Fall 2020

THE DEVELOPMENT OF A NATURALLY-DERIVED HYDROPONIC NUTRIENT SOLUTION FROM RECIRCULATING AQUACULTURE SYSTEM EFFLUENT USING MICROBIAL DIGESTION

Joseph Tetreault

University of New Hampshire, Durham

THE DEVELOPMENT OF A NATURALLY-DERIVED HYDROPONIC NUTRIENT SOLUTION FROM RECIRCULATING AQUACULTURE SYSTEM EFFLUENT USING MICROBIAL DIGESTION

BY

JOSEPH TETREAULT

Bachelor of Science, Lyndon State College, 2017

THESIS

Submitted to the University of New Hampshire

in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

in

Agricultural Sciences

September, 2020

This thesis was examined and approved in partial fulfillment of the requirements for the degree of Master of Science in Agricultural Science by:

Thesis Director, Stephen Jones, Research Associate Professor, Natural Resources and the Environment

Todd Guerdat, Affiliate Professor, Agriculture, Nutrition and Food Systems

Paula Mouser, Associate Professor, Civil and Environmental Engineering

On July 14th, 2020

Approval signatures are on file with the University of New Hampshire Graduate School.

ACKNOWLEDGEMENTS

This work was fully funded by the Northeast Regional Aquaculture Center. Additional support was provided by the United States Department of Agriculture National Institute of Food and Agriculture Hatch Project (Accession number 1010110) and the New Hampshire Agricultural Experiment Station (Scientific Contribution Number NH00648).

I would like to thank my academic advisor, Dr. Todd Guerdat, for his support and dedication to my graduate studies and professional development. I would like to express my gratitude towards the members of my thesis committee. Thank you to Dr. Paula Mouser for advising me on microbiology and the methodology of my research. Thank you to Dr. Steve Jones for guiding my scientific writing and thesis preparation. Thank you to my lab mates, Alexander Sitek, Ashutosh Rao, Sean Fogarty, and William Moore for supporting and assisting in my research. Thank you to my family for the continual support and motivation throughout my graduate studies.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

The United States imports more seafood than any other country in the world. Supporting the development of a sustainable aquaculture industry will allow the United States to meet domestic seafood demand and compete in international markets. However, conventional aquaculture production methods such as pond and net pen systems are limited in capacity to meet the market demands for variety and local production. Instead, recirculating aquaculture systems (RAS) are a promising option for domestic aquaculture expansion. RAS is a controlled environment agriculture production model which is location-independent, offers significant water conservation, and optimizes environmental conditions to maximize fish production year round. Similar to other animal agriculture production facilities, RAS effluents must be treated to prevent pollution in waterways, but the cost of effluent treatment is a primary obstacle for expanding the RAS industry in the United States. Terrestrial animal agriculture producers are able to offset operating costs through the re-utilization and monetization of manures as a fertilizer for land-based crops. Similarly, RAS effluents contain the nutrients required for plant production. However, the high water content of RAS effluents makes the treated waste stream better suited for reuse as a fertilizer in hydroponic cropping systems. The development of a naturally-derived fertilizer from RAS effluents would benefit the hydroponic industry by creating a circular nutrient economy, reducing reliance on finite mineral reserves currently used to make nutrient salts, and by enabling USDA Organic certification for producers to increase crop value and profit margins.

Highly dissolved plant essential macro- and micro-nutrients and low amounts of total organic carbon are two essential characteristics for a successful hydroponic nutrient solution. Additional treatment is required to mineralize particulate-bound nutrients and remove organic carbon before RAS effluent can be a viable hydroponic nutrient solution. Microbial digestion is a

commonly used treatment method to mineralize solids and remove organic carbon in municipal and terrestrial agriculture wastes. Using both aerobic and anaerobic microbial digestion treatment methodologies, the objectives of this research were to 1) characterize nutrient mineralization of RAS effluent, 2) characterize organic carbon mass reduction, 3) and evaluate the microbially-treated effluent relative to commercially available hydroponic nutrient solutions. The effluent from a pilot-scale RAS was collected and analyzed to develop a nutrient profile and to determine organic carbon concentrations before and after anaerobic and aerobic treatment in batch reactors. Bioreactors were operated until stabilization was observed in total suspended solids (TSS) concentrations. An evaluation of the nutrient profile and organic carbon concentrations before and after microbial digestion was used to determine the viability of developing a naturally-derived hydroponic nutrient solution from RAS effluent. Results indicated that both treatment methods significantly mineralized particulate-bound nutrients in RAS effluent and successfully reduced organic carbon concentrations. Anaerobic treatment resulted in a 76% reduction in the TSS concentration and a 47% reduction in the organic carbon concentration of the effluent. After anaerobic treatment, the percent of the total concentration that was dissolved increased by a factor of 3.13 for phosphorus, 1.36 for calcium, and 1.24 for manganese. Aerobic treatment resulted in a 62% reduction in the TSS concentration of the effluent. After aerobic treatment, the percent of the total concentration that was dissolved increased by a factor of 1.39 for phosphorus, 1.22 for aluminum, and 1.10 for boron. A significant degree of denitrification was observed in the anaerobic treatment. As a result of denitrification, the nutrient ratios of the anaerobically treated effluent were different than the nutrient ratios of the aerobically treated effluent. The mass reduction of nitrogen via denitrification must be considered when determining which treatment method to use to meet the nutrient needs of a specific crop. RAS waste treatment systems must maximize plant-available nutrient mass while reducing the mass of dissolved organic carbon (DOC). Additional research is needed to optimize bioreactor operating parameters and to support the development of a two-stage effluent treatment system employing both anaerobic and aerobic treatment processes to capitalize on the benefits of both treatment methods.

This research provides a framework for future research focusing on the optimization of RAS waste treatment for use in hydroponic cropping systems.

CHAPTER 1
THE DEVELOPMENT OF A CIRCULAR NUTRIENT ECONOMY IN THE CONTROLLED ENVIRONMENTAL AGRICULTURE INDUSTRY

1.1. Controlled Environmental Agriculture

Greenhouse hydroponic production and land-based recirculating aquaculture systems (RAS) are two prominent controlled environment agriculture (CEA) food production methods. Hydroponics is a crop culture technique commonly used in greenhouse-based production systems where roots are free floating in water or supported by soilless substrates (Resh, 2012). Greenhouse production allows for environmental conditions such as temperature, humidity, and lighting to be controlled for maximum yield in specific crops (Resh, 2012). Greenhouse CEA facilities can result in a faster growth rate at higher densities than traditional field agriculture, provide year-round production, and can be located in any region with the infrastructure to supply the required electrical demands (Treftz and Omaye, 2015). Current and future limitations to the greenhouse hydroponic industry include the lack of United States Department of Agriculture (USDA) Organic certification to increase crop value and the reliance on depleting reserves of mined minerals for fertilizer solutions (Crowder and Reganold, 2015).

Aquaculture is the farming of aquatic species (Timmons et al., 2018). Land-based RAS is an intensive fish production method focused on optimizing fish growth rate and stocking density by maintaining ideal water quality parameters (Timmons et al., 2018). Sophisticated water treatment methods allow for more then 99% of total system water volume to be re-used on a daily basis and, like greenhouse hydroponics production, RAS can provide year-round, fresh seafood to any location with the infrastructure to meet its operational demands (van Rijn, 1996; Gelfand et al., 2003). While the rapid removal of waste from a RAS system allows for increased water re-use rates, treating and disposing of the captured wastes adds to the overall operating costs that limit industry expansion (Miller and Semmens, 2002; Tsani and Koundouri, 2018). A waste treatment system that generates a product to serve as a naturally-derived hydroponic nutrient solution from discharged RAS waste would benefit both industries. Farmers in the RAS industry would be able to offset operation costs through waste monetization and hydroponic farmers could increase produce value through USDA organic certification while creating a circular nutrient economy independent of finite mineral reserves currently used to create crop fertilizers (USDA NRCS, 2013; Henckens et al., 2016).

1.2. Aquaculture Industry Overview

Over the past decade, employment in the capture fishing industry has plateaued and overfishing has reduced wild populations of many high value fish species (FAO, 2018). Recent projections indicate that several decades of conservation are needed to have even minimal impact on population recovery (Hutchings and Reynolds, 2004). Hutchings and Reynolds (2004) examined over 230 marine fish species and found that over half of the populations had declined by over 80% during the study period. Capture fishing of wild populations will not be able to meet the demand for seafood at a domestic or global level.

Aquaculture currently supplies 10 percent of the world's protein, and is one of the fastest growing food production industries (FAO, 2018). The United States is the world's largest importer of seafood and needs to develop a profitable and environmentally sustainable aquaculture industry to meet domestic demands and compete in international markets. Primary aquaculture methods include net-pen, raceway, and RAS. Net-pen aquaculture utilizes marine cages to produce large quantities of fish. These cages are often kept offshore or in river tributaries (Tovar et al., 2000;

Huiwen and Yinglan, 2007). Net-pen aquaculture benefits from the ability to keep fish in their natural or preferred habitat to maximize growth and rely on ecosystem services to provide favorable environmental conditions (Naylor et al., 2005; Huiwen and Yinglan, 2007). Net-pens are a proven method for fish production and are used world-wide to meet seafood demands. However, issues including marine and freshwater eutrophication caused by concentrated fish waste, detrimental effects of escaped net-pen fish on wild fish populations, and parasite outbreaks in penned populations have diminished public perception of net-pen aquaculture and resulted in increased government regulation regarding waste management (Tovar et al., 2000; Naylor et al., 2005; Huiwen and Yinglan, 2007).

Raceway aquaculture is characterized by flowing water through a channel or trough for fish culture (Masser and Lazur, 1997). Advantages of raceway culture over net-pen aquaculture include greater control over water quality parameters, higher stocking densities, and easier harvesting (Masser and Lazur, 1997; Funck et al., 2019). The single use of water in the flow-through design of raceway aquaculture results in large volumes of water required to operate the system and large volumes of waste discharged (Funck et al., 2019). Similar to net-pens, raceway systems release large masses of waste into the environment causing eutrophication and other negative impacts in natural waterways (Funck et al., 2019). Net-pen and raceway aquaculture have an important role in global seafood production. However, the increased control of water quality conditions, high system water re-use, and location independence of RAS are ideally suited for process optimization and integration with other CEA methods and is the aquaculture method of focus for the remainder of this review.

1.3. Recirculating Aquaculture System Design

A RAS design is comprised of different unit processes, with each unit providing a specific function for fish growth and system productivity (Losordo et al., 1999). The ability to optimize each component individually contributes to the overall efficiency and economic viability of RAS (Losordo et al., 1999; Timmons et al., 2018). Basic unit processes found in RAS include culture tanks for fish rearing, a waste removal system, a biofilter for nitrification, and a pumping station for continuous water recirculation (Losordo et al., 1999). The basic components of a RAS are shown in **Error! Reference source not found.**-1. A simple way to describe the function of each unit process in RAS is to follow the flow of feed, waste production, waste removal, and culture water treatment.

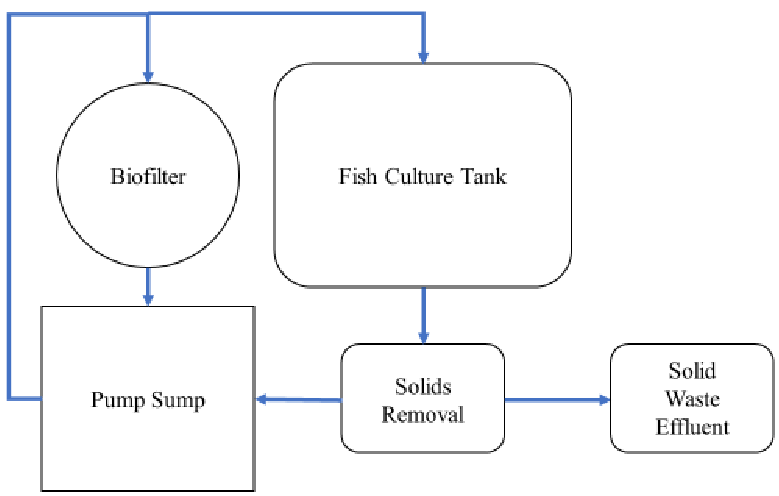

Figure 1-1. Basic components of a RAS. Fish culture tank water flows to the solid waste removal component. Solid waste is removed from the system and discharged into municipal treatment facilities or natural waterways. Culture water flows to the pump sump where it is recirculated to the biofilter for nitrification before fully treated water is returned to the fish culture unit.

Feed enters the system in the fish culture tanks. Tanks are sized to meet fish population stocking densities and are constantly aerated to provide sufficient dissolved oxygen (DO). Maintaining required DO concentrations is a primary limiting factor to fish stocking density in RAS, and DO concentration can be negatively affected by the accumulation of organic carbon rich waste that includes uneaten feed and fish feces (Masser et al., 1999). Efficient waste removal from the fish culture unit is vital for ensuring fish health and overall system productivity. There are multiple methods to ensure that waste flows out of the fish culture unit. Common characteristics between methods include a circular water flow in the fish culture unit to push waste into a drain at the center and bottom of the tank (Losordo et al., 1999). After being removed from the fish culture unit, solid waste effluent is separated from culture water and removed entirely from the system.

The effluent waste is removed from the system immediately after flowing out of the fish culture unit. Rotating mechanical micro-screens or granular media filters are commonly used for physical filtration of solid particulates that make up the effluent (Losordo et al., 1999). After solids removal, the clear culture water is biologically treated to transform compounds lethal to fish, primarily ammonia, into nontoxic derivatives. Dissolved ammonia (NH_3/NH_4^+), which is excreted through fish gills, is lethal to fish and can cause stunted growth and tissue damage in concentrations as low as 0.02 mg/L (Losordo et al., 1999; Timmons et al., 2018). A microbial biofilter is used to convert NH_3/NH_4^+ into nitrate (NO_3^-), a process called nitrification, which is safe for fish at significantly higher concentrations (Losordo et al., 1999). Maintaining a proper microbial ecosystem is essential to ensure complete NH_3/NH_4^+ conversion (Losordo et al., 1999). Effective solids removal prior to biofiltration is essential to remove organic carbon. Fast-growing heterotrophic bacteria that consume organic carbon can outcompete nitrifying bacteria and prevent successful biofilter operation (Losordo et al., 1999). Removal of waste and efficient conversion of

NH_4^+ to NO_3^- allow RAS to reuse over 99 percent of its water volume on a daily basis (Gelfand et al., 2003). In RAS, water conservation with high fish stocking density and growth rates is combined with optimization potential for each unit process. This allows RAS producers to meet specific needs of a fish species, and sets RAS apart from other forms of aquaculture in regards to suppling location-independent fresh seafood at domestic or international level (Badiola et al., 2012).

1.4. Current Limitations to Commercial RAS Success

Efficient waste removal is required to maintain ideal RAS operating conditions. Effluent from RAS is traditionally discharged into natural waterways or sent to municipal wastewater treatment systems (Miller and Semmens, 2002; Tsani and Koundouri, 2018). The effluent must be treated prior to discharge to meet guidelines established by the United States Environmental Protection Agency (EPA) (EPA, 2004). Solid wastes in RAS effluent contain high concentrations of nitrogen (N), phosphorus (P), total organic carbon (TOC), biochemical oxygen demand (BOD) and chemical oxygen demand (COD) (Guerdat et al., 2011; Guerdat et al., 2013). Untreated RAS effluent can cause eutrophication and negatively impact natural aquatic ecosystems (EPA, 2004). Effluent treatment costs, whether by in-house treatment and discharge or by disposing of waste to municipal treatment plants, is a limiting factor for the expansion of the RAS industry (Miller and Semmens, 2002; Sharrer et al., 2010; Tsani and Koundouri, 2018). Current effluent treatment strategies force RAS producers to internalize the cost of treatment, effectively increasing break-even operating costs resulting in increased prices for consumers and decreased profit margins for producers.

Adopting a capture and re-use effluent management model based on the terrestrial animal agriculture industry would allow RAS producers to monetize their effluent stream. Terrestrial animal agriculture farmers are able to offset production cost by selling waste as a field crop fertilizer (USDA, NRCS). The moisture content is the primary difference between RAS effluent and terrestrial agriculture waste. Moisture content in terrestrial animal agriculture waste is approximately 80 percent, while RAS effluent may have a moisture content that exceeds 95 percent (Sharrer et al., 2010; Timmons et al., 2018). Research has shown that RAS effluent contains the macro- and micro-nutrients required to grow plants, but the high moisture content may not be conducive to field application due to the hydraulic loading limitation of soils (Guerdat et al., 2013; Sharrer et al., 2010). A cropping system using a liquid fertilizing solution would allow for the most direct utilization of RAS effluent.

1.5. Hydroponic Industry Overview

Controlled greenhouse conditions allow producers to grow crops regardless of season and in a smaller land area than traditional field agriculture (Resh, 2012). Location independence, yield to cropping area ratio, and significantly less water required when compared to field agriculture has made hydroponic production popular in urban settings to provide a constant supply of fresh produce (Lages Barbosa et al., 2015). Operating costs associated with lighting, heating, and other environmental controls can make hydroponic greenhouses expensive to run but producers are still finding a market for hydroponically-grown produce (Treftz and Omaye, 2015). In 2014, the United States hydroponic industry sold approximately 500 million USD worth of produce and, based on the growing global population, the demand for agricultural products is expected to grow up to 70% by 2050 (Hunter et al., 2017). Depletion of mineral reserves, including phosphorus and iron, used in fertilizer production are also projected to negatively affect the agriculture industry in the coming decades and further complicate supplying food to a growing population (Henckens et al., 2016;

Yogev et al., 2017). Since shortages of mineral-based fertilizers occur, the hydroponic industry will benefit by reducing its reliance on this dwindling resource.

Organic certification from the United States Department of Agriculture (USDA) would also help hydroponic growers increase the value and demand of a crop. Allowing USDA organic certification for hydroponically grown produce has been debated, and reversed back and forth, for several decades (Morath, 2018). One argument against certification is that nutrients in traditional chemical hydroponic fertilizers are not organically derived and recycled from biological sources (USDA, 1997). A nutrient solution derived from an organic source, similar to manures used in terrestrial field-based agriculture, could help increase acceptance of organic certification for the hydroponic industry. Utilizing RAS effluent would generate a hydroponic nutrient solution from a naturally derived source and benefit both the hydroponic and RAS industries while creating a sustainable circular nutrient economy.

1.6. RAS Waste as a Hydroponic Fertilizer

Hydroponic production relies on liquid fertilizer solutions that serve as the sole nutrient source for the crop (Resh, 2012). Nutrient ratios in hydroponic fertilizer solutions can be customized to meet specific crop needs, but all nutrients must be dissolved in the solution to be accessible by plants (Crohn, 2004; Resh, 2012). In terrestrial agriculture, naturally occurring microbes in soil mineralize particulate nutrients found in manures and allow nutrients to be taken up by plants (Adesemoye and Kloepper, 2007). Total solids must be minimized and all nutrients must be dissolved into solution before RAS effluent can become a viable hydroponic fertilizer.

Previous research has found that the macro- and micro-nutrients required for plant growth are present in RAS effluent in sufficient quantities to support crop production (Seawright et al., 1998; Guerdat et al., 2013; Goddek et al., 2018). However, much of the total mass of certain nutrients are bound to particulates that must be mineralized before plant utilization is possible (Goddek et al., 2018). The nutrient presence and high moisture content make RAS effluent a potential natural fertilizer for hydropic crops but additional treatment of the effluent is required to develop an ideal solution to maximize plant utilization of the effluent. A treatment method designed to mineralize particulate bound nutrients and reduce total solids is needed to develop an organic nutrient solution from RAS effluent.

1.7. Microbial Wastewater Treatment

Domestic wastewater is biologically treated using microbial digestion to oxidize and remove organic matter from waste. Naturally-occurring microbes use organic substrates as a source of nutrients, energy, and carbon (Chen et al., 2008; Ersahin et al., 2011). The end product of microbial digestion is a reduction in total solids, organic carbon, COD, and BOD, as well as the mineralization of particulate bound nutrients (Parkin and Owen, 1986). Success of microbial digestion in domestic wastewater treatment has led producers in the terrestrial animal agricultural industry to begin applying microbial digestion techniques to animal waste to create a nutrient dense fertilizer (Othman et al., 2013). For RAS producers to develop a capture and re-use waste management strategy modeled after terrestrial animal agriculture producers, proven treatment methods such as microbial digestion must be adapted to meet the specific requirements involved with RAS waste treatment. Two primary types of microbial waste treatment used in the terrestrial animal agriculture industry are anaerobic digestion (AD) and aerobic digestion. Presence of oxygen differentiates the two treatment methods and allows the growth of specific microbial species (Bryant, 1987; Ersahin et al., 2011). Understanding the basic biological processes of these microbial digestion methods will allow for optimization to meet specific requirements for treating RAS waste.

1.8. Anaerobic Digestion Process

Obligate anaerobes grow in the absence of oxygen and can oxidize organic matter in a four-stage digestion process (Parkin and Owen, 1986; Fan et al., 2018). Hydrolysis is the first stage of AD. During hydrolysis, water molecules and enzymes separate chemically bonded complex organic matter such as proteins, lipids, and carbohydrates. These complex components are hydrolyzed into simpler monomer and dimer compounds including amino acids, sugars, and short and long chain fatty acids (Ma et al., 2018). Acidogenesis is the second stage and creates volatile fatty acids (VFA) and intermediate products of butyrate and propionate through fermentation of sugars and simple monomers created during hydrolysis (Anukam et al., 2019). The third stage of the AD process is acetogenesis, which occurs after VFA formation during fermentation and is the reduction of intermediate fermentation products into acetate, hydrogen, and carbonate (Anukam et al., 2019). Methanogenesis is the fourth and final stage of AD and results in the oxidization of acetate, hydrogen, and carbonate to methane (CH_4) and hydrogen gas (H_2). The end product of AD is a digestate comprised of inert solids, a treated effluent with a reduced mass of solids that can meet EPA approval for discharge into waterways, and valuable CH_4 and H_2 gases that can be collected and sold or used to produce power (Anukam et al., 2019).

Environmental operating parameters must be maintained to achieve the full benefits of AD. Maintaining an oxygen free environment is required during AD to prevent the influx of new electron acceptors into the system and because oxygen can disrupt the biochemical pathways utilized for enzyme production required for the reduction and oxidation processes (Botheju and

Bakke, 2011). Under batch conditions, waste transitions from an aerobic stage, where oxygen is present, to an anoxic stage, where electron acceptors such as NO3- and SO42 depleted, before reaching the anaerobic stage where digestion occurs in the absence of electron acceptors (Tchobanoglous et al., 2014).

Obligate anaerobes are pH sensitive. The different microbes at each of the four AD stages perform ideally at a slightly different pH levels (Cioabla et al., 2012). Targeted pH control can be used to for optimization of specific stages of digestion but maintaining pH between 6-8 will meet needs of the microbes across all stages (Cioabla et al., 2012). Rapid fluctuation in pH can cause microbial death and any manual changes must be done gradually to ensure system health (Zhou et al., 2019). Temperature range and fluctuation must also be managed during AD. Thermophilic temperatures, above 55 ^{o}C, have been shown to result in the fastest digestion (Ge et al., 2011). However, when making a financial decision regarding AD, the speed of digestion must be considered against the cost of constant heating. While AD can occur across a variety of temperatures, rapid temperature change can cause reduced digestion efficiency or system death (dos Santos et al., 2018; Anukam et al., 2019). When operated correctly, AD is a cost-efficient and effective treatment option for wastes with high organic contents.

1.9. Aerobic Digestion Process

Organic waste is oxidized during aerobic digestion through heterotrophic microbe aerobic respiration (Samer, 2015). Unlike obligate anaerobes, obligate aerobes require oxygen to support biochemical pathways for enzyme production (Samer, 2015). Microbial populations have four phases of growth under batch conditions (Maier et al., 2009). The extent of these phases is dependent on the amount of organic matter available to serve as terminal electron acceptors and nutrient availability to support other cellular functions (Maier et al., 2009). Lag phase is the first phase of microbial growth (Tchobanoglous et al., 2014). Little growth occurs during the lag phase as cells are physiologically adapting to the new growing conditions in the reactor (Maier et al., 2009). The exponential phase is the second phase of microbial growth, and is the phase with the highest growth rate (Maier et al., 2009). During the exponential phase, cells have become adapted to the environmental conditions and there is an abundance of organic matter in the waste for the cells to consume (Maier et al., 2009). The microbial population will grow at an exponential rate until the organic matter serving as an energy source or the availability of essential macro- and micro-nutrients becomes a limiting factor (Maier et al., 2009). Once there is not enough organic matter to support exponential growth, the stationary phase begins. The stationary phase is the third growth phase. This steady state phase is characterized by a cell growth rate that is equal to the cell death rate (Maier et al., 2009). Eventually, the organic matter becomes more limiting and the fourth growth phase begins. The death phase is the final phase of microbial growth, and begins once the death rate of cells is greater than the growth rate (Maier et al., 2009).

An aerobic waste treatment system operated as a batch reactor will experience each of the above phases of microbial growth. Without new influent to be treated or drainage of treated effluent, the death phase is inevitable and continuous reactor operation is not possible. The microbial growth phases are managed to ensure the most complete digestion of organic wastes is achieved (Tchobanoglous et al., 2014). Flow-through aerobic reactors are managed to maintain steady state operation (Tchobanoglous et al., 2014). This steady state is achieved by the balancing of the dilution rate and the potential growth rate of the microorganisms in the reactor, and it ensures that an appropriate number of microbes are present and constantly dividing to oxidize organic wastes (Maier et al., 2009). Additionally, as some cells die and lyse, a sub phase called the endogenous phase occurs simultaneously with the exponential phase (Maier et al., 2009). During the endogenous phase, dead and lysed cell tissue is aerobically oxidized to carbon dioxide (CO_2), water (H_2O), and nitrate-nitrogen (NO_3^-) (Maier et al., 2009). A properly maintained aerobic reactor results in reduced TOC and TSS concentrations and an increase in dissolved nutrient concentrations (Bryant, 1987).

Similar to AD, the aerobic digestion process can be significantly affected by rapid changes in reactor temperature, pH, or DO (Ugwuanyi et al., 2005). The temperature requirements of aerobic digestion are comparable to those of AD (Ugwuanyi et al., 2005). While treatment is most rapid in the thermophilic temperature range above 55 ^oC, digestion can occur at lower temperatures (LaPara and Alleman, 1999; Habermacher et al., 2016). Reactors can be operated at ambient temperatures to decrease operating costs associated with heating. Similarly, the pH during aerobic digestion can be maintained at various levels and it is the rapid fluctuation in pH that is harmful to the aerobic microbes (Ugwuanyi et al., 2005). A constant supply of oxygen is needed to support the aerobic microbes and facilitate aerobic digestion (Tchobanoglous et al., 2014). Aerobic reactors are considered easier to operate and less prone to disruption than anaerobic reactors. The cost of constant aeration required for aerobic digestion can increase the overall cost of treatment when compared to AD and the production of TSS as a by-product of aerobic treatment can result in additional waste removal requirements (Del Pozo & Diez, 2003; Maier et al., 2009). The cost of treatment versus the ease of operation is an important consideration when determining which treatment method should be used.

1.10. Microbial Digestion of RAS Effluent for Re-use as a Hydroponic Solution

Published research on the microbial digestion of RAS effluent for reuse as a hydroponic fertilizing solution is limited and this review cover the most recent and relevant publications to date (Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018). Monsees et al. (2017) evaluated microbial digestion as a treatment option for effluent from a combined hydroponic and RAS (aquaponic) facility. The nutrient mass of nine macro- and micro-nutrients was measured in the effluent prior to treatment. All of the nutrients were present in the effluent, but a fraction of each nutrient's mass was present in particulates and unavailable for uptake by plants without additional treatment. Lab-scaled anaerobic and aerobic reactors were used to treat the effluent. The anaerobic treatment was conducted for eight days and aerobic treatment was run for fourteen days. Both treatment methods significantly increased the amount of the total mass of specific nutrients that was dissolved in the effluent. However, the anaerobic treatment significantly reduced the mass of TN in the treated effluent. Monsees et al., (2017) recommended aerobic digestion as the better treatment option for developing a hydroponic fertilizer due its ability to increase total nutrient availability for plant uptake and retention of nitrogen.

Potential areas of refinement for future experiments that can be gained from this study include increasing the length of treatment time and increasing the monitoring of the biological activity occurring within the reactors. The anaerobic treatment in Monsees et al., (2017), was run for only eight days while the aerobic treatment was run for fourteen. Matching the length of anaerobic treatment time to the aerobic treatment time would provide of more accurate comparison of the nutrient mineralization capabilities of both treatments. Additionally, oxidative reduction potential (ORP) was not measured in either treatment method throughout the experiment. Electrical charges from ions provide ORP measurements and correspond to biological activities such as nitrification and biological sulfur removal (Gerardi, 2007). Wastewater treatment plants worldwide routinely measure ORP to determine the rate and extent of waste treatment (Zhang et al., 2020). Managing ORP in the microbial digestion of RAS effluent would allow for greater control and consistency in the treatment process by providing information on nutrient reduction, available electron acceptors, and stabilization of microbial activity.

Goddek et al. (2018) conducted on experiment on the anaerobic digestion of RAS effluent using up-flow anaerobic sludge blanket (UASB) reactors. The effect of reactor pH on nutrient mineralization and organic carbon reduction was determined. It was determined that a pH between 5.5 and 6.0 was ideal for nutrient mineralization, but that a more neutral pH between 6.5 and 7.0 resulted in better organic carbon removal. Goddek et al. (2018) recommended a multi-stage AD process for pH control to optimize nutrient mineralization and organic carbon reduction.

Potential areas of refinement for future experiments that can be gained from this study include adopting routine ORP monitoring and a more targeted approach for measuring organic carbon. Similar to Monsees et al. (2017), ORP was not reported throughout the experiment and routine measurements would allow for additional insight into if, and when, specific biological reactions were occurring in the reactors. Goddek et al (2018) reported organic carbon reduction in terms of total solids, COD, and cellulose reduction. Reporting change in organic carbon in terms of TOC and DOC would provide more insight into the treatment process. Carbon is essential for the reactions occurring during both anaerobic and aerobic digestion, and the ratio of carbon to nitrogen (C:N) is a parameter used to identify if there is enough carbon and nitrogen in the waste for optimum AD to occur (Hills, 1979). Without measuring TOC and total nitrogen (TN), the C:N ratio of RAS effluent cannot be determined. Additionally, the mineralization of carbon during treatment must be considered when evaluating the treated effluent as a hydroponic fertilizing solution. Measuring DOC in the treated effluent would indicate how much of the remaining organic carbon would be remain in the treated solution and effect ability of the final product to serve as a hydroponic fertilizing solution.

1.11. Future Research Needs

A more controlled evaluation of the treatment of RAS effluent using anaerobic and aerobic digestion for reuse as naturally derived hydroponic fertilizing solution can be designed to address the present gaps identified in recent published work (Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018). Goddek et al (2018) did not account for the effect of treatment on the final DOC concentration or the C:N ratio of the initial RAS effluent. Final DOC concentrations effect the potential of the effluent as a hydroponic fertilizing solution by increasing biofilm and pathogen potentials, and the C:N ratio provides insight into the suitability of the effluent for microbial digestion (Hills, 1979; Yaron & Römling, 2014). Neither Monsees et al. (2017) and Goddek et al. (2018) provided the specific TOC concentration or mass that was removed as a result of microbial treatment. Additionally, neither study reported ORP measurements throughout their experiments, which would provide insight into the specific biological reactions occurring within the reactors (Gerardi, 2007). Consistent ORP monitoring would provide increased control over the treatment process as fluctuations in ORP could identify the occurrence of specific *in situ* biological reactions and the stability of ORP could serve to indicate a diminished level of biological activity and the completion of the digestion process.

A lab-scaled experiment using batch reactors would address these knowledge gaps in the current literature and allow for scaled-up evaluations for larger operations. Accounting for the C:N ratio of the initial RAS effluent, TOC reduction, effect of treatment on the final DOC concentration, and routine ORP monitoring would provide a more accurate assessment of the microbial digestion of RAS effluent for reuse as a hydroponic fertilizer than any currently publish work. Determining the degree and rate of nutrient mineralization in RAS waste under anaerobic and aerobic batch conditions is the first stage in developing a flow-through treatment method that can be applied at the commercial scale.

1.12. Conclusions

Optimization to the CEA industry is required to meet the food production demands of the near future (Hunter et al., 2017). Hydroponics and RAS are two prevalent CEA methods that can provide location independent produce and protein are greater yields than traditional agricultural methods (Resh, 2012; Timmons et al., 2018). The economic success of both industries is currently limited by high operating costs (Miller and Semmens, 2002; Treftz and Omaye, 2015). The hydroponics industry is additionally facing mineral shortages for the production of fertilizing solutions (Henckens et al., 2016). The development of a naturally-derived hydroponic nutrient solution from RAS effluent would benefit both industries. The sale of treated effluent would offset production costs for RAS farmers and the hydroponic industry would be able to further justify USDA organic certification to increase crop value. A capture and re-use RAS effluent management strategy would also create a circular nutrient economy to reduce hydroponic dependence on depleting mineral reserves.

Microbial digestion is a commonly used waste treatment method in the municipal and agricultural sectors. Research has begun transitioning this method to RAS effluent treatment with the specific purpose of developing a hydroponic fertilizing solution (Goddek et al., 2018). Continued improvement to microbial digestion reactor monitoring, TOC reduction, and nutrient mineralization are needed before any treatment method can be applied at the commercial-scale to effectively develop of natural hydroponic nutrient solution from RAS effluent.

CHAPTER 2
ANAEROBIC MINERALIZATION OF RECIRCULATING AQUACULTURE DRUM SCREEN EFFLUENT FOR USE AS A NATURALLY-DERIVED FERTILIZER IN HYDROPONIC CROPPING SYSTEMS

2.1. Introduction

Global aquaculture has increased by 5.8% over the past decade, while employment in the capture fishing industry has dropped by 15% since 1990 (Moffitt and Cajas-Cano, 2014; FAO, 2018). One fifth of the world's protein is supplied by fish, half of which is produced through aquaculture (FAO, 2018). The United States is the world's largest importer of seafood, while ranking only 15th in overall seafood production (Moffitt and Cajas-Cano, 2014). The United States needs to develop an environmentally and economically sustainable aquaculture industry to meet domestic demand and compete in international markets.

Land based recirculating aquaculture systems (RAS) are a promising option to enhance the aquaculture industry and provide fresh seafood in non-coastal regions due to their location independence and water conservation (van Rijn, 1996; Gelfand et al., 2003). A well-maintained RAS typically reuses more than 95% of the system volume on a daily basis by utilizing effective waste treatment methods (Timmons et al., 2018). Mechanical micro-screen drum and bead or sand-based filters are commonly used for removal of wastes which contain high concentrations of organic carbon (OC) and nutrients (Cripps and Bergheim, 2000; Malone and E. Beecher, 2000). The rapid removal of waste allows for high rates of water reuse that set RAS apart from other forms of aquaculture. However, operating costs associated with treating and discharging captured waste effluent prevent RAS from achieving greater commercial success (Miller and Semmens, 2002; EPA, 2004; Sharrer et al., 2010; Tsani and Koundouri, 2018).

Adopting a capture and re-use waste management system similar to that utilized by terrestrial animal agriculture producers would allow RAS growers to turn the effluent into a commodity. Effluent treatment costs are typically internalized and result in decreased profit margins for growers and increased prices for consumers (Sharrer et al., 2010; Turcios and Papenbrock, 2014; Goddek et al., 2019). Terrestrial animal agriculture producers are able to offset production costs by selling manure as a crop fertilizer (USDA NCRS, 2013). While RAS effluent contains all of the nutrients required for plant growth, the high moisture content of the effluent is not conducive to field application prior to thickening (Sharrer et al., 2010). Effluent from RAS can be thickened to reduce moisture content, however, costs associated with this process make it difficult to monetize the waste stream (Sharrer et al., 2010). Due to its high moisture content, a RAS-based fertilizer solution would be more applicable to hydroponic cropping systems than land-based agriculture.

Plants grown hydroponically do not use soil to support the root zone and require liquid fertilizer solutions as the nutrient source for roots that are either floating in water or supported by soilless substrates (Resh, 2012). Hydroponic fertilizer solutions are optimized to meet the specific nutrient needs of individual crops and contain little or no OC, which can increase disease potential by fostering growth of pathogenic bacteria (Resh, 2012; Goddek et al., 2018). Nutrient salts in hydroponic fertilizers must be fully dissolved in order to be utilized by plants (Crohn, 2004; Adesemoye and Kloepper, 2007). In terrestrial agriculture, naturally occurring microbes in soil mineralize particulate-bound nutrients found in manures and allow nutrients to be taken up by plants (Eghball et al., 2002). Preliminary studies have shown that much of the nutrients in RAS effluent are bound in particulate form and cannot be immediately accessed by plants (Schneider et al., 2005; Guerdat et al., 2013; Goddek et al., 2018). Additional treatment of solid RAS waste is required to remove total organic carbon (TOC), reduce total suspended solids (TSS) and mineralize any particulate-bound nutrients before the effluent can become a viable fertilizing option in the hydroponic industry. Development of a naturally-derived nutrient solution from RAS effluent would benefit both aquaculture and hydroponic industries by offsetting operating cost associated with RAS waste management, enhancing produce value by meeting USDA requirements for organic crop certification, and developing a circular nutrient economy that is not reliant on finite reserves of mined minerals (Schneider et al., 2005; Henckens et al., 2016; Yogev et al., 2017). For capture and reuse waste management to be successfully adapted to the RAS industry, waste treatment processes from terrestrial animal agriculture must also be adapted to treat RAS effluent.

Anaerobic digestion (AD) is a commonly used process for agricultural and municipal wastewater treatment where natural metabolic processes of bacteria are used to breakdown organic matter (Chen et al., 2008; Ersahin et al., 2011). The goal for AD is a reduction in TOC, biochemical oxygen demand (BOD) and TSS (Parkin and Owen, 1986; Horan et al., 2018). The success of a municipal waste treatment method is determined by the extent to which solid wastes are reduced and by the method’s capacity for removing other targeted contaminants (Tchobanoglous et al., 2014). Goddek et al. (2019) states that the success of solid waste treatment methods with the intention of creating a hydroponic nutrient solution must also be measured by the ability to mineralize particulate-bound nutrients. The optimization of existing AD methods could allow for the specific treatment of converting RAS effluent into a naturally-derived hydroponic nutrient solution.

Several studies have characterized the nutrient profile of RAS effluent (van Rijn, 1996; Chen et al., 1997; Guerdat et al., 2013). However, there is little published research regarding nutrient concentrations, nutrient availability for plants, or effect of AD on nutrient mineralization for effluent from a coupled hydroponic and RAS production (aquaponic) facility (Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018). Additionally, none of the studies cited above reported the change and stabilization of the oxidative reduction potential (ORP) of the reactors used to mineralize the nutrients. Specific oxidation and reduction (redox) reactions occur within known ORP ranges (Gerardi, 2007). Electrical charges that form ions in wastewater are measured and can provide ranges that correspond with biological activities important to AD such as nitrification, denitrification, and biological sulfur removal (Dabkowski, 2006; Gerardi, 2007). Domestic wastewater treatment plants worldwide use ORP as parameter to measure the extent and rate of biological reactions in waste treatment processes (Zhang et al., 2020). Adopting ORP monitoring into the control of AD for particulate-bound nutrients in RAS effluent to be re-used as a hydroponic fertilizer would provide additional control and consistency in the treatment process. Fluctuations in ORP would potentially demonstrate the occurrence of specific *in situ* biological reactions, and ORP stabilization over time would imply a lack of biological reactions occurring and indicate treatment completion.

The objectives of this research were to determine the total concentration and plant availability of nutrients found in aquaponic effluent and to characterize the degree of nutrient mineralization achieved by AD in lab-scaled batch reactors. Secondary goals included comparing the nutrient profile of the treated effluent against recommended hydroponic nutrient needs and identifying optimization opportunities to refine the AD process for nutrient mineralization. The anaerobic microbial treatment of the effluent was expected to reduce total organic carbon (TOC), increase the percent of the total nutrient mass dissolved into the treated solution, and decrease the total suspended solids (TSS).

2.2. Materials and Methods

This project was conducted using effluent from the co-production of tilapia and hydroponic lettuce in the University of New Hampshire (UNH) Kingman Farm Recirculating Aquaponic Research Greenhouses (KFRAG) located in Madbury, New Hampshire, USA (see Figure 2-1). The UNH Kingman Farm is part of the New Hampshire Agricultural Experiment Station (NHAES). The KFRAG consists of three replicated greenhouses, each constructed and operated identically for the purpose of generating research data at the farm-scale. The facilities were operated under actual production conditions at feeding and waste production rates commensurate with industry feeding and waste production rates. The UNH KFRAG systems were in operation for at least 365 days prior to collecting samples for analysis and were operating under pseudo-steady-state conditions. For this study, pseudo-steady-state operation was achieved when all change in the system was due to the consistent and planned growth and harvest of fish and plants, and not due to any mechanical failures or other unexpected circumstances.

2.2.1. Aquaponic Facility Description

The Recirculating Aquaponic Greenhouses are a replicated coupled aquaponic research facility (**Error! Reference source not found.**). Each recirculating aquaponic system was housed in an 11.0 x 14.6 m high tunnel greenhouse (Nor'Easter Series, Rimol Greenhouse Systems, Hookset, NH, USA), and was covered using polycarbonate. The recirculating systems consisted of a single 3,000 L fish culture tank, a rotary drum screen filter (PR Aqua model RFM2014) fitted with 54 micron screens, one 1,300 L mixed media bed bioreactor (MBBR) used for nitrification, a 200 L pumping reservoir, a 300 L standpipe well, and three 12.6 m^2 deep water raft (DWR) hydroponic growing tables used for hydroponic lettuce (*Lactuca sativa*) production (see Figure 1). The combined system volume was 15,000 L. The only nutrient supplementation made to the system were daily additions of potassium carbonate (K_2CO_3) for biofilter management and a chelated diethylemetriamine pentaacetic acid (DTPA) iron (III) salt that was added as needed to ensure that sufficient iron (Fe) concentrations were met in the system for optimum lettuce growth.

Tilapia (*Oreochromis niloticus*) were stocked at 36 kg per m^3 and fed 3 mm floating feed (Finfish Silver, 40% protein, 10% lipid, Zeigler Bros. Inc., Gardner, PA, USA). The fish were fed 1300 g/day and a constant biomass approach to maintain a consistent feed rate was used. The total fish biomass in the culture tanks was measured bi-weekly and the number of fish in the culture tank was adjusted to ensure that 1300 g of feed each day would provide optimum fish growth based growth rates per DeLong et al. (2009).

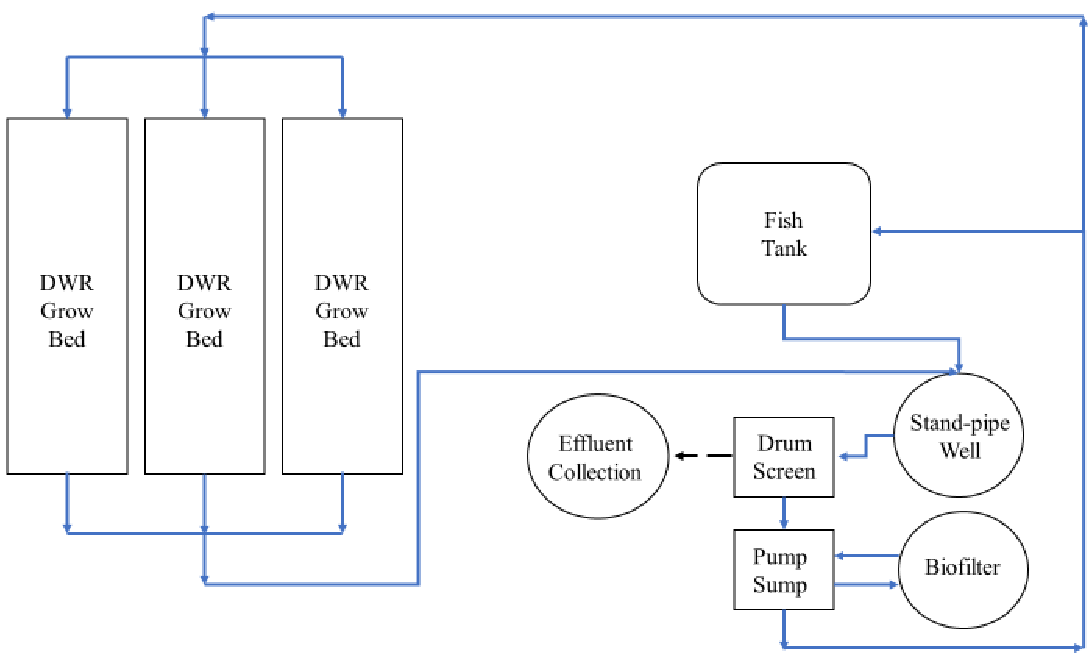

Figure 2-1. A flow schematic of the recirculating aquaponic facility at the UNH Kingman Farm Research Greenhouses located in Madbury, New Hampshire, USA. Composite samples were collected directly from the drum screen filter effluent pipe for 72 hours under pseudo-steady-state operating conditions.

2.2.2. Drum Screen Effluent Collection, Analysis, and Nutrient Characterization

Collection of effluent from the farm-scale KFRAG facility operating under actual production conditions provided effluent with comparable characteristics to the commercial aquaponic industry. The effluent from the rotary drum screen filter was captured and stored during a period of 72 hours for a total volume of 200 L. The collected effluent was aerated to preserve all nitrogen and prevent microbial denitrification during the collection period. The effluent was well mixed and then immediately sampled and analyzed. The effluent was filtered into particulate and aqueous fractions before all analysis using 1.5-micron filters. Analysis of TSS was conducted at the UNH Agricultural Engineering Laboratory (Method 2540D, APHA, 2012). A Fisher Scientific Accumet AB250 (MA, USA) was used for pH measurements.

A hydroponic nutrient profile of the effluent was determined using a commercial hydroponic fertilizer laboratory service (JR Peters Laboratory, Allentown, PA, USA). Both the particulate and the aqueous fractions of the effluent were analyzed for six macro-nutrients, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) and five micro-nutrients, Fe, manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), and aluminum (Al). The particulates analyzed were captured on the filters used to separate the particulate and aqueous effluent fractions. Filters were dried at 110 ^{o}C for a minimum of 72 hours before being ground and sent for analysis. Filter blanks were also dried and ground to ensure that no extraneous nutrients were considered in the experimental analysis. Solid nutrient analysis was conducted using combustion and an organic elemental analyzer. The filtrate consisted of the aqueous fraction of the filtered effluent and was analyzed using inductively coupled plasma atomic emission spectrometry. The concentration of each nutrient in the aqueous fraction of the effluent was reported in mg/L. Nutrients dissolved in the aqueous fraction of the filtered effluent were assumed to be plant available. Plant availability of each nutrient was determined by the percent of its total mass in the aqueous fraction. A hydroponic nutrient profile for the feed was also determined using the same methodology as the particulate-bound nutrients to identify the impact of feed on effluent nutrient characterization.

The OC and total nitrogen (TN) analyses were conducted by the United States Forest Service's Northeastern Forest Science Application Lab (Durham, NH, USA). Particulate and aqueous samples were prepared in the same manner as the samples used in the hydroponic nutrient profile analysis. Particulate samples of the effluent were analyzed for OC and TN using combustion. The percent of C and N that made up the total mass of the particulates was reported. Dissolved organic carbon (DOC) in the aqueous sample was analyzed using high temperature oxidation (HTO) and total dissolved nitrogen (TDN) was analyzed using HTO with chemiluminescent N detection as described in Merriam et al., (1996) with a Shimadzu TOC-5000 HTCO carbon analyzer (Shimadzu Scientific Instru-ments, Inc., Columbia, MD) and a Antek 720C chemiluminescent N detector (Antek Instruments,Inc., Houston, TX). The DOC and TDN concentration in the aqueous fraction of the filtered effluent were reported in mg/L.

The drum screen effluent contained all of the RAS waste greater than 54 microns, including nutrients in aqueous and particulate forms. The total nutrient concentration was determined as a means for characterizing and normalizing the total mass of each nutrient in the reactor, regardless of form, based on total nutrient mass and reactor effluent volume. The total concentration of each nutrient, in mg/L, was calculated by totaling the particulate and aqueous nutrient masses as a function of reactor effluent volume. The percent of the total concentration of each nutrient in the aqueous and particulate fractions was used to determine plant availability. Nutrients dissolved in the aqueous fraction of the effluent were assumed to be plant available.

Particulate nutrient mass fraction results from the analysis for N, P, K, Ca. Mg were reported as a percentage of the TSS mass and were calculated using the following equation:

$$M_{solids,\%} = [TSS] \times C_{\%} \tag{1}$$

where $M_{solids,\ \%}$ is the nutrient mass for the entire reactor volume (as mg/L) for nutrients reported as % of TSS, TSS is the concentration of the TSS of the drum screen effluent in the reactor (mg/L), and $C_{\%}$ is the mass of the nutrient as a percentage of the reactor TSS. Particulate nutrient mass fraction for S, Fe, Mn, Cu, Zn, Na, and Al were reported as mg nutrient/kg TSS, and the associated mass was calculated using the following equation:

$$\mathrm{M_{solids,f}} = \frac{[\mathrm{TSS}] \times \mathrm{C_f}}{\mathrm{V_{effluent}}} \tag{2}$$

where $M_{solids,\ f}$ is the nutrient mass for the entire reactor volume (as mg/L) for nutrient mass fractions reported as mg nutrient / kg TSS, TSS is the concentration of TSS in the reactor wastewater (kg/L), C_f is the nutrient mass fraction of TSS reported as mg nutrient / kg TSS, and $V_{effluent}$ is the volume of the drum screen effluent in the reactor (L).

2.2.3. Continuously Mixed Batch Reactor Design

Anaerobic treatment of the collected effluent was conducted using 20 L high density polyethylene (HDPE) anaerobic reactors (**Error! Reference source not found.**). The effluent was continuously mixed using an externally-mounted recirculating pump (Danner Supreme Mag-Drive 190 GPH, New York, USA) that pumped from the bottom center of the reactor and recirculated through a manifold with three equally spaced 0.64 cm outlets angled to ensure maximum circulation in a cylindrical holding tank (Timmons et al., 2018). A one-way check valve with a 0.023 bar cracking pressure was mounted on the lid of each reactor to allow for gas ventilation (e.g. methane and carbon dioxide) as needed. A 1.91 cm port was positioned 2.54 cm from the base of the reactor to allow for sampling. Two 1.91 cm ports for nitrogen gas venting were installed in the reactor lids to allow for nitrogen purging of the headspace during sampling to prevent oxygen infiltration.

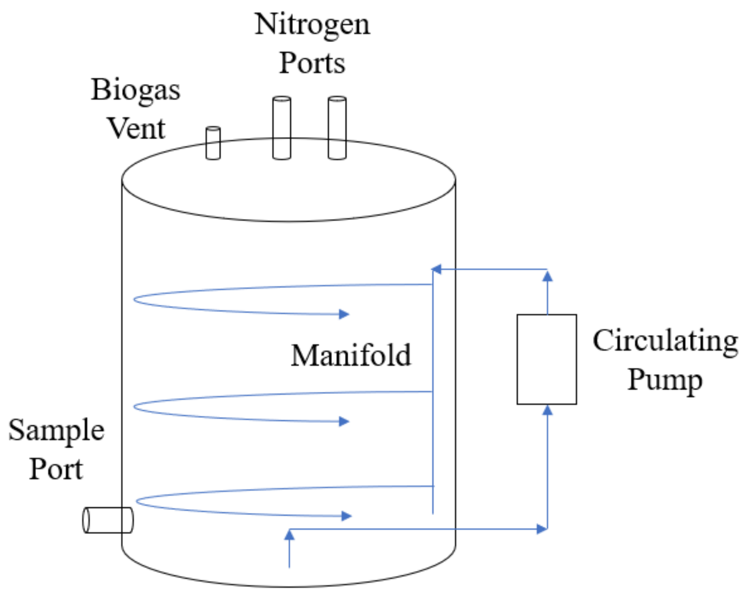

Figure 2-2. Anaerobic reactor schematic. Effluent to be treated was circulated using a small inline pump. Effluent was removed from the center of the reactor vessel and recirculated using a vertical manifold to allow for even mixing and prevent settlement of solid particles. Samples were removed from bottom port. Nitrogen gas administered and vented through top ports to prevent oxygen infiltration during sampling events. Biogas was vented passively using a ball check valve.

2.2.4. Experimental Design

One anaerobic microbial treatment was evaluated in this study with an abiotic control to provide a reference for microbial and physical effects of treatment. The treatment and control used the same batch of drum screen effluent, and the anaerobic treatment and abiotic controls were each conducted in triplicate at the same time. The anaerobic treatment was not inoculated and used only endogenous microbes present at the time of collection. The abiotic control was dosed with 0.05%

sodium azide (NaN_3) to inhibit microbial growth and biological activity. The purpose of the control was to confirm through comparison that anerobic conditions were met throughout the experiment in the AD reactors. The addition of NaN_3 inhibits microbial oxygen consumption, ORP reduction, denitrification, and sulfide production, which do not occur without microbial growth and activity (Barbot et al., 2010). Dissolved oxygen (DO) concentrations, ORP, total nitrogen (TN) concentrations, and total S concentrations were monitored in both the AD reactors and abiotic controls to ensure proper functioning of the reactor systems.

The experiment was completed when the AD reactors were determined to have reached stabilization for 7 days. Reactor stabilization was defined for this study as less than 10% fluctuation in TSS and ORP measurements between three samples taken at 48-hour intervals. Final analyses for TOC, N, P, K, Ca, Mg, S, Fe, Mn, Cu, and Zn were conducted after stabilization to determine the change in the plant availability of each nutrient after AD. Data collected throughout the study was compared with commercially-available fertilizer mixes to provide a reference for relative nutrient availability and identify nutrients where supplementation will be required.

2.2.5. Reactor Operation and Sample Analysis

Each reactor was filled with 15 L of drum screen effluent. Sample volumes of 50 mL were collected from each reactor every 48 hours. Sample analysis included temperature, DO, pH, TSS, and ORP. A Hach (Loveland, CO, USA) IntelliCAL ORP-REDOX probe was used for ORP measurement and a Hach HQ 40D was used for DO measurements. Temperature, DO, and pH were monitored to ensure ideal AD operating parameters, while stabilization in ORP and TSS was used to determine treatment completion. Reactors were operated at ambient temperatures and reactor pH was adjusted using 1 M hydrochloric acid (HCl) when the pH measured above 8 to maintain an appropriate environment for anaerobic microbes (Cioabla et al., 2012). Samples for solid and liquid carbon analysis and macro- and micro-nutrient analysis were collected at the end of the experiment to determine the increase of nutrient plant availability. The final carbon and nutrient analyses were conducted as described above in section 2.2. The change in the aqueous concentration of a specific nutrient after AD was calculated using the following equation:

$$\Delta M_{aqueous} = \frac{[B_{aqueous}]/[B_{total}]}{[A_{aqueous}]/[A_{total}]} \quad (3)$$

where $\Delta M_{aqueous}$ is the change in the percent of the total nutrient concentration that is in the aqueous fraction of the effluent. Additionally, $[A_{aqueous}]$ is the concentration, in mg/L, of a nutrient in the aqueous fraction in the untreated drum screen effluent, $[A_{total}]$ is the total nutrient concentration in the untreated drum screen effluent, in mg/L, $[B_{aqueous}]$ is the concentration, in mg/L, of a nutrient in the aqueous fraction after AD, and , $[B_{total}]$ is the total nutrient concentration after AD.

2.2.6. Statistical Analysis

A One-way Analysis of Variance (ANOVA) in JMP Pro version 14.1 Statistical Software (SAS Institute, Cary, NC) was used to determine if the temperature, DO, pH, and ORP was statistically similar or different between AD reactors and the abiotic controls. A Tukey's honestly significant difference (HSD) test was used to evaluate if the replicate reactors were statistically similar or different from each other within the AD treatment and the abiotic control. A pooled t-test was used to test the significance of the differences in TOC, TN, and total sulfur concentrations and in the percent of each nutrient's total mass in the aqueous fraction of the effluent after treatment. A p value < 0.05 was considered significantly different for all analyses.

2.3. Results

Drum screen effluent was collected from the KFRAG and analyzed for nutrients and plant availability. The nutrient profile data was used to calculate the total concentration of each nutrient in the reactors. The total concentration of each nutrient in each reactor was determined as the sum of the particulate-bound and aqueous nutrient masses in 1 liter of unfiltered reactor wastewater. Reactor operational data such as temperature, DO, pH, TSS, ORP were analyzed for the AD reactors and abiotic controls at 48-hour intervals. An analysis of starting and ending masses for TOC, TN, and S was also conducted for both the AD reactors and abiotic controls. An analysis of the nutrient profile was then conducted to determine the treatment effects in both the AD reactors and abiotic controls.

2.3.1. Feed and Effluent Nutrient Analysis

The nutrient profile of the 40% protein, 10% lipid finfish feed is reported in Table 2-1.

Table 2-1. The nutrient profile of Zeigler Bros. Inc., Finfish Silver, 40% protein, 10% lipid feed.

Nutrient	***Feed***
Macro-nutrients	
N^*	6.44
P^*	0.97
K^*	0.96
Ca^*	1.17
Mg^*	0.14
$S^\dagger$	1024
Micro-nutrients	
$Fe^\dagger$	209
$Mn^\dagger$	91.8
$B^\dagger$	5.9
$Cu^\dagger$	46.5
$Zn^\dagger$	89.6
$Mo^\dagger$	4.13
$Na^\dagger$	2051
$Al^\dagger$	0

*Reported as percent of total mass
†Reported as mg/L

The initial effluent had a pH and TSS concentration of 7.4 and 1347 mg/L, respectively. The TOC concentration of the initial effluent was 151 mg/L,18.83% of which in the aqueous form as DOC. The TN concentration of the initial effluent was 143 mg/L, with 88.54% in the aqueous form as TDN. The DOC:TDN ratio of the aqueous fraction of the effluent was 0.20:1 and the TOC:TN ratio of the effluent was 1.05:1. Macro- and micro-nutrient concentrations, and the associated aqueous and particulate fractions in the initial effluent, are reported in Table 2-1. Over 99% of the K, S, and Na in the effluent was aqueous. The macro-nutrients with the lowest percentage of their total mass in the aqueous form in the effluent were P (31.76%) and Ca (72.80%). The micro-nutrients with the lowest percentage of their total mass in the aqueous form in the untreated effluent were Fe (87.74%), Mn (80.43%), and Cu (80.04%).

Table 2-2. Total nutrient concentrations in the drum screen effluent collected from UNH KFRAG as a combination of the aqueous and particulate nutrient mass normalized to 1 L of effluent. The aqueous and particulate mass fractions for each nutrient are expressed as a percent of total mass. Nutrients found in the aqueous fraction were considered plant available.

Nutrient	Total Drum Screen Effluent (mg/L)	Aqueous (%)	Particulate (%)
TOC	151	18.83	81.17
Macro-nutrients			
N	143	88.54	11.46
P	5.13	31.76	68.24
K†	303	99.96	0.04
Ca	21.3	72.80	27.20
Mg	17.6	96.93	3.07
S	23.2	99.61	0.39
Micro-nutrients			
Fe†	1.78	87.74	12.26
Mn	0.16	80.43	19.57
Cu	0.15	80.04	19.96
Zn	0.74	94.20	5.80
Na	34.6	99.62	0.38

†Supplemented nutrient in KFRAG.

2.3.2. Reactor Sample Analysis

Temperature, DO, and pH were measured at 48-hour intervals in all reactors to evaluate the treatment effects and to ensure anaerobic conditions were maintained throughout the study period. The mean ± standard deviation (SD) of the temperature, DO, and pH in the AD reactors and abiotic controls are shown in Table 2-3-3. The temperatures in the replicate AD reactors were not significantly different from each other throughout the experiment (p = 0.6606). The temperatures in the replicate abiotic control reactors were not significantly different from each other throughout the experiment (p = 0.3669). The DO concentrations in the replicate AD reactors were not significantly different from each other throughout the experiment (p = 0.5520). The DO concentration in the replicate abiotic controls were significantly different from each other throughout the experiment (p = 0.0101). The pH in the replicate AD reactors was not significantly different from each other throughout the experiment (p = 0.9798). The pH in the replicate abiotic controls was not significantly different from each other throughout the experiment (p = 0.4230).

Table 2-3. The mean ± SD of the temperature, DO, and pH in the AD reactors and the abiotic control reactors during the study.

Parameter	AD Reactors	Abiotic Controls	p – value between treatments
Temperature (^{o}C)	22.6 ± 1.32	23.4 ± 1.17	= 0.0398
DO (mg/L)	0.96 ± 0.22	4.1 ± 1.04	< 0.0001
pH	7.5 ± 0.28	7.3 ± 0.13	= 0.0003

The change in TSS concentration over the entire study period is shown in Figure 2-3. The initial TSS concentration of the drum screen effluent was 1347 mg/L. In the AD reactors, the mean ± SD TSS concentration declined to 474 ± 101 mg/L by day 9 before stabilizing at 322 ± 94 mg/L on day 15. The overall average change in the TSS concentration in the replicate AD reactors were not significantly different from each other throughout the experiment (p = 0.3549). The mean ± SD TSS concentration was reduced by 76.17 ± 6.97% in the AD reactors. In the abiotic controls, the mean ± SD TSS concentration declined to 666 ± 320 mg/L by day 9 and stabilized at 511 ± 105 mg/L by day 15. The overall average change in the TSS concentration in the abiotic controls were significantly different from each other throughout the experiment (p = 0.0261). The mean ±

SD TSS concentration in the abiotic controls was reduced by 62.06 ± 7.77%. The mean overall average change in TSS concentrations measured at 48-hr intervals throughout the experiment in the AD reactors and the abiotic controls were not significantly different (p = 0.5460).

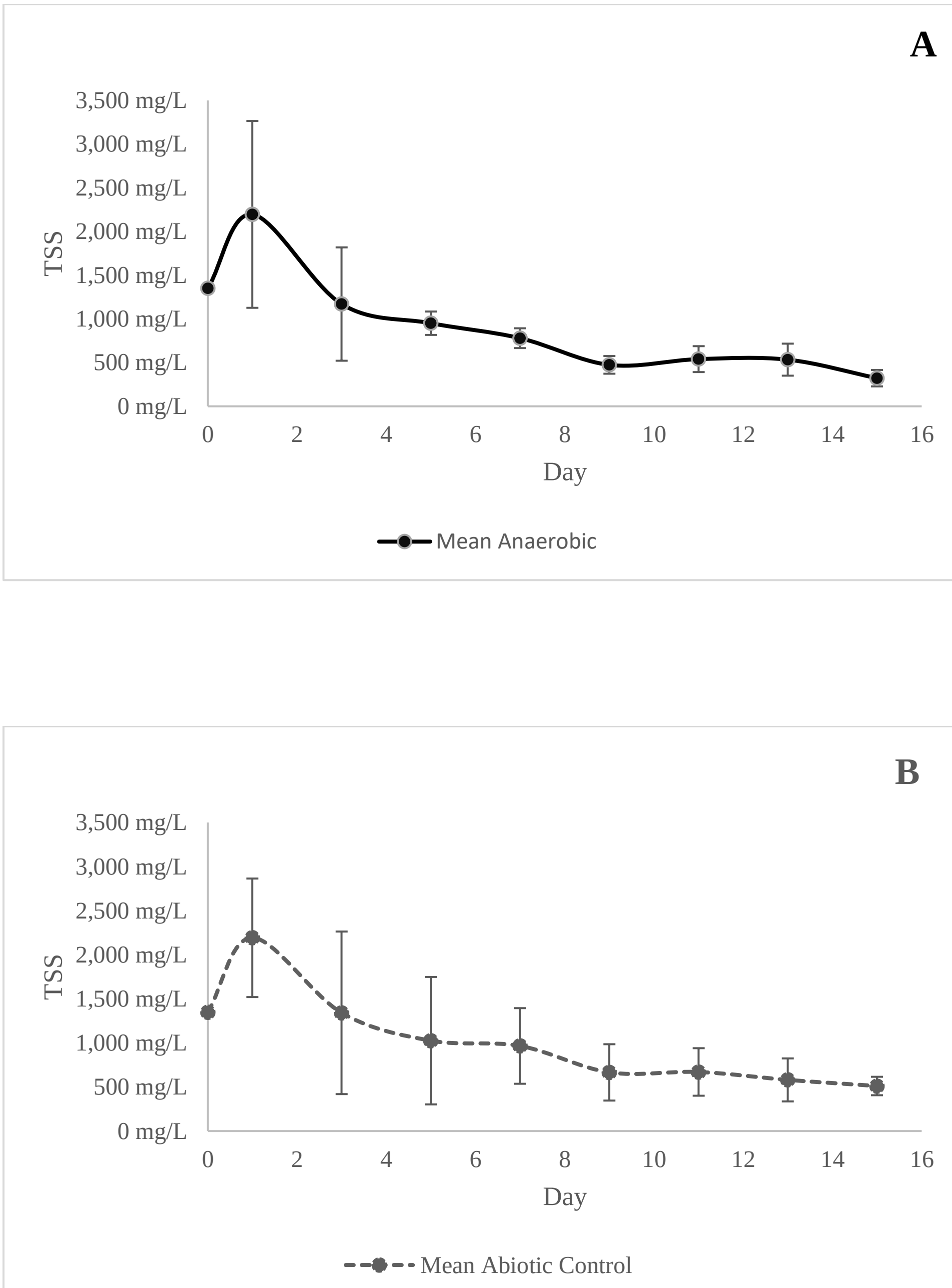

Figure 2-3. Mean TSS concentration within AD treatment (A) and abiotic control (B) replicates throughout the 15-day study period. Error bars indicate standard deviation between treatment replicates.

The change in ORP in the AD reactors and abiotic controls is shown in Figure 2-4. The mean ± SD ORP in the AD reactors decreased consistently until reaching -298 ± 16.6 mV on day 9, and then remained stable for the remainder of the study with a final measurement of -333 ± 16.1 mV. The overall mean ORP in each of the AD replicate reactors was not significantly different from each other throughout the experiment (p = 0.9781). The mean ± SD abiotic control ORP remained consistent at 85.7 ± 14.8 mV over the entire course of the study. The ORP of the abiotic control replicates was not significantly different from each other throughout the experiment (p = 0.1782). The mean ORP in the AD reactors was significantly lower than the mean ORP in the abiotic controls ($p < 0.0001$).

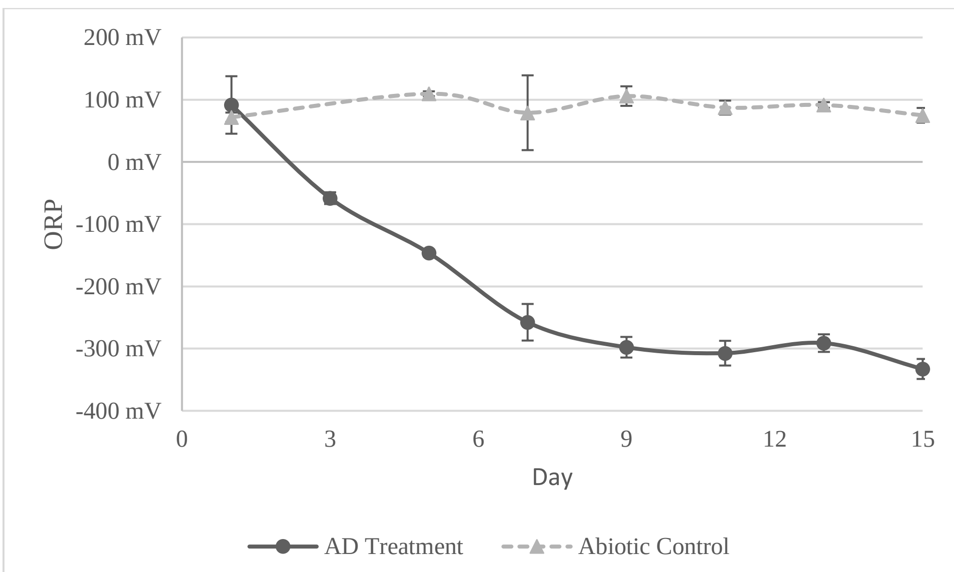

Figure 2-4. Mean ORP within the control and anaerobic replicates throughout the 15-day study period. Error bars indicate standard deviation between treatment replicates.

2.3.3. Carbon, Nitrogen, and Sulfur Mass Analysis

The TOC, TN, and total sulfur concentrations in the initial effluent and after treatment in the AD reactors and abiotic controls are shown in Figure 2-5. The final mean ± SD of the TOC, TN, and total S concentrations in the AD reactors were significantly lower than the concentrations in the initial drum screen filter effluent (p = 0.0030, p < 0.0001, p < 0.0001, respectively). In the initial effluent, the TOC, TN and total S concentrations were 151 mg/L, 143 mg/L, and 23.2 mg/L, respectively. The final mean ± SD TOC, TN, and total S concentrations mean ± SD in the AD reactors were reduced to 79.4 ± 19.2 mg/L, 17.83 ± 1.15 mg/L, and 3.39 ± 0.59 mg/L, respectively.

The final mean TOC and TN concentrations in the abiotic controls were not significantly different than the initial drum screen effluent (p = 0.3408 and 0.4179, respectively). In the abiotic controls, the final mean TOC concentration was 137 ± 22.5 mg/L and the final mean TN concentration was 139 ± 6.32 mg/L. The final mean total S concentration was significantly greater than the initial effluent at 25.29 ± 0.47 mg/L (p = 0.0015).

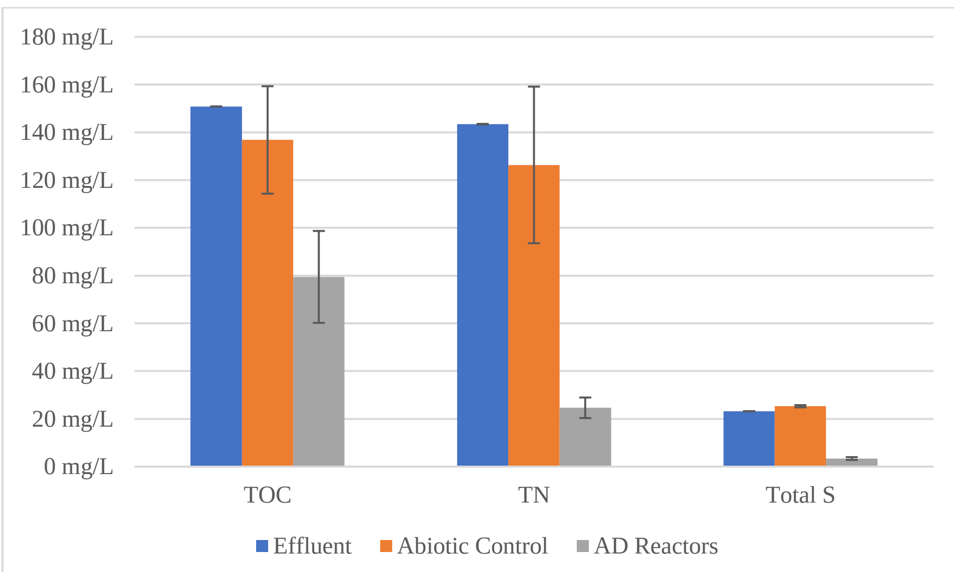

Figure 2-5. The TN, TOC, and total S concentrations in the untreated drum screen effluent and after treatment in AD reactors and abiotic controls. Error bars indicate standard deviation.

2.3.4. Final Nutrient Analysis

The change in the amount of the total nutrient concentration in the aqueous fraction of the treated effluent for TOC, N, P, Ca, Mg, Fe, Mn, and Cu are reported in Table 2-4. The TOC mass in the AD reactors were significantly lower after treatment, with an observed reduction of 47.42 ±

12.76%. The mean ± SD TOC concentration was reduced to was 79.4 ± 19.2 mg/L after AD. However, the mean ± SD percentage of the TOC dissolved in the aqueous fraction of the effluent after AD was 86.36 ± 3.00%. The percent of the remaining TOC that was in the aqueous fraction was significantly greater than the percent of TOC in the aqueous fraction of the initial effluent ($p = 0.0004$), which increased the plant available OC in the treated effluent increased from 28.4 mg/L to 68.6 mg/L. The TN concentrations in the AD reactors were significantly lower after treatment, being reduced by 89.30%. The was no significant difference ($p = 0.2125$) in the percent of the remaining TN in the aqueous fraction after AD when compared to the TN dissolved in the aqueous fraction in the untreated effluent. After AD, TN in the aqueous fraction of the effluent was comprised of 91.60% ammonia-nitrogen (NH_4-N), 7.32% nitrate-nitrogen (NO_3-N), and 1.08% urea.

The two macro-nutrients in the initial effluent with the lowest aqueous fraction percentage were P and Ca (31.76% and 72.80%, respectively). After AD, the percent of total P and total Ca concentration dissolved in aqueous portion of the effluent significantly increased ($p < 0.0001$ and $p < 0.0001$, respectively). After AD, the amount of aqueous P increased by a factor of 3.13 and the amount of aqueous Ca increased by a factor of 1.36. The micro-nutrients in the initial effluent with the lowest aqueous fraction percentage were Fe, Mn, Cu, and Zn (see Table 2-2). The percent of the total Fe and Mn concentrations in the aqueous fraction of the effluent increased significantly after AD ($p = 0.0003$ and $p < 0.0001$). The amount of plant available Fe and Mn respectively increased by a factor of 1.13 and 1.24, respectively. The percent of the total Cu and Zn concentrations in the aqueous effluent fraction after AD was not significantly different than the initial effluent ($p = 0.1628$ and 0.0696, respectively).

Table 2-4. Percent change of nutrient concentrations in aqueous form (plant available) in the aquaponic tilapia effluent after AD. Mean percent ± SD of total nutrients in aqueous form shown before and after anaerobic treatment are shown.

Nutrient	**Initial Effluent (% Aqueous)**	**Post-AD Treatment (% Aqueous)**	**Change in % Aqueous**
TOC	18.83	86.36 ± 10.8	4.59x
Macro-nutrients			
N	88.54	93.83 ± 4.23	1.06x
P	31.76	99.53 ± 0.20	3.13x
Ca	72.80	98.93 ± 0.45	1.36x
Mg	96.93	99.78 ± 0.07	1.03x
Micro-nutrients			
Fe[†]	87.74	98.91 ± 1.58	1.13x
Mn	80.43	99.52 ± 0.26	1.24x
Cu	80.04	91.61 ± 11.7	1.15x
Zn	94.20	86.85 ± 5.18	0.92x

[†]Supplemented nutrient in KFRAG

2.4. Discussion

Increasing the mass of nutrients available through capture and re-use agricultural waste management methods has become immediately important as the global scarcity of mined minerals is projected to have a detrimental impact on the agriculture industry in the coming decades (Cordell et al., 2009; Henckens et al., 2016). This study focused on the treatment effects of AD for improving the plant availability of nutrients from aquaponic/RAS effluent as compared to an abiotic control. Both the AD treatment and abiotic control affected the aqueous nutrient profile. Additionally, analysis of OC removal was conducted. The removal of OC is required for any agricultural waste treatment method designed to re-purpose the waste as a hydroponic fertilizing solution (Lee et al., 2006; Furtner et al., 2007). The following sections discuss the nutrient profile of the untreated aquaponic effluent, the operation of the AD reactors and abiotic controls, and the potential of the treated effluent for re-use as a hydroponic fertilizing solution. Suggestions for future research are also be provided.

2.4.1. Feed and Drum Screen Effluent Nutrient Profiles

The nutrient profile of the feed used would likely have a significant impact on the nutrient profile of the system effluent. The feed used in this study was chosen as it is commercially available and commonly used in the RAS industry. The drum screen effluent nutrient profile from KFRAG was similar to other reported aquaponic/RAS effluents (Guerdat et al., 2013; Monsees et al., 2017; Goddek et al., 2018). In these previous studies, P, K, Ca, Mg, Fe, Mn, Cu, and Zn were found to have a large percent of their total mass in the particulate fraction of the effluent, thus not immediately available for uptake by plants, across multiple studies characterizing aquaponic/RAS effluent (Guerdat et al., 2013; Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018). Excluding K, those were also the nutrients identified as the least plant available in the KFRAG drum screen effluent. Daily additions of soluble K_2CO_3 were made to the KFRAG systems for pH buffering and alkalinity adjustments for biofilter maintenance (Anderson, 2016). This increased the total mass and the plant availability of K at KFRAG compared to other aquaponic/RAS facilities (Guerdat et al., 2013; Monsees et al., 2017; Goddek et al., 2018). While Fe was identified as a nutrient primarily bound in particulates in the KFRAG effluent, the percent of total Fe in the solid fraction of the KFRAG effluent was less than other facilities (Goddek et al., 2018). The routine additions of soluble DTPA Fe (III) at KFRAG resulted in an increased percent of the total Fe mass dissolved in the aqueous fraction of the effluent.

The mass and plant availability of K and Fe are unique in the KFRAG effluent due to the additions of K_2CO^3 and DTPA Fe (III). System design and nutrient additions will result in some variation of the nutrient profile of the effluent at different aquaponic/RAS facilities. However, P, K, Ca, Mg, Fe, Mn, Cu, and Zn have been repeatedly shown to be bound in the particulate fraction of the effluent and needing treatment to maximize utilization by plants in a hydroponic cropping system (Guerdat et al., 2013; Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018).

The removal of OC is required to maintain adequate biofilter operation in aquaponic/RAS facilities (Chen et al., 1997; Guerdat et al., 2011). With OC removal being an essential step in cultured fish production, the effluent of aquaponic/RAS facilities, including KFRAG, contains high amounts of OC (Guerdat et al., 2011, 2013; Monsees et al., 2017). Pathogenic heterotrophic microbes can also utilize OC as an energy source in hydroponic systems (Lee et al., 2015; Yaron and Römling, 2014). A fertilizing solution containing OC could increase disease potential in a hydroponic system and potentially cause restrictions in the plumbing systems. The removal of OC from aquaponic/RAS effluent is essential before it can become a viable hydroponic fertilizing solution.

2.4.2. Anaerobic Operating Conditions

The DO concentration, temperature and pH were kept consistent throughout the experiment to prevent microbial inhibition (Celis-García et al., 2004; Cioabla et al., 2012; Bergland et al., 2015). The stabilization of TSS and ORP was used to monitor biological reactions and solids reduction, as well as determine experiment completion. With a mean ± SD temperature and pH of 22.6 ± 1.32 and 7.5 ± 0.28, respectively, the TSS and ORP of the AD reactors became relatively stable by day 9.

Optimization of temperature and pH has the potential to increase the rate and degree of nutrient mineralization in AD (Conroy & Couturier, 2010; Ge et al., 2011). A wide range of temperatures can be used for AD, but two of the most commonly used temperature ranges for domestic wastewater treatment are the mesophilic (35 ^{o}C) and thermophilic (55 ^{o}C) ranges (Bergland et al., 2015; Ge et al., 2011; Gebreeyessus and Jenicek, 2016). Hydrolysis has been identified as one of the slowest reactions to occur during AD (J. Ma et al., 2013). Ge et al., (2011) found that the hydrolysis rate in AD was nearly doubled when reactor temperature was increased from 38 ^{o}C to 55 ^{o}C. However, the same study also determined that fermentation and glucose consumption occurred more rapidly at 38 ^{o}C than 55 ^{o}C (Ge et al., 2011). Both the initial breakdown of large organic matter during hydrolysis and the mineralization of smaller particles during fermentation are vital to the treatment of RAS effluent. Further research dedicated to either identifying an optimal middle temperature to better facilitate both processes in a batch reactor or developing a multi-stage reactor with different temperature ranges could increase the AD rate of aquaponic/RAS effluent. While increasing temperature from the ambient range used for the AD of KFRAG effluent may increase digestion rate , the cost of reactor heating must also be considered against the decrease in reaction time (Ruffino et al., 2015).

A pH range between 6 and 8 has been shown to facilitate anaerobic microbial growth and allow for each stage of AD to occur (Cioabla et al., 2012). However, a more acidic pH between 5.5 and 6.5 has been shown to result in a greater degree of nutrient mineralization in aquaponic/RAS effluent (Conroy and Couturier, 2010; Goddek et al., 2018). While a lower pH resulted in a greater degree of mineralization in several studies, Goddek et al., (2018) reported a greater reduction in OC when the pH of an AD reactor was maintained between 6.5 and 7. With a mean ± SD pH of 7.5 ± 0.28, the AD reactors used in this study were above the ideal ranges reported for both nutrient mineralization and OC reduction. Similar to temperature control, further research dedicated to either identifying an optimal middle pH to better facilitate both processes in a batch reactor or developing a multi-stage reactor with different pH ranges could increase the degree of nutrient mineralization in aquaponic/RAS effluent during AD.

2.4.3. Abiotic Controls Confirm Microbial Mineralization in AD Reactors

The mean ± SD of the DO, ORP, TOC, TN and total S in the abiotic controls were stable throughout the experiment and significantly different than the AD reactors. These differences in reactor parameters confirmed that microbial activity was present and the cause of solids reduction and nutrient release in the AD reactors. The increase in the percent of each nutrient's total concentration that was dissolved after abiotic treatment was similar to the increase observed in the AD reactors. (results not shown). This was attributed to hydrolysis and the deflocculating properties of NaN_3, which has been shown to effectively reduce solids concentrations (Barbot et al., 2010). While NaN_3 was able to produce a similar degree of mineralization as AD through chemical reactions, its excessive sodium concentration and inhibition of cell growth eliminate it for use in the hydroponic industry regardless of the effect on increasing the percent of the total nutrient concentration that was dissolved (Barbot et al., 2010; Marschner, 2011). It is also important to note that while the microbial and chemical properties of treatment exhibited by the AD and abiotic treatments, respectively, resulted in some similarities in terms of mineralization effects, the reduction of TOC concentration was distinctively unique to the microbial respiration in the AD treatment. The TOC concentration in the AD reactors was reduced by 47.42% after treatment, while the TOC concentration in the abiotic controls was not significantly different than the untreated effluent. The 8.26% total sulfur concentration increase from the initial effluent in the abiotic controls was assumed to be a sampling error from uneven mixing prior to analysis. Abiotic control reactors provided evidence through comparison that AD conditions were met and microbially activity occurred throughout the experiment in the AD reactors.

2.4.4. AD Reactor Solids Reduction and Biological Activity

The stabilization of the TSS concentration in the AD reactors was used as the metric for determining the time at which the mineralization of the particulates was effectively completed given the environmental conditions in the AD reactors. Stabilization of the treated waste was defined as less than a 10% change in TSS concentration and ORP over 3 sampling periods at 48-hour intervals. The 76.17 ± 6.97% reduction of the KFRAG effluent over 15 days was greater than the TSS reductions previously reported from a similar study on the AD of aquaponic effluent (Delaide et al., 2018). Delaide et al., (2018) reported a 49.02% TSS reduction in aquaponic tilapia effluent after AD with a 15-day hydraulic retention time (HRT).

Change in ORP over time can be associated with specific biological activities (Gerardi, 2007). Biological reactions important to AD that can be monitored using ORP include denitrification and sulfide formation and fermentation (Gerardi, 2007). Denitrification occurs at ORP values between +50 to -50 mV and sulfide formation occurs at ORP values between -50 and -250 mV (Dabkowski, 2006; Gerardi, 2007). On day 3, the ORP in the AD reactors was within the range for denitrification and sulfide production, with mean ± SD measurements of -58.2 ± 9.43 mV. On day 9, ORP in the AD reactors began to stabilize at -298 ± 16.6 mV, which was below the range for all relevant reactions to occur within the reactors. The ORP of AD reactors was not routinely reported in other published studies on the anaerobic treatment of aquaponic effluent for re-use as a hydroponic fertilizing solution (Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018).

The ORP of each reactor used in this study was measured at the same time each TSS sample was taken. With an R^2 value of 0.94, a strong correlation between TSS concentration and ORP was identified during the AD of the effluent KFRAG (Figure 2-6). The correlation between the reduction and stabilization of TSS concentrations with the reduction and stabilization of ORP measurements indicate that ORP stabilization can serve as a metric for determining when a specific AD reactor has achieved maximum mineralization and solids reductions.

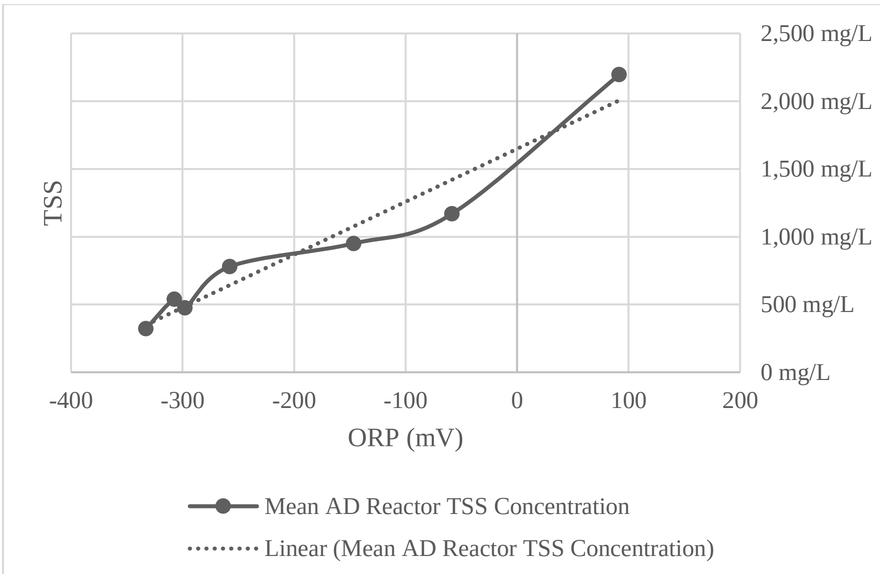

Figure 2-6. The mean AD reactor TSS concentration plotted against the mean AD reactor ORP during the study period. The R^2 value of the linear regression line is 0.94.

2.4.5. Treated Effluent Nutrient Profile

While AD resulted in the solids reduction, TOC removal, and nutrient mineralization needed for re-use as a hydroponic fertilizer, the treated effluent also had significantly reduced concentrations of N and S. Both are macro-nutrients required for plant growth and deficiencies in either nutrient can result in stunted growth and the disruption off physiological pathways (Marschner, 2011; Etienne et al., 2018). In plants, N serves a vital role in photosynthesis, and both N and S are primary components in proteins (Marschner, 2011; Etienne et al., 2018). The form of N after AD must also be considered when evaluating the effluent for re-use as a hydroponic fertilizing solution. Prior to AD, the effluent from KFRAG had a plant available TN concentration of 127 mg/L. Over 99% of the plant available TN in the untreated effluent was NO_3-N, the preferred form for uptake by hydroponic plants (Ikeda and Osawa, 1981; Shinohara et al., 2011). After AD, the mean plant available TN concentration of the effluent was 23.1 mg/L, and comprised of 91.60% NH_4-N, 7.32% NO_3-N, and 1.08% urea. Additionally, S is often considered an overlooked element in fertilizers and many crops have been identified as sulfur deficient in the past several decades (Gilbert, 1951; Scherer, 2001; Etienne et al., 2018). When evaluating the potential of using AD to develop a hydroponic nutrient solution, the reduction in total mass of N and S must be considered with the increased plant availability of other nutrients. As a means for estimating the effectiveness of the treated effluent as a fertilizing solution, the nutrient profile of the treated effluent was compared to nutrient recommendations for hydroponic lettuce and leafy green production (Table 2-5).

Table 2-5. Plant available concentrations of nutrients after anaerobic digestion compared against recommended concentrations for hydroponic lettuce and leafy green production ((fertilizer information retrieved from jrpeters.com)

Nutrient	Anaerobic (mg/L)	Jack's Hydroponic (mg/L)
Macro-nutrients		
N	23.1	150
P	5.11	39
K†	303	162
Ca	21.1	139
Mg	17.6	47
S	3.14	N/A
Micro-nutrients		
Fe†	1.8	2.3
Mn	0.16	0.38
Cu	0.137	0.113
Zn	0.74	0.11
Na	34.6	N/A

†Supplemented nutrient in KFRAG

The plant availability of the nutrients was increased after AD. However, only the K, Cu, and Zn concentrations in the KFRAG effluent met or exceed the recommended concentrations for hydroponic lettuce and leafy green production after treatment. It must also be noted that the concentrations of K and Fe are supplemented at KFRAG and the overall concentrations are not necessarily representative of other aquaponic facilities. In other studies on the nutrient profile of aquaponic effluent, K was shown to be present in lower concentrations and largely plant unavailable prior to treatment (Monsees et al., 2017; Goddek et al., 2018). Similarly, the addition of soluble Fe salts increased the plant availability of Fe in the untreated effluent at KFRAG in comparison to other published studies (Monsees et al., 2017; Goddek et al., 2018). While the mass and initial plant availability of Fe is not comparable between KFRAG and other aquaponic facilities, this study still demonstrated the ability AD to significantly increase the plant availability of Fe in aquaponic effluent.

The plant availability of the nutrients in aquaponic effluent can be increased through AD. After maximizing plant availability, the total concentration of many nutrients in the treated effluent was still below the recommended concentrations for hydroponic lettuce and leafy green production. The total concentration of each nutrient, not the plant availability of the nutrients, becomes the limiting factor for developing a hydroponic nutrient solution from aquaponic/RAS effluent through AD. Supplementation with traditional chemical fertilizers or concentration of the effluent is required to fully meet crop needs. A hydroponic fertilizer program comprised primarily of components derived from captured effluent supplemented secondarily by traditional chemical productions would reduce mined mineral dependency and provide aquaponic/RAS producers with alternative waste management strategies. However, a combined fertilizer would do little to progress the hydroponic industry closer to unopposed USDA organic certification. Increasing nutrient concentration through crystallization is a potential method to develop a fertilizer that can meet crop needs exclusively from coupled hydroponic and RAS waste (Schooley et al., 2017).

Crystallization of aqueous nutrient salts is currently being explored in the terrestrial animal agriculture industry to create highly concentrated and fully soluble fertilizers, prevent eutrophication as a result of runoff, and end reliance on finite mineral reserves (Schooley et al., 2017; Zhang et al., 2017). Continuing to adopt technologies developed for the terrestrial animal agriculture industry could increase nutrient concentration and meet crop needs in a fertilizer derived solely from the effluent of an aquaponic facility.

The relative ratios of nutrients between treated aquaponic/RAS effluent and commercial hydroponic fertilizing solutions must be evaluated when concentrating treated aquaponic/RAS effluent is being considered. The plant available nutrient ratios, on a part per million (ppm) basis, in the untreated KFRAG effluent, the KFRAG effluent after AD, and a commercial hydroponic fertilizing solution are shown in Table 2-6. The macro-nutrient ratios of the untreated KFRAG effluent is lower than the commercial solution and not ideal for plant production. The nutrient ratios of the KFRAG effluent after AD are more comparable to the commercial solution than the untreated effluent. This similarity between the nutrient ratios of the effluent after AD and the commercial solution is due largely to the TN reduction that balanced the ratio of N to the other macro-nutrients in the treated effluent.

Nutrient ratios of concern in the KFRAG effluent after AD are N:K and N:Na. Although K is an essential nutrient for plant growth, excess K can interfere with the uptake of other nutrients (Cooil and Slattery, 1948). The increased K concentration at KFRAG compared to other aquaponic/RAS facilities has been established (Monsees et al., 2017; Goddek et al., 2018). The high N:K ratio observed in the KFRAG effluent is unlikely to occur when a facility is not dosing with K_2CO^3 on a daily basis. Few plants need Na for growth, and similar to excess K, it can interfere with the uptake of required nutrients (Marschner, 2011). A hydroponic fertilizing solution containing excess Na could prevent plants from up-taking other nutrients. To maximize the potential of aquaponic/RAS effluent for re-use as a hydroponic fertilizing solution, the nutrient profile of anything added to the system must be considered in terms of how it will affect the effluent.

Table 2-6. The plant available nutrient ratios of the untreated KFRAG effluent, the KFRAG effluent after AD, and a commercial hydroponic fertilizing solution on a ppm basis is shown. All nutrients are compared to N. Macro-nutrients (and Na) are normalized to 10 ppm N and micro-nutrients are normalized to 100 ppm.

Nutrient	Untreated Effluent (ppm)	AD Reactors (ppm)	Jack's Hydroponic (ppm)
Macro-Nutrients			
N	10.0	10.0	10.0
P	0.12	2.21	2.60
K†	22.3	131	10.8
Ca	1.14	9.13	9.27
S	1.70	1.36	N/A
Na	2.54	15.0	N/A
Micro-Nutrients			
Fe†	1.04	1.20	1.53
Mn	0.09	0.11	0.25
Cu	0.08	0.09	0.08
Zn	0.47	0.49	0.07

†Supplemented nutrient in KFRAG

2.4.6. Organic Carbon Removal

The presence of OC could limit the adoption of treated aquaponic/RAS effluent as a hydroponic fertilizing solution regardless of increased plant availability and solids reduction. In a hydroponic system, OC build-up can result detrimental effects to the physical and physiological health of plants (Lee et al., 2006; Yaron and Römling, 2014). Heterotrophic bacteria feed on OC and can colonize into biofilms that physically disrupt irrigation water flow in a hydroponic system, consume DO in the root zone, and outcompete plants for nutrient uptake. Many heterotrophic bacteria are also pathogenic and can cause disease in crops and humans (Yaron and Römling, 2014). Beyond disruption of system operation and potential food safety concerns, OC has also been shown to have phytotoxic effects that reduce plant growth by negatively altering physiological functions (Garland et al., 1997; Lee et al., 2006).

The AD reactors used in this study reduced the mean ± SD TOC concentration of the KFRAG effluent by 47.42 ± 12.76%. This reduction is comparable to TOC reductions in other waste streams by AD and to the organic matter reduction reported in another study on the AD of aquaponic effluent (Delaide et al., 2018). The portion of the TOC concentration remaining after treatment that was dissolved in the aqueous fraction of the effluent was increased from 18.83% to 86.36 ± 10.8. Based on the results of this study, the DOC concentration increased from 28.4 mg/L to 68.6 mg/L as a result of AD. This study showed that AD can significantly increase the plant availability of nutrients and significantly reduce the TSS and TOC concentration of aquaponic effluent. However, AD also dissolved the majority of the remaining OC in the effluent, leading to a greater concentration of DOC after treatment than in the untreated effluent. While AD is a promising initial treatment option to increase nutrient availability and reduce solids, a secondary treatment process is required to remove remaining DOC before the effluent can become a viable hydroponic fertilizing solution.

Aerobic digestion is often used as a finishing process for wastes treated anaerobically (Borzacconi et al., 1999; Del Pozo and Diez, 2003). During aerobic digestion, organic matter is broken down and oxidized into CO_2 in a constantly aerated system (Maier et al., 2009). Aerobic digestion can achieve greater organic matter reduction in both industrial and aquaponic/RAS effluent treatment than AD (Borzacconi et al., 1999; Delaide et al., 2018). Sludge production from microbial growth and high treatment costs due to constant aeration are often limiting factors for large-scale applications aerobic digestion (Mata-Alvarez et al., 2000). However, multiple studies have shown greater than 90% COD reduction when aerobic digestion is used as a finishing process after AD has reduced the solids content of a waste stream and preformed initial OC reduction (Borzacconi et al., 1999; Del Pozo and Diez, 2003). An additional benefit of adding an aerobic treatment after the AD of aquaponic/RAS effluent is nitrification to convert NH_4-N to NO_3-N for improved plant growth performance (Ikeda and Osawa, 1981; Gerardi, 2007). Research on the incorporation of an aerobic stage after AD could enhance aquaponic/RAS effluent treatment and result in a final solution with reduced OC concentrations and the majority of TN in the ideal form for uptake by hydroponic plants (Ikeda and Osawa, 1981; Del Pozo and Diez, 2003).

2.5. Conclusions

Current projections predict the depletion of mined mineral reserves used for the production of fertilizers and an increased demand for agricultural products to feed the growing global population in the coming decades (Cordell et al., 2009; Henckens et al., 2016; Hunter et al., 2017). Based on these projections, a naturally-derived hydroponic nutrient solution has the potential to increase future food security by developing a circular nutrient economy to sustain the hydroponic industry independently of the finite mineral reserves that contain the nutrients required for fertilizer production. This study confirmed that AD reduced the TSS and TOC concentration of aquaponic/RAS drum screen effluent and simultaneously increased that plant availability of the nutrients. Additional data collected identified a strong correlation between the reduction and stabilization of TSS concentrations and the reduction and stabilization of ORP measurements throughout the treatment process within the AD reactors. Based on this correlation, ORP stabilization can provide an accurate assessment of when a specific AD reactor has achieved maximum solids reduction and nutrient mineralization.

After treatment, the nutrient ratios in the effluent were more comparable to the nutrient ratios of a commercially available hydroponic fertilizing solution than the untreated effluent. This is promising progress towards a treatment system for the development of a naturally derived nutrient solution from aquaponic/RAS effluent. The increased DOC concentration after AD makes the treated effluent unsuitable for use as a hydroponic fertilizing solution due to the negative effects that OC has on hydroponic production (Lee et al., 2006; Yaron and Römling, 2014). While AD can provide initial treatment to reduce solids and mineralize nutrients, a second stage of treatment is required to further remove OC before aquaponic/RAS effluent can be re-used as a hydroponic fertilizing solution. A finishing stage of aerobic digestion is often used on AD effluent to additional remove OC. Future research on the continued treatment of aquaponic/RAS effluent using aerobic digestion is needed to continue to development of a naturally-derived nutrient solution. Maximizing plant availability of aquaponic/RAS effluent in lab-scaled batch reactors is the first step to developing a treatment system for commercial operations.

CHAPTER 3
AEROBIC MINERALIZATION OF RECIRCULATING AQUACULTURE DRUM SCREEN EFFLUENT FOR USE AS A NATURALLY-DERIVED FERTILIZER IN HYDROPONIC CROPPING SYSTEMS

3.1. Introduction

The United States is the world leader in imported seafood (FOA, 2018). Increased seafood production would allow the United States to meet domestic demands. Over the past three decades the aquaculture industry has become a primary source for seafood production (FAO, 2018). Improvement and expansion of the aquaculture industry would allow the United States to better meet these domestic seafood demands and compete in international markets. There are multiple aquaculture production methods. Land-based recirculating aquaculture systems (RAS) are the most promising option for optimization in the aquaculture industry due to the inherent high water re-use rates, location independence, and controlled environment maximizing fish growth rates producing fresh seafood in areas with the infrastructure to meet the requirements of its industrial operations (van Rijn, 1996; Gelfand et al., 2003).While RAS has the potential to address the seafood production deficit in the United States, expansion of commercial RAS is currently limited by costs associated with the waste treatment despite the high water conservation rates (Miller and Semmens, 2002; Tsani and Koundouri, 2018).

The high nutrient and water content of RAS effluent makes it well-suited for treatment and reuse as a naturally derived fertilizer solution for hydroponic greenhouse production (Cripps and Bergheim, 2000; Guerdat et al., 2013; Goddek et al., 2019). Research has shown that RAS effluent contains the nutrients required for plant growth, but that the effluent also requires treatment to mineralize particulate-bound nutrients, reduce suspended solids, and remove organic carbon before it can become a viable hydroponic fertilizer (Monsees et al., 2017; Chapter 2). Developing a hydroponic fertilizing solution from RAS effluent would benefit the hydroponic and RAS industries, while creating a circular nutrient economy.

RAS operations can monetize the waste stream and offset operating costs by adopting a capture and re-use model of waste management similar to that of terrestrial animal agriculture producers (USDA NRCS, 2013). Additionally, hydroponic growers would benefit from a naturally derived nutrient solution enabling facilities to earn USDA Organic certification (Schneider et al., 2005; Yogev et al., 2017). Reusing nutrients from RAS effluent as a hydroponic fertilizer would create a circular nutrient economy, effectively enabling production systems to mitigate the agricultural industry's current reliance on mined minerals in the production of conventional inorganic fertilizers (Henckens et al., 2016). Phosphorus (P) and iron (Fe) serve as two examples of nutrients that are both essential for plant growth and have a diminishing mineral reserve (Marschner, 2011; Henckens et al., 2016; Cieślik and Konieczka, 2017). Additionally, both P and Fe are found in solid RAS waste, but the effluent requires treatment before complete utilization by plants is possible (Goddek, 2019; Chapter 2).

Aerobic digestion is a common method for domestic and agricultural wastewater treatment that has potential for adoption into the RAS industry (Samer, 2015; Goddek et al., 2019). Aerobic waste treatment utilizes heterotrophic bacteria and constant aeration to oxidize solid organic matter into CO_2 during respiration (Maier et al., 2009). Benefits of aerobic digestion over other waste treatment methods include ease of operation and increased rate of organic matter reduction, however the cost of constant aeration must be considered when determining a cost-benefit analysis of aerobic treatment against other forms of wastewater treatment (Chen et al., 1997).

Waste streams treated aerobically are typically evaluated based on the removal of specific contaminants and solids reduction (Tchobanoglous et al., 2014). Goddek et al. (2019), suggests that the success of a RAS effluent treatment method with the intention of reuse as a hydroponic fertilizer should also be based on the ability to increase the overall plant availability of nutrients in the effluent. While research has been conducted on the aerobic treatment of the particulates in the solid RAS effluents, few have evaluated the process in terms of mineralization across a broad spectrum of plant-essential macro- and micro-nutrients with effluent from a coupled hydroponic and RAS (aquaponic) production system (Conroy and Couturier, 2010; Monsees et al., 2017; Khiari et al., 2019). Plant-essential macro- and micro-nutrients are required for a plant to complete its lifecycle and cannot be replaced by any other nutrient, with the relative concentration for the macro-nutrients are significantly greater than concentration of micro-nutrients (Marschner, 2011).

The objectives of this research were to determine the nutrient concentrations of eleven plant-essential macro- and micro-nutrients in the effluent of an aquaponic system, characterize the degree of mineralization for those nutrients under aerobic conditions in triplicated lab-scaled batch bioreactors, and identify future needs for continued research in the aerobic digestion of aquaponic/RAS effluent. The aerobic microbial treatment of the effluent was expected to reduce total organic carbon (TOC), increase the percent of the total nutrient mass dissolved into the treated solution, and decrease the total suspended solids (TSS).

3.2. Materials and Methods

This project was conducted using effluent from the co-production of tilapia and hydroponic lettuce in the University of New Hampshire (UNH) Kingman Farm Recirculating Aquaponic Research Greenhouses (KFRAG) located in Madbury, New Hampshire, USA (**Error! Reference source not found.**). A full description of the production facility may be found in Chapter 2. The facilities were operated under actual production conditions, with feeding and waste production rates commensurate with industry production facilities. The UNH KFRAG systems were in operation for at least 365 days prior to collecting samples for analysis and were operating under pseudo-steady state conditions. For this study, pseudo-steady state conditions were defined as the continual function of desired activities without disruption. Feeding rates, as a function of fish biomass, remained constant and effluent production and water usage remained consistent for a minimum of two weeks prior to sampling.

3.2.1. Aquaponic Facility Description

The KFRAG was a replicated coupled aquaponic research facility (**Error! Reference source not found.**) comprised of three identically-constructed and operated freestanding greenhouses. Each recirculating aquaponic system was housed in an 11.0 x 14.6 m high tunnel greenhouse (Nor'Easter Series, Rimol Greenhouse Systems, Hookset, NH, USA), and were covered using polycarbonate. The recirculating systems consisted of a single 3,000 L fish culture tank, a rotary drum screen filter (PR Aqua model RFM2014) fitted with 54 micron screens, one 1,300 L mixed media bed bioreactor (MBBR) used for nitrification, a 200 L pumping reservoir, a 300 L standpipe well, and three 12.6 m^2 deep water raft (DWR) hydroponic growing tables used for hydroponic lettuce (*Lactuca sativa*) production (see Figure 1). The overall system volume was 15,000 L. The only nutrients supplemented into the system were potassium carbonate (K_2CO_3) for biofilter management and chelated diethylemetriamine pentaacetic acid (DTPA) iron (III) salt that was added as needed to ensure that sufficient iron (Fe) concentrations were met in the system for optimum lettuce growth.

Tilapia (*Oreochromis niloticus*) were stocked at 36 kg per m^3 and fed 3 mm floating feed (Finfish Silver, 40% protein, 10% lipid, Zeigler Bros. Inc., Gardner, PA, USA). The fish were fed 1300 g/day and a constant fish biomass was maintained allowing for a consistent feed rate. The total fish biomass in the culture tanks was measured bi-weekly and the number of fish in the culture tank was adjusted to ensure that 1300 g of feed each day would provide optimum fish growth rates per DeLong et al (2009).

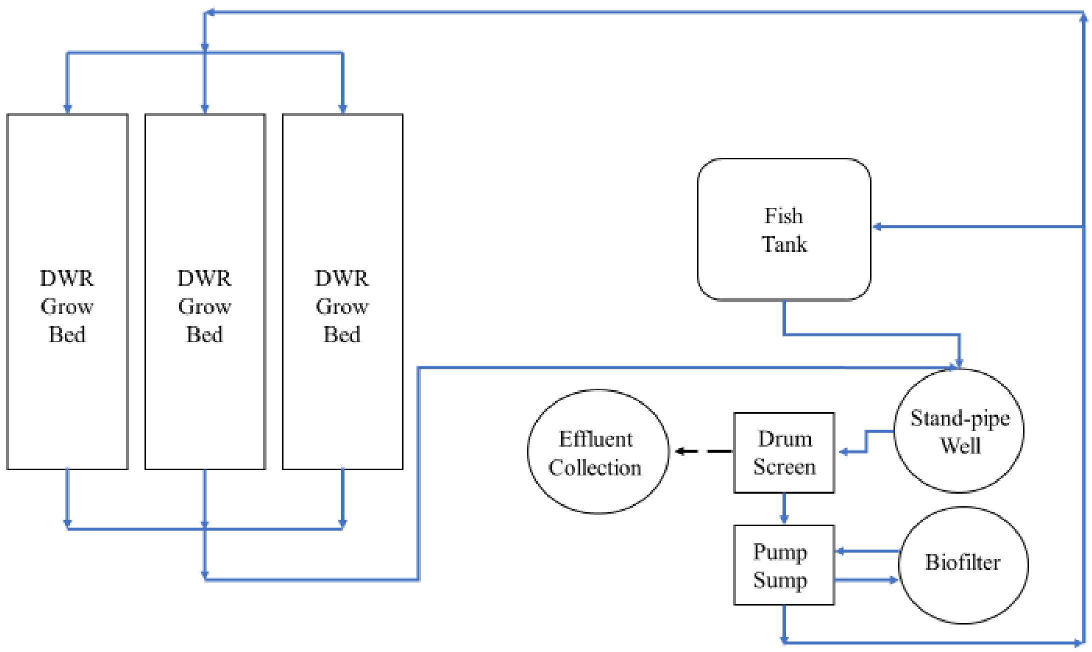

Figure 3-1. The flow schematic for each recirculating aquaponic greenhouse at the UNH Kingman Farm Research Greenhouses located in Madbury, New Hampshire, USA. Composite samples were collected directly from the drum screen filter effluent pipe for 72 hours under pseudo-steady-state operating conditions.

3.2.2. Drum Screen Effluent Collection, Analysis, and Nutrient Characterization

The collection of 200 L of rotary drum screen effluent was conducted over a period of 72 hours. After collection, effluent samples were filtered to divide the samples into particulate and aqueous fractions prior to analysis to develop a nutrient profile of the effluent. Effluent collection and filtration were conducted as described in Chapter 2. Analysis of TSS was conducted at the UNH Agricultural Engineering Laboratory (Method 2540D, APHA, 2012). A Fisher Scientific Accumet AB250 (MA, USA) was used for pH measurements.

A hydroponic nutrient profile of the feed used and drum screen effluent was obtained using a commercial hydroponic fertilizer laboratory service (JR Peters Laboratory, Allentown, PA, USA). The particulate and the aqueous fractions of the effluent were analyzed for six macro-nutrients (nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), and magnesium (Mg)) and five micro-nutrients (Fe, manganese (Mn), copper (Cu), zinc (Zn), sodium (Na), and aluminum (Al)). The TOC and total nitrogen (TN) concentrations in the effluent were determined based on analysis conducted by the United States Forest Service's Northeastern Forest Science Application Lab (Durham, NH, USA). Samples were prepared and analyzed as described in Chapter 2.

Total nutrient mass was calculated using particulate and aqueous analyses. Total reactor nutrient concentration was determined based on reactor volume to normalize comparison between reactors. The total concentration of each nutrient, in mg/L, was determined by adding the particulate and aqueous nutrient masses as a function of reactor effluent volume as calculated in Chapter 2. The percent of the total concentration of each nutrient in the aqueous and particulate fractions was used to determine plant availability. All nutrients in the aqueous fraction were defined as plant-available for the context of this study. Particulate-bound, aqueous, and total nutrient concentrations were calculated using equations described in Chapter 2.

3.2.3. Batch Reactor Design

Aerobic treatment of the collected effluent was conducted using 20 L high density polyethylene (HDPE) aerobic containers (**Error! Reference source not found.**). The effluent was continuously mixed using constant aeration from an air pump (Sweetwater Linear II Model SNL42, Pentair, Minneapolis, MN, USA) with a medium pore stone diffuser.

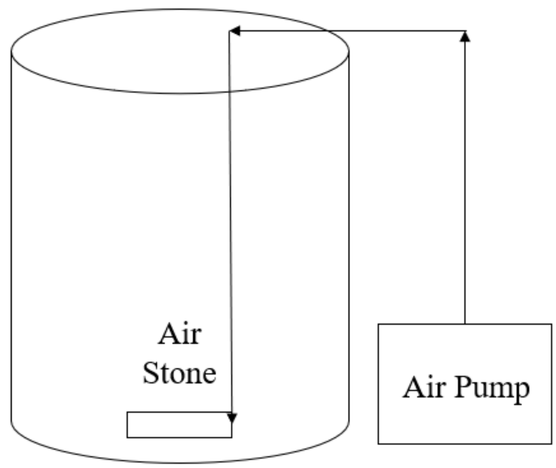

Figure 3-2. Aerobic reactor schematic. Effluent to be treated was continuously mixed using constant aeration. Samples were taken by removing the reactor lid to access the effluent.

3.2.4. Experimental Design and Reactor Operation

One aerobic microbial treatment was evaluated in this study with an abiotic control to provide a reference for microbial and physical effects of treatment. Both the treatment and control were replicated in triplicate at the same time. The treatment and control used the same batch of drum screen effluent. The aerobic treatment was not inoculated and used only endogenous microbes present at the time of collection. The abiotic control was dosed with 0.05% sodium azide (NaN_3) to inhibit microbial growth and biological activity (Barbot et al., 2010). The purpose of the abiotic control was to establish the effects of microbial treatment as compared to physical dissolution of the products in the effluent.

Reactors were filled with 15 L of drum screen effluent and operated continuously, and samples were collected as described in Chapter 2. A 50 mL sample was collected from each reactor every 48 hours and analyzed for temperature, dissolved oxygen (DO) pH, and TSS. A handheld DO meter (Model HQ 40D, Hach, Loveland, CO, USA) was used for DO measurements. All equipment was calibrated using manufacturer supplied solutions and at intervals prescribed in by standard operating procedures developed by the respective manufacturers. Reactors were operated at ambient temperatures and reactor pH was adjusted using sodium bicarbonate ($NaHCO_3$) when the pH measured below 7 to maintain an appropriate environment for anaerobic microbes (Cioabla et al., 2012).

Reactor operation was terminated when the treated effluent was determined to have reached stabilization. Reactor stabilization was defined as a change in TSS of less than 10% over 3 consecutive 48 hour sample periods in the aerobic reactors. After reactor stabilization, final nutrient and TOC analyses and were conducted identically to the analysis of the initial drum screen effluent to determine the change in plant availability after treatment. Change in plant availability was determined using Equation 3 in Chapter 2. The plant available nutrient concentrations after aerobic treatment were compared to a commercially-available hydroponic lettuce and leafy green fertilizing solution.

3.2.5. Statistical Analysis

A One-way Analysis of Variance (ANOVA) in JMP Pro version 14.1 Statistical Software (SAS Institute, Cary, NC) was used to determine if the temperature, DO, pH, and TSS was statistically similar or different between aerobic reactors and the abiotic controls. A Tukey's honestly significant difference (HSD) test was used to evaluate if the replicate reactors were statistically similar or different from each other within the aerobic treatment and the abiotic control. A pooled t-test was used to test the significance of the differences in TOC, TN, and total sulfur concentrations and in the percent of each nutrient's total mass in the aqueous fraction of the effluent after treatment. A p value < 0.05 was considered significantly different for all analyses.

3.3. Results

A complete nutrient profile was first developed via analysis of the particulate and aqueous fractions of the aquaponic drum screen effluent. The nutrient profile data were used to calculate the particulate and aqueous mass fractions for twelve different nutrients (TOC, N, P, K, Ca, Mg, Fe, Mn, B, Cu, Zn, Na, and Al). The effluent was then transferred to the experimental bioreactors and treated aerobically as a batch reaction. Operational data including temperature, DO, pH, and TSS were measured at 48-hour intervals. A final nutrient analysis was conducted after stabilization to determine the change in nutrient plant availability.

3.3.1. Feed and Effluent Nutrient Analysis

The nutrient profile of the 40% protein, 10% lipid finfish feed is reported in Table 3-1.

Table 3-1. The nutrient profile of Zeigler Bros. Inc., Finfish Silver, 40% protein, 10% lipid feed.

Nutrient	Feed
Macro-nutrients	
N*	6.44
P*	0.97
K*	0.96
Ca*	1.17
Mg*	0.14
S†	1024
Micro-nutrients	
Fe†	209
Mn†	91.8
B†	5.9
Cu†	46.5
Zn†	89.6
Mo†	4.13
Na†	2051
Al†	0

*Reported as percent of total mass

†Reported as mg/L

The initial effluent pH was 7.4, and a TSS concentration of 1217 mg/L. Lab closures caused by the COVID-19 outbreak prevented a complete TOC analysis of the drum screen effluent before and after aerobic treatment as the US Forest Service lab was subsequently closed, and remains closed at the time of writing this manuscript. The dissolved organic concentration (DOC) concentrations for the initial effluent and following treatment were analyzed. However, the organic carbon (OC) content of the particulate fraction was not analyzed for the initial effluent are after treatment as a result of the lab shutdowns. The initial DOC concentration of the drum screen effluent was 20.8 mg/L. The TN concentration of the effluent was 174 mg/L, with 96.36% of the concentration dissolved in the aqueous fraction of the effluent. The C:N ratio of the aqueous fraction of the effluent was 0.13:1.

The total macro- and micro-nutrient concentrations in the reactors, and the associated aqueous and particulate mass fractions in the initial effluent, are reported in **Error! Reference source not found.**. Over 98% of the total K, Mg, Zn, and Na was dissolved in the aqueous fraction of the effluent. The macro-nutrients with the lowest percentage of their respective total concentration in the aqueous effluent fraction were N (96.36%), P (68.11%), and Ca (92.78%). Micro-nutrients, Fe (94.57%), B (90.11%), Cu (94.60%), and Al (71.15%) had the least amount of the total concentration in the aqueous fraction of the effluent.

Table 3-2. Total nutrient concentrations in the drum screen effluent collected from UNH KFRAG as a combination of the aqueous and particulate nutrient mass normalized to 1 L of effluent. The aqueous and particulate mass fractions for each nutrient are expressed as a percent of total mass. Nutrients found in the aqueous fraction were considered plant available.

Nutrient	Total Drum Screen Effluent (mg/L)	Aqueous (%)	Particulate (%)
TOC	N/A	N/A	N/A
Macro-nutrient			
N	174	96.36	3.64
P	3.82	68.11	31.89
K†	402	99.61	0.39
Ca	30.4	92.78	7.22
Mg	21.8	98.33	1.67
Micro-nutrient			
Fe†	1.90	94.57	5.43
Mn	0.27	97.50	2.50
B	0.26	90.11	9.89
Cu	0.17	94.60	5.40
Zn	0.80	99.24	0.76
Na	34.5	99.72	0.28
Al	0.51	71.15	28.85

†Supplemented Nutrient in KFRAG

*Data not available due to lab shutdowns caused by COVID-19

3.3.2. Reactor Sample Analysis

All aerobic reactors and abiotic controls were measured for temperature, DO, and pH at 48-hour intervals to ensure ideal operating parameters were maintained during the experiment. The mean ± standard deviation (SD) of the temperature, DO, and pH in the aerobic reactors and abiotic controls are shown in Table 2-3. The mean temperature in the aerobic reactors was significantly lower than the temperature in the abiotic controls ($p = 0.0018$). The temperatures in the replicated aerobic units ranged from 20.6 to 21.9 ^{o}C, but were significantly different from each other over the course of the experiment ($p = 0.0420$). The temperatures in the replicated abiotic control units ranged from 20.7 to 22.7 ^{o}C, and were also significantly different from each other throughout the experiment ($p = 0.0011$). The mean DO concentration throughout the study period was not significantly different between the aerobic reactors and the abiotic controls (*p* = 0.3818). The DO concentration in the replicated aerobic units were not significantly different from each other throughout the experiment (*p* = 0.0691), however the mean DO concentration in the replicated abiotic control units were significantly different from each (*p* = 0.0035). The pH was not significantly different between the aerobic reactors and the abiotic controls (*p* = 0.3639). The pH in the replicated aerobic units and the replicate abiotic controls were not significantly different from each other throughout the experiment (*p* = 0.0543 and *p* = 0.9598, respectively).

Table 3-3. The mean ± SD of the temperature, DO, and pH in the aerobic reactors and the abiotic control reactors during the study

Parameter	Aerobic Reactors	Abiotic Controls	p – value Between Treatments
Temperature (^oC)	21.1 ± 0.41	21.6 ± 0.34	0.0018
DO (mg/L)	8.83 ± 0.20	8.85 ± 0.09	0.3818
pH	7.3 ± 0.18	7.4 ± 0.37	0.3639

The initial TSS of the effluent was 1217 mg/L. The change in TSS concentration over the entire study period is shown in **Error! Reference source not found.**. On day 15, the final mean TSS ± SD concentration in the aerobic reactors was 475 ± 5.20 mg/L. The overall average change in the TSS concentration of the replicate aerobic reactors were not significantly different from each other throughout the experiment (*p* = 0.9647). The aerobic reactors reduced the mean ± SD TSS concentration in the effluent by 60.96 ± 0.43% over the 15-day study period. The final mean ± SD TSS concentration in the abiotic controls was 538 ± 34.21 mg/L. The overall average change in the TSS concentration of the replicate abiotic controls was not significantly different from each other throughout the experiment (*p* = 0.5578). The final mean ± SD TSS concentration in the abiotic controls was 55.79 ± 2.81% lower than the initial effluent. The mean overall average change in TSS concentrations measured at 48-hr intervals throughout the experiment in the aerobic reactors and the abiotic control reactors was not significantly different ($p = 0.5527$).

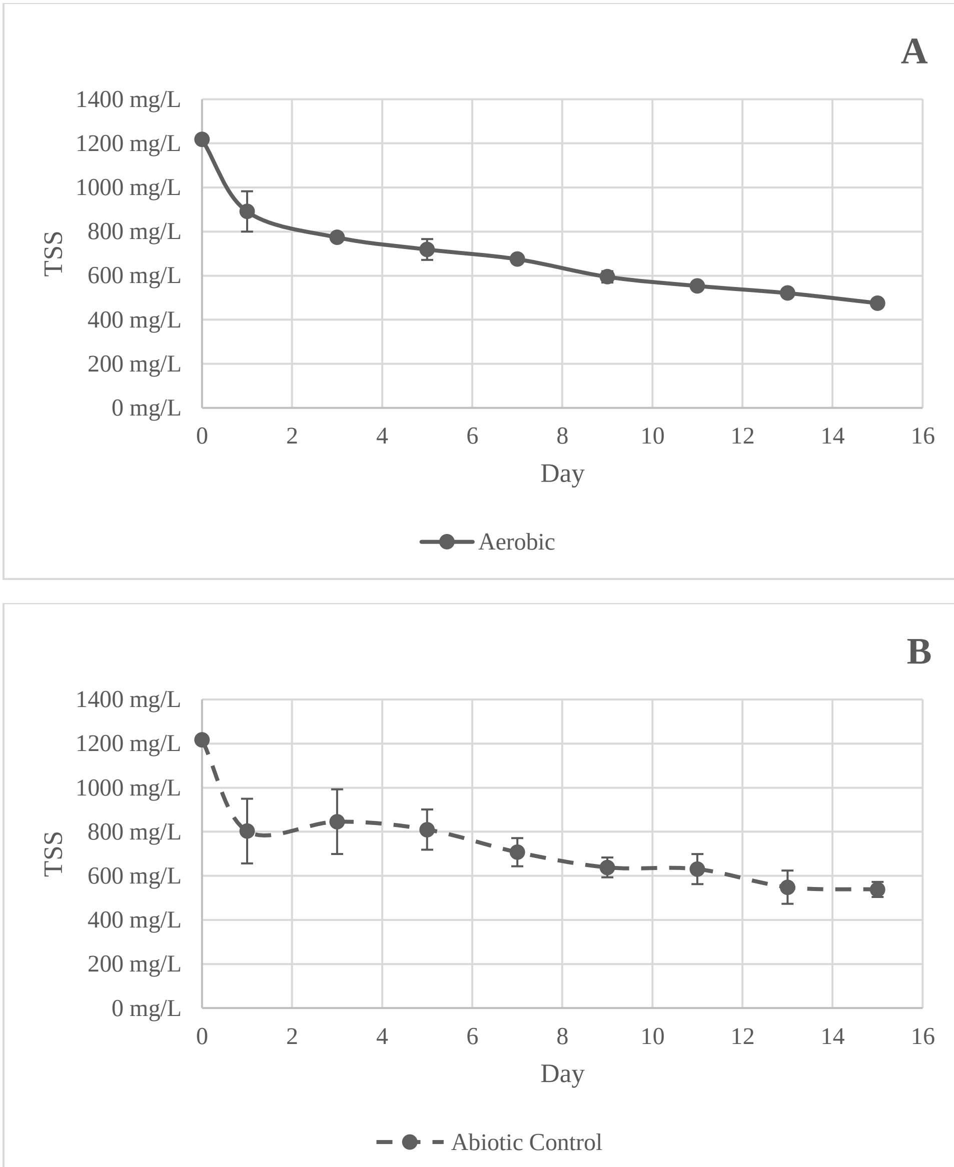

Figure 3-3. Mean TSS concentration within aerobic reactors **(A)** and abiotic controls **(B)** throughout the 15-day study period. Error bars indicate standard deviation between treatment replicates.

3.3.3. Total Organic Carbon Mass Analysis

Due to lab closures caused by the COVID-19 outbreak, particulate-bound OC data from KFRAG effluent was not available at the time of writing this manuscript. The mean ± SD of the DOC concentrations in the aerobic reactors and abiotic controls were 23.19 ± 4.86 and 36.54 ± 0.85, respectively (**Error! Reference source not found.**). The DOC concentration after aerobic treatment was not significantly different than the DOC concentration in the initial effluent (p = 0.4502). The DOC concentration in the abiotic controls was significantly greater than the DOC concentration in the initial effluent ($p < 0.0001$).

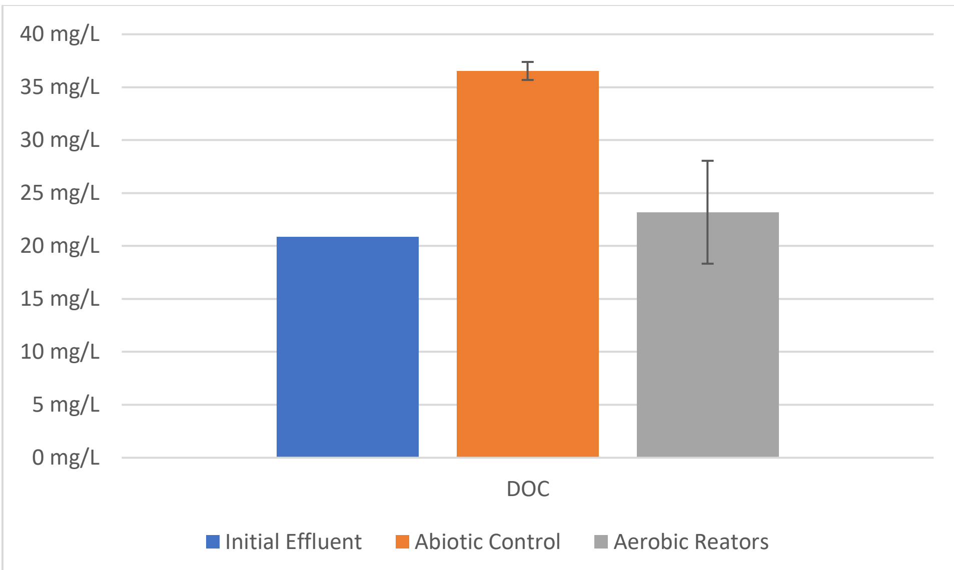

Figure 3-4. The DOC concentration in the initial drum screen effluent from KFRAG and in the abiotic controls and aerobic reactors after treatment. Error bars indicate standard deviation.

3.3.4. Final Nutrient Analysis

The change in the total concentration of the aqueous fraction in the treated effluent for N, P, Ca, Fe, B, Cu, and Al are reported in **Error! Reference source not found.**. In the initial effluent, N, P, and Ca were the three macro-nutrients with the lowest percent of their concentration in the aqueous form. After aerobic digestion, the percent of the total N, P, and Ca in the aqueous fraction of the waste was significantly increased ($p < 0.0001$, $p < 0.0001$, p = 0.0003, respectively). After aerobic treatment, the TN in the aqueous fraction of the effluent was comprised of 98.07% nitrate-nitrogen (NO_3-N), 1.06% urea, and 0.87% ammonia-nitrogen (NH_4-N). The micro-nutrients with the lowest percent of their total concentrations in the aqueous form were Fe, B, Cu, and Al. After aerobic digestion the percent of total B, Cu, and Al in the aqueous fraction of the waste was significantly increased ($p < 0.0001$, $p = 0.0003$, $p < 0.0001$, respectively). There was not a significant change in the percent of total Fe in the aqueous fraction of the effluent after aerobic treatment ($p = 0.1529$).

Table 3-4. Factor increase of total nutrient concentrations in aqueous form (and available for plant uptake) in tilapia effluent after aerobic digestion. Mean percent ± SD of total nutrients in aqueous form shown before and after aerobic treatment.

Nutrient	Effluent (% Aqueous)	Aerobic (% Aqueous)	Change in % Aqueous
TOC*	N/A	N/A	N/A
Macro-nutrients			
N	96.36	98.60 ± 0.1	1.02x
P	68.11	94.97 ± 1.1	1.39x
K†	99.61	99.84 ± 0.0	1.00x
Ca	92.78	96.59 ± 0.6	1.04x
Mg	98.33	99.4 ± 0.2	1.01x
Micro-nutrient			
Fe†	94.62	93.64 ± 0.9	0.99x
B	90.11	99.84 ± 0.1	1.10x
Cu	94.60	98.57 ± 0.6	1.04x
Al	71.15	86.74 ± 0.9	1.22x

†Nutrient supplemented at KFRAG

* Data not available due to lab shutdowns caused by COVID-19

3.4. Discussion

This study evaluated the effects of aerobic digestion on aquaponic effluent to develop a naturally derived nutrient solution for hydroponic plant production as compared to an abiotic control. Improved plant availability of nutrients and reductions in TSS and TOC were achieved using aerobic microbial treatment. The following sections discuss the nutrient profile of the untreated aquaponic effluent, the operation of the aerobic reactors and abiotic controls, and the potential of the treated effluent for re-use as a hydroponic fertilizing solution as compared to anaerobic treatment methods. Suggestions for future research are also discussed.

3.4.1. Feed and Drum Screen Effluent Nutrient Analysis

The nutrient profile of the feed used would likely have a significant impact on the nutrient profile of the system effluent. The feed used in this study was chosen as it is commercially available and commonly used in the RAS industry. Analysis of the aquaponic drum screen effluent from KFRAG supported results found in previous experiments on the nutrient profile of aquaponic/RAS effluent (Seawright et al., 1998; Guerdat et al., 2013; Delaide et al., 2018). Each of the nutrients analyzed for were present in the effluent. Several nutrients had large percent of their total their total mass in the particulate fraction of the effluent, thus not immediately available for uptake by plants. Additional treatment is needed before the nutrient can become plant available and an efficient hydroponic fertilizer. The nutrients that were the least plant available in the effluent from KFRAG were N, P, Ca, Fe, B, Cu, and Al, which corresponded to result of particulate-bound nutrients across multiple studies characterizing RAS and aquaponic effluent (Guerdat et al., 2013; Monsees et al., 2017; Goddek et al., 2018; Delaide et al., 2018; Chapter 2).

The primary nutrient difference at KFRAG compared to other aquaponic/RAS facilities found in the literature was found in K concentration and plant availability. Across multiple studies in the literature, K was found to be predominantly particulate-bound in aquaponic/RAS effluent (Monsees et al., 2017; Goddek et al., 2018). However, the total mass and the plant availability of K was greater at KFRAG than other aquaponic/RAS facilities due mostly to daily additions of soluble K_2CO_3 to the KFRAG systems for pH buffering and alkalinity adjustments for biofilter maintenance (Anderson, 2016; Goddek et al., 2018). Additionally, the mass and plant availability of Fe is also unique to KFRAG due to routine additions of soluble DTPA Fe (III). While Fe was still identified as a nutrient requiring mineralization at KFRAG, the percent of the total Fe in the aqueous fraction of the effluent was still greater than other facilities (Goddek et al., 2018). System design and nutrient additions can result in nutrient profiles unique to specific facilities, however, multiple macro- and micro-nutrients including P, Ca, Fe, Cu, and Al have been shown to require mineralization regardless of facility before becoming available for plant uptake (Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018; Chapter 2).

Similar to the effluent from other aquaponic/RAS facilities, the effluent from KFRAG contained high concentrations of OC (Guerdat et al., 2011; Delaide et al., 2018). Excess OC in a hydroponic system can result in biofilm proliferation (Lee et al., 2015). Biofilms are microbial masses that can adhere to nearly any surface in a hydroponic system. These biofilms create food safety concerns as they can be comprised of cells that are pathogenic to crops and humans (Elasri and Miller, 1999; Lee et al., 2015). Biofilms can form throughout a hydroponic system and have been shown to clog tubing and reduce irrigation water flow rate (Liu et al., 2017). Removing OC from the effluent to prevent biofilm film is required to develop a successful hydroponic nutrient solution.

3.4.2. Aerobic Operating Conditions

The pH and temperature of the aerobic reactors were maintained consistently throughout the experiment to prevent microbial inhibition. Zhou et al. (2019) recommended a pH range between 7.0 and 8.0 for aerobic digestion to result in optimum organic matter removal and microbial activity in wastewater. In a study to determine nitrogen mineralization in RAS effluent by aerobic treatment, Khiari et al. (2019) recommended that aerobic reactors be operated at a pH between 6.0 and 6.5 to limit nitrogen loss and nutrient precipitation. With a mean ± SD pH of aerobic reactors was 7.3 ± 0.18, the aerobic reactors used in this study with within the ideal range described by Zhou et al. (2019), but higher than the recommended values recommended for RAS effluent treatment to retain nitrogen (Khiari et al., 2019). Continued research on the effects of aerobic reactor pH between 6.0 and 8.0 may provide insight into optimization specific for the treatment of aquaponic/RAS effluent for re-use as a hydroponic fertilizing solution.

Aerobic digestion can occur across a variety of temperatures (LaPara and Alleman, 1999; Ugwuanyi et al., 2005; Habermacher et al., 2016). However, Ugwuanyi et al. (2005) found that increased temperatures can result in a greater degree of biodegradation. The aerobic reactors used in this study were kept at ambient temperature and had mean ± SD temperature of 21.1 ± 0.41. For practical applications, the cost of heating should be considered as a means for improving performance, especially in aerobic digestion where operating costs associated with constant aeration can also be a limiting factor.

3.4.3. Abiotic Controls Confirm Microbial Mineralization in Aerobic Reactors

The abiotic controls experienced a similar degree of TSS reduction and nutrient mineralization (results not shown) as the aerobic reactors. The mineralization observed in the abiotic controls was attributed to the deflocculating properties of NaN_3 (Barbot et al., 2010). Although NaN_3 resulted in mineralization degrees comparable to the aerobic, its toxicity and high sodium concentrations prevent its use as a treatment method for any waste to be re-purposed as a fertilizer (Barbot et al., 2010; Marschner, 2011). The purpose of the abiotic controls in this experiment was to confirm through comparison that the all mineralization occurring in the aerobic were a result of microbial digestion. The primary comparison made to confirm the absence of microbes in the abiotic controls and the presence of microbes in the aerobic reactors would have been the final TOC concentration in each reactor. No net change in TOC concentration was expected in the abiotic controls, while the TOC concentration in the aerobic reactors was expected to be reduced as a result of the oxidization of solid matter organic matter into CO_2 as a result of microbial respiration (Maier et al., 2009; Samer, 2015; B. Delaide et al., 2018). The TOC mass and overall reactor concentration in the initial drum screen effluent, aerobic reactors, and abiotic controls could not be calculated due to lab closured caused by the COVID-19 outbreak. The TOC concentration was to be calculated by totaling the DOC mass in the aqueous fraction of the effluent and the particulate-bound OC in the effluent. The DOC concentration in the initial effluent, aerobic reactors, and abiotic controls was analyzed prior to lab closures. The particulate-bound OC analysis was not able to be conducted, preventing the calculation of the overall TOC reactor concentrations.

Assumptions based on the DOC results were made to provide insight into how the TOC concentration was affected by the aerobic reactors and abiotic controls. After treatment, the DOC in the abiotic controls increased significantly from the initial effluent to 36.54 ± 0.85 mg/L. This increase suggests that a portion of the un-quantified particulate-bound OC concentration was dissolved into the aqueous fraction of the effluent by the NaN_3 in the abiotic controls. There was no significant difference in the DOC concentration in the initial effluent and after aerobic treatment. Chapter 2 found that TOC is predominantly particulate-bound in the untreated effluent at KFRAG, and that microbial digestion, albeit anaerobically, resulted in a TOC concentration that was predominantly in the aqueous fraction of the effluent as DOC. Based on this previous data, it can be assumed that the DOC concentration in the initial effluent made only a small fraction of the TOC concentration, but the DOC concentration in the aerobic reactors comprised the majority of the TOC concentration after treatment. If this assumption is correct, then the TOC concentration after aerobic treatment was likely lower than the TOC concentration of the initial effluent and abiotic controls, making microbial respiration responsible for the organic matter reduction and nutrient mineralization observed in the aerobic reactors.

3.4.4. Aerobic Reactor Total Suspended Solids Reduction

The stabilization of TSS concentrations was used as the metric for determining the completion of particulate mineralization in the aerobic reactors. Delaide et al. (2018), achieved a nearly identical TSS reduction of 60.81% in a similar study on the aerobic treatment of aquaponic effluent in reactors with a 15-day hydraulic retention time. In Delaide et al (2018), reduction in TSS was reported only in the initial aquaponic effluent and after treatment. The TSS concentration of the aerobic reactors were measured at 48-hour intervals throughout this experiment, which provided insight into the timeline of solids reduction in aquaponic/RAS effluent. The majority of TSS reduction occurred at the beginning of the experiment. By day 5, 67.19 ± 2.01% of the TSS reduction achieved throughout the 15-day experiment was completed. During the final 10 days of reactor operations only 32.81 ± 2.01% of the overall reduction in TSS concentration occurred. As the majority of the TSS concentration was removed from the effluent in early in the treatment process, consideration should be taken in regards to the benefit of treatment beyond day 5 compared to the cost of treatment beyond day 5.

3.4.5. Treated Effluent Nutrient Profile

The aerobic reactors used in this experiment significantly increased the plant available concentrations of N, P, Ca, B, Ca, and Al. The plant available nutrient concentrations after aerobic digestion were compared to nutrient recommendations for hydroponic lettuce and leafy green production to estimate the fertilizing potential of treated effluent (**Error! Reference source not found.**). While aerobic digestion was an effective means of increasing the plant availability of particulate-bound nutrients in the effluent, only N, K, Cu, and Zn met or exceeded the recommended concentrations for hydroponic lettuce and leafy green productions. The routine K and Fe supplementation of KFRAG should also be noted when comparing the nutrient profile of the treated effluent to a commercial fertilizer solution of the effluent from other aquaponic/RAS facilities. Due to K supplementation, the total concentration and plant availability of K was greater in the KFRAG effluent than the effluent analyzed in other studies (Monsees et al., 2017; Goddek et al., 2018). Routine additions of chelated Fe were made to the KFRAG aquaponic system as the mass of Fe in the feed used in this experiment was not sufficient to meet the needs of plants (Resh, 2012). The Fe concentration in the KFRAG effluent more closely resembled the concentration in Anderson (2016) where chelated Fe was also supplemented, then the plant available concentrations from other facilities (Monsees et al., 2017; Goddek et al., 2018). The limited mass of Fe in commercially available feeds and the limited mineralization of particulate-bound Fe by aerobic digestion shown in this study demonstrate the need for Fe supplementation in the aquaponic industry to fully meet the nutritional needs of the crops. Although the mass and initial plant availability of Fe is not comparable between KFRAG and other aquaponic facilities, this study still demonstrated that aerobic digestion did not significantly increase the plant availability of Fe in aquaponic effluent.

Table 3-5. Plant available concentrations of nutrients after aerobic digestion compared against the concentrations found in a commercial hydroponic lettuce and leafy green fertilizing solution (fertilizer information retrieved from jrpeters.com)**.**

Nutrient	Aerobic (mg/L)	Jack's Hydroponic (mg/L)
Macro-nutrients		
N	171	150
P	3.6	39
K[†]	402	162
Ca	29.3	139
Mg	21.7	47
Micro-nutrients		
Fe[†]	1.8	2.3
Mn	0.26	0.38
Cu	0.167	0.113
Zn	0.79	0.11
Na	34.5	0
Al	0.44	N/A

[†]Supplemented nutrient in KFRAG

Aerobic digestion was able to increase the overall plant availability for many of the nutrients in the effluent. However, the majority of the nutrient concentrations were still below what was present in the commercial solution. The plant availability of the nutrients was maximized after aerobic treatment, making the total concentration of each nutrient in the effluent the new limiting factor in developing a hydroponic nutrient solution. Supplementation with traditionally derived fertilizer salts is needed achieve the similar nutrient concentrations for hydroponic lettuce and leafy green production. Nutrient supplementation may affect USDA organic certification of hydroponic operations. The reuse of treated effluent would, however, begin to reduce to reliance on finite mineral reserves and provide aquaponic/RAS produces an alternative waste treatment option.

3.4.6. Organic Carbon Removal

As noted above, lab closures caused by the COVID-19 outbreak prevented a complete analysis of the TOC removal achieved by the aerobic reactors used in this study. The removal of

OC is required before any waste can be effectively utilized as a hydroponic fertilizing solution. Excess OC can result in biofilm accumulation caused by the colonization of pathogenic heterotrophic bacteria (Yaron and Römling, 2014). It has also been found that OC accumulation can have a phytotoxic effect on plants by negatively affecting physiological functions (Garland et al., 1997; J. G. Lee et al., 2006). Without a complete TOC analysis of the KFRAG effluent prior to and after aerobic treatment, the TOC removal achieved in this experiment cannot be determined. However, literature has shown that aerobic digestion can remove between 70% and 99% of the organic matter in agricultural and municipal waste, and that the degree of removal in aerobic digestion is greater than the degree of OC removal in other forms of microbial digestion (Borzacconi et al., 1999; Del Pozo & Diez, 2003; B. Delaide et al., 2018).

3.4.7. Combined Treatment Approach

Although previously published experiments have shown that waste treated using aerobic microbial digestion resulted in greater reductions in OC than anaerobic digestion, a combined treatment approach has consistently removed over 90% of organic matter while incorporating the benefits of both treatment methods (Borzacconi et al., 1999; Del Pozo and Diez, 2003; Delaide et al., 2018). Anaerobic digestion typically results in less sludge production after treatment and, without the need for constant aeration, often has a lower operating cost than aerobic treatment (Del Pozo and Diez, 2003; Tchobanoglous et al., 2014). In a study to characterize the mineralization of particulate-bound nutrients in aquaponic effluent using anaerobic batch reactors, Chapter 2 reported a mean ± SD reduction in TSS concentration of 76.17 ± 6.97% over 15 days. This was significantly greater than the mean ± SD reduction in TSS concentration of 60.96 ± 0.43% observed over the same time period in the aerobic reactors used in this experiment (p = 0.0198).

Chapter 2 also observed a mean ± SD TOC reduction of only 47.42 ± 12.76%, which is lower than experiments utilizing either solely aerobic treatment methods or a combination of anaerobic and aerobic digestion (Borzacconi et al., 1999; Del Pozo and Diez, 2003; Delaide et al., 2018). Delaide et al. (2018) reported a 68.48% TOC reduction in aquaponic effluent after aerobic treatment. A similar study reported a comparable organic matter removal rate of 74% after the aerobic treatment of cattle wastewater (Othman et al., 2013). Additionally, Mashal et al. (2017) found that the organic matter concentration in landfill leachate was reduced by 78% after aerobic treatment. A two-stage anaerobic to aerobic treatment approach was utilized in Borzacconi et al. (1999) and Del Pozo and Diez (2003). More than 90% of the organic matter was removed in both studies, suggesting that a combined approach may also be more effective at TOC removal in aquaponic effluent. More than 90% of the nitrogen mass was removed in the combined treatment approach reported in Del Pozo and Diez (2003). A combined anaerobic to aerobic treatment approach would result in a reduced nitrogen mass comparable to the reduction reported in the sole anaerobic treatment reported in Chapter 2.

The reduction in TN resulted in a balanced solution with nutrient ratios closely aligned to the nutrient ratios of commercial hydroponic solutions (Chapter 2). Although many of the nutrient concentrations in the anaerobically treated effluent were below the nutrient concentrations of the commercial solution, the similar nutrient ratios would for a concentrated anaerobically-derived solution to be comparable to commercial fertilizing solutions. The remaining plant available TN is comprised primarily of ammonia-nitrogen (NH_4-N) after anaerobic treatment (Chapter 2). Most hydroponically grown plants prefer nitrate-nitrogen (NO_3-N), which comprised 98.07% of the plant available TN after aerobic treatment, over NH_4-N (Ikeda and Osawa, 1981). Utilizing a combined anaerobic to aerobic approach to the mineralization of aquaponic effluent may achieve more complete TSS reduction and OC removal, as demonstrated in municipal treatment systems (Del Pozo and Diez, 2003). Additional benefits of a combined treatment approach may include matching the nutrient ratios of the treated effluent to the nutrient ratios of commercial solutions and maintaining the ideal form of nitrogen for hydroponic plant uptake. Additional research on a multi-stage treatment system utilizing anaerobic digestion to reduce solids and aerobic digestion to reduce OC and convert NH_4-N into NO_3-N is required to identify the optimal method to develop a naturally-derived hydroponic nutrient solution from aquaponic/RAS effluent.

3.5. Conclusions

This study demonstrated a decrease in TSS and an increase in the plant availability of several plant-essential macro- and micro-nutrients present in aquaponic/RAS effluent. Although nutrient availability was increased in the aquaponic effluent after aerobic treatment, the concentration of the majority of nutrients in the treated effluent was still below the recommended concentrations for hydroponic lettuce and leafy green production. The aerobically-treated effluent would require supplementation to match the concentrations of typically administered to hydroponic crops using commercially available fertilizer solutions. A hydroponic fertilizer solution developed from a mixture of naturally- and traditionally-derived nutrients would provide aquaponic/RAS produces an alternative to current effluent treatment methods and help offset the reliance on finite mineral reserves that is projected to negatively affect agricultural producers in the coming decades (Henckens et al., 2016; Hunter et al., 2017), and improve plant growth rates as compared to plants fertilized only with aquaponic nutrient solutions. However, a fertilizer solution that is not solely derived from capture and re-used effluent would not provide hydroponic producers an opportunity to earn the USDA Organic certification.

Future research is required for additional TOC removal in aquaponic/RAS effluent through aerobic digestion. Analysis lab closures caused by the COVID-19 outbreak prevented a complete analysis of the treated effluent developed for this experiment. Additional research evaluating the effects of a combined anaerobic and aerobic treatment approach would have on TSS reduction, TOC removal, plant availability of nutrients, and relative nutrient ratios in aquaponic/RAS effluent is needed to refine the development of a naturally-derived nutrient solution.

CHAPTER 4
CONCLUSION

The overall goal of this research was to evaluate the effectiveness of two microbial digestion waste treatment methods for developing a naturally-derived hydroponic nutrient solution from aquaponic/RAS effluent. Two lab-scaled experiments were conducted to determine the effect of microbially-mediated anaerobic and aerobic digestion on effluent from a farm-scaled coupled RAS and hydroponic facility. The first experiment characterized the mass of nutrients mineralized through anaerobic digestion and the second experiment characterized mass of nutrients mineralized through aerobic digestion. Reductions in total suspended solids (TSS) and total organic carbon (TOC) concentrations and an increase in the nutrient mass dissolved in the aqueous fraction of the effluent and available for utilization by hydroponic plants were used to evaluate the effectiveness of the two treatment methods. Both treatment methods were shown to be effective at significantly reducing TSS and increasing the plant available concentration of several macro- and micro-nutrients in RAS effluent. The TOC concentration was significantly decreased after anaerobic treatment. However, the effect of aerobic treatment on TOC concentration could not be determined due to lab closures resulting from COVID-19.

Previous studies on the microbial anaerobic (Monsees et al., 2017; Delaide et al., 2018; Goddek et al., 2018) and aerobic (Monsees et al., 2017; Delaide et al., 2018; Khiari et al., 2019) digestion of aquaponic/RAS effluent demonstrated similar findings in regards to the solids reduction, organic matter removal, and nutrient mineralization. An advancement to reactor operation achieved through this research was confirmation of oxidative reduction potential (ORP) stabilization as a metric for determining the completion of TSS mineralization in a specific anaerobic batch reactor. Additional insight into reactor TSS reduction was also achieved through this research. The TSS reduction and time of treatment for both experiments in this study were similar to Delaide et al. (2018). However, Delaide et al. (2018) only reported the overall percent reduction in TSS for anaerobic and aerobic treatment. With routine TSS analysis conducted throughout the operation of each reactor in both experiments, this research identified that rapid TSS reduction occurred in the early stages of the treatment process. Continued research on the cost of treatment against the benefits of maximum TSS mineralization would help to determine when treatment cost outweighs the benefits of developing a naturally-derived nutrient solution.

While several studies compared the nutrient profile of the treated effluent against commercially available nutrient solutions, none examined the nutrient profile on the basis of nutrient ratios (Monsees et al., 2017; Goddek et al., 2018). The anaerobic and aerobic treatments were effective at increasing the plant availability of nutrients found in aquaponic/RAS effluent. After treatment the total concentration of nutrients in the effluent became the limiting factor for meeting plant nutrient needs. The majority of nutrients in both treated solutions were still below concentrations present in a commercial hydroponic nutrient solution for lettuce and leafy green production. However, due to denitrification, the majority of the nutrient ratios in the effluent after anaerobic treatment were comparable to the nutrient ratios of the commercial solution. This would make a concentrated anaerobically-treated solution have a similar nutrient profile as commercial solution. With nitrogen being maintained, the nutrient ratios of the effluent following aerobic treatment did not resemble the nutrient ratios of the commercial solution. Nutrient supplementation would be needed in the aerobically-treated effluent to match the nutrient concentrations or ratios in the commercial solution.

Continued research is required to increase organic carbon removal in RAS effluent treatment. Although the nutrient profile suggests that the treated effluent could be used in either hydroponic or aquaponic production, the presence of OC may still prevent effective re-utilization. Pathogen proliferation, biofilm blocked irrigation tubing, and stunted growth can all result from excessive OC in an aquaponic or hydroponic system (Lee et al., 2006; Yaron and Römling, 2014; Lee et al., 2015). Both treatment methods used in this research resulted in significant, but not complete, OC reductions. Several studies have shown that a combined approach incorporating anaerobic treatment with and aerobic finishing stage can result in greater organic matter reduction than either treatment method operated on an individual basis (Borzacconi et al., 1999; Del Pozo and Diez, 2003). Continued research on a combined approach to increase OC removal is required to maximize the efficiency of the effluent as a nutrient solution.

The development of a naturally-derived hydroponic nutrient solution from RAS effluent would have a multi-faceted impact on the controlled environmental agriculture (CEA) industry. The CEA industry optimizes environmental growth parameters to provide year-round, location-independent vegetables, fruits, and seafood at maximum growth rates and yields. Increased utilization of CEA technologies will be required to meet the food demands of the growing global population and counter the reduction in farmable lands as a result or urbanization (Hunter et al., 2017). The CEA industry is currently limited by the cost of RAS solid waste disposal, the operating cost of technologies required maintain controlled environmental parameters, and a reliance on finite mineral reserves for crop fertilizing solutions (Treftz and Omaye, 2015; Hunter et al., 2017; Tsani and Koundouri, 2018). The capture, treatment, and reuse of RAS effluent as a hydroponic nutrient solution would enable a new integrated CEA model similar to that of terrestrial agriculture. Reliance on finite mineral reserves would be diminished, hydroponic producers would progress towards United States Department of Agriculture (USDA) organic certification for an added value crop to reduce operating costs, and the monetization of the effluent from RAS would allow producers to offset waste treatment costs.

The continued optimization of CEA food production processes to meet future demands is resulting in an increase in commercial aquaponic facilities (Goddek et al., 2019). Aquaponics is the co-production of crops and seafood through a combination of hydroponic and RAS technologies (Goddek et al., 2019). Recently published research on crop nutrient requirements in aquaponic systems indicate that the nutrient profile of the treated RAS effluent from Chapter 2 and 3 may be more appropriate for re-use in an aquaponic system than a traditional hydroponic system (Delaide et al., 2016). Delaide et al. (2016) found similar growth in lettuce grown under recommended hydroponic nutrient conditions and lettuce grown in aquaponic culture water with significantly lower nutrient concentrations. The same study also reported significantly greater lettuce growth in aquaponic culture water supplemented with nutrients to match a commercial solution than lettuce grown with the commercial hydroponic solution. Additional research is needed to identify why aquaponic lettuce growth at low nutrient concentrations is similar to hydroponic lettuce growth at greater nutrient concentrations. However, the results of Delaide et al. (2016) suggest that an aquaponic system supplemented with either anaerobically or aerobically treated effluent would result in greater lettuce growth rates than a hydroponic system operated with the same nutrient concentrations.

The data collected in this study can be used to begin designing treatment systems at a larger scale to meet the needs of a commercial facility. The specifications of the RAS or aquaponic system supplying the effluent is an important consideration for future research. Nutrient masses and ratios in the effluent are dependent on multiple factors including feed, fish species, and crop presence and variety. (Seawright et al., 1998; Guerdat et al., 2013; Monsees et al., 2017). Effluent nutrient characterizations from commercial RAS and aquaponic facilities with outputs other than tilapia and lettuce are required to further evaluate the nutrient variations between RAS and aquaponic facilities and between aquaponic facilities with different crops. Continued research is also needed to improve the environmental operating parameters of the treatment process and to address the potential benefits of a combined anaerobic to aerobic treatment approach. The optimization of the effluent treatment process has the potential to improve the RAS, hydroponic, and aquaponic industries, meet future food production needs, reduce reliance on finite mineral reserves for fertilizer production.

LIST OF REFERENCES

399 F.3d 486 (2nd Cir. 2005), 03-4470, Waterkeeper Alliance, Inc. V. U.S. E.P.A. (2004). Vlex.

Adesemoye, A., and Kloepper, J. (2007). Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl Microbiol Biotechnol., 85 (1), 1-12.

Anderson, T. (2016). Biological responses of lettuce to hydroponic and aquaponic conditions. Cornell University.

Anukam, A., Mohammadi, A., Naqvi, M., and Granström, K. (2019). A Review of the Chemistry of Anaerobic Digestion: Methods of Accelerating and Optimizing Process Efficiency. Processes, 7, 504. https://doi.org/10.3390/pr7080504

Badiola, M., Mendiola, D., and Bostock, J. (2012). Recirculating Aquaculture Systems (RAS) analysis: Main issues on management and future challenges. Aquacultural Engineering, 51, 26–35. https://doi.org/10.1016/j.aquaeng.2012.07.004

Barbot, E., Seyssiecq, I., Roche, N., and Marrot, B. (2010). Inhibition of activated sludge respiration by sodium azide addition: Effect on rheology and oxygen transfer. Chemical Engineering Journal, 163, 230–235.

Bergland, W., Dinamarca, C., and Bakke, R. (2015). Temperature Effects in Anaerobic Digestion Modeling. 261–269.

Borzacconi, L., López, I., Ohanian, M., and Viñas, M. (1999). Anaerobic-Aerobic Treatment of Municipal Solid Waste Leachate. Environmental Technology, 20(2), 211–217.

Botheju, D., and Bakke, R. (2011). Oxygen Effects in Anaerobic Digestion–A Review. ResearchGate. http://dx.doi.org/10.2174/1876400201104010001

Bryant, C. (1987). Lagoons, Ponds, and Aerobic Digestion. Journal (Water Pollution Control Federation), 59(6), 401–403. JSTOR.

Celis-García, M., Ramírez, F., Revah, S., Razo-Flores, E., and Monroy, O. (2004). Sulphide and Oxygen Inhibition over the Anaerobic Digestion of Organic Matter: Influence of Microbial Immobilization Type. Environmental Technology, 25(11), 1265–1275.

Chen, S., Coffin, D., and Malone, R. (1997). Sludge Production and Management for Recirculating Aquacultural Systems. Journal of the World Aquaculture Society, 28(4), 303–315.

Chen, Y., Cheng, J., and Creamer, K. (2008). Inhibition of anaerobic digestion process: A review. Bioresource Technology, 99(10), 4044–4064.

Cieślik, B., and Konieczka, P. (2017). A review of phosphorus recovery methods at various steps of wastewater treatment and sewage sludge management. The concept of “no solid waste generation” and analytical methods. Journal of Cleaner Production, 142, 1728–1740.

Cioabla, A., Ionel, I., Dumitrel, A., and Popescu, F. (2012). Comparative study on factors affecting anaerobic digestion of agricultural vegetal residues. Biotechnology for Biofuels, 5, 5-39.

Conroy, J., and Couturier, M. (2010). Dissolution of minerals during hydrolysis of fish waste solids. Aquaculture, 298(3), 220–225.

Cooil, B., and Slattery, M. (1948). Effects of potassium deficiency and excess upon certain carbohydrate and nitrogenous constituents in guayule1. Plant physiology, 23(4), 425–442.

Cordell, D., Drangert, O., and White, S. (2009). The story of phosphorus: Global food security and food for thought. Global Environmental Change, 19(2), 292–305.

Cripps, S., and Bergheim, A. (2000). Solids management and removal for intensive land-based aquaculture production systems. Aquacultural Engineering, 22, 33–56.

Crohn, D. (2004). Nitrogen mineralization and its importance in organic waste recycling. National Alfalfa Symposium, 5-13, December, 2004, San Diego, CA, UC Cooperative Extension, University of California, Davis

Crowder, D., and Reganold, J. (2015). Financial competitiveness of organic agriculture on a global scale. Proceedings of the National Academy of Sciences, 112(24), 7611–7616. https://doi.org/10.1073/pnas.1423674112

Dabkowski, B. (2006). Applying oxidation reduction potential sensors in biological nutrient removal systems. Hach Company.

Del Pozo, R., and Diez, V. (2003). Organic matter removal in combined anaerobic–aerobic fixed-film bioreactors. Water Research, 37(15), 3561–3568.

Delaide, B., Goddek, S., Keesman, K., and Jijakli, M. (2018). Une méthodologie pour quantifier les performances de digestion aérobie et anaérobie des boues, pour le recyclage des nutriments en aquaponie. Biotechnologie, Agronomie, Société et Environnement, 22(2), 106-112–112.

Delaide, B, Goddek, S., Gott, J., Soyeurt, H., Jijakli, M., (2016). Lettuce (Lactuca sativa L. Var. Sucrine) Growth Performance in Complemented Aquaponic Solution Outperforms Hydroponics. Water, 8(10), 467.

DeLong, D., Losordo, T., and Rakocy, J. (2009). Tank Culture of Tilapia. Southern Regional Aquaculture Center, 282.

dos Santos, S., Morals Chaves, S., and van Haandel, A. (2018). Influence of temperature on the performance of anaerobic treatment systems of municipal wastewater. Water Sa, 44(2), 211–222. https://doi.org/10.4314/wsa.v44i2.07

Eghball, B., Wienhold, B., Gilley, J., and Eigenberg, R. (2002). Mineralization of manure nutrients. Journal of Soil and Water Conservation, 57(6), 470–473.

Etienne, P., Sorin, E., Maillard, A., Gallardo, K., Arkoun, M., Guerrand, J., Cruz, F., Yvin, J.-C., and Ourry, A. (2018). Assessment of Sulfur Deficiency under Field Conditions by Single Measurements of Sulfur, Chloride and Phosphorus in Mature Leaves. Plants (Basel), 7(2).

FAO. (2018). The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals. Rome. Licence: CC BY-NC-SA 3.0 IGO.

Fan, Y., Klemeš, J., Lee, C., and Perry, S. (2018). Anaerobic digestion of municipal solid waste: Energy and carbon emission footprint. Journal of Environmental Management, 223, 888–897. https://doi.org/10.1016/j.jenvman.2018.07.005

Funck, M., Simões, J., Ferreira, M., Martins, E. de F., Oliveira, F. A. da S., Dos, L., Rodrigues, S., Melo, M., and Filho, K. (2019). Water Quality and Effluents Generated during Rainbow Trout Culture in a Raceway System. Biomedical Journal of Scientific and Technical Research, 16(5), 1–12. https://doi.org/10.26717/BJSTR.2019.16.002921

Furtner, B., Bergstrand, K., Brand, T., Jung, V., and Alsanius, B. (2007). Abiotic and Biotic Factors in Slow Filters Integrated to Closed Hydroponic Systems. European Journal of Horticultural Science, 72(3), 104–112. JSTOR.

Garland, J., Mackowiak, C., Strayer, R., and Finger, B. (1997). Integration of waste processing and biomass production systems as part of the KSC Breadboard project. Advances in Space Research, 20(10), 1821–1826.

Ge, H., Jensen, P., and Batstone, D. (2011). Relative kinetics of anaerobic digestion under thermophilic and mesophilic conditions. Water Science Technology, 64 (4), 848-853.

Gebreeyessus, G., and Jenicek, P. (2016). Thermophilic versus Mesophilic Anaerobic Digestion of Sewage Sludge: A Comparative Review. Bioengineering, 3(2).

Gelfand, I., Barak, Y., Even-Chen, Z., Cytryn, E., Van Rijn, J., Krom, M. D., and Neori, A. (2003). A Novel Zero Discharge Intensive Seawater Recirculating System for the Culture of Marine Fish. Journal of the World Aquaculture Society, 34(3), 344–358.

Gerardi, M. (2007). ORP Management in Wastewater as an Indicator of Process Efficiency. Interstate Water Report.

Gilbert, F. (1951). The place of sulfur in plant nutrition. The Botanical Review, 17(9), 671–691.

Goddek, S., Delaide, B., Joyce, A., Wuertz, S., Jijakli, M., Gross, A., Eding, E., Bläser, I., Reuter, M., Keizer, L. C., Morgenstern, R., Körner, O., Verreth, J., and Keesman, K. (2018). Nutrient Mineralization and Organic Matter Reduction Performance of RAS-based Sludge in Sequential UASB-EGSB Reactors. Aquacultural Engineering, 83, 10-19.

Goddek, S., Joyce, A., Kotzen, B., and Burnell, G. (2019). Aquaponics Food Production Systems: Combined Aquaculture and Hydroponic Production Technologies for the Future. Springer Open.

Guerdat, T., Losordo, T., Classen, J., Osborne, J., and Delong, D. (2011). Evaluating the effects of organic carbon on biological filtration performance in a large scale recirculating aquaculture system. Aquacultural Engineering, 44(1), 10–18.

Guerdat, T., Losordo, T., Delong, D., and Jones, R. D. (2013). An evaluation of solid waste capture from recirculating aquaculture systems using a geotextile bag system with a flocculant-aid. Aquacultural Engineering, 54, 1–8.

Habermacher, J., Benetti, A., Derlon, N., and Morgenroth, E. (2016). Degradation of the unbiodegradable particulate fraction (XU) from different activated sludges during batch digestion tests at ambient temperature. Water Research, 98, 206–214.

Henckens, M., van Ierland, E., Driessen, P., and Worrell, E. (2016). Mineral resources: Geological scarcity, market price trends, and future generations. Resources Policy, 49, 102–111.

Hills, D. (1979). Effects of carbon: Nitrogen ratio on anaerobic digestion of dairy manure. Agricultural Wastes, 1(4), 267–278. https://doi.org/10.1016/0141-4607(79)90011-8

Horan, N., Yaser, A. Z., and Wid, N. (2018). Anaerobic Digestion Processes. Springer. 978-981-10-8129-3

Huiwen, C., and Yinglan, S. (2007). Management of marine cage aquaculture. Environmental Science and Pollution Research - International, 14(7), 463–469. https://doi.org/10.1065/espr2007.05.423

Hunter, M., Smith, R., Schipanski, M., Atwood, L., and Mortensen, D. (2017). Agriculture in 2050: Recalibrating Targets for Sustainable Intensification. Bioscience, 67(4), 386–391.

Hutchings, J., and Reynolds, J. (2004). Marine Fish Population Collapses: Consequences for Recovery and Extinction Risk. BioScience, 54(4), 297–309. https://doi.org/10.1641/0006-3568(2004)054[0297:MFPCCF]2.0.CO;2

Ikeda, H., and Osawa, T. (1981). Nitrate- and Ammonium-N Absorption by Vegetables from Nutrient Solution Containing Ammonium Nitrate and the Resultant Change of Solution ph. Journal of the Japanese Society for Horticultural Science, 50(2), 225–230.

JMP® Pro, Version 14.2.0, (2018) SAS Institute Inc., Cary, NC, USA.

Khiari, Z., Kaluthota, S., and Savidov, N. (2019). Aerobic bioconversion of aquaculture solid waste into liquid fertilizer: Effects of bioprocess parameters on kinetics of nitrogen mineralization. Aquaculture, 500, 492–499.

Lages Barbosa, G., Almeida Gadelha, F., Kublik, N., Proctor, A., Reichelm, L., Weissinger, E., Wohlleb, G., and Halden, R. (2015). Comparison of Land, Water, and Energy Requirements of Lettuce Grown Using Hydroponic vs. Conventional Agricultural Methods. International Journal of Environmental Research and Public Health, 12(6), 6879–6891. https://doi.org/10.3390/ijerph120606879

LaPara, T., and Alleman, J. (1999). Thermophilic aerobic biological wastewater treatment. Water Research, 33(4), 895–908.

Lee, J., Lee, B., and Lee, H. (2006). Accumulation of phytotoxic organic acids in reused nutrient solution during hydroponic cultivation of lettuce (Lactuca sativa L.). Scientia Horticulturae, 110(2), 119–128.

Lee, S., Ge, C., Bohrerova, Z., Grewal, P., and Lee, J. (2015). Enhancing plant productivity while suppressing biofilm growth in a windowfarm system using beneficial bacteria and ultraviolet irradiation. Canadian Journal of Microbiology, 61 (7), 457-466.

Liu, J., Luo, Z., Liu, K., Zhang, Y., Peng, H., Hu, B., Ren, H., Zhou, X., Qiu, S., He, X., Ye, P., Bastani, H., and Lou, L. (2017). Effect of flushing on the detachment of biofilms attached to the walls of metal pipes in water distribution systems. Journal of Zhejiang University-SCIENCE A, 18(4), 313–328. https://doi.org/10.1631/jzus.A1600316

Losordo, T., Masser, M., and Rakocy, J. (1999). Recirculating Aquaculture Tank Production Systems A Review of Component Options. 12.

Ma, J., Frear, C., Wang, Z., Yu, L., Zhao, Q., Li, X., and Chen, S. (2013). A simple methodology for rate-limiting step determination for anaerobic digestion of complex substrates and effect of microbial community ratio. Bioresource Technology, 134, 391–395.

Ma, Y., Gu, J., and Liu, Y. (2018). Evaluation of anaerobic digestion of food waste and waste activated sludge: Soluble COD versus its chemical composition. Science of The Total Environment, 643, 21–27. https://doi.org/10.1016/j.scitotenv.2018.06.187

Maier, R., Pepper, I., and Gerba, C. (2009). Environmental Microbiology (2nd ed.). Academic Press.

Malone, R., and Beecher, L. (2000). Use of floating bead filters to recondition recirculating waters in warmwater aquaculture production systems. Aquacultural Engineering, 22, 57–73.

Marschner, H. (2011). Mineral Nutrition of Higher Plants—2nd Edition (3rd ed.). Academic Press.

Mashal, A., Al-Laqtah, N., and Abu-Dahrieh, J. (2017). Biological Treatment of Landfill Leachate using Sequential Batch Reactor. 12.

Masser, M., and Lazur, A. (1997). In-pond raceways. Southern Regional Aquaculture Center, 170, 1–8.

Masser, M., Rakocy, J., and Losordo, T. (1999). Recirculating aquaculture tank production systems. SRAC Publication No. 452.

Mata-Alvarez, J., Macé, S., and Llabrés, P. (2000). Anaerobic digestion of organic solid wastes. An overview of research achievements and perspectives. Bioresource Technology, 74(1), 3–16. https://doi.org/10.1016/S0960-8524(00)00023-7

Merriam, J., Mcdowell, W., and Currie, W. (1996). A high-temperature catalytic oxidation technique for determing total dissolved nitrogen. Soil Science Society of America, 60, 1050–1055.

Miller, D., and Semmens, K. (2002). Waste management in aquaculture. West Virginia University Extension Service Publication No. AQ02-1. USA, 8.

Moffitt, C., and Cajas-Cano, L. (2014). Blue Growth: The 2014 FAO State of World Fisheries and Aquaculture. Fisheries, 39(11), 552–553.

Monsees, H., Keitel, J., Paul, M., Kloas, W., and Wuertz, S. (2017). Potential of aquacultural sludge treatment for aquaponics: Evaluation of nutrient mobilization under aerobic and anaerobic conditions. Aquaculture Environment Interactions, 9, 9–18.

Morath, S. (2018). Hydroponics: The End of Organic? Natural Resources and the Environment. 33(1), 1-4.

Naylor, R., Hindar, K., Fleming, I., Goldburg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D., and Mangel, M. (2005). Fugitive Salmon: Assessing the Risks of Escaped Fish from Net-Pen Aquaculture. BioScience, 55(5), 427–437. https://doi.org/10.1641/0006-3568(2005)055[0427:FSATRO]2.0.CO;2

Othman, I., Anuar, A., Ujang, Z., Rosman, N., Harun, H., and Chelliapan, S. (2013). Livestock wastewater treatment using aerobic granular sludge. Bioresource Technology, 133, 630–634. https://doi.org/10.1016/j.biortech.2013.01.149

Parkin, G., and Owen, W. (1986). Fundamentals of Anaerobic Digestion of Wastewater Sludges. Journal of Environmental Engineering, 112(5), 867–920.

Rakocy, J., Masser, M., and Losordo, T. (2006). Recirculating aquaculture tank production systems: Aquaponics-Integrating fish and plant culture. Southern Regional Aquaculture Center, 454.

Resh, H. (2012). Hydroponic Food Production: A Definitive Guidebook for the Home Gardner and the Commercial Hydroponic Grower (7th ed.). CRC Press.

Ruffino, B., Campo, G., Genon, G., Lorenzi, E., Novarino, D., Scibilia, G., and Zanetti, M. (2015). Improvement of anaerobic digestion of sewage sludge in a wastewater treatment plant by means of mechanical and thermal pre-treatments: Performance, energy and economical assessment. Bioresource Technology, 175, 298–308.

Samer, M. (2015). Biological and Chemical Wastewater Treatment Processes. Wastewater Treatment Engineering. Intech Europe.

Scherer, H. (2001). Sulphur in crop production—Invited paper. European Journal of Agronomy, 14(2), 81–111.

Schneider, O., Sereti, V., Eding, E., and Verreth, J. (2005). Analysis of nutrient flows in integrated intensive aquaculture systems. Aquacultural Engineering, 32(3–4), 379–401.

Schooley, K., Bourgier, V., and Lawson, R. (2017). Evaporation and Crystallisation Challenges. World Fertilizer.

Seawright, D., Stickney, R., and Walker, R. (1998). Nutrient dynamics in integrated aquaculture–hydroponics systems—ScienceDirect. Aquaculture, 160, 215–237.

Sharrer, M., Rishel, K., Taylor, A., Vinci, B., and Summerfelt, S. (2010). The cost and effectiveness of solids thickening technologies for treating backwash and recovering nutrients from intensive aquaculture systems. Bioresource Technology, 101, 6630–6641.

Shinohara, M., Aoyama, C., Fujiwara, K., Watanabe, A., Ohmori, H., Uehara, Y., and Takano, M. (2011). Microbial mineralization of organic nitrogen into nitrate to allow the use of organic fertilizer in hydroponics. Soil Science and Plant Nutrition, 57, 190–203.

Tchobanoglous, G., Stensel, H., Tsuchihashi, R., and Burton, F. (2014). Wastewater Engineering: Treatment and Resource Recovery (5th ed.). Mcgraw-Hill Publishing Company.

Timmons, M., Guerdat, T., and Vinci, B. J. (2018). Recirculating Aquaculture 4th Edition (4th edition). Ithaca Publishing Company LLC.

Tovar, A., Moreno, C., Mánuel-Vez, M. P., and García-Vargas, M. (2000). Environmental impacts of intensive aquaculture in marine waters. Water Research, 34(1), 334–342. https://doi.org/10.1016/S0043-1354(99)00102-5

Treftz, C., and Omaye, S. (2015). Comparisons between hydroponic and soil systems for growing strawberries in a greenhouse. 6.

Tsani, S., and Koundouri, P. (2018). A Methodological Note for the Development of Integrated Aquaculture Production Models. Frontiers in Marine Science, 4 (406).

Turcios, A., and Papenbrock, J. (2014). Sustainable Treatment of Aquaculture Effluents—What Can We Learn from the Past for the Future? Sustainability, 6(2), 836–856.

Ugwuanyi, J., Harvey, L., and McNeil, B. (2005). Effect of digestion temperature and pH on treatment efficiency and evolution of volatile fatty acids during thermophilic aerobic digestion of model high strength agricultural waste. Bioresource Technology, 96(6), 707–719

USDA. USDA Natural Resources Conservation Service, Agricultural waste management field handbook. (2013). 134.

Van Rijn, J. (1996). The potential for integrated biological treatment systems in recirculating fish culture—A review. Aquaculture, 139(3–4), 181–201.

Yaron, S., and Römling, U. (2014). Biofilm formation by enteric pathogens and its role in plant colonization and persistence. Microbial Biotechnology, 7(6), 496–516.

Yogev, U., Sowers, K., Mozes, N., and Gross, A. (2017). Nitrogen and carbon balance in a novel near-zero water exchange saline recirculating aquaculture system. Aquaculture, 467, 118–126.

Zhang, T., Jiang, R., and Deng, Y. (2017). Phosphorus Recovery by Struvite Crystallization from Livestock Wastewater and Reuse as Fertilizer: A Review. In Physico-Chemical Wastewater Treatment and Resource Recovery. Intech Open.

Zhang, X., Wang, X., Feng, W., Li, X., and Lu, H. (2020). Investigating COD and Nitrate–Nitrogen Flow and Distribution Variations in the MUCT Process Using ORP as a Control Parameter. ACS Omega, 5(9), 4576–4587.

Zhou, Y., Zhang, J., Zhang, Z., Wang, P., and Xia, S. (2019). PH dependent of the waste activated sludge reduction by short-time aerobic digestion (STAD) process. Science of The Total Environment, 649, 1307–1313.