducted for five consecutive weeks whereas the seedling process of Pak Choy started in the first week after the start up the aquaponics system. Parameters for Pak Choy's growth such as plant height (cm), chlorophyll content and root length (cm) were collected on weekly while fresh weight (g) and dry weight (g) were collected at the end of the research .In collecting plant parameters data, plant height and root length were measured by using a ruler. The plant height were measured from root to shoot tip while root length were measured from the plant base to root tip. Chlorophyll content of Pak Choy's leaves were measured using a SPAD meter. After 5th week, the plant were placed into a forced-air oven at 80°C for 12 hours to determine its dry weight before measured using an analytical balance (Jones et al. 1991). Data on dry weight were taken as it is the precise measurement of biomass without large weight fluctuations caused by changes in water content (Peterson et al. 1993).

Red Tilapia (*Oreochromis sp.)* growth and development

In obtaining data on fish Body Weight (g) and Total Length (cm), ten (10) the fish from each replicate were randomly selected and recorded weekly. For its body weight parameter, the fish were brought to the lab to weight it using an analytical balance. In obtaining data on total length, the length of the fish were measured using a ruler ranging from head to the tail of the fish. The data of the 10 fish were calculated as mean representing the replicate.

Data analysis

All the data were analyzed using IBM SPSS Statistic version 14 at 95% confidence level. Independent Sample T-test used to compare the mean of every data collected. Microsoft Excel also was used to form the graph of each parameter that has been calculated and analyzed by SPSS software which was the overall mean, standard deviation, and significant difference.

RESULTS AND DISCUSSION

Red Tilapia (Oreochromis sp.) Performance

The Red Tilapia (*Oreochromis* sp.) was successfully cultured in 49 days beginning on 16th January 2018 to 6th March 2019. Growth of the red tilapia depends on the feeding rate which in this experiment the feeding rate were fixed at 7% of the fish body weight per day and the fish were fed three times daily (El-Sheriff & El-Feky,2009). The average body weight from all the tank was collected and their feeding has been calculated. The feeding rate was fixed based from 7% percentage every week. The growth performance of red tilapia fingerlings were observed from its body weight and body length. Figure 4.1 and Figure 4.2 showed the graphs for total length and body weight of the fish.

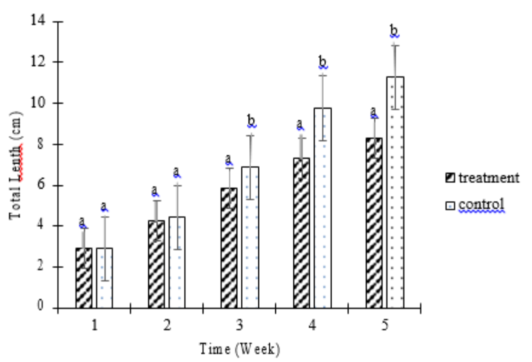

Figure 4.1: Comparison of means of total length of fish by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

The bar chart in Figure 4.1 illustrates the comparison of means of the total length of fish by week between control and treatment. From the graph, it shown that the control treatment showed a significantly higher in total length compared to treatment with *Nitrosomonas* sp. (B 43) from week 1st to week 5th.

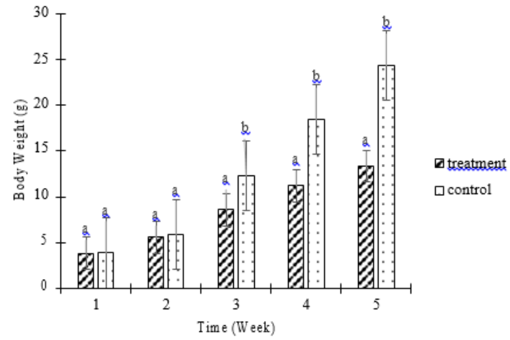

Figure 4.2: Comparison of means of body weight of fish by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

The bar chart in Figure 4.2 illustrates the comparison of means of fish body weight by weeks between control and treatment. Based on Figure 4.2, it showed that the mean body weight of fish for control were significantly higher than bacteria treatment. This factor because the nitrite content in treatment showed a significantly higher content from control (Figure 4.8). High content of nitrite ($N0^{-}_{2}$) in the treatment with water recirculating in the tank would result in exaggerated concentration of the nitrite each week which would cause methemoglobinemia in the fish. Methemoglobinemia is a condition in which a higher than normal amount of methemoglobin, a form of hemoglobin which cannot carry oxygen is found in the blood (Svobodoba et al. 2005). This factor explains the growth performance of Red Tilapia (*Oreochromis sp*.) in *Nitrosomonas sp*. (B 43)treatment which showed a significantly low in body length and body weight compared to control. Nitrite is toxic to fish compared to ammonia and nitrate, which it becomes harmful even at low concentrations for several fish species (Thangam et al. 2014).

Pak Choy (*Brassica chinensis*) Performance

The Pak Choy (Brassica chinensis) has been successfully cultivated in the aquaponics system for 42 days after a week of germination in a peatmoss and sprout of its third leaf before the plants were transferred into the aquaponics system. The plant height, root length and chlorophyll content of Pak Choy were recorded starting from the day it transferred into the aquaponics system at 23rd January 2019 till 6th March 2019. The plant were harvested and its fresh and dry weight were determined. Figure 4.3, Figure 4.4 and Figure 4.5 show the graphs of plant height, root length, and chlorophyll content of Pak Choy (*Brassica chinensis*). The graph of Fresh Weight and Dry Weight of Pak Choy (*Brassica chinensis*) supplemented with *Nitrosomonas sp*. (B43) and control was shown in Figure 4.6 and Figure 4.7

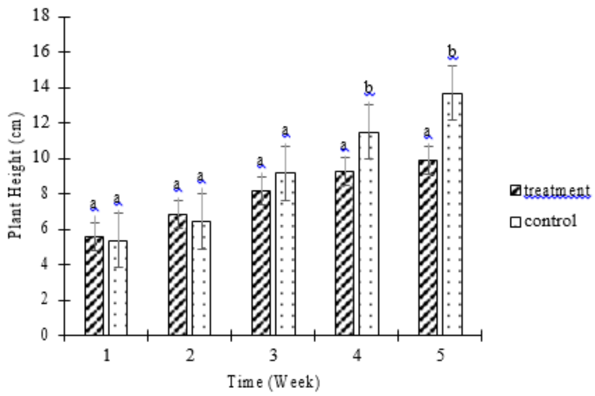

Figure 4.3: Comparison of means of plant height of Pak Choy by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

Based on Figure 4.3 it showed that mean of Pak Choy height for control was higher than bacteria treatment. This is because the plant in the bacterial treatment did not received enough nutrient for its growth. Pak Choy is one of the leafy vegetables which tend to absorbs high levels of NO_3^- in its suitable ratio (Chen et al. 2005). As stated by Chen et al. 2004, the main cause of high NO_3^- accumulation in vegetable leaves are due to high NO_3^- in N fertilizer applied to the plant (Chen et al. 2004). This statement is in parallel with data shown from Figure 4.10 in which the nitrate (NO^-_3 content) were significantly lower in treatment of *Nitrosomonas sp* (B43) compared to control.

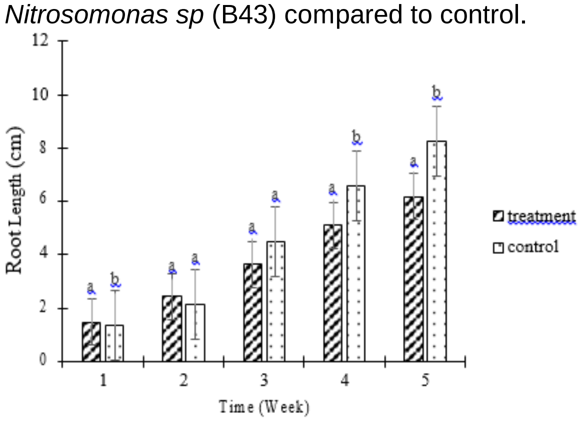

Figure 4.4: Comparison of means of root length of Pak Choy by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

Figure 4.4 a showed mean of root length of Pak Choy for control was higher than bacteria treatment. The result from Figure 4.4 is in parallel with result from Figure 4.10 which shows the nitrate content were higher in the control. The higher nitrate content in the control indicates a higher nitrogen influx conversion from NO^-_2 to NO^-_3 higher than treatment. This can further be explained as nitrogen uptake by Pak Choy was determined by nitrogen flux rate which is located at the plant root surface, the root morphology and the size of the root system. Based on mechanistic-mathematical model, Barber (1995) demonstrated that nitrogen uptake increased in linear as root growth rate and root radius mean increased with a proportionally nitrogen influx rate increased at the plant root surface.

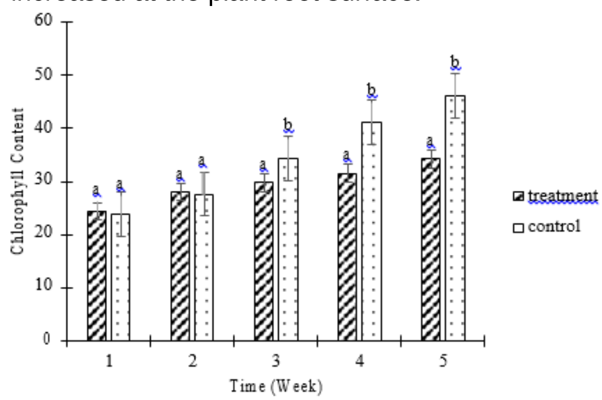

Figure 4.5: Comparison of means of chlorophyll content of Pak Choy by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

Based on Figure 4.5 it showed mean of chlorophyll content of Pak Choy for control was higherthan bacteria treatment. From the graph, there was a significant difference between control and treatment of the mean of chlorophyll content of Pak Choy starting on week 3. The significant difference can be relate to nitrate content which was higher in control as nitrate resulted in significant plant growth. Plant can absorb nitrogen in the form of nitrate better than nitrite for its growth (Rakocy et al. 2006). Chlorophyll content in plant measured is an indicator to precise measurement for growth of plant and quantity of nitrogen absorbed by the plant (Garbin & Dillenburg, 2008). Yavuzcan et al. 2017 stated that nitrate and ammonia were the most usual forms of nitrogen uptake up by the plants.

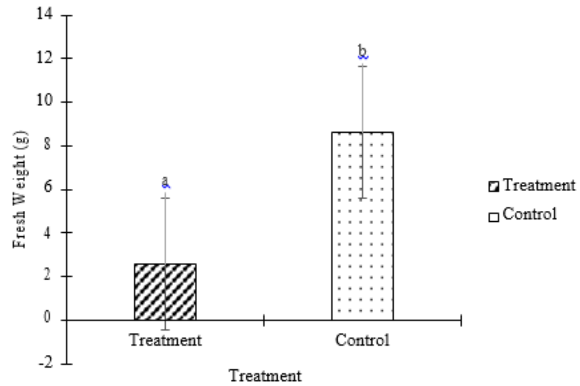

Figure 4.6: Comparison of means of fresh weight of Pak Choy by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

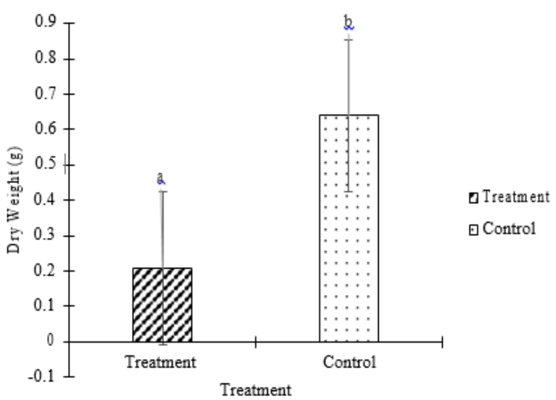

Figure 4.7: Comparison of means of the dry weight of Pak Choy by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

From Figure 4.6 and Figure 4.7, both dry weight and fresh weight of *Brassica chinensis* (Pak Choy) shows a significantly high biomass in control compared to treatment which was the result of efficient photosynthesis where the energy from the sun converts carbon dioxide and water to carbohydrates and oxygen (Sace & Fitzsimmons, 2013). The plant can easily grow when nitrate is higher than nitrite in which the plant need nitrogen in nitrate form for its growth. This statement is in parallel with Crawford, 1995 where he stated that plants need to transport enough nitrate to meet up total demand for nitrogen requirement thus the nitrate uptake system in plants should be versatile and robust.

Water Quality

In the aquaponics system, water quality parameters are an important indicator in identifying hazard related to the welfare risk assessment of assorted cultivation operations; thus, aquaponics systems are not different from aquaculture. Fish raised in aquaponics systems needs a sensible and optimum water quality conditions parameters such as dissolved oxygen (DO), ammonia (NidentifyingH_3) content, nitrate (NO_3^-) content, and nitrite (NO_2^-) content. The parameters should be among acceptable species-specific limits. The table and graph below show the analyzed data.

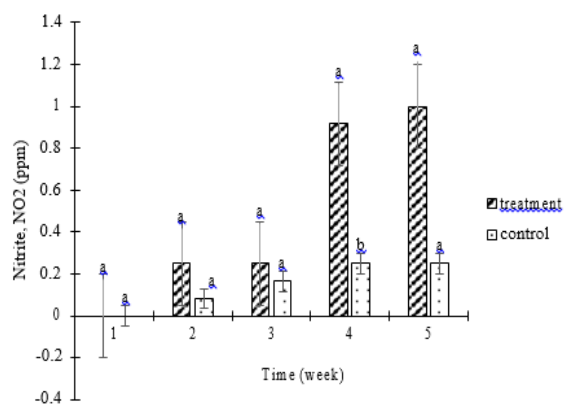

Figure 4.8: Comparison of means of nitrite content by week Error bar indicates standard the error of means. Different alphabet showed significance between control and treatment.

Figure 4.8 showed nitrite spike in treatment than control due to ammonia conversion to nitrite by nitrifying *Nitrosomonas sp*. bacteria added in the aquaponics system than existing nitrifying bacteria in the control tank. There was an imbalance in the system that a sudden spike occur in nitrite. This is related to *Nitrosomonas sp*. added to the treatment resulted with the nitrite spike. High amount of nitrite would be fatal to the fish and affect the growth and increase the chances to diseases of the fish (Yavuzcan et al. 2017). Higher total nirite (NO^{-}_{2}) content in treatment and low ammomium (NH^{+}_{4}) and nitrate

(NO-3) content in control (Figure 4.9 and

Figure 4.10) indicates that there was an abundance of *Nitrosomonas sp* bacteria in the treatment followed by scarcity of *Nitrobacter sp* in the treatment. Even though the important role of plant in avoiding NO^{-}_{3} accumulation in aquaculture tank thru the NO^{-}_{3} uptake proportional to the growth of Pak Choy, the plant uptake rate increased and along with the plant growth and biomass. However, a significantly higher growth in plant in control compared to treatment confirms the high nitrite in treatment are result of abundance of *Nitrosomonas sp* which produces NO^{-}_{2} that shows a significantly low content of NH^{+}_{4} in treatment. The production of NO^{-}_{2} in the treatment outpaced the natural existing *Nitrobacter sp* class bacteria that produces NO^{-}_{3}.

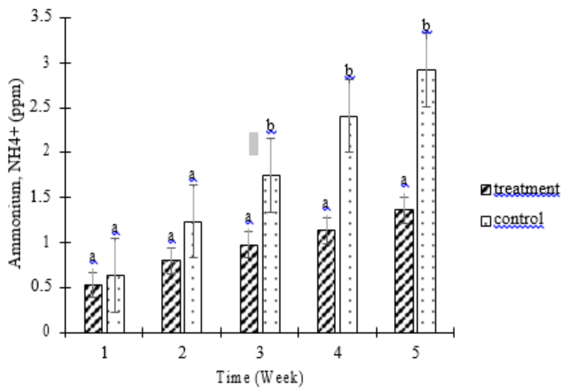

Figure 4.9: Comparison of means of ammonium content by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

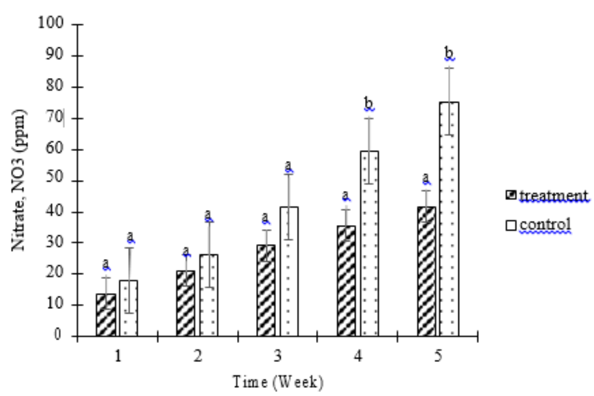

Figure 4.10: Comparison of means of nitrate content by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

Figure 4.9 showed mean of ammonium content for control was higher than treatment with *Nitrosomonas sp*.(B 43). Ammonia existed in two forms of equilibrium in the aquatic atmosphere which is un-ionized ammonia (NH_3) and ionized ammonium ion (NH^{+}_{4}). Total ammonia concentration refers to addition of the concentrations of un-ionized ammonia and ionized ammonium. The equilibrium between the NH_3 and NH_4+ varies with respect to the varied factors ,most importantly the concentration of hydrogen ions (pH) and temperature. (Yavuzcan et al. 2017). The abundance of *Nitrosomonas sp.* bacteria is correlated with ammomium oxidation in the treatment tank resulting in lower ammomium content concentrations in the treatment. During nitrification, *Nitrosomonas sp* will oxidize ammonia to nitrite (NO^{-}_{2}) and others which can be explained in the overall reaction of nitrification and cell biomass formation:

$$55\ NH_4{+} + 5\ CO_2 + 76\ O_2 \rightarrow C_5H7NO_2 + 54\ NO^{-}_{2} + 52\ H_2O + 109\ H^{+}$$

(Haug and McCarty, 1972)

However, Figure 4.10, showed that nitrate content was significantly lower in the treatment compared to control despite of low ammonium in the treatment. This is .due to the low nitrite conversion in the treatment which nitrifying flux of NO^{-}_{2} to NO^{-}_{3} depends on naturaly existing *Nitrobacter sp* in the tank.

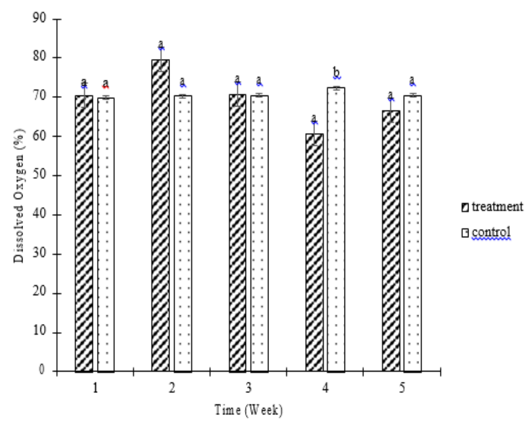

Figure 4.11: Comparison of means of dissolved oxygen by week. Error bar indicates standard error of means. Different alphabet showed significance between control and treatment.

Based on Figure 4.11, dissolved oxygen was lower in the bacteria treatment compared to control at week 4th. During this week *Nitrosomonas sp.* start to reproduce which requires oxygen. This is in parallel with statement by Geets et al. 2006 where he and his colleague observed that wastewater treatment plants that depends on the activity of *Nitrosomonas sp*., were characterized by dissolved oxygen (DO) changes and nutrient supply. When the oxygen provides was insufficient in fulfilling the minimal energy demands of necessity functions, it will result with suffocation. This may occur in nature when fishes that usually live in an optimum dissolved oxygen level were confronted with low dissolved oxygen environment. However, suffocation was rare in environment where oxygen deficiency happens in natural. Fish from such habitats possess an efficient capability in oxygen extraction and often possess alternative modes of oxygen uptake with a lot of various habitats available. However, several species can also survive under a minimal energy needs through anaerobic pathways (Hochachka 1982). In contrast to survival, a wide range of dissolved oxygen levels will affects the maximal rate of oxygen uptake by certain fish species (Fry, 1971). Therefore, it can be conclude that dissolved oxygen availability might have an effect to the fishes ecology via the availability of energy needed for locomotion, growth, and reproduction than through its direct effects on survival (Fry, 1971).

CONCLUSION

In conclusion, the addition of *Nitrosomonas sp.* (B43) at $8x10^5$cfu/ml into a 200L aquaponics system of cultivating 30 tails of red tilapia and 12 Pak Choy showed that the growth was significantly lower when supplementing with the Nitrosomonas sp. (B43).However treatment added with *Nitrosomonas sp*. (B43) in $8x10^5$cfu/mL at 200 liters shows a significantly higher conversion of ammomium to nitrite than control. Inoculum density of *Nitrosomonas sp*. (B43) $8x10^5$cfu/mL at 200 liters was shown as an optimum inoculum density for converting ammonia to nitrite as ammonium content was significantly low compared to control. As a recommendation for further study, this study can be repeated by adding another nitrifying bacteria such as *Nitrobacter* under optimum condition to increase the conversion of nitrite ($NO^-{}_2$) to nitrate ($NO^-{}_3$). It is also recommended to conduct bacteria isolation before and after addition of nitrifying bacteria. This is because nitrifying bacteria exist naturally in aquaponics.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

The authors would like to thank the faculty of Bioresources and Food Industry (FBIM), Universiti Sultan Zainal Abidin (UniSZA) for the facilities and such a great support on this study.

AUTHOR CONTRIBUTIONS

Add contribution of each author (with abbreviated name) here. For example WEP designed and performleed the experiments and also wrote the manuscript. EW, OA, and IDJ performed animal treatments, flow cytometry experiments, tissue collection, and data analysis. AS and MR designed experiments and reviewed the manuscript. All authors read and approved the final version.

REFERENCES

Barber, S. A. (1995). Soil nutrient bioavailability: a mechanistic approach. John Wiley & Sons

Chen, B. M., Wang, Z. H., Li, S. X., Wang, G. X., Song, H. X., & Wang, X. N. (2004). Effects of nitrate supply on plant growth, nitrate accumulation, metabolic nitrate concentration and nitrate reductase activity in three leafy vegetables. Plant Science, 167(3), 635-643.

Chen, W., Luo, J. K., & Shen, Q. R. (2005). Effect of NH4+-N/NO3−-N ratios on growth and some physiological parameters of Chinese cabbage cultivars. Pedosphere, 15(3), 310-318.

Crawford, N. M. (1995). Nitrate: nutrient and signalfor plant growth. The plant cell, 7(7),859.

Diver, S., & Rinehart, L. (2000). Aquaponics-Integration of hydroponics with aquaculture. Attra.

El-Sherif, M. S., & El-Feky, A. M. I. (2009). Performance of Nile tilapia (Oreochromis niloticus) fingerlings. II. Influence of different water temperatures.International Journal Agriculture Biology, 11(3), 1814-9596.

FAO (2001). FAO (Food and Agriculture Organization (FAO)) Integrated Agriculture-aquaculture: A Primer, Issue 407. ISBN 9251045992.

Fry, F. E. J. (1971). The effect of environmental factors on the physiology of fish. Fish physiology, 1-98.

Garbin, M. L., & Dillenburg, L. R. (2008). Effectsof different nitrogen sources on growth, chlorophyll concentration,nitrate reductase activity, and carbon and nitrogen distribution in Araucaria angustifolia. Brazilian Journal of Plant Physiology, 20(4), 295-303.

Geets, J., Boon, N., & Verstraete, W. (2006). Strategies of aerobic ammonia-oxidizing bacteria for coping with nutrient an oxygen fluctuations. FEMS microbiology ecology, 58(1), 1-13.

Haug, R. T. and P. L. McCarty. (1972). Nitrification with submerged filters. J.Water Pollut. Control Fed. 44:2086.

Hall, P. O., Holby, O., Kollberg, S., & Samuelsson, M. O. (1992). Chemical fluxes and mass balances in a marine fish cage farm. IV. Nitrogen. Marine Ecology Progress Series, 81-91.

Hochachka, P. W. (1982). Anaerobic metabolism: living without oxygen. A companion to animal physiology, 138-150.

Iannotti, M. (2018). The Spruce: How to Grow Bok Choy.Downloaded from https://www.thespruce.com/how-to-grow- bok-choy-4125560. Accessed on 4 October 2018.

James, N.(2014).Farmer`s weekly. Retrieved from The history of the red tilapia:https://www.farmersweekly.co.za/animalbs/ aquaculture/the-history-of-the-red-tilapia/. Accessed on 2 October 2018.

Joel F. (2007). Backyard Aquaponics. http://www.backyardaquaponics.com/guide-to-aqua ponics/plants/. Accessed on 8 October 2018.

Jones Jr, J. B., Wolf, B., & Mills, H. A. (1991). Plant analysis handbook. A practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Publishing, Inc..

Krom, M. D., Ellner, S., Van Rijn, J., & Neori, A. (1995). Nitrogen and phosphorus cycling and Transformations in a prototype'non-polluting'integrated mariculture system, Eilat, Israel. Marine Ecology Progress Series, 25-36.

Loh, R. (2015). Water quality explained. How it can affect your axolotl's health. In 40th World Small Animal Veterinary Association Congress, Bangkok, Thailand, 15-18 May, 2015. Proceedings book (pp. 480-482).World Small Animal Veterinary Association.

Lovshin, L. L. (2000). Criteria for selecting Nile tilapia and red tilapia for culture. Tilapia Aquaculture in the 21st Century. Rio de Janeiro, Brazil: American Tilapia Association and Departamento de Pesca e Aqüicultura.

Myers, C. (Ed.). (1998). Specialty and minor crops handbook (Vol. 3346). UCANR Publications

Okimoto, D. K. (2004). Aquaponics export conducts workshops in American Samoa.

Penczak, T., Galicka, W., Molinski, M., Kusto, E.&Zalewski, M. (1982). The enrichment of a mesotrophic lake by carbon, phosphorus, and nitrogen from the cage aquaculture of rainbow trout, Salmo gairdneri. Journal of Applied Ecology 19,371–393.

Peterson, T.A., T.M. Blackmer, D.D. Francis, & J.S. Schepers. (1993). Using a Chlorophyll Meter to Improve N Management. Nebguide G93-1171A. Cooperative Extension Service,University of Nebraska, Lincoln.

Piedrahita, R.H. (2003). Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture 226, 35–44.

Rakocy, J.E. & Hargreaves, J.A. (1993). Integration of vegetable hydroponics with fish culture: a review. In: Wang. J.K (Ed.), Techniques for Modern Aquaculture. American Society of Agricultural Engineers, St. Joseph, MI, USA, 112-136.

Rakocy, J. E., Masser, M. P., & Losordo, T. M. (2006). Recirculating aquaculture tank production systems: aquaponics—integrating fish and plant culture. SRAC publication, 454(1),16. Research, 1(12), 236-250.

Schneider, O., Sereti, V., Eding, E.H., & Verreth, J.A.J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. Aquacultural Engineering 32, 379–401.

Shpigel, M., Neori, A., Popper, D. M., & Gordin, H. (1993). A proposed model for “environmentally clean” land-based culture of fish, bivalves and seaweeds. Aquaculture, 117(1-2), 115-128.

Sulaiman Hud (2018). Aquaponics. Malaysian

Institute of Sustainable Agriculture (MISA).
Svobodova, Z., Machova, J., Poleszczuk, G., Hůda, J., Hamáčková, J., & Kroupova, H. (2005). Nitrite poisoning of fish in aquaculture facilities with water-recirculating systems. Acta Veterinaria Brno, 74(1), 129-137.

Thangam, Y., Perumayee, M., Jayaprakash, S., Umavathi, S., & Basheer, S. K. (2014). Studies of ammonia toxicity on haematological parameters to freshwater fish Cyprinus carpio (Common carp). International Journal of Current Microbiology and Applied Sciences, 3(12), 535-542.

Vos, J.and Biemond, H. (1992). Effects of nitrogen on the development and growth of the potato plant. 1. Leaf appearance, expansion growth, life span of leaves and stem branching. Ann. Bot. 70, 27–35.

Yavuzcan Y. H., Robaina, L., Pirhonen, J., Mente, E., Domínguez, D., & Parisi, G. (2017). Fish welfare in aquaponic systems: its relation to water quality with an emphasis on feed and faeces—a review. Water, 9(1), 13.