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VARIATION OF TOTAL AMMONIUM NITROGEN IN AN AQUAPONICS SYSTEM FITTED WITH BIO-FILTER MEDIA

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ABSTRACT

Without vital information on maintenance of water quality balance, the concept of aquaponics becomes challenged and unprofitable. A study was conducted to investigate the viability of biofilter media to an aquaponics setup. NH₄Cl was used at varying concentrations (0.3, 0.6 and 0.9 mg/L), in setups with and without biofilter media. A noticeable change in water quality and plant parameters happened after day 3. After 10 days NH₃ and NH₄⁺had reduced to 0mg/L. More NH₄Cl was added. After day 14 setups with biofilter media had more NH4⁺ and less NH3 in contrast to setups without biofilters. NH₃ ranged between 0.0046±0.003 mg/L to 0.011±0.008 mg/L in setups with biofilters; and 0.006±0.006mg/L to 0.014±0.01mg/L in setups without biofilters. NH₄⁺ ranged between 0.20±0.1mg/L to 0.50±0.34 mg/L in setups with biofilters; 0.26±0.1 to 0.46±0.4 in setups without biofilters. DO was constant in all setups with a mean 9.88±0.12 mg/L. Water temperature ranged between 16.1°C to 18.4°C. pH ranged between 7.8±0.01 to 8.11±0.9. There was an increase in the number of leaves per tank. Without biofilter media the conditions tended toward basic and so shifted the balance of NH4⁺ to more NH3. Biofilter media provide more surface area for multiplication of bacteria which in turn act on NH₄⁺ to other forms like nitrates which are beneficial for plant growth and thud cleaning the aquaponics system. The balance in water quality parameters favours plant and fish growth hence profitability to the farmer.

Keywords: Urban fish farming, Aquaponics, Biological filters, Ammonium, Ammonia

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1. INTRODUCTION

1.1. Introduction to World Aquaculture

Aquaculture, or the cultivation of aquatic species such as fish, molluscs, crustaceans, and aquatic plants, increased by 14 percent from 1990 to 2018 owing to rising demand for fish and other goods, which is now up by 122 percent at the current time (FAO, 2020). Fulfilling the need for edible fish has resulted in the development of aquaculture production, allowing for the availability of much-needed fish food. In 2018, inland aquaculture generated 51.3 million tonnes of aquatic animals, accounting for 62.5 percent of all farmed food fish output worldwide (FAO, 2020). This refers to aquaculture produced from inland natural water sources such as rivers and lakes, as well as fish farms.

Global aquaculture trends show and anticipate a future in which natural stocks alone will not be able to supply the nutritional demands of the world's growing population (currently at about 7.5 billion people) (FAO, 2020; Dauda, Ajadi, Tola-Fabunmi, & Akinwole, 2018). Aquaculture accounted for 17.9% of total fish output in Africa, 17.0% in Europe, 15.7 percent in the Americas, and 12.7% in Oceania. The value is expected to rise as culture technologies improve and there is a large demand for aquaculture goods (FAO, 2020). The figures forecast aquaculture as a feasible source for global fish supply to bridge the gap left by stalled/declining wild fish supplies versus the fish demand (FAO, 2020). More production is being driven by technical advancements, growing global incomes, reduced loss and waste, and increased knowledge of the health benefits of fish (Areerachakul, 2018). Therefore, aquaculture presents an opportunity for food provision, species conservation and income generation to many economies worldwide.

1.2. Aquaculture in Uganda

Aquaculture in Uganda was started to provide reliable animal protein to rural households in the 1940s (MAAIF, 2020; Ssebisubi , 2010). Over decades the sector has shifted from mainly subsistence food provision to extensive and intensive commercial enterprise since the year 2000 (MAAIF, 2020; FAO, 2020). Over the years, the aquaculture sub-sector has been a major contributor to the Agricultural GDP, recent figures at 12% and more than 120,000MT of fish food to the country (MAAIF, 2020). There has been an increasing rate of production showing that farmed fish practices are a major contributor to food supply and national development, and the industry is predicted to continue growing.

The strength of Uganda's aquaculture sector is built on export commerce for fish and fish products; revenue/job generation, local fish production (local market), and household food security (Bolman, Pieter van Duijn, & Rutaisire, 2018). Unpredictable wild fish harvests, combined with an ever-increasing human population, have exacerbated the demand for food (fish food) in the country (MAAIF, 2020). With an annual growth rate of 3% (>42 million people) over the previous decade, 2011 to 2020 (UBOS, Uganda Bureau of Statistics, 2020), and annual fish consumption of 12.5 Kg of fish per year, the demand for food (fish) is increasing by the years. The trend is particularly common in metropolitan regions where people are concentrated and farming space limited (Obiero, et al., 2019). The deficit in fish food demand was estimated to be 14.5 Kg of fish per person per

year (Obiero, et al., 2019), and as a result, there is a clear need for aquaculture and aquaculture goods to fill the gap.

Major fish production from earthen ponds (about 25,000 earthen ponds) and about 3,000 cages (MAAIF, 2020). Fish production from other systems like aquaponics and tanks has no readily available records at a national scale. This is because aquaponics and other forms of tank fish farming are mainly operated for subsistence or backyard food provision, household income, hatchery production, and experimental purposes (Kiweewa, 2021), and thus no known records are available. Small space urban food provision has been studied to be a sustainable way of food provision to service growing urbanisation and real estate growth which impact on agricultural land (Miernicki, Lovell, & Wortman, 2018).

To enhance urban food provision, the Government of Uganda through the Ministry of Agriculture Animal Industry and Fisheries (MAAIF), Department of Aquaculture Management and Development, advocate for urban and green agriculture as a way of modernising agriculture services in the country. Aquaponics is one of the suitable alternatives.

Aquaponics fish farming can be adopted as a viable fish production technology (method of production) for reliable fish supply in urban areas (Nalwoga, 2019), where farming space is limited due to real estate settlements and other commercial activities.

Aquaponics farming maximizes plant and fish production from the same system, saves on water for production, uses up small spaces of living like backyards and verandas (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014), hence saving nutrient input into the environment. Closed water systems like aquaponics save on water consumption during production; and so, they reduce production costs and promote a sustainable environment. They promote urban fish farming where space is not easily available for agriculture production, as well as, allowing fish farming where the outside environment is unfriendly to fish or plants, for example, extreme cold, too hot or where water is scarce.

One of the challenges of aquaponics fish farming is balancing water quality and fish output due to increased waste accumulation and minimal care. As a result, a study to investigate the viability of the addition of an extra component, bio-filters, to manage water quality fluctuations while maximising system output.

1.3. Importance of aquaponics fish farming in Uganda

In Uganda, high population growth and a rise in real estate are factors that have contributed to the shrinkage of agricultural space in urban areas. This implies more mouths to feed and reduced space to grow food, hence innovations are advocated for to increase urban agriculture production while maintaining a green environment. The National Development Plan III 2020/21-2024/25 highlights agriculture modernisation and innovation as drivers to improved and increased urban agricultural production. Increased investment in aquaculture is one of the ways to match up this production, however, it is constrained by space and water availability most common in urban areas. These factors coupled with dwindling wild fish catches have given room for the need and practice of aquaponics fish farming in Uganda.

An aquaponics demonstration farm was set up in Kyanja by the Kampala Capital City Authority Urban Agriculture Department to show that urban fish farming can be practised in small urban spaces (KCCA, 2016). The demonstration farm shows the possibility of aquaponics in providing income from selling the plants and fish while maintaining the system for successive culture cycles. In small scale farming cycles where nutrients are frequently utilised by the plants, clean water is maintained throughout the culture cycle through balancing nutrients (Nuwansi, Verma, Chandrakant, Prabhath, & Peter, 2021); water is topped up to counter evaporation. Water recycling provides the farmer with lower maintenance costs for the setups as compared to earthen ponds.

Aquaponics practised in schools and community projects provide socio-economics skills alongside farming knowledge for improved household sustenance and fighting hunger (Kiweewa, 2021). Water Governance Institute (WGI) introduced aquaponics in 2015 as a system for women's household projects and income development on a small-scale basis in Hoima district in western Uganda. The project was rolled out to cover the district in 3 years and thus improved local nutrition (Water Governance Institute, 2017). The development of these projects improves community food output and increases financial stability. Also, food accessibility, improving supply chains by shortening the distance from farm to table. Since the food component is also utilised by the farmer, incidences of malnutrition are reduced by the consumption of fish and plants for home meals. Water recycling implies lower irrigation costs to grow crops and less pollution of natural water systems with aquaculture effluents hence benefits to the environment.

The main component in the aquaponics system is the culture facility/setup: the sections that contain the fish and the sections for plant grow beds. To construct these sections or facilities, farmers make use of options like recycled plastic tanks, lined wooden tanks, and prefabricated metal tanks. These materials help in the greening of the environment by recycling.

1.4. Problem Statement

Given all its benefits, aquaponics fish farming is more technological and scientific than the basic approach to raising fish and plants. Farmers should have basic functioning knowledge to operate the system for benefits and profitable output. Water quality should be examined regularly to ensure fish development and well-being because it affects both fish and plant development (Andriani, Dhahiyat, Zahidah, & Zidni, 2017). Without this knowledge, farmers or operators responsible for aquaponics farms stand to lose if the balance between fish and plant development does not meet required optimum ranges.

Water quality, being the most important aspect in the system, must be monitored for changes to keep the system in balance. Ammonia and nitrogen compounds (NH₃, NH₄⁻, NO₃⁻, NO₂⁻) are the most critical parameters that may disrupt the growth balance (Elmonem, Maather, Marwa, & Amira, 2015). The compounds are introduced into the water system by the fish mostly through gills. Other sources include fish excreta and uneaten feed.

Ammonia levels in culture water above 1mg/L are hazardous to fish. Fish are agitated at this level, and their eating rate decreases. Although some fish like tilapias can withstand extremes of 2mg/L, reduced feeding, retarded growth and gill damage are signs of distress to the fish (Francisco, Roberto, Diego, & Marcelo, 2013; Effendi, Widyatmoko, Utomo, & Pratiwi, 2020).

If left unmanaged, increased ammonia levels may result in decreased fish development and deaths. As a result, it is necessary to analyse variations in ammonia levels in water to maintain optimal development levels for fish and eventual plant growth. Without this information, the concept of promotion of commercial aquaponics in urban areas for increased food provision becomes challenged and unprofitable.

1.5. Justification

In aquaponics systems where fish are raised at high stocking densities, more feed is given to the fish. More feed is directly linked to more waste, hence high production of ammonia leading to imbalances in fish growth. Bio-filters are then added to create an environment for the nitrifying bacteria to multiply and act on the ammonia to reduce adverse ammonia fluctuations and improve system functionality/output.

This study examined the variation of ammonium at different concentrations alongside selected water quality parameters in aquaponics systems fitted with biofilter media in sump tank. The study provides information on changes of levels of ammonium (NH_4^+) for the biofilter type at different concentrations of ammonia (representing fish biomass), and effects on selected growth parameters of plants in the aquaponics systems. Although tilapias can tolerate ammonia levels of up to 2 to 3 mg/L, 0.5 mg/L which has been studied as the critical level for optimum growth. At increasing levels, tilapia will tolerate the high ammonia levels, but growth rate is reduced at these rates. The experiment explored reduction of ammonium over number of days using biofilter media in a simulated study.

The investigation delivers information on water quality parameter interaction in association with the biofilters (bio balls inserted into the sump tank). The biofilter media used are easier to manage mainly for commercial aquaponics. Additional information will be available to farmers and technical personnel involved in aquaponics to inform decisions as they start up commercial systems. In the end, improved systems will in turn boost fish production.

1.6. Research objectives

Ammonification of water is a challenge to many aquaponics farmers causing reduced fish growth and in the end losses to the farmers. To gain experience in measurement of TAN variations in aquaponics systems, a study to monitor variation of TAN levels in an aquaponics system fitted with biofilter media (bio balls in sump tank) was conducted. Nitrification levels were monitored at the start and during the experiment time. Plant response in form of wet biomass increase and number of leaves added was also monitored at the different nitrification levels in the experiment systems. The experiment was a simulated experiment since no fish were added, ammonia seeding was done to substitute animal additions.

The main objective of the study was to explore changes in levels of ammonium in aquaponics systems fitted with biofilter media in sump tank, as compared to those without biofilter.

Specifically, the study looked at:

• Changes in concentration of ammonium, ammonia, nitrite and nitrate in an aquaponics setup fitted with biofilter media and another setup without biofilter media.

- Changes of pH, water temperature, alkalinity, and dissolved oxygen in the aquaponics setups.
- Change in plant growth represented as wet biomass at the start and end of experiment in relation to TAN removal.

The results were used to deduce the ammonia removal efficiency of the biofilter media, and recommendations made on the success or usefulness of the bio-filters for the aquaponics system.

2. LITERATURE REVIEW

What are aquaponics systems?

Aquaponics is the culture method where plants and fish are grown together in a soilless recirculating system. It combines hydroponics, where plants are grown in the water column, and aquaculture (fish farming), in a single system (Nuwansi, Verma, Chandrakant, Prabhath, & Peter, 2021). The plants utilise the effluent from the fish culture unit for their growth in a symbiotic relationship between plant roots and bacteria to utilise nutrients from the water column (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014; Thorarinsdottir, 2015).

When the fish are fed, excess feed, algae and excreta from fish break down or decompose to form nutrient-rich water. The water is pumped through plant beds to feed the roots, which in turn utilise the nutrients for their growth (Thorarinsdottir, 2015). The nutrient-free water is then filtered and returned to the fish growing unit. The aquaponics system then becomes profitable because no new materials are required in a short time of the cycle and no synthetic chemical substances are used (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014). The aquaculture effluent is diverted through plant beds and not released to the environment like conventional growing practices. The same nutrients from the fish growing units are supplied to the plants to form a sustainable loop and non-chemical source. This makes the aquaponics system an environmentally sustainable practice for urban and peri-urban areas where space for both plant and fish culture are limited.

Common types of aquaponics systems include Nutrient Film Technique (NFT), Media Bed, and Deep-Water Culture (DWC) systems. In commercial leafy greens productions, NFT systems are most used because they provide the farmer with many growing options including vertical placement (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014). The NFT system is not recommended for flowering plants because of weight concerns. In the media bed type of aquaponics, plants are potted then placed in gravel support basin beds. The plant roots grow out of the porous pots to access the water. It is recommended for fruiting plants but not easy to scale-out since it requires more space for production. Clogging with residues is also a common problem in this system. Deepwater culture requires that plants are suspended via porous material that floats on water basins known as raft or float systems. Plants draw nutrients directly from the water column (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014; Bolman, Pieter van Duijn, & Rutaisire, 2018).

There should be a balance between the nutrients in the water and the number of fish in the system to help plants grow. Choosing the type of culture for the aquaponics system will then rest on the farmer funding for start-up costs and space available for the set-up. For any of the systems chosen, the farmer is tasked with a balance of the system components in order to maximise set-up output. Natural growing conditions are an important consideration for any type of fish or plant chosen, to help them grow effectively within a favourable environment.

Components of an aquaponics systems

Aquaponics systems contain a fish culture section (aquaculture), plant growing section (hydroponics), filter tank and sump tank (Figure 1).

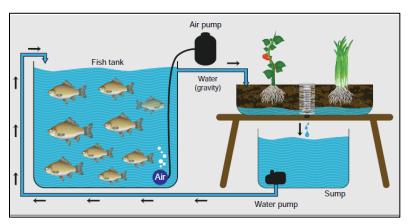


Figure 1: Simple Aquaponics unit, adopted from (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014)

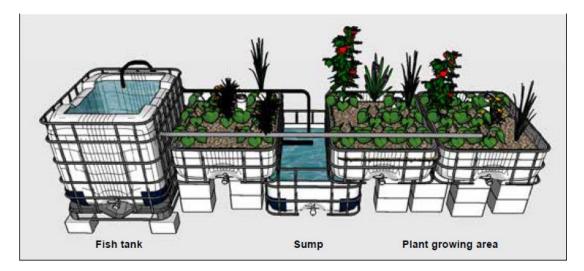


Figure 2: Media bed aquaponics unit, adopted from (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014)

Culture tanks are the tanks where the fish live during their growth to the desirable market size. When evaporation occurs, water is topped up, therefore a reservoir tank becomes necessary.

As the fish grow their requirements for space, food, water quality, lighting, flow, and other factors will change, therefore calculations for space of fish should consider these factors too. Fish stocking density for aquaponics is calculated at 12 to 25 Kg of fish per 1000 litres of water for extensive to intensive systems respectively (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014). It is

important to maintain good water quality, adequate oxygen levels, ideal flow rates, and waste removal to balance the system.

The biofilter is a part of the system where bacteria grow to break down ammonia from the harmful form to less toxic nitrates which are used by plants for growth. The biofilter component can help to reduce the quantity of debris in the system.

The aerator introduces air in the growing system for the fish to be able to grow well. At low stocking density especially in subsistence and extensive culture, less or equal to 12kgm⁻³, natural aeration is opted for, while in intensive systems, aerators are added to increase concentration of dissolved oxygen for the fish and aid the removal of carbon dioxide (CO₂) in the culture tank (Areerachakul, 2018).

Solid Waste filter is important for maintaining good water quality for the fish to live and grow. Plumbing modifications provide for efficient waste removal and may include mechanical filters installed to the plumbing sections via holding tanks to remove building wastes. The filters are regularly cleaned to remove solid waste build up. Mesh fibre mats, filter pad material or recycled foam pads can be used as for solid waste removal.

Biological Filter (bio-filter) may be a separate tank with substrates like plastic chips, bio balls, PVC ribbons, in-built filters; that help beneficial bacteria to grow at high densities (Ako & Baker, 2009). The bacteria take advantage of bacterial processes to convert toxic by-products of fish feeding into non-toxic forms that can be used by plants. the substrates are the least expensive and lightest weight products that will allow for water and air to pass through without clogging.

The sump tank is a reservoir that generally contains no plants or fish. The tank holds water momentarily before it is pumped back into the culture system. Biofilter substrate can be added to sump (sump biofilter) to clean the culture water before it flows to plants.

Water Pump can be electrical or solar powered, used for moving water among the various system components. Pumps are essential in intensive fish culture systems. Source Water tank contains water source utilized in the aquaponics system for example groundwater, surface runoff, rainwater collected, or national grid. To prevent algae-build up in source water, the water can be covered to reduce light penetration.

Water test kit is important for water quality monitoring regularly. The kit can be comprised of simple water tests which are color-coded test kits with watercolor corresponding to the color scale provided in the kit, titrations or probes. Results are read off with guidelines for water quality management on leaflets. The kits include tests for pH, ammonia, nitrite, nitrate, hardness. Metered probes are used for dissolved oxygen, pH, alkalinity, temperature, and carbon dioxide.

Plumbing requirements include plastic and PVC fittings which are opted for because they are less toxic and non-corrosive, however they can build up algae and clog. Hence regular cleaning is essential. A mesh covering is necessary to control fish escaping from the culture tank.

Table 1 shows a breakdown of costs for a simple aquaponics system that can raise 100 fish with 10 to 20 leafy/vegetable plants. Choice of materials for use in the setup entirely depends on the farmer's

choice and the options available. Recycled plastic tanks are cheaper but should be cleaned thoroughly of any chemicals or foreign materials before use. Perforated plastic cups can be used instead of mesh cups. Plant holders can be substituted with punctured Styrofoam boards; however, problems like clogging and waste build up should be considered. For the first growing cycle, profits may not be realized, however, as growing cycles increase profits also increase. Inputs like tanks, planters, plumbing materials may not be replaced. This means the system yields as the cycles increase (a cycle is considered at 6 to 7 months for catfish or Nile tilapia rearing in warm weather).

Item	Units	Unit cost (Ug. Shs)	Amount (Ug. Shs)
Tank (1,000L) with metal guard	1 pc	300,000	300,000
Drum tank (200L, reservoir)	1 pc	30,000	30,000
Catfish seeds- BW=@10g	100 pcs	300	30,000
Fish feeds- Floating pellets, complete formula, 35% & 30% CP	150 Kg	3,000	450,000
Water- growing medium	Monthly cost	50,000	50,000
Plumbing accessories (taps, valves, washers, PVC pipes, PVC glue, joints)	Assorted	100,000	100,000
Plant holder	1 pc	100,000	100,000
Garden Gravel (1kg)	1 Kg	2,000	2,000
Vegetable Seedlings	10 pcs	1,000	10,000
Mesh cups/planters (10)	10 pcs	1,000	10,000
Labour for set up	1 personnel	100,000	100,000
		Total	1,182,000

Table 1: Cost evaluation of a low-tech aquaponics systems

Adopted from The Kyanja Bulletin (KCCA, 2016) Note: Prices of items and farm produce used in this document are estimated market prices subject to change at any time.

An overview of water quality in aquaponics

Water is the main factor that supports the aquaponics system for fish, bacteria, and plant life. The nutrients in the system determine the success of survival for the organisms and affect the final outputs from the system. Some of the major water quality parameters for aquaponics are dissolved oxygen (DO), total nitrogen concentrations (ammonia, nitrites, and nitrates), pH and water temperature Table 2 (Danner, Mankasingh, Anamthawat-Jonsson, & Thorarinsdottir, 2019; Nalwoga, 2019). There are other determining factors for a successful aquaponics operation but the five in table 2 are crucial often with major incidences like plant failure in case of low nitrate levels and fish deaths in cases of high ammonia levels.

Water Quality Parameter	Quality Ranges
Dissolved Oxygen	5-8 mg/L
Ammonia	0 to 0.5 mg/L
Nitrites	0 mg/L
Nitrates	5-150 mg/L
pH	6-7 units
Water temperature	18 - 30°C

Table 2: Key water quality parameters in aquaponics systems

Culture water used in aquaponics operations supports the natural habitat for the growth of fish and plants influenced by nutrient availability and water quality (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014). Variations in these parameters affect the normal growing conditions of fish and as such should be regularly checked to keep the water system balanced for longer time. Dissolved oxygen concentrations of 3 mg/L or less are stressful and eventually deadly to fish (Al Tawaha, Wahab, Jaafar, Zuan, & Hassan, 2021). Loss of fish function affects the overall performance of an aquaponics system since fish will not be able to feed and avail the much-needed nutrients for plant growth.

When organic matter decomposes, ammonia is the first type of nitrogen released, and it is the primary nitrogenous waste expelled by most fish and freshwater invertebrates. Fish excrete ammonia primarily through their gills, although it can also be found in trace levels in their urine (Lysakovska, 2015). Unionized ammonia (NH₃) and ionized (NH₄⁺), also known as ammonium ion, exists in two forms in the culture water. Unionized ammonia is hazardous to fish, whereas ionized ammonia may cause indirect effects via toxicity, especially at very high concentrations (Ling & Chen, 2005; Francisco, Roberto, Diego, & Marcelo, 2013). Total Ammonia Nitrogen is the sum of the hazardous form and the less-toxic ionic form of ammonia (Sallenave, 2016).

When the culture water is at a pH of less than 7.0, fish can tolerate greater TAN levels (Sallenave, 2016). At dissolved oxygen (DO) levels lower than 3 mg/L and water temperature above 28°C, warm water fish affect this tolerance (Sallenave, 2016). Thus, TAN becomes toxic to the fish and will then require regular water quality checks.

Ammonia excreted by fish in aquaponics systems, on the other hand, is removed by nitrifying bacteria, which convert ammonia to nitrate nitrogen in a two-step process known as nitrification. *Nitrosomonas spp* bacteria first convert (oxidise) ammonia and ammonium to nitrite (NO_2^-) (Ling & Chen, 2005). This process necessitates the presence of oxygen and lowers pH. *Nitrobacter spp* bacteria convert nitrite (NO_2^-) , which is likewise hazardous to fish, to nitrate (NO_3^-) . In this stage oxygen is required and will also lower the pH. The non-toxic nitrate is utilised by plants for growth as fertilizer, see Figure 3 below (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014). Nitrates are the assimilable form from ammonia through the plant roots. Hence cleaning the aquaponics system and giving growth benefits to fish which provide the waste.

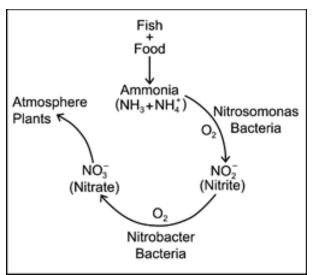


Figure 3: The Nitrogen cycle.

Effect of stocking density on ammonia production in aquaponics fish farming

Farmers frequently choose to increase the quantity of fish per unit growing space to profit from their fish farming enterprises (Al Tawaha, Wahab, Jaafar, Zuan, & Hassan, 2021). The approach is used in intensive culture systems where fish are stocked at high density to maximize productivity while using less water than lower stocking densities, which may not give as much desired output in terms of economic advantage (Sabwa, Manyala, Masese, & Fitzsimmons, 2021). Stocking fish at lower density helps to keep lower metabolic wastes in the system which improves fish survival rates but may not offer better production returns as compared to high stocking densities of the same system (Sabwa, Manyala, Masese, & Fitzsimmons, 2021). High densities, on the other hand, have a detrimental influence on fish growth and survival rates owing to the build-up of metabolic wastes such as faeces, the degradation of fish social interaction, and the worsening of water quality. This is relatable to a study by (Ali, Stead, & Houlihan, 2006), who recorded that increased stocking density from 15 fish per 100 L tank to 75 fish led to increased ammonia secretion and eventual slow growth of the fish.

In an aquaponics study with tilapia stocked at 8, 10 and 12 Kg/m³, survival rates of fish were higher (97%) in the treatment with lower stocking density (Al Tawaha, Wahab, Jaafar, Zuan, & Hassan, 2021). The lettuce grew better in the higher stocking densities, due to the high levels of nutrients that were available for the plants to grow (Al Tawaha, Wahab, Jaafar, Zuan, & Hassan, 2021). A balance of fish stocking density and water quality should be maintained to achieve better fish output and longevity of the culture cycle. Increased secretion of ammonia leads to increase in pH and reduced dissolved oxygen levels, due to the nitrification process which transforms ammonia to nitrites and nitrates (Dauda, Ajadi, Tola-Fabunmi, & Akinwole, 2018).

In aquaponics systems 12 to 25 Kg per 1000 litre of tank media is recommended (or 24 to 50 fish per 1000 litres at 500g of market size) (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014). Increasing the number of fish per unit of growing media implies an equivalent improvement in the rearing conditions or else growth of fish is impaired (Thorarinsdottir, 2015). Additional components

like bio-filters, aerators, and water pumps to improve system functionality are added for system efficiency. This is due to increase in the quantities of waste produced compared to the water renewal rate hence effects on the growth performance of fish.

Biofilters in aquaponics

Biofiltration is the process of removing ammonia and nitrite from aquaponics systems. To clean the water and remove or detoxify toxic waste products and uneaten feed, a filtering (biofilter) scheme is essential. The relationship between the fish component and the hydroponic component of an aquaponics system is biofiltration (Elmonem, Maather, Marwa, & Amira, 2015). The biofilter is usually installed between the culture tank and the hydroponic tanks.

Biofilters are classified into four types: recirculated suspended solids (activated sludge and bio floc systems), aquatic plant filters, fluidized bed filters, and fixed film biofilters (submerged and moving bed). They are meant to increase surface area for bacterial colonisation and action on the nitrite to nitrate to provide nutrients to plants while the fish are growing. Selection of a biofilter also depends on size and energy needs of the culture unit (Areerachakul, 2018). Without a healthy and functional biofilter, waste products from the fish production component accumulate, insufficient amounts of plant nutrients are created, and the system fails to work effectively. Aquaponics systems with low stocking density and floating raft system may not need a bio-filter. The plants will have sufficient beneficial bacteria to transform the water nutrients into growth.

Bio balls are classified as fixed film biofilters that can be used in aquaponics at greater stocking density where biofilters are necessary. They are plastic balls (1-5 cm in diameter) with holes and structural gaps that allow bacteria to colonize and grow, creating ideal conditions to flourish and hence aid in the conversion of ammonia to nitrite and nitrate (Somerville, Cohen, Pantanella, Stankus, & Lovateli, 2014). Because bacteria do not require light, they can be housed in dark containers to maximize their functionality. A steady flow of water permits the bacteria in the bio balls to continue extracting ammonia at the same pace. It also guarantees that the bacteria have a constant supply of oxygen, which they require to survive. Substitutions for biofilter media include perforated bottle caps in sump tank, old plastic pieces, old mesh nets, old fishing nets, perforated bottle caps, plastic filings, polished gravel, and many other materials.

3. METHODOLOGY

3.1. System design

The study applied recirculation aquaculture system of aquaponics with media bed culture method. Three identical systems of 3 aquaria each (size: $33 \times 21 \times 18$ cm and volume 20 litres) were assembled as system tanks in the biofilter media setups. Tank 1 was the planter, tank 2 the culture tank, and tank 3 the sump tank where the biofilter media was placed at 40 bio balls per tank as well as a 110W power suction motor to draw water from the sump to the planter.

Another set of three identical systems of 2 aquaria each (size: 33 x 21 x 18 cm and volume 17 litres) were used in set up without biofilter media. Water inflow was maintained at 450 mL per minute and no water exchange was carried out during the study. The aquaria/tanks were arranged at different heights with help of wooden planks to allow for gravitational flow of the water. Approximately 2 kg of poly-clay balls were added to the planter section of the setups. Water was left to run through the system for two days to build up dissolved oxygen and to test system functionality.

3.2. Treatments for the study

Since fish were not to be used in the systems (fishless cycling), Ammonium Chloride (NH4Cl) powder was used to create different concentration of ammonia/ammonium guided by values from, (Loan, Con, Hong, & Ly, 2013; Ali, Stead, & Houlihan, 2006). It was noted that the lowest ammonium production level to be 1.8mg/L for 15 fish in 65-liter tank. Warm water fish (for example tilapia) can tolerate higher than 1mg/L, therefore concentrations of 0.3mg/L, 0.6 mg/L and 0.9mg/L were considered for the study. For each concentration, two setups (no replication) were considered with one having biofilter media in sump tank, while the other did not have biofilter media giving a total of six setups.

Prior to the introduction of NH₄Cl, water samples were collected from each setup culture tank, analysed, and recorded for lowest initial values of the water quality parameters for two days. Ammonium solution for the different setups was prepared by dissolving NH₄Cl 15.1, 30.3 and 45.4 mg, in one liter of water to get a concentration of 0.3, 0.6 and 0.9 mg/L in setups without biofilters which had approximately 17 liters of water. For setups with biofilter media which contained 20 liters of water, quantity of NH4Cl was 17.8, 36.7 and 53.4 mg in one liter of water to achieve concentrations of 0.3, 0.6 and 0.9 mg.

NH₄Cl solutions were added on day 3 when the setups had the lowest value of NH_4^+ -N and left to run through the setups for 24 hours. After 24 hours, a culture of nitrifying bacteria was introduced into the biofilters with a liquid medium for aquaria (Sera bio Nitrivec) 10 ml per 25 L of water (at 8ml and 6.8ml for 20 liters and 17 liters respectively). Thereafter, pre-germinated seedlings of lettuce (*Lactuca sativa*) were weighed (wet biomass), number of leaves taken and thereafter planted directly into the planters (aquaria with poly-clay balls) at 5 seedlings per planter tank (approximately 1.2g per plant). There was no addition of artificial nutrients for the lettuce during the experiment. At the lowest value of NH_4^+ and NH_3 recorded in the systems, more NH_4Cl was added. The experimental setup was based at Hólar University, Department of Biology and Aquaculture in the research centre at Veriđ, Saudarkrokur, Iceland for 22 days.

3.3. Data collection

The primary objective of the experiment was to determine how well the biofilters perform the nitrification process. Data collection was carried out daily between 10 and 11 am. Samples were analysed within 15 to 20 minutes after collection.

Dissolved oxygen, water temperature and pH were measured *in situ* using EcoSense ODO 200 DO probe, ExTech 3240 waterproof thermometer and OxyGuard handheld pH probe. Water samples for testing ammonium, nitrites and nitrates and alkalinity were collected in 100ml cleaned, and thereafter sealed sample bottles, taken to the laboratory for analysis using Palintest 7500 kit. Since un-ionised ammonia (NH₃) could not be directly measured by the Palin test, concentration of daily NH₃ was calculated as a factor of measured NH₄⁺ from Palin test, at a given water temperature and pH using TAN calculator by the formula:

Un-ionised Ammonia (mg/L) = NH_4^+ value x value for temperature and pH.

See the schematic representation of the experimental setups below in figures 5 to 8.

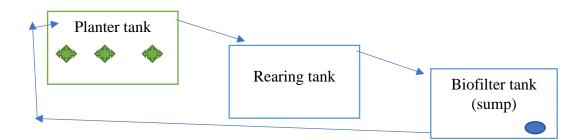


Figure 4: schematic of Aquaponics setup with bio-filter

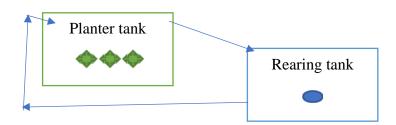


Figure 5: Schematic of Aquaponics setup without biofilter

Water flow direction
 water pump
 Plant growing basin with poly clay balls
 Aquaria

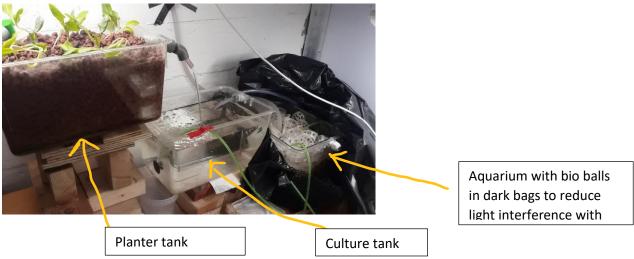


Figure 6: Aquaponics setup with biofilter media



Figure 7: Aquaponics set up without biofilter media

3.4. Statistical analysis

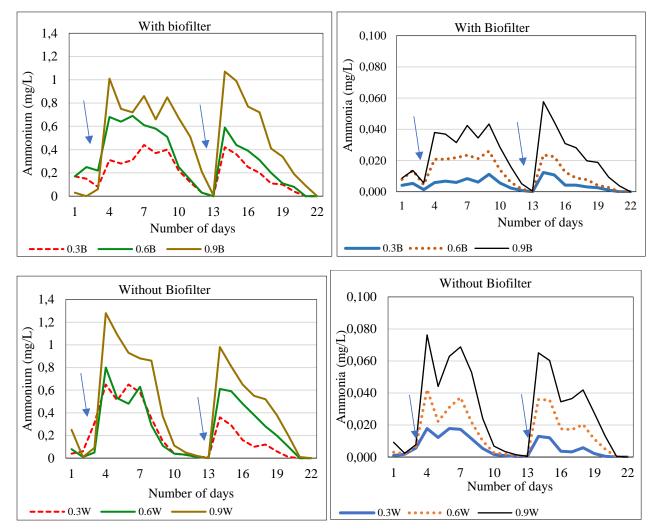
Data was organised into tables using Ms - Excel 2010 software. Water quality and plant growth data were analysed using descriptive statistics.

Since there were no replicates, plant growth data were expressed as values over number of days of initial and final weight measurements of wet weight and number of leaves per plant using Ms -Excel Analysis tool Pak 2010.

4. RESULTS

4.1 Water quality parameters

Water quality sampling and analysis was done for 22 days; initial ammonium chloride solution was added at day 3 when the ammonium in the setups was lowest (zero). The concentration of ammonium increased sharply at day 4, thereafter reducing further over the days, until day 12. More ammonium chloride was added at day 13 in all setups when the lowest value of ammonia and ammonium was recorded (0.00 mg/L). Further sampling was done until the concentration of ammonium dropped to zero.

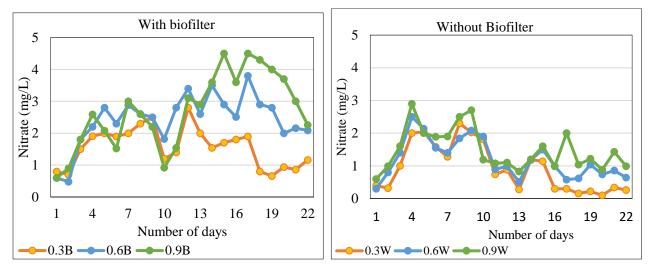


Variation of Ammonium and Ammonia

Figure 8 (a, c): Variation of Ammonium over the number of days; (b, d) Variation of ammonia over the number of days

Mean concentration of ammonium (NH₄⁺) after introduction into the set ups was between 0.20 ± 0.1 mg/L to 0.50 ± 0.34 mg/L in setups with biofilters; 0.26 ± 0.1 to 0.46 ± 0.4 in setups without biofilters. Set ups with biofilters had longer time for the drop of ammonia to zero in the initial addition than second addition. In all setups the initial addition of ammonium took 9 days to drop to zero while the second addition took 7 days to drop to zero (Figs. 9a, c) zero. By day 4 and day 14 there was higher concentration of ammonium which reduced over the number of days. Setups with 0.9mg/L had the slowest decrease compared to 0.3 and 0.6mg/L in both treatments.

Mean concentration of ammonia (NH₃) ranged between 0.0046 \pm 0.003 mg/L to 0.011 \pm 0.008 mg/L in setups with biofilters; and 0.006 \pm 0.006mg/L to 0.014 \pm 0.01mg/L in setups without biofilters (Figs 9b, 9d). Setups at concentration 0.9mg of NH₄Cl added, had higher values of ammonia in both set ups with and without biofilters. Setups with biofilters had low NH₃ concentration compared to setups without biofilters.



Variation of Nitrate

Figure 9 (a, b): Variation of Nitrate among the treatments over the number of days

Mean concentration of nitrate (NO₃⁻) ranged between 1.8 ± 0.71 to 2.26mg/L in setups with bio filters; and 1.68 ± 0.9 to 1.80 ± 0.8 mg/L in setups without biofilters. A high concentration of NO₃⁻ was recorded after day 12 for setups with biofilters, while those without biofilters had reducing concentration of nitrates from day 10 to the end of the study. There was more NO₃⁻ concentration at 0.9mg/L in both setups.

Variation of Nitrite

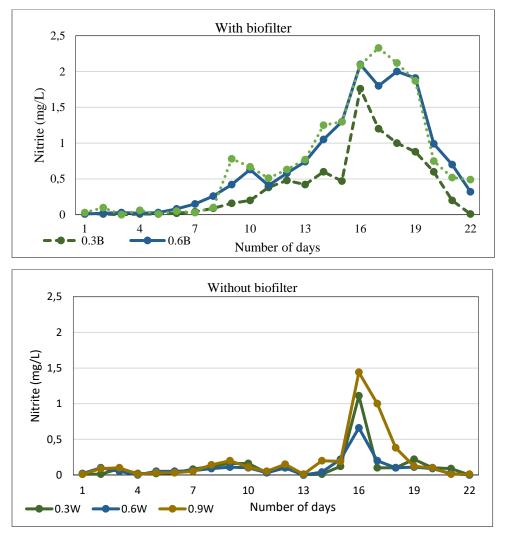
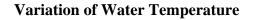


Figure 10 (a, b): Variation of Nitrite among the setups over the number of days

Mean concentration of nitrite (NO₂⁻) was between 0.12 ± 0.15 to 0.24 ± 0.28 mg/L in setups with biofilters. Setups with biofilters had a high concentration of NO₂⁻ after day 8; with high level at day 15 (1.7mg/L at 0.3, 2.0mg/L3 at 0.6 and 2.34 mg/L at 0.9). Mean concentration varied between 0.06 ± 0.03 to 0.08 ± 0.05 mg/L in setups without biofilters whose concentration increased after day 15 (0.66 mg/L at 0.3, 1.11mg/L at 0.6mg/L and 1.44mg/L at 0.9). Thereby reducing further by day 22.

Variation of Dissolved Oxygen (DO)

Dissolved oxygen was constant throughout the study time (9.7mg/L to 10mg/L) in all the setups with a mean 9.88±0.12 mg/L.



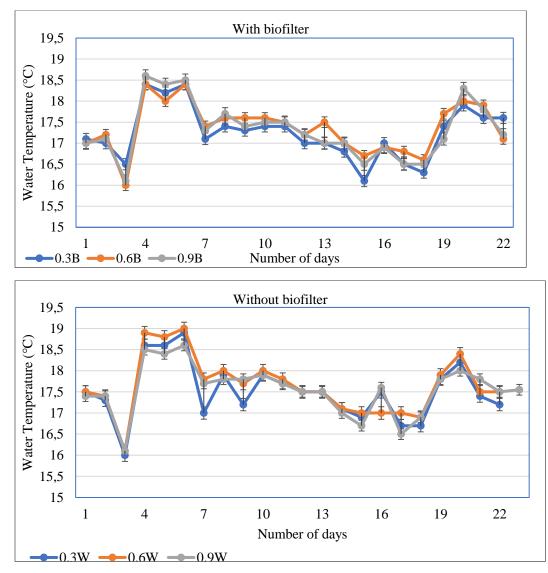
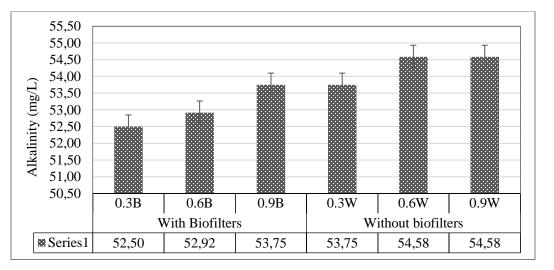


Figure 11: (a, b) Variation of Water temperature over the number of days

Water temperature ranged between 16.1° C to 18.4° C ($17.2\pm0.5^{\circ}$ C) in setups with biofilters; and 16° C to 19° C ($17.7\pm0.6^{\circ}$ C) in setups without biofilters. Day 3 showed the lowest temperature, which spiked on day 4, reduced again on day 7 and evened out on the rest of the days. Setups with biofilters had slightly lower temperature than those without biofilters.

Variation of pH

Mean pH was almost constant in all setups with ranges between 7.8 ± 0.01 to 8.02 ± 0.9 in setups with biofilters; and 7.75 ± 0.1 to 8.14 ± 0.11 in setups without biofilter media.



Variation in Alkalinity

Figure 12 : Variation in Alkalinity concentration in the setups over the number of days

Mean variation of alkalinity ranged between 53.75 ± 7.3 mg/L at concentration 0.9mg/L and 52.5 ± 7.4 mg/L at 0.3mg/L. At concentration 0.3mg/L in setup with biofilter there was a notable low alkalinity, and the high level of alkalinity was recorded among the setups without biofilters (between 53.75 ± 7.1 at 0.3mg/L and 54.58 ± 6.1 mg/L at 0.9mg/L).

4.2 Plant growth characteristics

Increase in number of leaves

Number of leaves were recorded as counts of leaves per plant. Before planting the seedlings number of leaves was taken as initial number while final number of leaves was considered at the end of the experiment. At the end of the study, there was an increase in the number of leaves per tank. At concentration 0.9 mg with biofilter plants had 9 leaves compared to other setups which had 8 leaves.

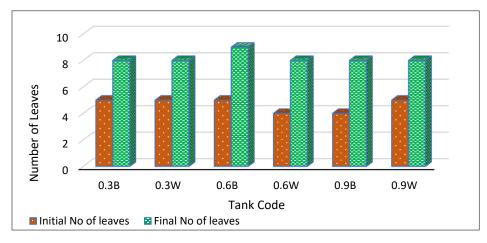
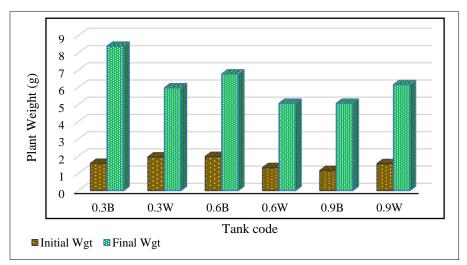


Figure 13: Increase in number of leaves



Variation of plant weight among the setups at different concentrations

Figure 14: Variation of plant weight in the set ups with biofilter (B) and without biofilter (W)

There was increase in plant weight throughout the setups. At concentration 0.3 with biofilter the plants had 8.3g as final weight generally increased more than the other setups.

5. DISCUSSION

5.1 Water quality parameters

Total ammonia nitrogen (TAN) is the total amount of nitrogen in NH_3 and NH_4^+ forms. As the ammonia is ionized to ammonium, there is a shift in balance of the forms which also affects the pH and water temperature. The lower the pH, the higher the ionized Ammonium - NH_4^+ , and the higher the pH, the higher the unionized Ammonia - NH_3 (Andriani, Dhahiyat, Zahidah, & Zidni, 2017).

 $NH_4^+ \leftrightarrow NH_3 + H^+$ Low pH \leftrightarrow High pH

From the experiment pH was constant in all setups with and without biofilter media at 7.7 to 8.1 pH units. As the pH remained in the higher ranges, more ammonia was present in the water. It should be noted that NH_3 levels beyond 0.5mg/L are considered toxic to fish (El-Sayed, 2019); however, in all the setups the concentration of NH_3 was below 0.1mg/L (Figs. 9b, 9d), indicating they were in the safety zone for growth of fish.

Ammonium is less toxic to fish but has adverse effects at levels beyond 1mg/L (Francisco, Roberto, Diego, & Marcelo, 2013). NH₄⁺ levels in the experiment were below 1.4 (Figs. 9 a, c) and continued to decrease over time (0.01mg/L reduced per day). This shows there was nitrification happening in the setups.

Set ups with bio-filters had a higher concentration of NH_4^+ (0.20±0.1mg/L to 0.50±0.34mg/L) than NH_3 (0.0046±0.003 mg/L to 0.011±0.008mg/L) as compared to setups without biofilters which had low concentration of NH_4^+ (0.26±0.1mg/L to 0.46±0.4mg/L) and high concentration of NH_3 (0.006±0.006mg/L to 0.014±0.01mg/L). Biofilters provide a larger surface area for action on ammonium chloride to form other forms like nitrites and then nitrates (Danner, Mankasingh, Anamthawat-Jonsson, & Thorarinsdottir, 2019). Since there were no biofilters, contact surface for bacteria was reduced hence there was a shift to toxic NH_3 in setups without biofilters.

The nitrification process is an aerobic process in which oxygen is consumed to facilitate for the conversion of ammonium (NH_4^+) into nitrite and then into nitrates. Dissolved oxygen in all set ups was constant at 9.7 to 9.8 mg/L (5mg/L is considered optimum especially for tilapia farming). Aeration in the setups was provided by air stones throughout the setups, this could have kept the dissolved oxygen at constant rate and therefore supported the formation of nitrites and nitrates.

Setups with bio-filter media had more nitrates (NO₃⁻) and nitrites (NO₂⁻) after day 10 (Fig. 10 a; 11a), as compared to setups without biofilters (Fig. 10 b; 11b). The first form of ammonia after oxidation is NO₂⁻, then NO₃⁻ (Danner, Mankasingh, Anamthawat-Jonsson, & Thorarinsdottir, 2019). The concentration of nitrates formed was more than nitrites after day 10 in both setups with and without biofilters. NO₂⁻ are the first unstable form of NH₃/NH₄⁺ oxidation which quickly transforms to NO₃⁻. Therefore NO₂⁻ formed could have transformed immediately to NO₃⁻ as immediately as it was formed. With biofilter media the action of bacteria on NH₄⁺/NH₃ is more evident than without biofilter media. This could explain the high levels in biofilter setups than those without biofilter media. NO₂⁻ is also toxic to fish and should be maintained as low as possible.

In the experiments levels were above 0mg/L, the recommended for aquaponics, however, the rate of transformation to NO_3^- could have kept the levels at low rate of toxicity. Nitrates are the least toxic form of nitrogen to fish and can range from 50 to 90mg/L (Ako & Baker, 2009). In the experiment, levels were below 5mg/L in all setups with and without bio filter media, which may be considered favourable for fish.

The pH in an aquaponics system should range from 6.5 to 7.5 for fish and plant growth, and between 8.0 to 8.3 for cycling bacteria (Danner, Mankasingh, Anamthawat-Jonsson, & Thorarinsdottir, 2019). From the experiment, all setups had pH ranging between 7.78 to 8.1 units. These levels are in the range suitable for aquaponics. Maintaining a suitable pH for plants, fish and bacteria is necessary for the proper function of an aquaponics system at suitable pH and temperature (10°C to 28°C for tilapia).

Lower levels of alkalinity and lower pH values in the treatment with a biofilter than in the treatment without a biofilter, may be owing to the biofilter. The bio balls as a biofilter allows more ions to be produced (NH_4^+ , NO_3^- , CI^- , NO_2^- and CO_2). As the water circulates through the system, and levels of nitrate reduced by plants, the alkalinity stabilizes and pH raises (Areerachakul, 2018). Although alkalinity may not pause high risk to growth of fish, it affects other factors that support fish growth.

5.2 Plant growth characteristics

The outcome of the NH_4^+/NH_3 breakdown, that is nitrate (NO_3^-), is utilized by the water plants for growth (Ako & Baker, 2009). This is evident with the increase in the number of leaves and average increase in the plant weights. Media beds were studied to provide more stability for root growth to the aquaponic plants. In the experiment media beds were used, and as a result increased surface area for nutrient uptake hence growth and increase in plant weight (Molovan & Băla, 2015).

6. CONCLUSION

There was reduction of ammonium in the setups with and without biofilters, however in setups with biofilters the concentration of nitrates was higher than the setups without biofilters implying that when biofilter media is added to the aquaponic system it promotes a favourable environment for bacteria growth/multiplication. As a result, the rate of toxic ammonia is reduced to ionised ammonium which is less toxic. Favourable rates of oxygen in aquaponics tank will support oxidisation to form nitrites and then nitrates which are the assimilable nutrient for plant growth in aquaponics.

In addition to biofilter media, to maintain a balanced growth setup for plants, fish and bacteria, the farmers should be equipped with test kits (and interpretation manuals) to regularly monitor changes in ammonia/ammonium, nitrates, nitrites, pH, water temperature and alkalinity. A maintained balance of parameters would then guarantee optimum aquaponics output in the form of plants and fish and support food production in urban areas.

In the experiment bio balls were used as biofilter media but farmers can replace bio balls with perforated bottle caps, shredded plastic, old fishing nets, old (but clean) sponges among others. Biofilter media should be cleaned after each growing cycle to limit transfer of harmful pathogens to other fish.

The study was carried out without replication, hence further studies with replications can be done to provide more evidence on the subject.

The study was carried out in an enclosed space, on a small scale without natural environment interruption. The same study can be replicated in an open environment for agreement or comparison of results.

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