



BIOREMEDIATION AND RECYCLING OF TREATED WASTEWATER IN AN AQUAPONICS SYSTEM

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Abstract

This study highlighted bioremediation of aquaponics wastewater and its re-use by providing a breeding compartment for the fish, plant and bacteria. The aquaponics wastewater from the fish, bio-remediated water from the bio-filter and water from the plant pots that recycled back to the fish tanks were characterized. An aquaponics setup was made to have three fish tanks and nine plant pots with three different bed media of gravel, periwinkle and palm kernel shell to hold the plant without soil. As the fishes grew, nitrite and nitrate concentrations significantly increased from 0.00 to 10.66 mg/L and from 0.00 to 30.13 mg/L respectively. The plants show a significant increase to a maximum height growth of 22.86 cm for periwinkle, 20.24 cm for gravel, and 30.48 cm for palm kernel shells bed in each of the beds. However, the palm kernel shells bed yielded the best plant growth measured up to 30.48 cm height. Atomic absorption spectrophotometer (AAS) analysis shows that plants used up essential nutrients like zinc, iron, and potassium from the bio-remediated water after the ninth week. For kinetic study, Line Weaver-Burke plot of reciprocals of the data was plotted to examine the possibility of the reaction fitting into the Michaelis-Menten model. V_{max} was obtained to be 19.12 % which indicates the minimum concentration of substrate at which there will be maximum oxidation. K_s obtained to be 0.97 indicates the substrate concentration at which half the bacteria's active sites are occupied by a substrate. This confirmed that the reaction in the system follows the first order reaction so the behavior of the system's reaction can be predicted over a desired time interval; Microbial nitrification shows Michaelis-Menten kinetics.

Keywords: aquaponics, bioremediation, fish wastewater, lettuce, nitrate, nitrite

Introduction

The potential environmental and human health impacts associated with the generation of waste are fast becoming an issue of public concern, several negative environmental impacts have been associated with aquaculture as related to

the Ecosystem Cifuentes-Torres *et al.*, 2021). The bioremediation process employed the use of nitrifying bacteria to solve the ammonia problem in the wastewater from aquaponics. The remediation produced nitrogen in the form of nitrate which is essential for plant growth.

Biological filters are commonly used during Ammonia removal in recirculating aquaculture systems (RAS) through biological nitrification which changes ammonia to nitrate. Ammonia exists in an aqueous solution in two forms: NH_3 and NH_4^+ . Although both forms may be toxic to aquatic organisms such as fish. Unionized ammonia (NH_3) is the more toxic form at low concentrations (Meade, 1985). Nitritation is a requirement for many recent rising novel biological nitrogen processes, such as partial nitritation-anammox and nitritation-denitrification, as nitrite is the substrate, or intermediary product, of both anammox and denitrification (Liu *et al.* 2016).

Bioremediation is the use of microorganisms or microbial processes to degrade environmental contaminants which are among new technologies (Boopathy, 2000). Bioremediation can be applied in so many ways which include clean-up of groundwater, soils, lagoons, sludge, and process-waste streams (Boopathy, 2000). Eutrophication and stress can be caused by the accumulation of nitrogenous toxicants and other nutrients, which always is unfavourable to the animals but extremely favorable to the disease-causing agents (Krishnani *et al.* 2006). Hence, the need to introduce bioremediation.

The type of production in which plant and fish breeding are obtained by incorporating hydroponics to closed recirculation systems where water is used over and over again (recycled) and intensive fish farming is generated in an Aquaponics system (Edwards, 2015). Nutrient film technique (NFT), deepwater or floating raft method and media-based systems (Rakocy *et al.*, 2006) are the three methods described in the literature that are used by hydroponics systems and adapted to an aquaponics

systems.

Aquaponics system is based on the mutual benefit of three living things where the coexistence of fish, plant and nitrating bacteria in the same environment occurs and the production of animal and vegetables products are achieved for human consumption (Goddek *et al.*, 2015; Armar-Klemesu, 2000)(Bekcan *et al.*, 2017; . Considering the integration of secondary species benefitting from unused parts of nutrients or wastes of the main breeding species, more efficiency from the unit area is likely to be achieved. In the aquaponics system, a lot of trials had been done on vegetable production by conversion of the ammonia formed by the wastes of fish and nutrition to nitrite and then nitrate to be used by plants via nitrifying bacteria (—Rakocy *et al.*, 2006).

Effluents-rich nutrients from the aquaculture component are distributed from the hydroponic component where a proportion of these nutrients are used up by the plants before the water is then returned to the fish tanks (Martins *et al.*, 2010). For a complete nitrogen cycle to occur, resources like nitrogen source, microorganisms, and plants should be adequate in supply, for all this to occur in a single process aquaponics system was considered (Tyson *et al.*, 2011). The nitrite, nitrate and ammonia content in the effluent of a fish tank is used for the growth of the plants, several vegetables can be grown in water culture using nutrients either provided by aquaculture effluents (Diana, 2009). In closed re-circulating systems with very little daily water exchange (less than 2 percent), dissolved nutrients accumulate in concentrations similar to those in hydroponic nutrient solutions (Seawright *et al.*, 1998). Dissolved nitrogen, in particular, can occur at very high levels in recirculation systems. Waste nitrogen is being excreted by fishes in the form of ammonia directly into the water

through their gills. This ammonia is then converted to nitrate by bacteria, ammonia and nitrite are very toxic to fish but nitrate is harmless which makes it the most accepted form of nitrogen for growing higher plants such as fruiting vegetables (Rakocy, 2012). Several benefits such as recovery of dissolved waste nutrients by plants, mitigation of discharge to the environment by the extension of the use of water through plant uptake, and reduction of water exchange rate are obtained from an aquaponics system' (Martins *et al.* 2010). Also, the operating costs of an aquaponics system in arid climates and heated greenhouses can be reduced by minimizing the water exchange rate (Rakocy and Bailey, 2003). Substantially, less water quality monitoring is required in an aquaponics system than separate hydroponic systems (Lennard and Leonard, 2006). Also, Large capital investment, skilled management and moderate energy are required to keep an aquaponics system running (Rakocy and Bailey, 2003).

However, despite the above-named benefits, excess waste water from aquaculture causes adverse effects on the environment, wastage of important nutrients and also decrease in food productivity. Moreover, it has been calculated that the reserves of plant nutrients will decrease in the following 60 years. This will consequently increase the cost of production of this nutrient (Cifuentes-Torres *et al.*, 2021). Therefore, it is pertinent to incorporate aquaponics system which would aid the reuse of wastewater from aquacultural operations. Hence, this study focuses on the bioremediation of aquaponics wastewater, its re-use by fish and plant for growth.

Materials and Methods

The main equipment for the aquaponics system was procured and coupled in the environment of Chemical Engineering

laboratory, University of Ilorin. The aquaponics system was supported on a frame constructed to hold three (3) fish tanks (black plastic containers) each having the dimension of 0.3 by 0.45 by 0.3 m, nine (9) plant pots (translucent plastic containers) each having the dimension of 0.2 by 0.15 by 0.15 m, and a 50 L plastic storage tank. Other equipment employed include: a biofilter (made up of bio balls), a pump (which was used to propel the bio remediated water to the plant pots), sprinklers (regulated the distribution of water to the plant), valves to allow for flow control, digital pH meter, a 100 W solar panel to provide energy, nitrite and nitrate multi-tester. The materials that were used for this study include palm kernel shells, periwinkle shells, gravel (contained in three plant pots for each of the media respectively to a total of nine ports as stated earlier above), ammonia test strips, sprinklers, bio balls. All the supporting media were treated as done by (Oladimeji *et al.*, 2018). Both the palm kernel shells (PKS) and the periwinkle shells (PWS) which are by-products were separately sorted to eliminate debris and autoclaved at 100 for 1 h to reduce the microbial load of these materials. It was then rinsed in clean water and sun-dried for 12 hours before placing each in planting troughs of the aquaponics system (filled up to 50 % capacity). Lettuce seeds were purchased and planted in a nursery for two (2) weeks before transplanting to the aquaponics system. One hundred and fifty juveniles of African catfish (*Clarias gariepinus*) were purchased. The three fish holding tanks contained fifty (50) fishes each. Thereafter, fishes were introduced into the fish tanks and water circulate through the biofilter (bio ball was used) for about two weeks to allow the nitrifying bacteria (nitrosomonas and nitrobacter) to grow and develop naturally before introducing the plants. After which, the cycle began as shown in Figure 1.

Measurements of plant and fish growth were taken at intervals of two weeks. Atomic Absorption Spectrophotometer (AAS) was used to characterize the fish wastewater at

the start of the project (that is 0 week) and subsequently every two weeks. Also, at the end of 8 weeks the grown lettuce.

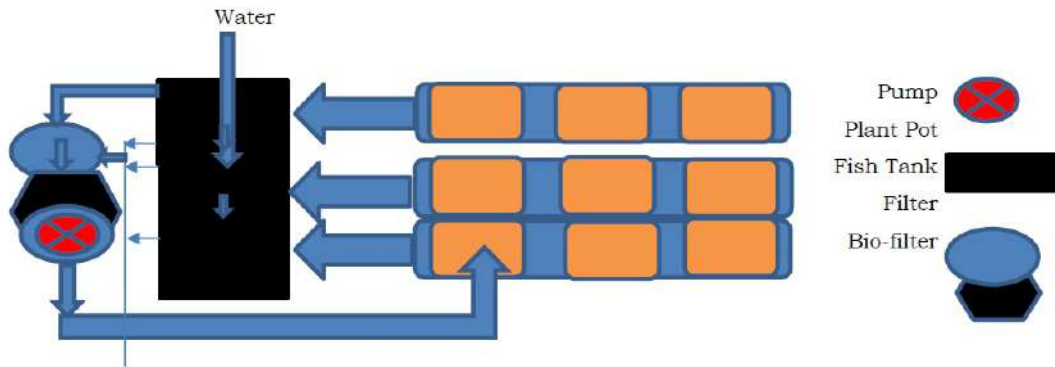


Figure 1: A Simple Representation of the Cycle in an Aquaponics System.

The fishes were fed with popular commercial diet Coppens (52 % crude protein, 15 % crude Fat, 2.5 % calcium, 0.7 % sodium, 1.5 % phosphorus, 1.2 % crude fiber, 8.2 % moisture, 9.5 % ash). The bio remediated water was then characterized after the first two (2) weeks for heavy metals using AAS. Also, water quality parameters such as temperature, pH, ammonia (NH_3), nitrate (NO_3) and nitrite (NO_2) in the system were checked weekly using a digital pH meter, while ammonia, nitrite and nitrate were checked with test strips. The temperature was maintained between 20-30°C (ambient temperature) and the pH range was between 6.5 to 8.5 in the bio-filter with an agitation range of 100-130 rpm. The kinetics for the conversion of nitrogen/ammonia to nitrate was investigated using Michaelis-Menten plot within the period of study.

Data were analyzed by performing linear regression analysis. With this, the Michaelis-Menten curve was converted to a straight line so that it could be expressed as a degree of statistical confidence Lineweaver and Burk, 1934; Charley *et al.*,

1980). The equation of the straight line is given by:

$$\frac{1}{V} = \frac{K_s}{V_{\max}} \frac{1}{S} + \frac{1}{V_{\max}} \quad (1)$$

Thus, the intercept on the y-axis is $\frac{1}{V_{\max}}$ and the slope of the line $\frac{K_s}{V_{\max}}$.

Results and Discussion

Plant Performance

There was appreciable increase in the heights of surviving plants in the three different media beds. A study by (Lennard and Leonard, 2006) also confirmed that different growth media could affect the nutrients uptake of plants in an aquaponics system. Consequently, this could diminish or increase the growth rate of the plants. Figure 2 shows that within eight weeks, Lettuce grew 30.48 cm high where the palm kernel shells were used, followed by periwinkle with 24.86 cm height and lastly gravel media beds with the height of 20.24 cm. The highest plant with palm kernel shells medium may be due to the shape and the fibrous nature of the shell which probably aided retention of the water-containing nutrients (Mader, 2012), hence,

might have more available nutrients for plants to use. The periwinkle shells are not fibrous but have depressions capable of retaining water containing nutrients which may have aided the plant yield observed. Unlike the palm kernel shell and the periwinkle shell, gravel is neither fibrous nor does it have depressions to aid water retention, therefore, little nutrients were

available for plant development. Mader, (2012) had reported in his work that the growth of the lettuce plant (*Lactuca sativa*) was significantly higher with coconut husk media compared to that of gravel. It was also observed that the lowest growth experienced using gravel bed was due to the inability of the gravel to retain sufficient nutrients needed for the lettuce plant to grow.

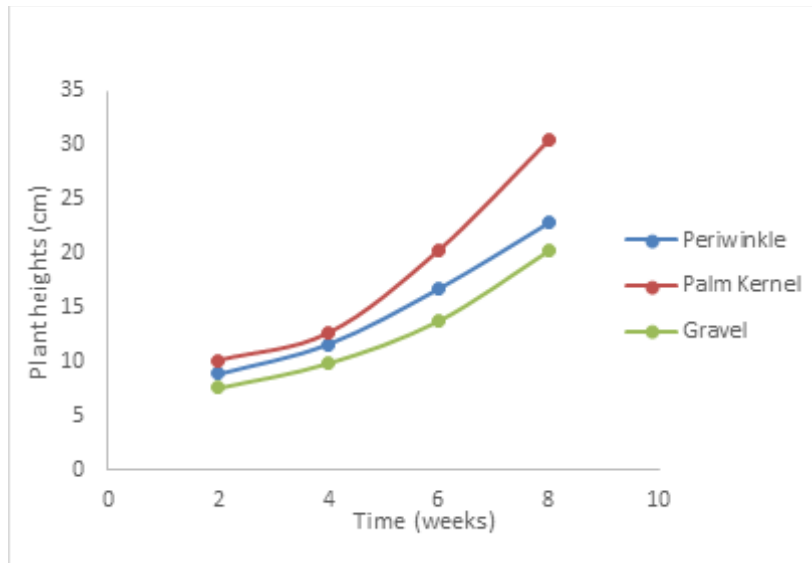


Figure 2: Plant Heights in Different Media Beds

Fish Growth

Table 1 shows fishes gradually increased from an average initial weight of 0.070 kg up to 0.087 kg within eight weeks which influenced the significant increase in the concentrations of nitrites and nitrates. The average weight increase in the fishes within the first interval of two weeks was observed to be 0.004 kg. This steady increase resulted in more production of ammonia which

directly influences the concentration of nitrites and nitrates produced.

Atomic Absorption Spectrophotometer (AAS)

This analysis was carried out on three (3) different water samples; one from the fish tank, another from the bio-filter and lastly from the plant pot compartment. The results obtained show variation in the level of essential elements in these compartments.

Table 1: Fish Development over Time

Time (wks)	Fish weight (kg)
2	0.075
4	0.079
6	0.083
8	0.087

It was observed that the essential nutrients such as Manganese (Mn), Potassium (K), Zinc (Zn), and Iron (Fe) in the plant pots compartment reduced significantly to Mn: 0 mg/L, K: 0.4m g/L, Zn: 0.01 mg/L, Fe: 0.04 mg/L compared to their concentrations in the bio-filter and fish tank. This significant reduction of nutrient consequently

increased the growth of the plant. This observation indicates that the plants had made use of the nutrients for growth. However, most of the heavy metals such as Lead (Pb), Cadmium (Cd), Chromium (Cr) were either present in negligible quantities or absent as shown in Table 2.

Table 2: Atomic Absorption Spectrophotometer Analysis

Water Sample	Na	Ca	K	Mn	Pb	Ni	Zn	Fe	Cu	Cd	Cr
Plant (pot)Trough (mg/L)	3.5	ND	0.4	0	0	0	0.01	0.04	0	0	0
Bio-filter(mg/L)	5.7	0.2	0.5	0	0	0	0.02	0.09	0	0	0
Fish Tank(mg/L)	5.8	0.3	0.5	0.01	0	0	0.03	0.11	0	0	0

The result from Atomic Absorption Spectrophotometer (AAS) analysis performed on the Lettuce grown was presented in Table 3. The zinc, magnesium, calcium and potassium contents were obtained to be 0.04, 0.32, 0.4 and 5.7 mg/l, respectively which are well within the World Health Organization (WHO) standard for

edible vegetables which are (< 5), 40, 180 and 50 mg/l for the contents of zinc, magnesium, calcium and potassium respectively (Ali *et al.*, 2012). These results suggest that the aquaponics system can produce edible plants (Cifuentes-Torres *et al.*, 2021). The obtained result was also influenced by the fish feed from proximate analysis as shown in Table 4.

Table 3. Atomic Absorption Spectrophotometer analysis of the Lettuce Leaf

Sample	Mg (mg/L)	Ca(mg/L)	K(mg/L)	Zn(mg/L)
Lettuce leaf	0.32	0.4	5.7	0.04

Table 4. Proximate Analysis of the Fish Feed

Component	Crude Protein	Crude Fat	Crude Fiber	Ash	Calcium	Sodium	Phosphorus
Percentage	52 %	15 %	1.2 %	9.5 %	2.5 %	0.7 %	1.5 %

Data Analysis and Kinetic Study

Oladimeji *et al.* (2018) had earlier reported that their findings showed that nitrogenous toxicity was highest in the fish tank and least at the plant trough outlet which they

reported to be an indication of the actions of the nitrobacteria present in the system. Figure 3 shows the data obtained from the weekly concentrations of ammonia, nitrite and nitrate. The value of pH and temperature

were also recorded for a total period of nine (9) weeks; various concentrations of ammonia in Figure 3a shows the trend of ammonia concentration in the bio-filter over the period of study. It was observed that ammonia concentration gradually reduced; this reduction was due to the nitrification process where ammonia was gradually broken down into nitrite and finally nitrate. Figure 3b shows the data for nitrite. The concentrations of nitrite which is the first product gradually increased over time till maximum conversion was reached from the sixth to the ninth week and oxidation rate

gradually became steady; forming a lag phase. Figure 3c shows the data for nitrate and since the formation of nitrate is dependent on the concentration of nitrite available, the nitrate concentrations follow a similar trend where nitrate concentration gradually increased before reaching maximum conversion between the sixth and ninth week causing a steady oxidation rate. The lag phase in the first two week in the nitrite and nitrate trends shows that there has not been evident conversion of ammonia to nitrite and nitrate.

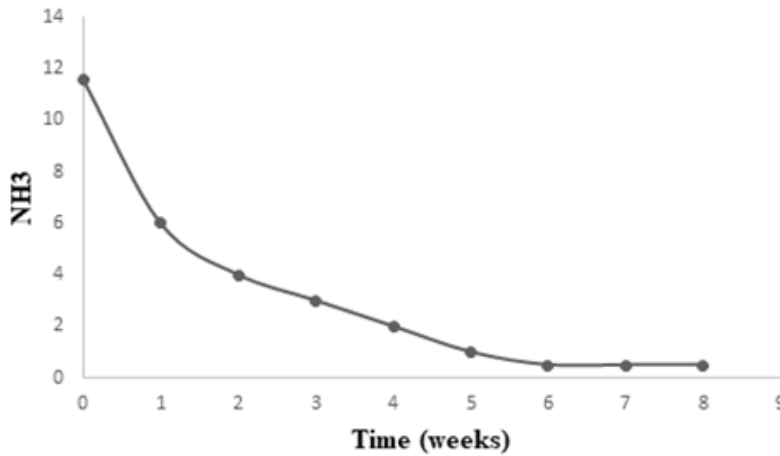


Figure 3a. The Trend of Ammonia Concentration over Time

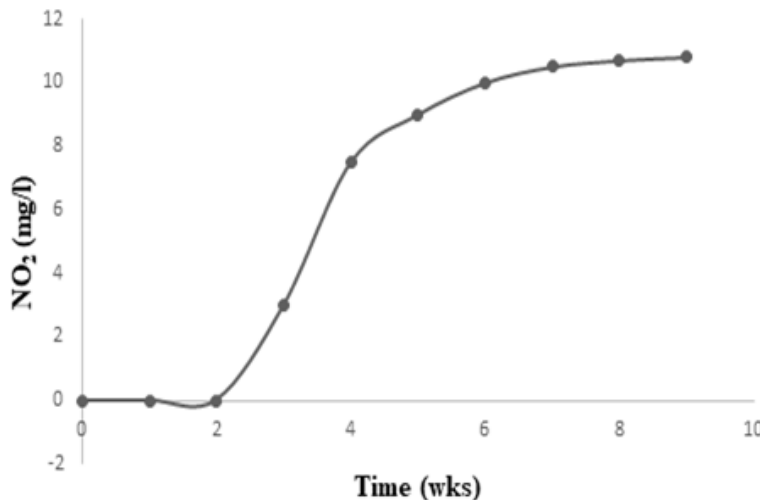


Figure 3b: Trend of Nitrite Concentration over Time

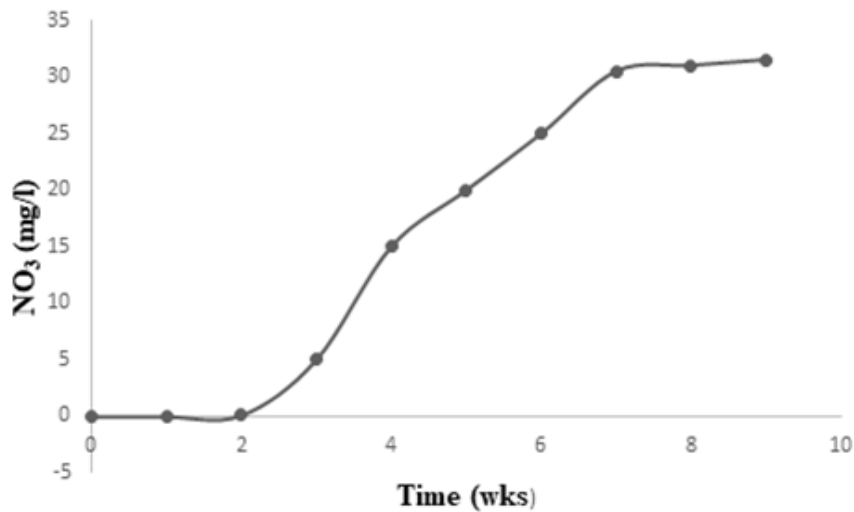


Figure 3c: Trend of Nitrate Concentration over Time

Kinetic Studies

The kinetic study of (Charley *et al.*, 1980) was adopted such that linear regression analysis was performed on the data from the bio-filter water analysis. The analysis could be carried out directly if the kinetics were zero or first order. Though, to examine the possibility of the reaction fitting into the Michaelis Menten model, it was first necessary to perform a Line Weaver-Burk plot of reciprocals of the data. If the relationship between oxidation and substrate was a zero order, the correlation coefficient would be zero. i.e. V was independent of S .

After treatment using the technique of Lineweaver and Burke, a straight line was obtained as shown in Figure 4. The equation of this line was used to generate the curve in Figure 5.

K_s and V_{max} were calculated. However, in practice K_s and V_{max} were derived directly and more accurately from the equation of the line in Figure 4. Charley *et al.*, (1980) had earlier stated that K_s values for nitrification generally vary only from 0.61 to 2.5 mg/l NH_3 . This implies that the K_s value 0.97 in

this study (from Equation 2) is within the range, indicating efficient oxidation of ammonia. It also indicates the substrate concentration at which half the bacteria's active sites are occupied by the substrate. A high K_s means a lot of substrates must be present to saturate the bacteria, meaning the bacteria has a low affinity for the substrate. On the other hand, a low K_s means only a small amount of substrate is needed to saturate the bacteria, indicating a high affinity for substrate as shown in this study. (Charley *et al.*, 1980) thought conceivably that ammonia oxidation could be more efficient (i.e. K_s is low) at a given condition which is in agreement with the conditions used in this work that are favourable for ammonia oxidation efficiency. The Values of rate (V) from the oxidation of ammonia after each week, the concentration of substrate S (NH_3) in mg/L, the inverse of substrate oxidation rates and substrate concentrations i.e. $1/V_E\%$ and $1/S$ respectively are shown in Table 5. The value of V_{max} obtained to be 19.12 (From Equation 3) indicates the minimum concentration of substrate at which there will be maximum reaction.

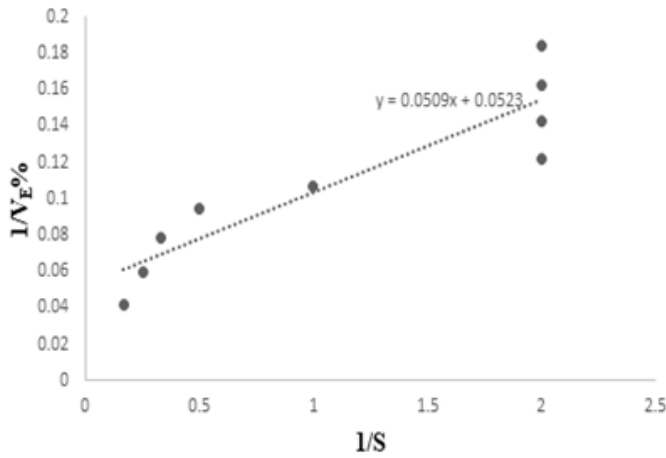


Figure 4: Typical Lineweaver-Burk Plot.

$$\text{Slope} = \frac{K_s}{V_{\max}} = 0.0509 \quad (2)$$

$$K_z = 0.97$$

$$Y(\text{intercept}) = \frac{1}{V_{\max}} = 0.0523 \quad (3)$$

$$V_{\max} = 19.12\%$$

From Michaelis and Menten equation 4 below

$$V = V_{\max} \frac{S}{(K_s + S)} \quad (4)$$

$$V_T = V_{\max} \frac{S}{(K_s + S)}; V_T \text{ is theoretically derived V.}$$

$K_s = S$, saturation constant equal to substrate concentration at $0.5V_{\max}$ (mg/L)

$$V = 0.5V_{\max} \frac{K_s}{(K_s + K_s)}$$

$$V = 4.78 \text{ mg / (1wk)}$$

Table 5. Experimental and Theoretical Values of Rate V

Time (Weeks)	NH ₃ (mg/l) Substrate (S)	Rate V _E (mg/(1wk))	V _E %	1/V _E %	1/S
0	11.6	0.00	0.00	0.00	0.086
1	6.00	5.40	23.87	0.041894	0.17
2	4.00	3.80	16.8	0.059524	0.25
3	3.00	2.87	12.69	0.078802	0.33
4	2.00	2.4	10.61	0.094251	0.5
5	1.00	2.12	9.37	0.106724	1.00
6	0.50	1.85	8.18	0.122249	2.00
7	0.50	1.59	7.03	0.142248	2.00
8	0.50	1.39	6.15	0.162602	2.00
9	0.50	1.23	5.44	0.183824	2

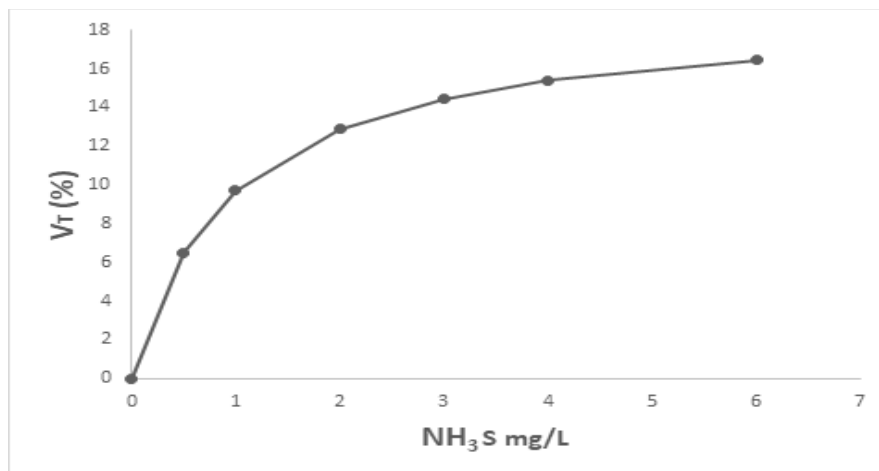


Figure 5. Typical Mathematically Derived Michaelis-Menten Plot

Conclusion

This study concludes that palm kernel shell (PKS) and periwinkle shell (PWS) are suitable media beds in the aquaponics production of lettuce. Availability of these materials as wastes makes them a good choice for media beds. In addition, relatively clean water is recycled back to the fish tank as the plants used up essential nutrients from the bioremediated water to enhance their growth.

References

- Ali, Z.N., Abdulkadir, F.M., Imam, M.M (2012). Determination of some heavy metals in spinach and lettuce from selected markets in Kaduna metropolis. *Nigerian journal of chemical research*, 17, 23-29
- Armar-Klemesu, M. (2000). Urban agriculture and food security, nutrition and health. *Growing cities, growing food. Urban agriculture on the policy agenda*, 99-118.
- Bekcan, S., Atar, H. H., and Yavuzcan, H. (2017). Formation Of Bacteria (Rhizobium Spp.) In The Roots Of Bean Plants Grown In Aquaponics System. *Appl Biol Sci J.* (2), 35-38.
- Boopathy, R. (2000). Factors limiting bioremediation technologies. *Biores Technol*, 74(1), 63-67.
- Cifuentes-Torres, L., Correa- Reyes, G and Mendoza-Espinosa, L.G. (2021). Can Reclaimed water be used for sustainable food production In Aquaponics?. *Crop and product physiology, Frontiers in plant science*. <https://doi.org/10.3389/fpls.2021.669984>
- Charley, R., Hooper, D., and McLee, A. (1980). Nitrification kinetics in activated sludge at various temperatures and dissolved oxygen concentrations. *Water Res*, 14(10), 1387-1396
- Edwards, P. (2015). Aquaculture environment interactions: past, present and likely future trends. *Aquaculture*, 447, 2-14
- Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K., Jijakli, H., and Thorarinsdottir, R. (2015). Challenges of sustainable and commercial aquaponics. *Sustainability*, 7(4), 4199-4224.
- Krishnani, K. K., Parimala, V., Gupta, B., Azad, I., Meng, X., and Abraham, M. (2006). Bagasse-assisted bioremediation of ammonia from

- shrimp farm wastewater. *Water Environ Res*, 78(9), 938-950.
- Lennard, W. A., and Leonard, B. V. (2006). A comparison of three different hydroponic sub-systems (gravel bed, floating and nutrient film technique) in an aquaponic test system. *Aquaculture Inter*, 14(6), 539-550.
- Lineweaver, H., and Burk, D. (1934). The determination of enzyme dissociation constants. *J American Chem Soc*, 56(3), 658-666.
- Liu, X.; Kim, M., Nakhla, G. (2016) Operational Conditions for Successful Partial Nitrification in a Sequencing Batch Reactor (SBR) Based on Process Kinetics. *Environ. Technol.*, 38 (6), 694–704.
- Mader, J. (2012). Plant growth in aquaponics system through comparison of different plant media. *Senior Honors Project, Lynchburg College, Virginia, USA*, 23.
- Martins, C., Eding, E. H., Verdegem, M. C., Heinsbroek, L. T., Schneider, O., Blancheton, J.-P., d'Orbcastel, E. R., and Verreth, J. (2010). New developments in recirculating aquaculture systems in Europe: A perspective on environmental sustainability. *Aquacultural Engineering*, 43(3), 83-93.
- Meade, J.W. (1985). Allowable ammonia for fish culture. *Prog. Fish-Cult.* 47, 135–145.
- Oladimeji, A. S., Olufeagba, S. O., Ayuba, V. O., Sololmon, S. G., and Okomoda, V. T. (2018). Effects of different growth media on water quality and plant yield in a catfish-pumpkin aquaponics system. *J. King Saud University-Science*.
- Rakocy, J., and Bailey, D. (2003). Initial economic analysis of aquaponic systems. *aquaculture Europe*, 58-64.
- Rakocy, J. E., Masser, M. P., and Losordo, T. M. (2006). Recirculating aquaculture tank production systems: aquaponics—integrating fish and plant culture. *SRAC publication*, 454, 1-16.
- Rakocy, J. E. (2012). Aquaponics-integrating fish and plant culture. *Aquaculture production systems*, 1, 343-386.
- Seawright, D. E., Stickney, R. R., and Walker, R. B. (1998). Nutrient dynamics in integrated aquaculture–hydroponics systems. *Aquaculture*, 160, (3-4), 215-237.
- Tyson, R. V., Treadwell, D. D., and Simonne, E. H. (2011). Opportunities and challenges to sustainability in aquaponic systems. *Hort Technol*, 21(1), 6-1