MICROBIAL FLORA AND FAUNA OF AQUAPONICS SYSTEM

Dissertation submitted to the University of Kerala
in partial fulfillment of the
requirements for the award of the degree of

BACHELOR OF SCIENCE IN ZOOLOGY (2021-2024)

DEPARTMENT OF ZOOLOGY
TKM COLLEGE OF ARTS AND SCIENCE
KOLLAM-05

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CERTIFICATE

This is to certify that the dissertation entitled………………………………………………………………. is a bonafide work done by ………………………………………………………..........under my supervision as partial fulfillment of the requirements for the ***Degree of Bachelor of Science in Zoology*** and this report has not been submitted earlier for the award of any degree or diploma or any other similar titles anywhere.

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I do hereby declare that this dissertation entitled **……………………….**is a bonafide work done by me under the supervision of Smt. Remya Babu R., Guest Lecturer , Department of Zoology, TKM College of Arts and Science, Kollam as partial fulfilment of the requirements for the award of ***Degree of Bachelor of Science in Zoology***. No part of this has been presented earlier for any degrees or diploma of any university.

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CONTENTS

INTRODUCTION

Urban population expansion, climate change, declining arable land, limited freshwater sources, and food security are some of the major issues the globe is now dealing with (FAO, 2007). These issues could be helped by aquaponics, a commercially feasible food production method that combines hydroponic plant growth with aquaculture, or the production of fish, in a soilless recirculating water system. Certain types of microbes, such as heterotrophs and nitrifies, clean up fish waste and leftover fish food in aquaponics systems by mineralizing them into a steady source of nutrients that stimulate plant growth. But developing commercially viable aquaponics systems has been difficult due to the need to balance water quality requirements for each aquaponics compartment to function at its best (Rakocy, 2012).

According to Goddek et al. (2016), aquaponics is a sustainable hybrid of hydroponics and aquaculture that uses the nutrient-rich wastewater from aquaculture to fertigate crop output. Over time, aquaponics has expanded and changed to suit the demands, systems, and designs of individual farmers. An aquaponics system can develop with or without a medium and have a coupled or decoupled structure. Whereas a decoupled system prevents water from returning to the fish after plant irrigation, a coupled system returns water to the fish after plant irrigation. Open aquaponics, domestic systems, demonstration aquaponics, commercial aquaponics, and large-scale systems are examples of more specialized designs (Goddek et al., 2016; Palm et al., 2018).

The nutrients given to a hydroponic system for producing vegetables are comparable to those found in aquaculture leftovers (Rakocy, 2012). Since fish become harmed by excessive ammonia levels, ammonia and solid waste expelled from gills and faeces must be removed from traditional fish tanks. If ammonia is not removed, it may quickly accumulate to hazardous levels. However, in a recirculating system, the ammonia is eliminated and the microorganisms in the leftover transform it into nitrite and then nitrate, which is the ideal type of nitrogen for vegetables to thrive (Rakocy, 2012).

There are several advantages to aquaponics that cannot be obtained with a stand-alone hydroponic or aquaculture system. Aquaponics transfers dissolved nutrients from aquaculture water to plants, extending the water's useful life and lowering the water exchange rate. This lowers operating costs and the need for water quality monitoring since daily feed replenishes the nutrients lost in the water. When nutrients run out in a hydroponic system, water needs to be replaced; however, in an aquaponics system, nutrients are continually supplied (Rakocy, 2012) The fact that aquaponics depends on bacteria and the products of their metabolism is one of its key characteristics. Fish faeces, which has a high ammonium content, and plant fertiliser, which ought to have a low ammonium and high nitrate combination, are connected by bacteria (Somerville et al., 2014).

A growingly important aspect of agriculture, both nationally and globally, is food safety in agricultural systems (U.S. FDA Food Safety Modernization Act, 2011). Over the past 20 years, aquaponics has become more and more popular as an agricultural system. However, because of its novelty, food safety and appropriate harvesting techniques have only just started to be addressed for aquaponics farmers (Hollyer et al., 2009). Additionally, a number of recent studies have examined food safety and the concentrations of microorganisms that serve as food safety indicators in both the produce and the water in commercial aquaponics systems (Rakocy, 2004). However, Munguia-Fragozo et al. (2015) note that the whole microbial population in the various AP system compartments has not been characterised. The microbial communities in the various subunits of AP systems—such as the settler, fish tank, bio filter, drum filter, and hydroponic systems—may differ significantly, making them intriguing to study with the ultimate goal of improving process steering. These subunits can also have different possible designs and optimal conditions.

Most bacterial fish diseases are secondary infection. Fish prone to bacterial infection is due to high stocking density, improper transport, polluted water and wound infection. As a result, water management and disease prevention are necessary, especially in aquaponics system since high organic matter and high ammonia could cause fish stress and prone to diseases. In the present study, total viable count of bacteria in the fish tank of aquaponics was analyzed and will provide insight into the microbial load present in the fish tank.

AIM AND OBJECTIVES

To conduct a preliminary analysis of microbiota, present in the fish tank of aquaponics system.

Objectives:

- To calculate the Total viable count of microbes, present in the water of fish tank of aquaponics system.
- To ascertain the Total plate count of Vibrio, present in the water of fish tank of aquaponics system.

REVIEW OF LITERATURE

There are concerns related to food safety with aquaponics due to limited research, but it has slowly increased in the past 5 years (Stivers, 2016). Concerns with aquaponics stem from the uncertainty of potential sources of contamination as it is difficult to pinpoint where in the system foodborne pathogens enter, making it difficult to come up with methods to reduce contamination. Produce can become contaminated with pathogenic microorganisms by contact with soil or improperly composted manure, irrigation or post-harvest washing with contaminated water, or contact with infected food handlers (Beuchat and Ryu, 1997). Produce irrigation utilizes a plethora of water sources, including ground water, surface water, rainwater, and municipal water (Lennard, 2017). Each one has a different concern in relation to foodborne pathogens contaminating the produce, and many food outbreaks have been linked to the water sources used on plants during growth. This concern has been addressed in the Food Safety Modernization Act (FSMA) Produce Safety Rule developed by the FDA (FDA, 2019).

One component of aquaponics is aquaculture, in which fish break down feed and excrete nutrients for plants utilization. Some systems utilize the fish in addition to harvesting produce as a source of income while other systems are at a low stocking density to provide nutrients to the plants and the fish are not harvested for a profit. There have been many types of fish utilized in aquaponics and found successful based on various factors including climate and demand. Some fish species currently used in aquaponics are Nile tilapia (*Oreochromis niloticus*), hybrid tilapia (*Oreochromis urolepis hornorum and Oreochromis mosambicus*), koi carp (*Cyprinus carpio*), hybrid carp (*Ctenopharyngodon idella and Aristichthys nobilis*), hybrid striped bass (*Morone chrysops* and *Morone saxatilis*), and goldfish (*Carassius* spp.) (Selock, 2003). Rainbow trout (*Oncorhynchus mykiss),* Australian barramundi (*Lates calcarifer*), and Murray cod (*Maccullochella peelii peelii*) as well as crustaceans such as red claw crayfish (*Cherax quadricarinatus*) have also been utilized in aquaponic systems (Adler et al., 2000; Diver and Rinehart, 2000).

In aquaponics, the recycling of nutrients is driven by microbes which help to convert fish waste into plant biomass. Thus, making the function of the microbial ecosystem paramount (Skar et al., 2015). The microbial communities play an important role in different components of the aquaponics system (Schmautz et al., 2017). For example, the bio filter in the RAS component plays an important role in the nitrification process while the hydroponics component contains microbes associated with plant roots. Moreover, the solid waste in the system is decomposed by microbes (Leonard et al., 2002, Joyce et al., 2019). The microbial processes such as the biological degradation of solids can increase the biological oxygen demand. A feature of aquaponics is the reliance on the bacterial ecosystem. Most fish species retain 20–30% of ingested dietary nitrogen (Schreier et al., 2010), and 70–80% is re-leased into the water as waste. Digested protein is excreted mostly as ammonium(NH_4^+) through the gills (Tyson et al., 2011; Wongkiew et al., 2017). NH_4^+can also accumulate because of bacterial decomposition of organic matter such as protein and nitrogenous compounds in uneaten feed (Roosta and Hamidpour, 2011). Ammonium nitrogen (NH_4^+-N) excreted by fish provides the major form of nitrogen essential for plant growth (Roosta and Hamidpour, 2011; Wongkiew et al., 2017). Biological nitrification of the nutrient-rich fish tank water forms nitrate (NO_3^-) which is assimilated by plants (Tyson et al. 2011). Nitrification converts NH_4^+, which becomes harmful to fish at increasing pH, to NO_3^-, thereby maintaining water quality. In addition, in weakly buffered water, nitrification can decrease the pH value of the system water, while bacterial denitrification can increase water pH. Therefore, the composition of the bacterial ecosystems essential to aquaponics farming (Rakocy et al., 2004; Wongkiew et al., 2017). Comprehensive reviews on the relative distribution of microorganisms have been published in the context of RASs and with emphasis on these systems bio filtration (Rurangwa and Verdegem, 2015; Schreier et al., 2010). However, those reviews only considered recirculating systems without plants. In addition, aquaponics systems include a hydroponic unit (gravel media beds and/or deep-water culture rafts) and may include an additional sump and water treatment systems. These more complex systems potentially have more diverse microbial communities, with the microbiome in the different compartments likely to differ. Knowledge of the microbial diversity and functional distribution of these microorganisms in aquaponics systems is key to understanding microbial community dynamics and to enhance system performance (Bartelme et al., 2018,2017; Munguia-Fragozo et al., 2015; Schmautz et al., 2017).

Aquaponics Water

In an aquaponics system, water quality has a significant impact on food safety and nutrient concentration, as water is a highly variable input. If compromised, water quality would significantly change causing the death of fish, plants, and microflora. Water quality can be affected chemically, physically, or biologically and it needs to be monitored to meet the criteria of use. The water source has an effect on these factors, as it can contain high quantities of minerals impacting fish growth and microflora of the fish tank. Knowing the water chemistry can support nutrient management and manipulation of the water for fish growth and produce production (Lennard, 2017). Monitoring biological content, specifically *E. coli*, is suggested by the FDA Produce Safety Rule as aquaponics water without solids is viewed as agricultural irrigation water and sampling is required if the water can come into contact with the edible produce eaten in raw, which is an indicator of being contaminated by foodborne pathogens (FDA, 2019; Stivers, 2016).

Microorganisms in aquaponics systems

Nitrifying microorganisms

In aqueous media, ammonia nitrogen can be found in two chemical forms: a non-ionized form (NH_3) that is highly toxic to aquatic organisms and an ionized form (NH_{4+}) that has lower toxicity to aquatic organisms. Together they form the total ammonia nitrogen (TAN), wherein the ratio between the two forms is mainly controlled by pH, and to a lesser extent by temperature and salinity of water. Ammonia in aquaponics is the product of protein digestion by the fish, released into water mainly through their gills, and accumulates to toxic concentrations if left untreated. Elevated ammonia concentration causes convulsions, coma, and death in fish and other aquatic animals through displacing K_+ and depolarizing neurons which leads to the activation of NMDA type glutamate receptors and influx of excessive Ca_+, followed by cell death in the central nervous system (Randall and Tsui, 2002).

Concentrations >1mg NH_3/L has shown toxicity to carp species (Abbas, 2006). Ammonia can be oxidized in bio filters by a communities of microorganisms called nitrifiers. These organisms are aerobic chemolithotrophs that obtain their energy from the oxidation of inorganic nitrogen compounds (ammonia and nitrite) and grow very slowly. Therefore, these organisms can be outcompeted by heterotrophic bacteria if organic carbon, mostly present in biosolids suspended in the water, is allowed to accumulate in the system. Solid forms (sand grains, stones, plastic elements, etc.) are used as substrates to provide surface area for bacterial attachment and biofilm formation, which retains nitrifying bacteria in the system (Goddek et al., 2019).

The nitrification process is facilitated by ammonia oxidizing bacteria (AOB) and archaea (AOA) and nitrite oxidizing bacteria (NOB) (Stein, 2019). In this process, AOB (such as *Nitrosomonas* and *Nitrosospira* genera) and AOA (such as *Nitrososphaera*) catabolize ammonia to nitrite, which serves as the substrate for NOB (such as *Nitrobacter* and *Nitrospira*) to produce nitrate. For more than a century, nitrification was thought to be a two-step process with metabolic labor divided between ammonia oxidizers and nitrite oxidizers. However, oligotrophic bacteria capable of complete oxidation of ammonia to nitrate were postulated to exist in the environment (Costa et al., 2006), and ten years later, complete ammonia oxidizing *Nitrospira* (comammox *Nitrospira*) were discovered from biofilm samples (Daims et al., 2015; Van Kessel et al., 2015). Nitrate (NO^-_3), the end product of nitrification, has significantly lower toxicity to fish, as compared to nitrite and ammonia. At very high concentrations (> 150-300 mg/L depending on the fish species), nitrate limits the capacity of oxygen-carrying molecules in fish blood, similar to the toxicity mechanism of nitrite. However, unlike nitrite, nitrate cannot easily penetrate through the fish gill membrane, and low toxicity of nitrate is mainly attributed to its low permeability (Wongkiew et al., 2017).

Heterotrophic microorganisms

Heterotrophic microbial communities are important constituents of aquaponics systems. These microorganisms play significant roles in nutrient recycling and biodegradation of organic matter. Heterotrophic bacteria in aquaponics and RAS mainly originate from fish feces and are key organisms for sludge treatment and water quality. These microorganisms are actively involved in the biodegradation of organics and removal of bio solids in aquaponics; thus, lowering the turbidity and improving water quality. Microbial degradation of organics in aquaculture mainly includes fine solids (particles < 30 μm) which cannot be removed by sieving (Goddek et al., 2019).

Another major role of heterotrophic bacteria in aquaponics could be phosphorous cycling. Different phosphatase producing strains of *Bacillus, Pseudomonas*, and *Enterobacter* enhance phosphorous availability to plants by mineralizing organic phosphorous (mainly phytates) into orthophosphates (da Silva Cerozi and Fitzsimmons, 2016). Phosphorus has a fundamental role in nucleic acid production and ATP formation and can induce rapid growth of buds and flowers and stimulate root development. Strains of *Bacillus* and *Pseudomonas* can also ameliorate the deficiency of ferric ion non-availability by producing bacterial siderophores (e.g. organic iron-chelating compounds) (Goddek et al., 2019). Despite their importance for efficient performance of aquaponics, control of heterotrophic bacteria can be a major difficulty in systems with an unbalanced C/N ratio. Since bio solids serve as a substrate for the growth of heterotrophic microorganisms, an increase in the concentration of organics in the water may eventually result in increased oxygen consumption and poor water filtration.

Pathogen Transmission Through Water

Agricultural irrigation water has been identified as a risk factor for fresh produce contamination with foodborne pathogens during production and especially in recirculating aquaculture systems (EFSA, 2014). Water utilized in an aquaponics system is often either ground water, municipal water, or surface water; rainwater is also often used as a supplemental source (Lennard, 2017). There has been recent investigation on foodborne pathogens in irrigation water on produce and its potential effects, specifically a study found lettuce and cabbage was contaminated with foodborne pathogens that was irrigated with sewage-contaminated water (Ackers et al., 1998; Ceuppens et al., 2015; Decol et al., 2017; Wachtel et al., 2002). Many foodborne pathogens thrive in water, as it provides nutrients, neutral pH, and high available water needed for metabolism and cellular function allowing bacteria, viruses, and protozoa to grow in the environment (Craun et al., 2003). According to the EPA, pathogens are the leading cause for contamination in 480,000 km of rivers and shorelines, and 2 million ha of lakes (EPA, 2010). A wide array of foodborne pathogens has been found in ground water environments including *Salmonella* spp., *E. coli* and other fecal coliforms, and *Staphylococcus aureus* whereas surface water environments have contained *Yersinia entericolitica*, *E. coli*, *Cryptosporidium*, *Clostridum perfringence*, *Campylobactor* and *Salmonella* spp. The most common microorganisms found in ground water and surface water include *E. coli* and fecal coliforms (Pandey et al., 2014). Indicator organisms like fecal coliforms and generic *E. coli*, have been used to estimate pathogen loads in ambient bodies of water. But by using indicator organisms, it becomes difficult to identify the source of contamination and therefore prevention cannot be performed (Pandey et al., 2014). All three types of water sources, ground water, municipal water, or surface water, and are utilized in aquaponics, therefore monitoring the water is essential in identifying the likelihood of contaminating produce.

Water Sources Associated with Foodborne Pathogens

As mentioned in the previous section, many water sources used in an aquaponics include ground water, municipal water, surface water, and supplemented with rainwater. Ground water and rainwater are the most suitable in aquaponics as they are less likely to have high amounts of minerals that could impact fish growth and survival, while low in pathogens (Lennard, 2017; Rakocy et al., 2004). In a survey conducted on the water sources in aquaponics across the United States, 90% of the facilities use potable water, well water, or piped water due to its accessibility. Of those facilities, 39% of producers use drinking water supplemented with rainwater. Surface water was used by 8% of producers (Love et al., 2014; Rakocy et al., 2004). Other water sources can be used, but should be tested prior to usage to ensure they do not contain high amounts of minerals, salts, and pathogens (Lennard, 2004).

Aquaponics water requires a balance of microflora to transform ammonia while combating input of foodborne pathogens. Since surface water is open to the environment, it can become easily contaminated with foodborne pathogens carried by birds or mammal, this includes *E. coli*, *Salmonella* spp., *Vibrio*, and *Shigella* (Cabral, 2010). By utilizing surface water, these microorganisms could contaminate the system producing unsafe produce for consumers. In addition to foodborne pathogens, water allows for high microbial loads of other microorganisms that could affect the overall fish tank microbiota and nitrogen cycle.

Aquaponics Water Treatment

The most popular method for controlling microorganisms of aquaponics water is UV, but ozone and hydrogen peroxide are also used for water treatment (Glaze et al., 1987). Water treatments are still being researched as there are many challenges to overcome for ensuring water safety while preserving the beneficial microorganisms and nutrients required for a successful aquaponics system. Through the USDA Aquaponics Agricultural Practices Pilot water likely to contaminate the edible portion of the produce must undergo a water treatment, such as UV treatment, chlorination, or ozonation.

Ozone is commonly used in the water industry as a method to disinfect drinking water. In 1975, the FDA recognized ozone treatment as a good manufacturing practice for the bottled water industry, with the minimum treatment concentration of 0.1 ppm of ozone in water solution in an enclosed system for at least 5 minutes (FDA, 2019). Ozone is an unstable O_3 molecule which easily breaks down, deactivating microorganisms. Ozone disinfects water through direct reaction with the ozone molecule and indirect reaction with the radical species formed when ozone is decomposed in water (Glaze et al., 1987). Once ozone oxidizes the microorganisms, it transforms into oxygen resulting in a safe byproduct to the water and environment. Ozone can also be used as a hurdle with UV or hydrogen peroxide as a more effective of treatment at lower concentrations.

UV treatment is the most common form of water disinfection in aquaponics with the target microorganism being *E. coli*. UV light acts as an antimicrobial agent by penetrating bacteria and damaging its DNA to the point of inactivation. In an aquaponics system, UV treatment requires a balance of exposure to the water to inactivate *E. coli* but does not exceed exposure to the point of damaging beneficial bacteria that assist in the nitrogen cycle (Rico et al.,2007).

Moriarty et al. (2018) tested whether UV treatment had a significant effect as an antimicrobial on coliforms, that were often the indicators of fecal contamination and the presence of *E. coli*. When used in water with 90-95% transmittance, the UV light is able to deliver a dose of UV radiation between 180 mJ/cm^2 at 26 L/min and 30 mJ/cm^2 at 170 L/min. The results had a significant reduction of coliforms in the water used in an aquaponics system containing lettuce (Moriarty al., 2018). In an additional study performed by Elumalai et al., (2017) there was no significant difference between a UV and non-UV treated model aquaponics system in accordance to coliforms and aerobic plate counts, and there was no detected *E. coli* in either system. The main concern with UV treatment in aquaponics water is whether UV is able to penetrate into the water. If there are too many solid particles in the water, it will inhibit UV light from being able to penetrate into the water for disinfection.

Chemical treatment such as chlorine or chloramine is common in disinfecting water used for municipal purposes (CDC, 2015). Similar treatment has been suggested to be used on the water in aquaponics systems by the USDA before applying it to the plant to kill potential pathogens in the water. This method cannot be used in a coupled aquaponics system as the chlorine and chloramine could be potential fatal to the fish but could be used in a decoupled aquaponics system at low concentrations as it could also be detrimental to the plants (Sallenave, 2016).

Limited research has been performed on this method as there are many negative effects that could occur with the addition of chlorine. Hydrogen peroxide is a viable chemical that could be added into the water as it has been found to be effective in reducing to low amounts of *E. coli* (Glaze al., 1987). Hydrogen peroxide is often used together with other antimicrobials as a hurdle to increase the efficiency of other disinfectants (EPA).

Microbial Water Testing Parameters and Methods

As aquaponics is still a novel method for produce production, guidelines are limited on water quality monitoring. There has been much discretion as to how aquaponics water is viewed, either as biological amendment or agricultural irrigation water. If the solids are removed from the system, it can be determined that the water is no longer manure and therefore agricultural irrigation water (Stivers, 2016). The FDA created the Final Rule on Produce Safety as part of FSMA in 2016. It details a minimum standard for the microbial quality of water used for agricultural irrigation purposes (FDA, 2019). As long as the water is not likely to come into contact with the edible portion of the produce it does not need to be monitored, but if water is likely able to splash on the edible portion of the leafy greens, the water must be monitored (Stivers, 2016).

In the FSMA Produce Safety Rule, states two criteria must be upheld. The water must contain no generic *E. coli* that would be transferred from direct or indirect contact with the produce, including water used on food contact surfaces, water used to directly contact produce during or after harvest, and water used for sprout irrigation. Therefore, untreated surface water cannot be used for any of these purposes. The second criteria being agricultural irrigation water that is directly applied to the growing produce (other than sprouts) must have a geometric mean (GM) of 126 or less and a statistical threshold (STV) of 410 or less CFU of generic *E. coli* per 100 mL of water (FDA, 2019). If water does not meet this criterion, corrective actions must be made within one year. Generic *E. coli* must be measured throughout a year using one of the approved methods (FDA, 2019). Ground water initially must be sampled four times in the first year and once annually thereafter and surface water initially must be sampled 20 times over 2 to 4 years and a minimum of 5 times after the standard has been set. This difference is due to the higher risk and potential contamination of surface water compared to ground water. These rules are not as strict for water that is transported onto the plant through drip irrigation where it does not come into contact with harvestable portion of the plant, but there is no set limit for irrigation water used in this aspect (FDA, 2019).

E. coli and coliforms are used as indicator microorganisms to indicate the likelihood of other pathogens being present in the water without the cost of testing an entire panel of pathogens. Total coliforms are commonly tested in drinking water, present in the environment, and generally harmless. Fecal coliforms are a subgroup of total coliforms and are found in the intestines and feces of humans and animals. *E. coli* is an even smaller subgroup of fecal coliforms, many strains are considered harmless, but a few can cause illness to humans through consumption.

In 2017, the FDA compiled a list of methods used to test *E. coli* and coliforms in the water which suggested using the EPA Method 1603: *Escherichia coli* in Water by Membrane Filtration Using Modified membrane-Thermotolerant *Escherichia coli* Agar (Modified mTEC), but also approved other EPA Methods including: 1103.1, 1604, 9213 D, 9222 B, D 5392-93 (FDA, 2019). In some studies, the EPA Method 1604 was found to work well and accurately for *E. coli* detection in aquaponics water, as there tends to have a large number of solids in the water and when used the EPA Method 1603, the solids hindered *E. coli* isolation.

Aquaponics is still a novel method for growing fish and produce and with that comes many concerns on the safety of food being produced in this system. If bacterial diversity is not balanced or operational conditions are not suitable for bacterial metabolism, the water quality in the aquaponics system may fluctuate, creating a harmful environment for fish and plants. Though proper monitoring of the water and water source in addition to a food safety plan, it will provide enough support to the industry to ensure the produce is safe to consume. More research is needed to determine the validity of the safety of aquaponics, but through microbial studies and surveys more information will assist the government to make appropriate decisions for producer or processors to produce food.

MATERIALS AND METHODS

EXPERIMENTAL SETUP

Water samples for microbial analysis was obtained from aquaponics maintained and operated by Department of Aquatic biology and fisheries. The water quality was monitored per week and had the following ranges during the sampling period:

Temperature, 28–30 ^{0}C; pH, 7.4–7.6; Ammonia, 0.1–0.4mg/L; DO, 5.12 mg/L

ENUMERATION OF COLONY FORMING UNITS

Water samples from aquaponics fish tank were collected aseptically in sterile Falcon tubes (TARSON) 50 ml capacity and stored in ice pack for analysis. The isolation of bacterial strains was done according to (Yaylacı, 2022). The water samples were serially diluted by ten-fold dilution using Normal saline. The bacteria were enumerated by spread plating on Nutrient Agar (Himedia). After 24 h of incubation at 30±2°C, the colonies were counted to determine the total viable bacteria. For enumerating vibrio colonies, the same were plated on to TCBS agar (Himedia) and incubated at 30±2°C for 24h and the colonies were counted.

STATISTICAL ANALYSIS

The relevant data were processed and analyzed manually and MS Excel Office 2016 version was used for computer-based analysis.

Fig 1. Aquaponics

Fig 2.Water samples in sterilized falcon tubes

Fig 3.Sterilised nutrient agar plates

Fig 4.Spreading of samples

RESULTS

Proper management of aquaponics systems can result in reduced water consumption and effluent. However, if water quality is not carefully monitored, issues may arise, particularly with dangerous amounts of ammonia or nitrite. Conversely, high-quality water may shield against the development of harmful illnesses. Low dissolved oxygen can also happen during the culture stage due to excessively high stocking densities, high feeding rates, poor aeration, low water flow, and high organic loads that result in a lot of bacteria in the system. It's crucial to assess the total viable bacteria in the water in addition to regularly checking its properties. Aerobic plate counts were used to quantify the total microbial burdens.

Total plate count known as the spread plate count, total viable count, total bacterial count, aerobic colony count, or aerobic plate count has been in use in the water quality analysis (Gleeson et al., 2009). Table 1 and Table 2 shows the viable count of bacteria cultured in nutrient media and TCBS. TCBS is specifically used for enumeration of *Vibrio sps*. The detection from samples of aquaponics water tested positive for *Vibrio sps.* The average number of microbes in fish tank of aquaponics water was found to be 4.3±0.73 and especially of *Vibrio sps* was found to be 2.95±0.49. Total plate count on samples of aquaponics did not exceed the maximum limit required by SNI 7388:2009.

Dilution	CFU	LVC	TOTAL VIABLE COUNT
10^{-4}	200000	5.3	4.3±0.73
10^{-3}	20000	4.3	
10^{-2}	2000	3.3	

Table 1. Viable count of bacteria in different dilutions (Nutrient agar)

Dilution	CFU	LVC	TOTAL VIABLE COUNT
10^{-2}	2000	3.3	2.95±0.49
10^{-1}	400	2.6	

Table 2. Viable count of bacteria in different dilutions (TCBS agar)

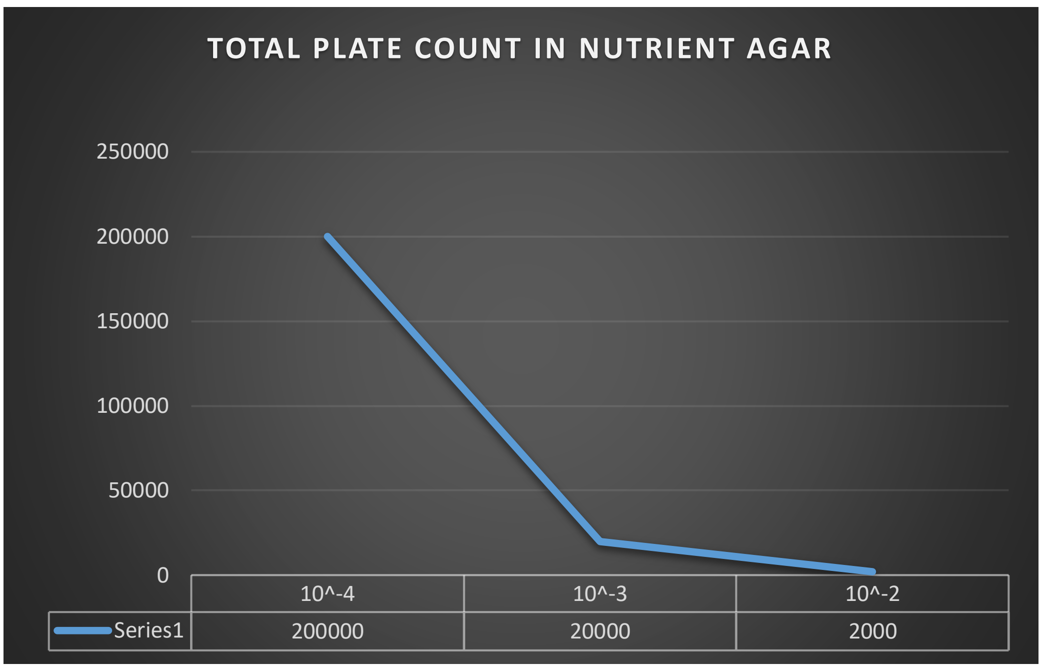

Fig 5. Total plate count in different dilutions

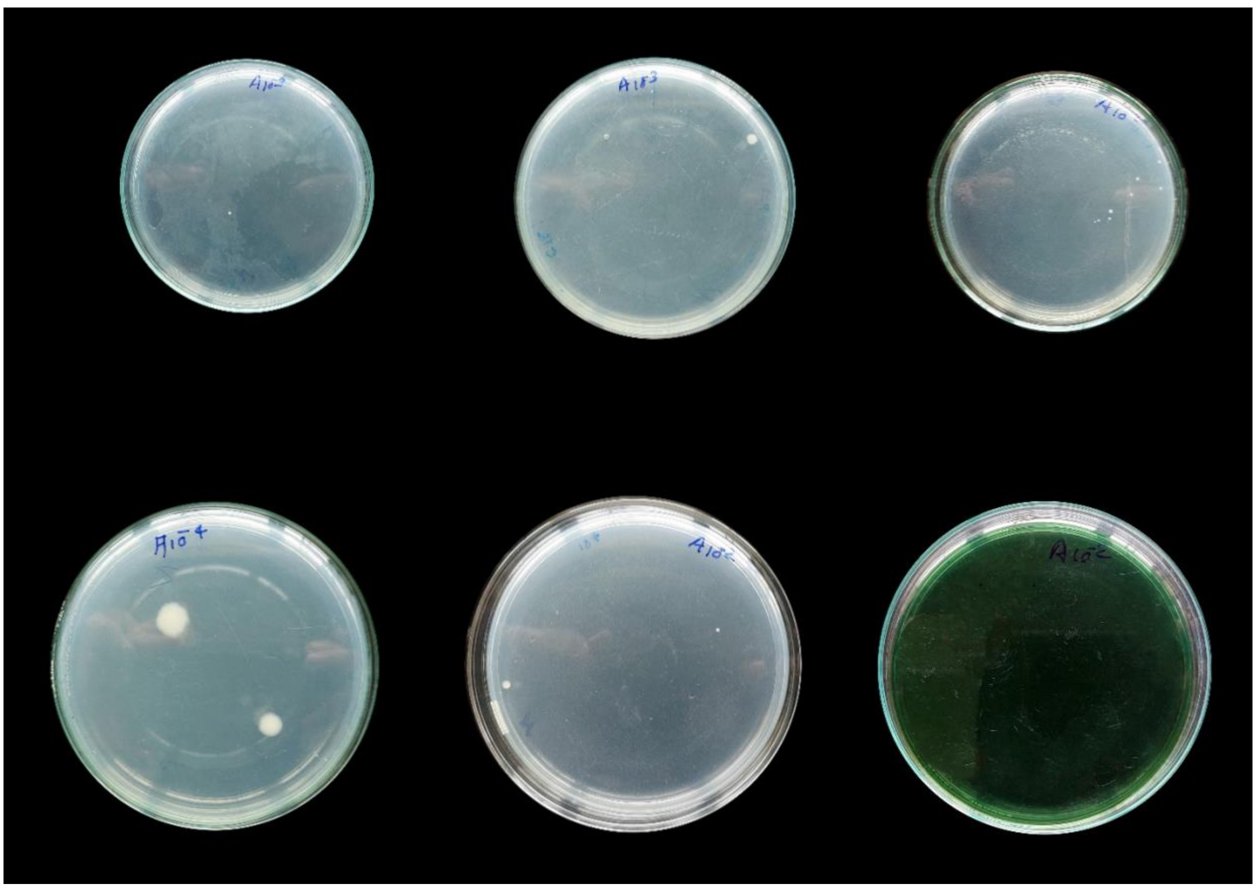

Plate 1. Microbial colonies formed in different dilutions

DISCUSSIONS

Studies examined pathogen transmission through water, risk factors for infection, variables that impacted spread and disease severity, shedding rate of infectious organisms by diseased fish or plants, and doses required to cause infection. A wide variety of fish and plant pathogens were found to spread through aquaculture and hydroponics systems, thereby also posing a threat to aquaponics systems. Waterborne transmission occurs when the infectious organism is released into the aqueous environments by the diseased fish or plant, then is taken up by a previously healthy organism to cause infection. Fish typically shed pathogens through bodily fluids, like mucus, which enter the new host via a variety of portals, such as the skin (Bricknell, 2017). Plants shed pathogens from their root systems and the infectious agents adsorb to the roots of healthy plants (Marquardt and Führling, 1994). In this study detection of *Vibrio sps* implicated that there was a point of entry of pathogen through diseased fish.

Aquaponics are mechanically sophisticated and biologically complex which sometimes fails due to poor water quality leading to fish stress, diseases and off-flavor in poorly managed systems (Masser, 2012). As fish are stocked at high density within the recirculation system they are more at risk in becoming stressed and prone to disease. It is necessary to monitor fish health continuously as if a disease outbreak does occur, it can spread extremely rapidly throughout the culture tank (Emperor Aquatics, 2013).

Microbial load of fish tank of aquaponics valued as 4.3. This is consistent with the study of Jun *et al*. (2009), who reported microbial load of aerobic heterotrophic bacteria in the aquaponics which fluctuated between 0.01 and 8.7×10^5 cfu/ml. Though microbes can serve as food source to fishes, some nutrients can also be obtained through the sediment sources, hence, high microbial load can be inimical to health. Though fishes feed on some microorganisms, high level of contamination with the presence of these indicator organisms could be alarming and could be linked to neglecting good management practices. Since the water source is from open surface water, it is likely to become contaminated with mammal and bird feces (Lennard, 2017). Warm blooded animal feces are likely to carry pathogenic and non-pathogenic *E. coli*, contaminating an open water source like surface water (FDA, 2019).

Once the fish tank is contaminated from the water input, it is difficult to decrease or remove the microorganisms in the water for irrigation if no mitigation steps are established. Additionally, an aquaponics system has an ideal environment for microbial growth, e.g. temperature, pH, oxygen, and nutrients (Hou et al., 2017). This allows microbes to grow throughout the system once it is introduced into the fish tank. One way to reduce the microorganisms in the system is through solids removal. By removing the solids, microorganisms are also removed resulting from the microbial attachment on the solids (Wu et al., 2019).

Bartelme et al. (2018) outlined that plant growth promoting microorganisms (PGPM) or microflora may play a major role in the plant's ability to uptake nutrients in an aquaponic system. Although many studies have investigated PGPM in soil environments, there are minimal studies published on PGPM in soilless environments, due to the sterility and lack of necessity for PGPM in hydroponics. Therefore, there is an immense opportunity to investigate PGPM in aquaponics. Bartelme et al. (2018) summarized that species such as *Pseudomonas, Bacillus, Enterobacter, Streptomyces, Gliocladium,* or *Trichoderma* could increase nutrient availability for plants. For example, the addition of *Pseudomonas fluorescens* Pf-5 is known to increase siderophore production in roots found in soil. Siderophores are structures which bind to iron and facilitate its transport into plant roots. Therefore, Pf-5 may be a valuable PGPM in remedying common iron deficiencies in aquaponics systems (Bartelme et al., 2018, Goddek et al., 2015). Schmautz et al. (2017) sampled different locations of an aquaponics system and found that although similar microorganisms were present in all locations, their populations differed. It was found that the biofilter had high levels of *Rhizobiales* and Actinobacteria, while roots had high levels of *Burkholderiales, Flavobacteriales,* and *Pseudomonadales*. *Pseudomonas* spp. is a significant microorganism because it is capable of producing antimicrobial properties as a method of expansion, which simultaneously protects the surface area they are growing on from disease, such as root rot caused by *Pythium* (Schmautz et al., 2017). This may be a factor in the ability of aquaponics plants to mitigate waterborne diseases. It has been suggested that PGPM of different species can have a synergetic effect on plant growth when more than one are present at the same time; however, future studies are needed to clarify these mechanisms (Avis et al., 2008).

Speculation of increased plant performance from PGPM has led to the development of PGPM culturing. Considering multiple studies on plant improvement through microflora addition, it is suggested that microflora play a key role in the success of plants in aquaponics systems. There is however, few studies which investigate the key mechanisms and species of microflora, which allow this to happen in aquaponics systems. Future research on such matters could have significant value in terms of application for increased crop production with lower requirements of nutrients.

SUMMARY

The detection result from samples of aquaponics showed a microbial load of 4.3±0.73 and was positively contaminated with *Vibrio sps*. Microbial analysis of the aquaponics tank water is extremely necessary in order to detect the kind of bacteria being transferred into the aquaponics. Bacteriological analysis of the aquaponics tank water is important to detect the presence of microorganisms that might create health hazard and death of fish in the fish tank. This can serve as a guide to monitor and protect aquaponics system as intensified in this study.

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