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INOCULATING FISH SLUDGE FROM AQUAPONICS WITH MICROBES TO ENHANCE MINERALISATION OF PHOSPHORUS

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Thanks to the almighty God for thus far, he has brought me stemming back to 2018 when this masters just began and giving me the life and ability to study this course to completion in good health amidst the alarming situation of COVID-19 world pandemic.

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PREAMBLE

I arrived in South Africa in February 2020, weeks before the declaration of COVID-19 as a world pandemic, and here some preparatory work in organizing for my study had already commenced. This involved refining the experimental design, logistic planning for the experiments, which included securing a supplier for the tilapia fish. The required number of fish as per the experimental design was already available awaiting transfer from the farm to the experimental tanks. The experimental tanks were checked to ensure that they are in good condition for use, and a plan for cleaning them up was in the process. A supplier for the commercial fish feed was already identified, having the required pellet size as stated in the methodology. The procurement process for the necessary equipment to run the experiment was being undertaken.

With regards to analytical work, internal and external laboratories within Stellenbosch had already been contacted. Determination of microbial community with the sludge using the RISA method was to be performed at Sporatec laboratory at the department of genetics, this is a university laboratory which issued quotation according the number of sludge samples. Bemlab which is a private laboratory from where the macronutrient analysis was to be done was also contacted and a reservation made.

Unfortunately, at the verge of getting started with the experiment, all University facilities were closed following a nationwide lock down in South Africa due to COVID-19 bringing a halt to the progress of the study. The inability to conduct the proposed experiment later turned my thesis rather into a literature review thesis other than an experimental thesis. This expanded my literature review to cover in depth aquaponics rather than focus on only mineralization of Phosphorus. No data were collected at all for this study; hence the objectives and hypothesis of this study were not tested nor were the research questions of this study answered. Not collecting data also makes this study lack a results chapter even if it provides a description of a methodology that would have been used in case the study was not halted by the subsequent lock downs. Moreover the methods of analysis described in this study are based from literature due to failure to access the laboratories mentioned herewith and the discussion chapter for this write up is based more on expected results rather than factual.

ABSTRACT

The solid wastes from recirculating aquaculture are rich in nutrients, and these nutrients may cause harmful effects to the environment upon direct discharge from the aquaculture system. On the contrary, these nutrients are essential for plant growth. Phosphorous can be recovered from these solid wastes through mineralization. This biological process is achieved through the activity of microorganisms that break down the complexes in which these nutrients are bound into readily available forms. This study describes an experimental setup to assess whether the mineralization of Phosphorus may be enhanced by bioremediation with *Trichoderma harzanium. Trichoderma* is thought to have application aquaculture sludge treatment because of its compatibility with organic manure and its aerobic properties. The sludge comprising of uneaten feeds and feces is obtained from two drum filters, each connected to three *Oreochromis niloticus* tanks. The two composite sludge samples are subjected to three treatments; i) a control without *Trichoderma harzanium*, 1 and 2g *Trichoderma harzanium* / kg sludge. For each treatment on each sludge composite, four replicates of digesters are set up. The amount of Phosphorus is analyzed using acid digestion and spectrophotometry. Chemical oxygen demand is determined using the closed reflux method, and total suspended solids are determined using method 2540 as adopted from APHA protocols. The dominant microbial community determined using the RISA method. The results from this experiment are expected to show an increase in the amounts of Phosphorus recovered from the fish sludge in the presence of *Trichoderma* as compared to mineralization using the available indigenous microorganisms within the sludge. This will help in solving the complementation of aquaponics water with nutrients for the growth of plants in the soilless systems. This will also promote the application of aquaponics technology further as the fish effluents are capable of producing nutrients sufficient to favor plant growth.

Keywords. Aquaponics, Phosphorus, fish sludge, *Trichoderma harzanium*

1. Introduction

Aquaculture (farming of aquatic organisms) is one of the fastest-growing food production sectors in the world and accounted for 115 million tonnes of fish by 2018 (FAO 2020). This high tonnage is because of the increasing global population that is projected to reach 10 billion by 2050, and thus demands for sustainable food production will increase. However, the increased stocking densities in aquaculture units pose a threat to the quality of the rearing water due to the increased unused amounts of feed and fish excreta deposited. Accordingly, Recirculating Aquaculture Systems (RAS) that operate on the principle of reuse of water are popularly gaining use. RAS ensures maintenance of the quality of rearing water by mechanical and biological filtration before reuse. Mechanical filtration traps solid wastes from uneaten feed and feces of the aquatic organism, which concentrate together to form sludge. Additionally, disposal of this sludge has counterproductive effects to the environment as it contains not only organic matter but also nutrients such as nitrogen and phosphorous. Such a diversity of nutrients would trigger the formation of algal blooms when directly discharged to the environment. Consequently, a liquid effluent is produced, abundant in plant nutrients as a result of putrefying organic matter, fish manure, and excreted nitrogenous wastes from fish (Rakocy et al. 2006).

The advent of aquaponics technology has counteracted this environmental pollution concern from aquaculture wastes. The system that integrates aquaculture and hydroponics is gaining increasing interest as a promising model for sustainable food production. This is an example of a semi-closed system where wastewater from fed aquaculture is used to grow crops in the absence of soil. This kind of production reduces the emissions of wastes, especially nutrients (nitrogen and phosphorous) to the environment; most importantly, up to 80% of phosphorous is lost in this sludge as it binds to the particulate organic matter (POM). Moreover, nitrogen, phosphorous, and potassium are the three major nutrients essential for plant growth and typically constitute the industrially manufactured NPK (Nitrogen, Phosphorous, and Potassium) fertilizers in agriculture. Phosphorous is a vital nutrient because it is a major component of nucleic acids and adenosine triphosphate. To maintain crop growth, the deficiency of P must be met. Lately, the natural stocks of P have declined, yet it is a non-renewable resource (Van Vuuren et al. 2010). This exerts pressure on this natural resource as its demand largely exceeds its supply. Therefore, aquaculture sludge is not a waste but a potential for nutrient recovery.

In aquaponics, the nutrients from both the liquid and solid waste streams are essential to crops. However, the nutrients in the solid stream, especially Phosphorus, are not available for crop growth as they remain bound in the particulate organic matter. Therefore, the solid waste must be decomposed to release the bound nutrients. This is achieved through the mineralization of sludge to transform phosphorous into an ionic form of orthophosphates ($H_2PO_4^-$ and HPO_4^{2-}(Pi) soluble to the plants (Becquer et al. 2014). Mineralization can be explained as biological processes aided by the activity of microorganisms, mainly bacteria that utilize the organic matter present in the sludge as a food source. In so doing, nutrients formerly bound on the organic matter are released in forms easily absorbed by plants.

There have been several interventions to improve the recovery of nutrients from the solid waste stream. Among them is a recent study by Monsees *et al*. (2017) on aerobic and anaerobic mineralization of fish sludge using indigenous microorganisms within the fish sludge, and the phosphorous concentration recovered from these two processes was good. Unfortunately, different plant species have different nutrient requirements. In this particular study, even when phosphorous was recovered under aerobic conditions, it was not sustainable for the industrial production of tomatoes. Goddek *et al*. (2018) used anaerobic reactors that operate under low pH to increase the recovery of nutrients; however, anaerobic digestion needs a long startup period that is not fully realized. In another study, the amounts of nutrients from aquaponics were complemented with macronutrients and micronutrients so as to increase plant growth. This leaves a gap to explore techniques that enhance the recovery of more amounts of phosphorous from the sludge.

This current study explores bioremediation using exogenous microbes to enhance the microbial community within the sludge in the bioreactor and to increase the efficiency of phosphorous removal. An example of such microbes is the phosphorous solubilizing microorganisms (PSO) in the vicinity of the soil rhizosphere for plants. These solubilize phosphorous making it available for uptake by plants in soil agriculture (Bashir et al. 2018). Some species of PSO, such as *Trichoderma harzianum* have been used in soil agricultural experiments to enhance crop yield. Moreover, this fungus is also compatible with organic manure, bio-fertilizers such as *Bacillus subtilis* and Phosphobacteria through symbiotic associations (Kamal et al. 2018a). *Trichoderma* thrives in the presence of oxygen and the sludge is saturated with oxygen to enable oxidation of organic matter. In the long run the amount of organic matter reduces in the system resulting in reduction in the amount of sludge to be disposed. The liquid supernatant after this process is used as the hydroponic solution for plant growth. This saves costs of complementing aquaponics water with nutrients, so as to meet the varying nutrient requirements for different plants, and also the expense of a stand-alone hydroponic system.

General objective

The main objective of this study is to inoculate *Trichoderma harzianum* into fish sludge, obtained through a tilapia fish culture, to enhance mineralization of phosphorous initially bound in the sludge.

Specific objectives

1. To evaluate the amounts of Phosphorus, present in the sludge before mineralization and that present in the supernatant after mineralization;
2. To assess the sludge reduction performance after oxidation of organic matter in terms of chemical oxygen demand (COD) oxidation and total suspended solid (TSS) reduction;
3. To conduct morphological identification of major microbes initially present in the fish sludge as they also mineralize phosphorous, to ascertain whether upon addition of the fungus significant difference is observed.

Hypothesis

It can be **hypothesized** that addition of commercial strain of *Trichoderma harzianum* into the fish sludge in a bio-digester under aerobic conditions shall enhance the mineralization of phosphorous.

2. Literature review

2.1 Background

Sustainability, environmental ethics, and responsible utilization of earth in the face of population growth and rapid urbanization continue to pose serious challenges. In 2015, the world's population was projected to increase by more than one billion people within the next 15 years, reaching 8.5 billion in 2030. By the same projection, a more significant majority of those people (66%) are predicted to live in cities by the year 2050 (UN 2015). From a food production point of view, such population growth threatens to undermine global food security, and as such, the likely threat due to unsustainable food production systems cannot be underestimated (FAO 2012; UNDP 2016). Consequently, the desire to respond to both present and future challenges related to sustainability and environmental degradation has gained ground, and as such better systems of food production/agriculture have been developed. At present, in aquaculture, aquaponics has been presented as a promising remedy to these challenges.

2.2 Aquaponics

2.2.1 Definition of aquaponics

Aquaponics is a system that integrates aquaculture and hydroponics. Mostly, this system has a recirculating aquaculture unit and a plant growing section without soil (Diver 2006; Rakocy et al. 2012). This is the modern aquaponics technology however; some low technology systems using ponds are also in existence. In such a system, the effluents from the fish unit serve as nutrients for plant growth in the hydroponic unit. These effluents are transformed into absorbable products by the help of microorganisms. Therefore, fish, microorganisms and plants are the role players in aquaponics.

2.2.2 Benefits of aquaponics

2.2.2.1 Contribution to food security

Rapid growth in the human population raises an increasing demand for food, especially animal proteins, across the globe. This has stirred up the agriculture sector with agribusinesses such as aquaculture. For sustainable food production, these sectors require the availability of land, water, and nutrients (Conijn *et al.*, 2018). These nutrients, such as Phosphorus, are being depleted more than their regeneration(Van Vuuren et al. 2010; Cooper et al. 2011). While water has other domestic and industrial uses, globally, 70% of this water is used in agriculture (OECD 2008). On a commercial scale, this could potentially evolve into a water crisis accompanied by climate change (Mancosu et al. 2015). As mitigation, technologies such as aquaponics, which enables the production of high densities of fish and large quantities of vegetables using small volumes of water and land, are receiving favorable appraisal. This technology also favors food production even in arid and semi-arid areas (Goddek and Körner 2019), where the climate is not favorable for agriculture. Similarly, production can be effected on a small piece of land hence providing a sustainable strategy to end hunger and eradicate poverty at the same time (UN SDG, 2017).

2.2.2.2 Contribution to environmental ethics

With aquaculture being a fast-growing industry in the world accounting for 115 million tonnes of fish by 2018 (FAO 2018), it also creates environmental concerns (eutrophication) if its wastes are not correctly discharged into the environment. Accordingly, technologies with minimal environmental impact are of importance (Rijn., 2013). For instance, in aquaponics, the nitrifying bacteria transform the toxic ammonia released by the fish into nitrite, which is then transformed into nitrate (less toxic) that is absorbable by plants (Zou *et al.*, 2016). This technique not only supports plant growth but also maintains good water quality required by the fish as these plants act as a biological filter (Espinosa Moya *et al.*, 2016). Relatedly, the solid wastes, which are a combination of feces and uneaten feed, are trapped at the mechanical filter as sludge. This sludge is organic matter rich in nutrients essential for plant growth and can be mineralized, making phosphorus bioavailable for plant use. Eventually, less of this mineral gets into the environment.

2.2.2.3 Economic aspects of aquaponics

Even when aquaponics was earlier imposed as a solution to environmental challenges of nutrient loading and sustainable food production, this technology is of economic importance as two products are obtained from one system. There is a growing interest in the economic viability of aquaponics as an emerging business sector basing on the fact that it integrates the growing of aquatic organisms together with plants (Quagrainie et al. 2018). This can be evaluated by looking into the internal costs such as fish feed, labor, electricity, juveniles, seedlings, water etc. versus the outcome after sales (Engle 2016). These production costs, however, are controlled by the size and design of the system being used. Additionally, the technological complexity of the designs raises the costs of initial installations. Therefore, not enough reliable data on the economic evaluation of aquaponics is available (Turnšek *et al*., 2019)

A study carried out by Asciuto *et al*. (2019) on cultivating lettuce (*Lactuca sativa*), and Nile tilapia (*Oreochromis niloticus*) in a pilot plant showed that aquaponics farming is profitable having obtained a cost-benefit ratio which is greater than one and this implies that the business is feasible. Furthermore, in the cultivation of snakehead (*Channa striata*) with water spinach (*Ipomoea aquatic*), the production volumes of these species were higher than in normal aquaculture and hydroponic systems. A higher-income was realized than in typical farming systems, which underlines the economic viability of aquaponics (Bich *et al*., 2020). These two studies are based on small pilot plants while in the latter locally available materials from the farm were used, which lowers farm input costs. However, this is not always constant as the amount of revenue obtained also depends on other factors like market demand, consumer preference, price, the match between nutrient input and output. More importantly, the choice of fish species has to be considered because high-value species fetch much revenue even when the taxes are factored in (Bosma *et al*., 2017). Moreover, the suitability of the fish species to culturing in aquaponics is also essential. Therefore, there is a need to balance between the species suitable for aquaponics and its value in the market.

In summary, Gooley and Gavine (2003) highlighted the following as the key advantages of aquaponics; 1) increased productions and farm profits as you harvest both the fish and plants cultivated yet using small volumes of water for production, 2) being able to use fish farm wastes as valuable nutrients for the crop production, 3) reusing of fish farm wastes reduces the environmental impacts of intensive fish farming methods, 4) and farm diversification into higher-value fish species and crops. Therefore, aquaponics is undoubtedly a solution to sustainability issues that continues to attract research interests globally.

2.2.3 Production from aquaponics over the years to total aquaculture production

Notwithstanding the benefits of aquaponics as an emerging technology, data on production statistics, especially on how much fish has been produced from this system compared to aquaculture, is scarce or even non-existent. Moreover, the level of vegetable production in aquaponics compared to hydroponics over the years has not yet been documented. However, the number of RAS, aquaponics, and hydroponics publications are graphically presented in ***Figure 1***

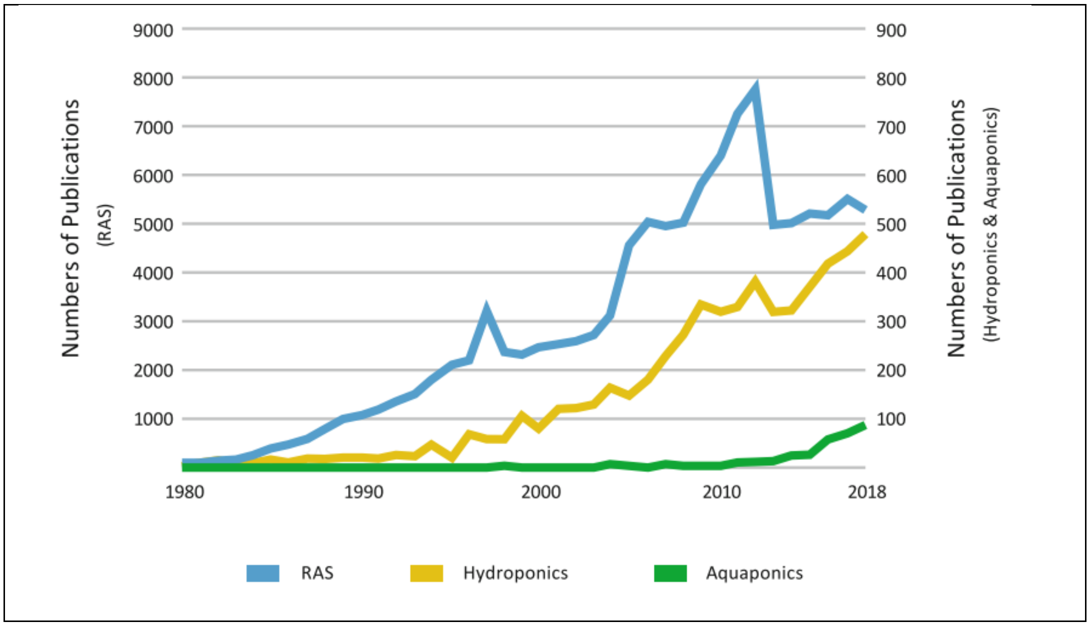

Figure 1. Pear reviewed publications in RAS, aquaponics, and hydroponics over the years 1980-2018. These data were collected from the Scopus database 2019. Adopted from Goddek et al. (2019).

It is evident from Figure 1. Pear reviewed publications in RAS, aquaponics, ***and*** hydroponics over the years 1980-2018. These data were collected from the Scopus database 2019. Adopted from Goddek et al. (2019).that aquaponics is the least researched field compared to recirculating aquaculture (RAS) and hydroponics. In stark contrast, between 2010 and 2018, this budding technology received increasing research, as seen in the rise in the number of publications. This presents a need to have in-depth studies in aquaponics technology to evaluate species suitability.

2.2.4 Species used in aquaponics

Aquaponics technology integrates both aquatic animals and plant species in the system. These species are highly diverse and only limited by water quality parameters, strongly influenced by fish feed and nutrients for the case of plants and the plant cultivation area (Knaus and Palm., 2017).

A number of fish species as shown in **Figure 2** have been used in aquaponics such as tilapia (*Oreochromis sp.*), catfish (*Siluriformes*), goldfish, Koi carp, common carp (*Cyprinus carpio*), rainbow trout *(Oncorhynchus mykiss*), Asian sea bass (barramundi) (*Lates calcarifer*) and many others (Rakocy et al. 2012). Important among the many species is tilapia (*Oreochromis sp.*), as reported in a survey carried out by Love *et al*. (2015a), involving 257 correspondents where 69% of these correspondents voted tilapia as the species they raise mostly among the many other species. Similarly, a national survey carried out in South Africa on 44 respondents showed tilapia as the most commonly used species for aquaponics with a high score of 82%, as compared to trout *(Oncorhynchus mykiss*) having 30% and barbell catfish (*Clarius gariepinus*) 18% (Mchunu et al. 2018). Some specific tilapia species like the red tilapia have been investigated for their potential in aquaponics (Kotzen and Appelbaum 2010). Some other studies show the use of African catfish (*Clarius gariepinus*) (Endut et al. 2009). Among all these, tilapia is the most preferred fish species for aquaponics production.

The preference for tilapia is justified by its ability to withstand low dissolved oxygen levels (0.1-0.5 mg/l) (Palm *et al*., 2014; El-Sayed., 2020). In addition, tilapia has a fast growth rate and reproduces several times (Day et al. 2016; Fanuel et al. 2016).This together with its ability to be stocked at high densities (crowding) has economic advantages leading to its preference. Moreover, high density fish stocking is a prerequisite for generation of more effluent for use by the plants in the aquaponics systems. As the fish are stocked at high densities, the animal get stressed, however tilapia has proven to withstand stress due to overcrowding. Notably, tilapia is capable of living under varying environmental conditions such as salinity and temperature (El-Sayed 2020). The normal temperature range for its growth is 20-35^{o}C; this is however species- dependent. This is in agreement with the findings of Xie *et al*. (2011) who reported 25-34^{o}C as the optimal temperature for proper physiology and growth of tilapia, hence making it relatively tolerant to stress. Meanwhile, even when it is a fresh water fish, tilapia can grow in brackish water and to some extent sea water, but this is species-dependent (Chourasia et al. 2018). As earlier mentioned that feed has an influence on the species for culture, tilapia eats a wide variety of food types, including planktonic foods (microphagous) which makes the species tolerant to suspended solids.

With regards to plants, leafy vegetables such as lettuce, spinach, kale, swiss chard, pak choi, flowers and fruits such as tomatoes are among the species commonly grown in aquaponics. Some of the vegetable varieties are shown in **Figure 3**. A survey carried out in South Africa indicated that over 75% of the raised plants in aquaponics were leafy vegetables (Mchunu *et al*. 2018). This could be attributed to their fast growth rate; for instance lettuce takes 2-3 weeks to get ready for harvest. In addition, leafy vegetables have low nutrient requirements (Nozzi et al. 2018) and they have a high demand in the market (Bailey and Ferrarezi 2017). Interesting to know is that the fish species can have an influence on the growth performance of the vegetables (Knaus and Palm, 2017ab) and this was elaborated by looking at the type of feed and physiological condition of fish species. Therefore, it is of importance to review when and how long these species have been used in aquaponics.

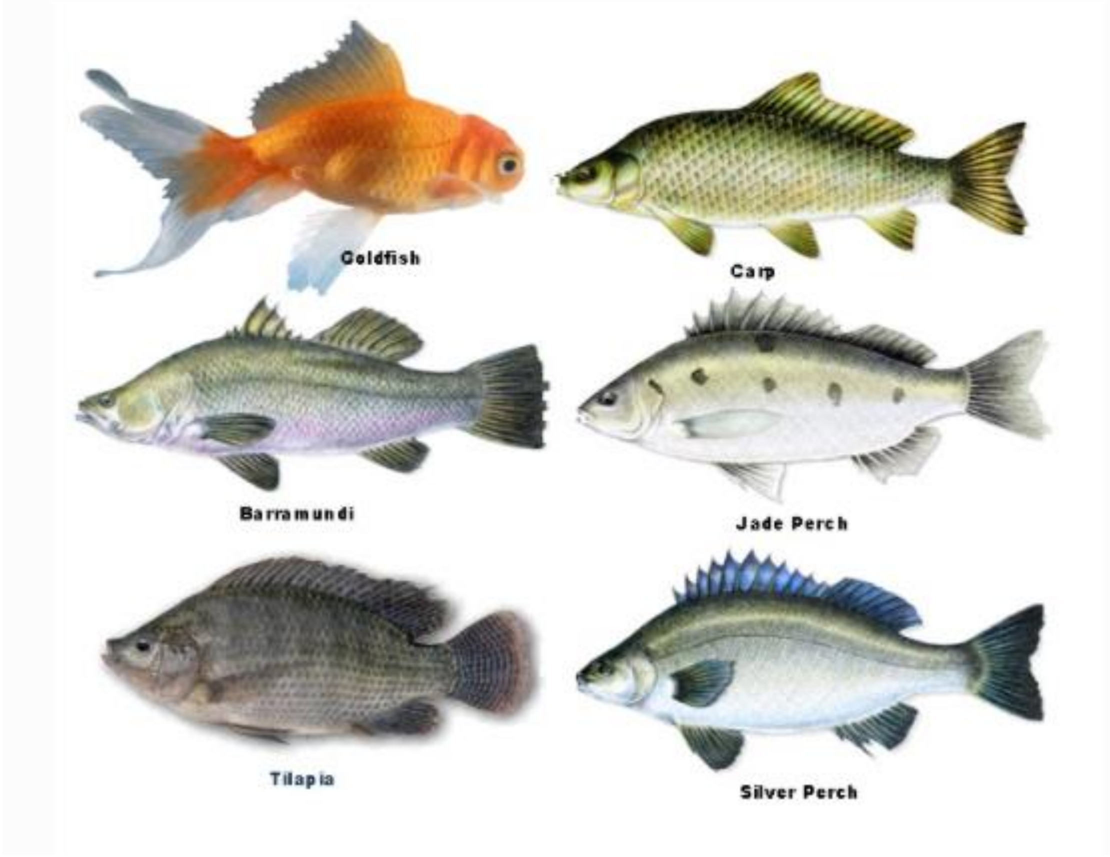

Figure 2.Fish species commonly cultured in aquaponics systems. **Source**: www.endlessfoodfactory.com

Figure 3. Vegetable species used in aquaponics source: www.pinterest.com and Aquaculture-hydroponics-greenhouse.blogspot.com

2.2.5 History of aquaponics

Aquaponics is reported to have started 1500 years ago in southern China and to have spread all over South East Asia (Goddek et al. 2019). The Chinese cultivated rice in paddies in polyculture with fish. These systems can be best described as integrated farming, although there are other forms of integrated farming which do not involve the combination of plants for instance fish and livestock farming (***Figure 4***).

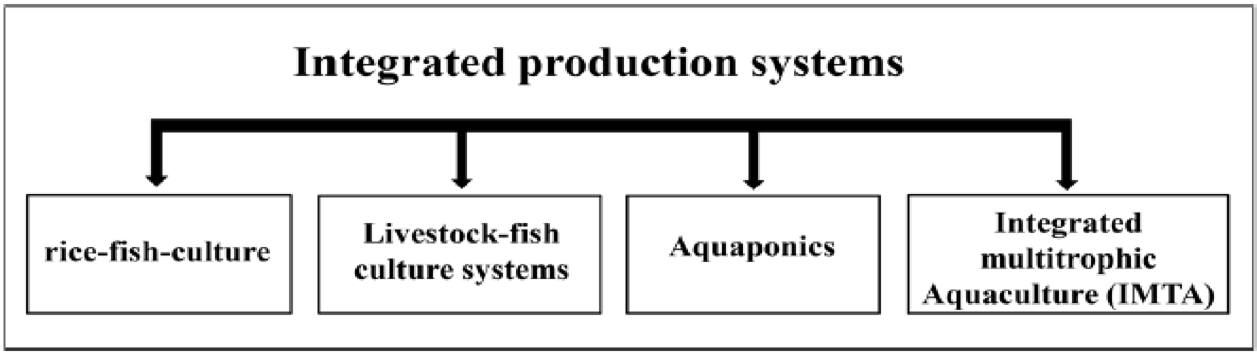

Figure 4. Integrated food production systems. Aquaponics falls under the category of integrated production systems **source:** (Palm et al. 2018)

The rice-fish cultures are outdoor systems; hence, they are seriously affected by the change in environmental conditions where freezing winters would halt farming. In ancient Mexico, the Aztecs had *chinampa* systems that utilized water-based cultivation techniques where plants were grown above the water level while the fish grew in irrigation channels from which sediments were collected for use in the plants (Crossley 2004). In recent times, research on aquaponics shifted to more modern techniques in areas with limited fresh water such as Australia (Gooley and Gavine

2003). It is reported that modern aquaponics started around the 1970s, following research into the science of this technology at various institutions. For instance, experiments at the University of Virgin Island used raft hydroponic systems to grow lettuce together with production of tilapia for research on aquaponics (Rakocy, 1988). Lately, the appreciation of aquaponics in Europe is on an upward trajectory where recirculating aquaculture systems with solid removal unit have been added to the fish production unit and fitted to the hydroponic unit for vegetable production (Thorarinsdottir 2015).

2.2.6 Types of aquaponics

2.2.6.1 Pond aquaponics

Pond aquaponics was first introduced in East Asia growing catfish (*Clarius gariepinus*) and tilapia (*Oreochromis sp.*) with vegetables. The fish are usually in mono or polyculture and very few technicalities are involved in the system. Important to mention is that a raft system is installed on the surface of the pond, meaning that there is a direct transfer of nutrient rich water to the plants. The plants in turn filter the water making it ammonia-free for the fish (Espinosa Moya et al. 2016; Shakil Rana et al. 2018).This method is very cost-effective in terms of farm inputs but also a larger profit can be realized.

Figure 5. Pond aquaponics in which plants are growing floating on the surface of an open pond. They receive direct nutrients from the water in the pond and in the long run filter out ammonia from the water. Source: (Pavlis 2018)

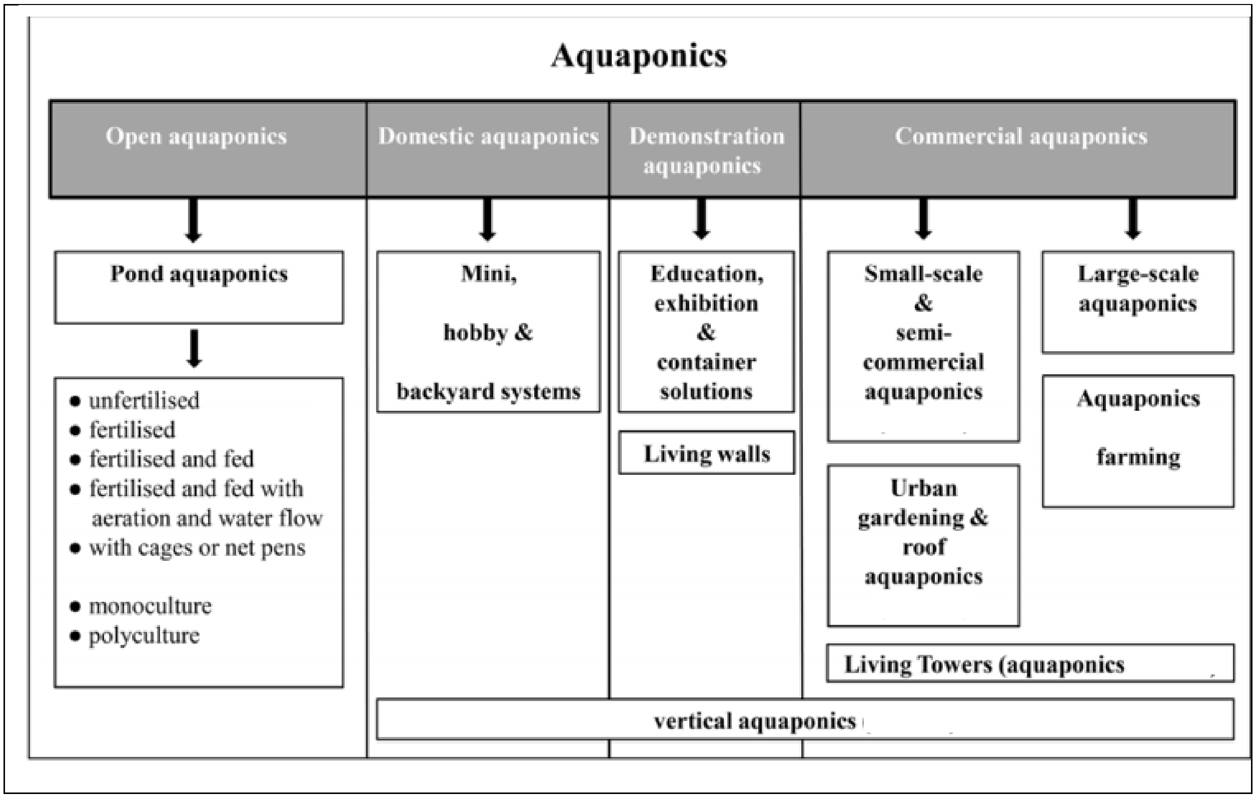

Figure 6. The different types of aquaponics systems ranging from low technology to high technology system. Adopted from Palm et al., (2018)

2.2.6.2 Domestic aquaponics

The main focus of this kind of aquaponics is to produce for home consumption, from which the term backyard farming originates. Small tank volumes with extensive stocking of fish and small plant areas are set up (single fish reservoir and a small hydroponic unit). Plants may float directly on the fish water tank or some designs are such that the aquaculture unit is separated from the hydroponic unit (Palm et al. 2018). Domestic aquaponics is a cost effective method of applying small farm inputs with medium technology and low stocking densities.

Figure 7. A small backyard aquaponics system having a small separate fish tank and a small hydroponic unit where the vegetables are growing. Source: https://www.urbanorganicyield.com

2.2.6.3 Small- scale aquaponics

This is a semi-commercial type of aquaponics described by advancement in technology of the functional units and is purposed for retail markets. The system has fish rearing tanks, a mechanical filtration unit and a biological unit, which optimizes fish and plant production, the plants can be grown in a facility set up with the nutrient film technique or deep water culture. Some small scale aquaponics systems use media bed with substrates such as gravel and sand for plant production (Palm et al. 2018). These media beds have a tendency of clogging after a while and this leads to costs of cleaning the system, but on the other hand these substrates are advantageous in the sense that they provide surface for formation of bacterial biofilms, hence aiding biological filtration of nutrients needed by the plants (Maucieri et al. 2018). This makes a cost-effective design, as there is no need to install a separate biological filter in the system. Another advantage of small-scale aquaponics is seen in the raft system where low energy costs apply as water flow is by gravity and a single pump can be used to circulate water. This design also minimizes space and can be installed for example on rooftops.

Figure 8. A small-scale commercial aquaponics system with few fish tanks and a hydroponic system situated in a green house. Source: www.ecofilms.com

2.2.6.4 Large scale aquaponics

In large scale aquaponics the components are similar to those of the semi-commercial aquaponics, however, set up is for big production where the number of fish tanks are multiple (Rakocy et al. 2012). Mostly the plant production area is in controlled greenhouses (Palm et al. 2018). These systems are big and may cover a land area above 500m^2 as seen in the system of the University of Virgin Islands (Rakocy et al. 2012). This applies advanced technology with computerization and monitoring of water quality. High investment costs are incurred which demerit this technique, however to increasing production output is possible through a stocking density at maximum capacity. The target markets for such a system are for example grocery stores, wholesalers, and restaurants (Love et al. 2015b). System designs have lately been classified into coupled and decoupled designs, and this applies to the mentioned aquaponics types with exception of pond aquaponics (Forchino et al. 2017).

Figure 9. Large-scale commercial aquaponics system with a square fish tank and plant growing area beside it in a greenhouse structure. Source: http://www.herbanfarms.com/index.html

2.2.6.5 Coupled aquaponics systems design

This is a system design consisting of a fish tank, mechanical filter, biological filter, hydroponic unit and a pump connected in series to form a single loop (Palm et al. 2019). In such a system, water flow from the fish tank through the filters to the plant section is by gravity (Lastiri et al. 2016). This water is in turn pumped back to the fish tank. This type of water flow is unidirectional as seen in ***Figure 6***. Coupled aquaponics systems are applicable to backyard aquaponics, small scale and commercial systems. The system variation is seen with the number of fish tanks connected to the system. This spells out advantages of such a system design as being affordable in terms of costs and efficient use of resources like feed, water and energy under the different types of aquaponics (Palm et al. 2019). Moreover, coupled systems can also be used in a wide range of geographical locations, with varying climatic conditions and limited resources. It enables the adjustment of the system design to suit the local conditions, hence making it affordable. Other benefits of coupled aquaponics systems are seen in the welfare of the fish itself under culture. Baßmann *et al*. (2017) investigated the welfare of African catfish (*Clarius gariepinus*) cultured together with cucumber (*Cucumis sativus*) and compared it to catfish culture under RAS. Fewer injuries were observed in the aquaponics fish as compared to the RAS fish. This is attributed to the filtering ability of plants that help in water clarity and thus proper vision by the fish in the water, reducing territorial fights as compared to the RAS system with increased water turbidity. However, coupled designs are challenged by establishing the right component ratios between the feed input, stocking densities of the fish, water treatment and hydroponic unit. This results in possible consequences of nutrient deficiency in the plants as usually the plant growing area is larger as compared to the fish unit providing the nutrients. Moreover, plant and fish may have different growth requirements: for instance, fish require a pH of around 7-9 , whereas plants require a lower pH of about 5.0-6.5 (Goddek *et al*., 2015ab; Tyson *et al*., 2008). In such a case its becomes difficult to regulate the water pH in the fish tank, and later adjust it for plant growth in the hydroponic system (Sace and Fitzsimmon 2013). Hence, the growth conditions of one part are a disadvantage to the other part. To solve such a problem, a decoupled system design is relevant as the fish and hydroponic unit are independent from each other (Goddek et al. 2016a).

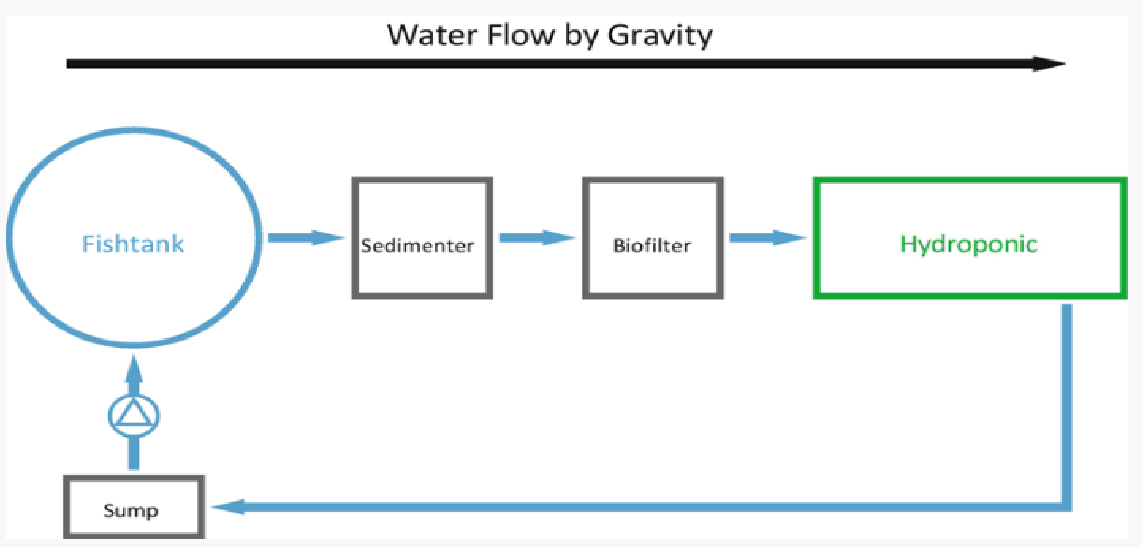

Figure 10. Basic technical system design of a coupled aquaponic system with fish tank, sedimentation tank, biofilter, hydroponic unit and a sump where the water is pumped or airlifted back to the fish tanks and flows by gravity along the components. Adopted from Palm et al. (2019)

2.2.6.6 Decoupled aquaponics systems designs

This technology emerged as a solution for the above challenges in coupled aquaponics systems (Blanchard et al. 2020). It is a system design such that the aquaculture unit is separate from the hydroponic unit. The RAS water that leaves the fish unit to the hydroponic unit does not return back. Additionally, the sludge collected from the fish unit is placed in a bioreactor, mineralized and pumped to the hydroponic side for plant growth (Goddek and Vermeulen 2018). Bioreactors have significant advantages for increased nutrient recovery from the sludge, thus helping to close the nutrient cycling loop within aquaponics systems. Kloas *et al*. (2015) describe modern decoupled aquaponics system growing tomatoes and fish in a green house in 2 independent recirculating units. One important merit of such a system is that optimal growing conditions can be achieved for each unit, most importantly pH as it varies for each unit (Goddek et al. 2016a). Decoupled system designs also reduce the risk of disease outbreak as the water from the fish unit does not return back. A study shows that mineral fertilizers may be introduced into the hydroponic unit on top of minerals obtained from the fish effluent as a way to optimize plant growth conditions (Kloas *et al*. 2015). This is because liquid effluent provides 50% of the nutrients for plant growth. To mitigate this, there is need to increase the efficiency of nutrient recovery from the solid wastes of the fish and uneaten feed with the help of microorganisms.

Figure 11. A decoupled aquaponics system design where A is the fish-rearing tank, B is the nutrient sump, C is the digester of mineralization vessel and D id the hydroponic system. **Source**:astfilters.com

2.2 Nutrients in an aquaponics system

2.2.1 Source of nutrients in aquaponics

Nutrients in an aquaponics system are obtained from uneaten feed in the fish rearing tank and feces together with dissolved wastes released by the fish (Ayoola Olusegun et al. 2016; Kokou and Fountoulaki 2018; Dauda et al. 2019). Fish mainly release ammonia (unionized) as a waste and this becomes toxic to the fish, hence the need to be removed (Romano and Zeng 2013). Ammonia is a nitrogenous waste and this makes it a potential source of nitrogen highly needed by the plants (Dauda et al. 2019). The uneaten feed is a source of macronutrients and micronutrients. Mineral elements are added as ingredients during aquafeed manufacture and the quantities of the elements present in the feed depend on the nutritional requirements of the fish species together with other factors like the stage of growth (Bureau 2001). The nutritional requirements of carnivorous fish differ from omnivorous fish, which are in turn differing from those of herbivorous fish. Therefore, also the uneaten feed will contain nutrients that vary with the type of aqua feed applied.

2.2.2 Examples of nutrients in an aquaponics system

In an aquaponics system, plants require both macro and micronutrients for their growth. There are various essential nutrients for plant growth (***Table 1***). Of these, nitrogen (N), Phosphorus (P) and potassium (K) are the major nutrients required for plant growth (major macronutrients). Other macronutrients include calcium (Ca), sulfur (S), and magnesium (Mg). Boron (B), chlorine (Cl), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo), and nickel (Ni) are categorized as micronutrients or trace elements (Bittsanszky et al. 2016).

Table 1. Essential macro and micronutrients for the 3 basic components of an aquaponics system. M symbolizes macronutrients and µ symbolizes micronutrients, dash (-) means the nutrient is absent in that part of the aquaponics system.. Source: Bittsanszky et al. (2016)

Elements	**Fish tank**	**Plants**	**Biofilter**
Boron	-	µ	-
Calcium	M	M	M
Chlorine	M	M	µ
Cobalt	µ	µ	µ
Copper	µ	µ	µ
Chromium	µ	-	-
Fluorine	µ	-	-
Iodine	µ	-	-
Iron	µ	µ	µ
Magnesium	M	M	µ
Manganese	µ	µ	µ
Molybdenum	µ	µ	µ
Nitrogen	M	M	M
Nickel	-	µ	-
Phosphorous	M	M	M
Potassium	M	M	M
Sodium	M	-	µ
Sulfur	M	M	M
Zinc	µ	µ	µ
Selenium	µ	µ	µ
Silicone	-	µ	-

Carbon, oxygen, and hydrogen are not mineral elements but are essential for biomass production in both the fish and plants. Oxygen is important for respiration in both fish and microorganisms within the biofilter. The bacteria, in so doing releasing the bound minerals within it, break down the sugars containing carbon.

Very little has been researched on the nutrient requirements of the microbial communities within the biofilter even when they play and important role in organic matter breakdown and conversion of toxic ammonia into less toxic forms (Munguia-Fragozo et al. 2015).

2.2.2 The role of nitrogen, phosphorous and potassium in plants

2.2.2.1 Nitrogen

Nitrogen contributes to the plant biomass as a component of proteins, nucleic acids, chlorophyll and co-enzymes (Malcolm Hawkesford et al. 2012). Plants take up nitrogen in the form of nitrate which is assimilated into amino acids. These amino acids when joined together form the protein structure of plants. Fish release ammonia as a waste and this is very toxic to them. However, with the help of bacteria in the RAS component of the system, the toxic ammonia is converted to nitrite by *nitrosomonas* bacteria. The nitrite is finally converted by *nitrobacter* to nitrate, the form of nitrogen that is easily absorbable by plants and constitutes inorganic nitrogen (Crab et al. 2007; Zou et al. 2016). Low bioavailability of nitrogen greatly impacts on plants through stunted growth, narrow leaves that appear pale green and even yellowing termed chlorosis (Hawkesford *et al*., 2012). This nitrogen deficiency can be corrected by the application of external fertilizers in crop agriculture. It is estimated that over 109 million metric tons of industrially made nitrogen fertilizers are being used globally per year (FAO 2017). Unfortunately, only 50% of this fertilizer is utilized by the plants, the rest is washed into the environment causing counterproductive effects to the environment.

2.2.2.2 Potassium

This is also an essential macronutrient that plays a role in osmoregulation, a process important for cell wall extension and stomata movement. Moreover, potassium in its ionic form together with its accompanying anions contributes to the osmotic potential of the plant cell regulating flow of water within the plant. It also balances the charge on organic acid anions to enable pH stability that suits enzyme activity. Globally 38 million metric tones of potassium fertilizer is used in agriculture annually (FAO, 2017). These large quantities of industrially produced potassium could potentially be scaled down by mineralizing potassium from the uneaten feed from the fish rearing unit.

2.2.2.3 Phosphorus

Phosphorus is a component of adenosine triphosphate (ATP), which enables energy transfer. Phosphorus is also a component of nucleic acids and phospholipids and facilitates the transfer of sugar molecules to the leaf of the plants. ATP is also responsible for regulation of plant metabolic pathways like in the chloroplasts (Hawkesford *et al*., 2012). Deficiency of Phosphorus affects shoot growth while the roots continue to elongate, and also hinders leaf expansion. Globally, 45million metric tonnes of phosporus fertilizer were used in 2017 to be able to meet the mineral deficiency (FAO, 2017). Such high usage not only inflates agricultural production costs but could also be detrimental to the environment. It is also important to note that the global phoshorus stocks are getting depleted, yet it is a non-renewable resource (Van Vuuren et al. 2010). Incidentally, aquaponics presents an opportunity to utilize the phosphorus present in the fish feed together with the uneaten feed that accumulates to form a sludge. However, this form of Phosphorus is not readiliy available to the plants.

2.2.3 Standard levels of macronutrients in an aquaponics and hydroponic systems

Nutrient levels in a hydroponic system are usually set formulated according to the optimal requirements of the plant (Martin Steyn and Du Plessis 2001). These vary among the multiple plant species. As for nutrients from the wastes in aquaculture, no research has reported the optimal yield for a particular system design. Various experiments report different values of nutrient yield from aquaponic wastes. The average values of some macronutrients (**Table 2**). Bittsanszky *et al*. (2016) investigated the macronutrient concentration in aquaponics water. In this study, the hydroponic data were calculated as averages from concentrations in nutrient solutions described by (Libia I. Trejo-Téllez and Fernando C. Gómez-Merino 2012) and Murashige and Skoog (1962).

Table 2. Macronutrient concentrations in aquaponics and hydroponics systems. **Source:** Bittsanszky et al. (2016)

Nutrient	Hydroponics (mg/L)	Aquaponics (mg/L)	Concentration ratio (hydroponic/Aquaponic)
$P(PO_4^{3-})$	6.6 ± 1.0	36.9 ± 6.2	5.59
N	10.6 ± 2.1	321 ± 130	30.3

K^+	50.8 ± 11.9	340 ± 101	6.7
Ca^{2+}	129.6 ± 18.5	160 ± 10	1.2
Mg^{2+}	20.9 ± 1.1	40.9 ± 3.3	2
SO_4^{2-}	88.3 ± 12.2	134 ± 53	1.5

The nutritional concentration of the macronutrients is lower in the aquaponics system than in the hydroponics system that is optimized for plant growth (**Table 2**). Importantly still, the mineral requirements of a plant vary with the species, stage of growth and the nature of the plant itself. For instance, fruit bearing plants require more nutrients than leafy vegetables in a hydroponic system (Suhl et al. 2016; Bailey and Ferrarezi 2017). Complementing aquaponics water with macro and micro nutrients shows an increased growth performance of vegetables (Delaide et al. 2016; Ru et al. 2017). Unfortunately, this increases the internal costs of production. Appropriate amounts of nitrogen are usually present in the liquid effluent after biofiltration, yet phosphorous and potassium are limiting in the aquaponic process water. The limiting minerals are however bound in the RAS solids which constitute uneaten feed and feces, concentrated together to form a sludge. Interestingly, when this sludge is controllably degraded by microorganisms, it can release the phosphorous together with potassium. This makes these nutrients available for use by the plants in the hydroponic solution. This process is termed mineralization of the sludge.

2.3 Mineralization of fish sludge

2.3.1 Definition and types of mineralization

Mineralization is a biological process aided by the activity of microorganisms, mainly bacteria, that utilize carbohydrates from the sludge as a source of energy to breakdown the organic matter, releasing the bound nutrients. Mineralization not only solves the problem of adding nutrients into the aquaponic water but also reduces environmental nutrient loading in the long run. A study that estimated the nutrient load in tilapia wastes observed that 18% of the feed is not consumed; yet among what is consumed, only 50% and 84% of phosphorous and nitrogen, respectively, are digestible (Montanhini Neto and Ostrensky 2015). The rest of the nutrients is excreted in fecal matter making aquaculture sludge a nutrient-rich source.

Aerobic mineralization is a biodigestion process in the presence of oxygen. The heterotrophic bacteria oxidize organic matter within the fish sludge. It is of importance to ensure sufficient supply of oxygen in such a system so as to support the activity of the microorganisms. Moreover, this is seemingly an expensive process as it requires constant pumping of air that utilizes power.

Anaerobic mineralization is a kind of sludge digestion facilitated by facultative anaerobic bacteria performing biochemical processes leading to nutrient release (Delaide et al. 2019). It is a simple method that does not require high running costs since no air supply is required. This gives anaerobic digestion a comparative economic advantage over aerobic digestion.

Anaerobic and aerobic mineralization has aided the investigation of the amount of nutrients released after the process. Secondly is the sludge reduction performance. Lastly is the effect of these nutrients on plant growth. (Monsees et al. 2017; Delaide et al. 2018; Goddek et al. 2018).

2.3.2 Current research on mineralization

Monsees *et al*. (2017) investigated the nutrient mobilization of fish sludge under aerobic and anaerobic conditions. The results showed an increased yield in phosphorous and potassium concentrations for both treatments. It is reported that under aerobic treatment, the phosphate concentration obtained is not sufficient for growth of tomatoes industrially and requires supplementation with fertilizers (Suhl et al. 2016). This corresponds with the fact that nutrient requirements for plants vary with the species and stage of growth amidst other factors. This evidence shows that it is worthwhile to improve the efficiency of sludge mineralization.

In a similar study, Goddek *et al*. (2016b) examined the growth and performance of lettuce using the supernatant from aerobic and anaerobic mineralization. The outcome of this study showed better growth with the supernatant from anaerobic mineralization as compared to aerobic. This improved growth stems from the fact that anaerobic conditions generate ammonium, the ammonium ion is acidic making the pH in the reactore acidic yet this low pH is important for nutrient uptake. Anaerobic mineralization however requires a long startup period and does not favor large scale nutrient requirements.

Goddek e*t al*. (2018) investigated mineralization and organic reduction performance of aquponics sludge in upflow anaerobic sludge blankets (UASB). It can be noted from these findings that nutrient mobilization increased with low PH inside the reactor although this did not apply to nitrogen because nitrogen mineralization occurs at high PH. Similarly, the sludge reduction performance is higher under high PH than in acidic conditions. Eventually, this creates antagonism between nutrient mobilization and organic sludge reduction. Notwithstanding the added costs, this problem could be solved by installing two separate reactors. Therefore, PH is one of the key parameters to monitor during mineralization of Phosphorus as it has a substantial impact on how much of phosphorous is released during mineralization. Under aerobic conditions, the PH of the solution is lowered after respiration and nitrification. Carbon dioxide as the end product of respiration contributes to the low PH (Delaide et al. 2019). This supports nutrient mobilization of Phosphorus together with potassium. In the same way, lowering PH below 6 in the bioreactor increases mineralization and nutrient mobilization, hence more phosphorous is recovered at low PH (Goddek et al. 2018). It is therefore important to ensure that PH is constantly maintained below 6 throughout the mineralization process.

Enhancing the efficiency of the microbial community in the fish sludge is of interest since it is responsible for the biochemical breakdown of the organic matter and eventually releases phosphorous in a form soluble to plants. In soil agriculture, phosphorous solubilizing microorganisms are applied to the plant growth area such that they enable nutrient mobilization within the plant (Goswami et al. 2019).

2.4 Bioremediation of aquaculture sludge

2.4.1 Phosphorus solubilizing microorganisms (PSO) as agents of bioremediation

PSO are microorganisms capable of solubilizing inorganic Phosphorus from insoluble compounds play the role of bioremediation of Phosphorus from aquaculture sludge. These comprise of bacteria, fungi, algae and actinomycetes (Alori et al,. 2017). The bacteria, which form the majority of the microbial population here, may be aerobic or anaerobic strains. These bacteria are of varying species but are majorly of the genus *Pseudomonas* spp, *Bacillus* and *Agrobacterium* (Babalola et al. 2005). Among the fungi are *Aspergillus, Penicillium, Trichoderma, Saccharomyces, Rhizopus* and many others. In crop agriculture, PSOs are commonly found in the soil rhizosphere with a few existing out of it (Vazquez et al. 2000).

There is a growing interest in PSOs by agriculturalists due to its potential to enhance plant growth. PSOs play a role of biofertilizer formation by transforming the insoluble phosphorous content within organic materials to soluble forms improving its bioavailability to plants (Zhu et al. 2012). Phosphorous solubilizing bacteria have also been reported to increase the dry matter partitioning and biomass accumulation in maize (Amanullah et al. 2019**).** This is therefore an environmentally friendly approach for sustainable food production in soil agriculture.

2.4.1.1 Mechanism of phosphorous release by PSOs

Microorganisms are capable of solubilizing and mineralization of phosphorous through a number of mechanisms. These stem from release of organic acids, siderophores, anions, protons and hydroxyl ions. Other mechanisms are enzymatic while others release phosphorous through substrate degradation (Arcand et al. 2006) and (Sharma et al. 2013) and (Mcgill and Cole,. 1981). Phosphorus solubilization occurs for inorganic phosphates. Here organic acids are released by PH lowering, these chelate the cations bound to the phosphate such that phosphorous is released by forming soluble complexes with the metal ions associated with the insoluble phosphorous. At low PH, monovalent phosphate ions are released ($H_2PO_4^-$) through anion exchange, and as the PH increases divalent and trivalent forms are released (HPO_4^{-2} and HPO_4^{-3} respectively). Examples of organic acids used are acetate, citrate, oxalate, and malate (Sharma et al. 2013). Phosphorous mineralization occurs for organic phosphate and this is aided by the activity of enzymes such as phosphatase that dephosphorylates phospho-ester bonds (Nannipieri et al. 2011). This is followed by phytases causing release of phosphorous from phytate degradation, and lastly by phosphonatases and C–P lyases, that cleave the C–P bond of organophosphonates (Behera et al. 2014).

In soil agriculture, PSOs have been isolated through a number of microbiological techniques and have been further packaged for commercial use by farmers. A number of strains have been used, among which is *Trichoderma*.

2.4.2 *Trichoderma* and its role in phosphorus mineralization

Trichoderma is a fungal genus with a vast number of species such as *T. koningii, T. harzianum, T. reesi, T. polysporum, T. viride* (Kubicek et al. 2019). Among these, *T. viride* and *T. harzianum* are most commonly used as biofertilizers (Kamal et al. 2018b). The latter is capable of improving plant mineral absorption in crops. Even when *Trichoderma* is naturally present in soil, these species have also been commercialized which makes ease of their application by farmers. *Trichoderma* not only improves uptake of micronutrients such as zinc, iron, and copper but is also capable of solubilizing phosphorous making it available for plants. Additionally, *Trichoderma* is compatible with organic manure and biofertilizers such as *Bacillus subtilis, Rhizobium* and *Phosphobacteria*. *Trichoderma* can be used in different types of crops among which are some aquaponic crops like tomatoes, cauliflower, peas, and peppers. Besides improving plant growth as a biofertilizer, *Trichoderma* has portrayed the ability to control plant diseases through symbiotic relationships with plants. It also releases secondary metabolites including growth hormones and proteolytic enzymes.

Singh *et al*. (2015) demonstrates an experiment to determine the optimal physical parameters for growth of *Trichoderma*. Here different species of *Trichoderma* were grown at varying PH between 4 and 8 which yielded differences in biomass production of the fungal species for the different PH values. The highest biomass production was in the PH range of 5.5-7.5. Importantly, the PH that favours mineralization of phosphorous (Goddek et al. 2018) falls within the optimal range of biomass production for *Trichoderma*. In the same study Singh *et al*. (2015) tested biomass production of *Trichoderma* at varying temperatures in the range of 20-35^{o}C and the outcomes shows 25-30^{o}C as the most suitable temperature for this fungus. This temperature is similar for some tropical fish species like tilapia (Xie *et al*., 2011). These authors also tested the effect of aeration at different agitation speeds. From the above study it is clear that *Trichoderma* can thrive under the same conditions as for aerobic mineralization of sludge to ensure that Phosphorus is transformed into an available form. The mechanism of phosphorous solubilization is by either enzyme activity or organic acid release (Tandon et al. 2020). In this study, *Trichoderma* was subjected to alkaline and drought conditions (abiotic stress) where at high pH, it produces organic acids for solubilizing the tri-calcium phosphate and in drought conditions phosphatase enzymes are released that solubilize phosphorous.

3. Materials and Methods

3.1 Fish culture system

3.1.1 Setting up the recirculating aquaculture system

The preparatory work of this study before the actual mineralization process involves setting up the recirculating aquaculture system (RAS), stocking fish, and feeding the fish so as to accumulate sludge. The sludge is collected from the mechanical filters and fed into the mineralization experiment.

The experiment is carried out at Welgevallen experimental farm (Stellenbosch University, South Africa). This farm is stocked with numerous aquaponics tank systems. Each of these systems consists of three tanks fitted with a drum filter and a biofilter and an air pump that supplies oxygen to the fish in the tank. A total for six tanks made of plastic with a capacity of 1000 L of water fresh are used for rearing the fish.Three tanks are connected to one biofilter and drum filter and the other are connected to another biofilter and drum filter. Prior to the start of the fish feeding, the tanks are thoroughly cleaned of all dirt clogging and the biofilter, the biofilter is made up of thread and mesh-like material which provides surface for biofilm formation. This rearing water is fresh water from a municipal water supply in Cape Town. This water is reused in the fish rearing tank after passing through the biofilter and the water temperature is 25^{o}c upon supply. It is heated to 28^{o}c using a simple water heater. The purpose of heating is to maintain temperature between 25^{o}C and 35^{o}C basing on the changes in South African weather and the period meant for the fish culture was in autumn hence slightly colder temperatures.

3.1.2 Stocking fish

In this study, tilapia (*Oreochromis niloticus*) of about 200-300 g body weight, outsourced from a local farm within Stellenbosch municipality, are used. Each tank is filled with 1000 L of fresh water and stocked with 50 fish. Therefore, the total number of fish for this study is 300 fish. Prior to stocking, the fish are first acclimated for 15 min to ensure a uniform temperature of 28^{o}C and checked for possible presence of any fish parasites.

3.1.3 Feeding fish

The fish are fed on a commercial diet branded sea master, manufactured by specialized aquatic feeds (Pty) LTD. Still St. 7200 Hermanus. Western Cape - South Africa. These are floating pellets of 4-5 mm diameter, consisting of 34% protein among other nutrients. The nutritional composition of this feed (**Table 3**). The fish are fed twice a day (morning and evening) by hand feeding at a feeding level of 2% of its body weight (FAO 2013). The fish in all the six tanks are fed on the same type of diet for a period of two weeks in order to accumulate enough sludge in the drum filter.

Table 3. The nutritional composition of the aqua feed used in culturing the fish. Source: Specialized aquatic feeds (Pty) Ltd.

Nutrients	Nutrient composition (%)
Moisture	9.4
Protein	34.0
Energy	13.1
Starch	22.9
Fat	8.0
Fiber	4.8
Calcium	0.9
Total Phosphorous	0.9

3.2 Mineralization experiment

3.2.1 Microbial inoculum for this study

The microbial inoculum for this experiment is *Trichoderma harzanium* branded Eco-T and manufactured by Madumbi Sustainable Agriculture Pty Ltd South Africa. This is a wettable powder hence can be mixed in water and contains 2×10^9 spores/g and 1g/kg of soil is applied. In this study, taking the same amount of sludge of 2kg and incorporating two varying quantities of *Trichoderma harzanium* thus 2 g / 2 kg sludge and 1 g / 2 kg sludge and 0 g / 2 kg sludge.

3.2.2 Aquaculture sludge

Before inoculation of the fungus, samples of sludge are collected. For every three fish tanks connected to one drum filter yields a composite sludge sample. This gives two composite sludge samples which are immediately analyzed for dominant microbial community present in the sludge, amount of Phosphorus, total suspended solid (TSS) content and chemical oxygen demand (COD) of this raw sludge. At this very point, the pH, temperature, and dissolved oxygen in the sludge are checked. As all these analyses are being done, the sludge is transferred into the digesters to set up the mineralization experiment this sludge is mixed with abit of water from the fish rearing tank s as to reduce on the viscosity (Monsees et al. 2017)but also the sludge contains 96% water already (Gómez et al. 2019).

3.2.3 Setting up digesters

After two weeks of culturing the fish, two composite sludge is collected from the drum filters of the two RAS system. Upon collection of sludge, it is right away transferred to the digesters in a space of less than 24 hr to avoid any microbial degradation already. Small plastic buckets of 5 L volume are used as digesters, which are fitted with a lid and a small pipe to supply oxygen for aerobic digestion of the sludge. The two composite samples of sludge as obtained from the two drum filters are further partitioned, the sludge from system one is portioned in three buckets, one having 2 g / 2 kg *Trichoderma* inoculum, the other having 1 g / 2 kg sludge of *Trichoderma* and the other having zero inoculum per 2 kg sludge. For each of these quantities of inoculum, the digesters are set in four replicates. For composite sludge from system two, the sludge is portioned in three buckets, one having 2 g / 2 kg *Trichoderma* inoculum, the other having 1 g / 2 kg sludge of *Trichoderma* and the other having zero inoculum per 2 kg sludge. This makes three treatments for every composite sludge sample and each treatment replicated four times gives twenty four (24) digesters. The buckets are covered and air supplied using a small pump to keep the sludge aerated and the experiment is allowed to stand for 42 days at a temperature of 25^{o}C and a pH of between 5.5 and 7.5 by adding to allow microbial mineralization of the sludge during which the liquid supernatant collects on top of the solid sediments. A sludge retention time of 10 days is considered to give the microorganism a regeneration time before it starts its degradation activities.

3.2.4 Sampling

Supernatant samples are collected for the various analyses. On the day of sampling, aeration is turned off to let the sludge to settle such the solids are at the bottom and the liquid supernatant can be drawn out. Sampling for water quality parameters is done once weekly from week two up to week six. Sampling for phosphorus analysis is done three times thus in week 2, 4 and week 6. These samples are immediately taken to Bemlab on the very day of sampling. This is a private laboratory outside Stellenbosch University where macronutrient analysis is done. Upon receipt, the samples are stored under temperatures of 18 ^{o}C to avoid further microbial actions on this.

3.3 Monitoring water quality parameters

The pH, temperature, and dissolved oxygen levels during the initial preparation of feeding the fish are checked on a daily basis every morning at 9, 00 am using the Hach multi-meter (HQ40d, HACH Lange, Loveland, CO, USA). The nitrite and ammonia levels in the fish tanks are checked every morning at 9,00am using a nitrite and ammonia test kit branded salifert, it is important to check if the conditions in the fish tank are favorable for the growth of tilapia.

At the start of the mineralization experiment, temperature, pH, conductivity and dissolved oxygen of the fresh sludge before transferring into the digester is checked. After 10 days of sludge retention time, supernatant samples are collected weekly and in each of these weeks, the pH, temperature, and dissolved oxygen are checked. The water quality parameters are checked from the water laboratory within Welgavalen experimental farm.

3.4 Analyses on sludge and supernatant

3.4.1 Determination of phosphorous content in fresh sludge and phosphate in the supernatant after mineralization

Determination of phosphorous follows two procedures 4500-P (B and E). The first procedure is a digestion method that enables its release from the organic matter. In this case digestion is aided using perchloric acid ($HClO_4:2H_2O$), hence the perchloric acid method. The two composite samples of sludge are sampled from the two RAS systems in four replicates. From these, 2 g of sample is taken from each and placed in an erlenmeyer tube to which concentrated HNO_3 is added to acidify the sample to methyl orange. This is followed by another addition of 5 ml of concentrated HNO_3. The sample is then evaporated on a hot plate to about 20 ml. Here 10 ml of each concentrated HNO_3 and $HClO_4$ is added to the flask, cooling the flask between additions. The sample is heated and evaporated gently on a hot plate until dense white fumes of $HClO_4$ appear. The sample is cooled after digestion followed by addition of drops of phenolphthalein indicator and NaOH until the solution just turns pink. A calibration curve is to be made by digestion of a series of standards containing orthophosphate. At this point, the total Phosphorus of the sludge sample is obtained.

The ascorbic acid method is used to determine the phosphate (PO_4^{3-}) content in the supernatant after mineralization. A master mix of 100 ml is prepared containing 50 ml of 5N H_2SO_4, 5 ml of antimony potassium tartrate solution, 15 ml of ammonium molybdate and 30 ml ascorbic acid solution. A standard phosphate solution is prepared by diluting 50 ml stock phosphate solution in 1000 ml with distilled water, and from this 0.25, 0.50, 0.75, 1.0 and 1.25 mg/L are pipetted.

The procedure is such that 50 ml of supernatant from each of the treatments is measured into a 125 ml flask. For the three treatments, the reactors are sampled in three replicates. This is followed by addition of 1 drop of phenolphthalein indicator and upon development of a red color, 5N H_2SO_4 solution is added dropwise to discharge the color. To this sample, 8 mL of the master mix is added and the absorbance of each sample at 880 nm is read from a spectrophotometer (iCAB 6000, Thermo Fisher Scientific). A sample blank is prepared by adding all reagents except ascorbic acid and antimony potassium tartrate to the sample. The absorbance of the blank is subtracted from that of the sample. A calibration curve from about 6 standards is prepared including the blank sample, and a graph of absorbance vs. phosphate concentration is plotted. The concentration of orthophosphate is calculated using the equation below.

$$mg\ PO_4^{3-}/L = \frac{mg\ P(in\ approximately\ 58ml\ \ final\ volume)\times 1000}{ml\ sample} \quad \text{Equation 1}$$

The equation is used to calculate the amounts for Phosphorus from each of the reactors following each of the treatments.

3.4.2 Determining TSS and COD of the sludge

3.4.2.1 Determining total suspended solids (TSS) of fresh sludge

This method is adopted from APHA protocols, 2017 method number 2540. This procedure is used to determine the total suspended solids in the fresh sludge before the mineralization experiment. Thereafter, the sludge in the digesters is sampled at the end of the mineralization and checked for the TSS in it. In this method, a well homogenized sample of sludge is filtered through a pre-weighed standard-glass fiber filter and the filter together with the residue are dried to a constant weight in an oven at 103-105ºC. The filter paper together with the dried sample of sludge are weighed on an analytical balance. The increase in weight of the filter is the TSS of the sludge. The TSS reduction performance is the ability to degrade organic matter into soluble contents in the bioreactor. Hence sludge sampling is done at the beginning and at the end of the mineralization experiment. For each sampling, two composite sludge samples in four replicates each are used and a known volume of sample is to be measured aiming at a solid residue of 2.5-200 mg after filtering through a commercially prepared glass-fiber filters with applied vacuum. The filter is washed 3 times with reagent grade water while ensuring complete drainage between washings and suction to ensure that all traces of water are removed. The glass fiber filter paper is then carefully removed from the filtration apparatus and transferred to an inert weighing dish. At this point, the filter paper is dried in an oven at 103-105^{o}C for 1 h. This is followed by cooling in a desiccator to ambient temperatures and then weighing the filter paper again. The TSS of the sample is calculated as follows.

$$TSS\ mg/L = \frac{(\boldsymbol{R-F})\times \mathbf{1000}}{\boldsymbol{sample\ volume}\ (\boldsymbol{ml})} \qquad \textit{Equation 2}$$

R represents the final weight of the filter + dried residue in mg; F represents the weight of the filter in mg.

3.4.2.2 Determination of Chemical Oxygen Demand (COD) using the closed reflux method

In this method, the organic matter in the sludge is to be oxidized by a boiling mixture of chromic and sulfuric acid and after digestion, the remaining unreduced $K_2Cr_2O_7$ is titrated with ferrous ammonium sulfate to determine the amount of $K_2Cr_2O_7$ consumed and the oxidizable matter is calculated in terms of oxygen equivalent.

Sample collection is done in glass bottles and 6 samples are collected from the 6 tanks and each of these samples is homogenized as they contain high level of suspended solids. One gram of sample is diluted with 50 ml water each and the analysis is run in triplicates hence a total six analyses is done. From these samples, 2.5 ml each is pipetted off and placed in the digestion ampules to which 1.5 ml of standard potassium dichromate 0.02 M is added and 3.5 ml sulfuric acid is carefully run down the vessel such that an acid layer is formed beneath the sample-digestion solution layer. These ampules are sealed and inverted to ensure thorough mixing of the solutions, after which they are placed in a block digester preheated to 150°C and refluxed for 2 h behind a protective shield. The ampules are cooled at room temperature and placed on a test tube rack from which the contents are transferred to a bigger vessel. To this mix, 2-3 drops of ferroin indicator are added and stirred rapidly using a magnetic stirrer while titrating with standardized 0.10M standard ferrous ammonium sulfate (FAS) titrant until a sharp color change from blue-green to reddish-brown is obtained. This indicates the end point of the reaction. A blank with an equal volume of water and reagents is also refluxed and titrated the same way. The COD of the reaction can be calculated through the following equation.

$$\text{COD as mg } O_2 / L = \frac{(S-B)\times M\times 8000}{ml\ Sample} \qquad \text{Equation 3}$$

S is the volume of FAS in mL used for the sample, B is the volume of FAS in mL used for the blank, and M is the molarity of FAS, 8000 is the milliequivalent weight of oxygen in 1000 ml/L.

3.4.3 Determination of dominant microbial community in the fish sludge using ribosomal intergenic spacer analysis (RISA)

This analysis is to take place at Sporatec laboratories, which is a Stellenbosch University facility. In this case, six sludge samples in triplicates are analyzed giving 18 samples.

The DNA from the sludge sample is extracted using the FastDNAR SPIN Kit for Soil (MP Biomedical, USA), according to the manufacturers' protocol (Fakruddin 2015). A direct lysis method is used to extract DNA from bacteria and fungi in the sludge samples. This is followed by running a PCR to amplify the region of the rRNA gene operon between the 16S and 23S region. This region is known as the intergenic spacer region. Two primers S-D-Bact-1522-b-S-20 (eubacterial rRNA small subunit, 5'TGCGGCTGGATCCCCTCCTT3') and L-D-Bact-132-a-A-18 (eubacterial rRNA large subunit, 5'- CCGGGTTTCCCCATTCGG-3') are used to amplify the gene by annealing to the conserved region of the 16S and 23S rDNA (Bailón-Salas et al. 2017).When the PCR is complete, the fragments are run in polyacrylamide gel and appropriate amounts of loading dye are added and loaded the gel. This is followed by gel electrophoresis. Upon completion, the gel is stained for 15 min with gel star nucleic acid stain. The gel is removed and placed onto a UV trans illuminator and viewed. The resulting PCR product is a mixture of fragments contributed by several dominant community members. This provides a community-specific profile, with each DNA band corresponding to a bacterial and also fungal population on the original assemblage.

3.5 Statistical analysis

Descriptive statistics like means are used to explain the variations in the water quality parameters (pH, temperature, dissolved oxygen). After mineralization, one-way ANOVA using R-software (R×643.4.2) is used to check if there is a significant difference between the digesters of three treatments in terms phosphorus content in the final supernatant. Then a cross comparison is made on the amounts of Phosphorus in the reactors between sludge from system one and sludge from system two. Principal component analysis (PCA) is used to evaluate the similarities between the microbial communities; profiles are encoded on the basis of the presence or absence of bands.

4. Discussion and Conclusions

4.1. Discussion

This section explains the anticipated results had this experiment been conducted as planned. The discussion in this section is based on what is reported in literature.

In this study, it is expected that the optimal temperature for growth of the fungus *Trichoderma* is between 25-30°C as also reported by Singh *et al*., 2015. Similarly, the optimal temperature for rearing tilapia is 25-34°C (Xie et al., 2011).

The pH of the reactor is expected to lower during the digestion process as *Trichoderma* breaks down sugars in the organic matter through respiration. The resulting low pH of 6 is important as it increases the mobilization of nutrients in the process (Singh et al. 2015; Goswami et al. 2019). The expectation of higher mineralization of Phosphorus in the reactor at a lower pH of 6 by the fungus was corroborated by Goddek et al. (2018).

In this study, it is expected that to obtain an organic matter reduction after aerobic digestion of the sludge, *Trichoderma* release enzymes that break down the sugars in the sludge. As a result, organic matter content is reduced and soluble elements are released. The organic performance has been demonstrated by using the equations for mass balance (Delaide et al. 2018). In this particular study, the total suspended solids (TSS) reduction and organic matter oxidation (COD) were high under aerobic conditions giving 60.8% and 68.5% respectively than compared to anaerobic conditions. Similarly, an experiment in which fish sludge from marine RAS was inoculated with the polychaete *Abarenicola pusilla* resulted in a high organic matter removal of 85.37% (Gómez et al. 2019). It would therefore be expected that a high reduction in organic matter would be achieved if this current experiment was done.

The mineralization performance of phosphorous is expected to be high through the phosphorous mineralizing ability of *Trichoderma harzanium* in this study as Trichoderma is to be added to the already existing indigenous microorganisms in the sludge. Lananan et al. (2014) investigated removal of Phosphorus and ammonia through symbiotic bioremediation of microalgae and the indigenous microorganisms in the fish waste. This study yielded a high percentage removal of phosphorous (99.15%) and ammonia from aquaculture wastewater as compared to the non-symbiotic experiment using microalgae alone. However, this study was performed on the liquid effluent and not on solid sludge. The ability to transform Phosphorus into soluble forms is also a factor of pH as earlier mentioned and also reported by Conroy; Couturier, (2009). In chapter three, the method describes inoculation of *Trichoderma harzanium* at three concentrations. This is expected to show a significant difference in the amounts of Phosphorus obtained after the digestion however since the experiment was not done, this is not conclusive.

The amounts of nutrients mineralized can also vary depending on the method of analysis used, even if the experimental conditions are well maintained. The methodology described in this thesis remains a bit unclear as the standard protocols for analysis are obtained from literature rather than the laboratory where the actual experiment was meant to be. The subsequent lock downs did not create room for being able to consult the laboratories of Bemlab and sporatec laboratories so as to have an up to date method of analysis of the parameters as stated in the study objectives. The perchloric digestion method used to determine organic phosphorous content in the fresh sludge as described in the methodology is suitable for sediment analysis hence applicable to sludge analysis, as the acid is a strong oxidizing agent. The orthophosphate in the supernatant determined by the ascorbic acid is a spectrophotometric method. Such a method is fast and can determine even low levels of phosphorous although it has interferences like with nitrites, making the results less accurate.

4.2 Conclusion and recommendations

Managing aquaculture sludge is of great concern in aquaculture. This can be achieved through bioremediation to be able to reduce the effects of aquaculture wastes on the environment. In this particular study, the use of *Trichoderma harzanium* in removal of organic phosphorous and conversion into orthophosphates offers a possibility of recycling nutrients from RAS solid wastes and shall increase the mineralization of phosphorous. This can be achieved by understanding the symbiotic relationship between the native microbial communities within the sludge with the foreign strain added. However, little research has so far been done on mineralizing phosphorous from fish solid wastes. This review only explains about unlocking phosphorous from the fish solid wastes but did not look into the effect of the supernatant after mineralization on plant growth, which should be the subject of future research into bioremediation. Moreover, in this study *Trichoderma harzanium* is to be applied on the sludge making it suitable for a decoupled aquaponics system where the supernatant from the plant growth section do not return to the fish tank and also decoupled systems have a mineralization unit embed as it. At the moment, it is not certain whether this fungus can be inoculated in the RAS system. This can be an area of research to check if the fungus can be inoculated in the RAS system without causing any harm to the fish.

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