Optimization of Operational Parameters in Biofloc Technology

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DEDICATION

“Dedicated to my biggest support system, my mother, Uzma Tallat, my caring sisters, Mariam and Sara, and my friends whose unconditional support and assistance has led me to this magnificent achievement”

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LIST OF ABBREVIATIONS

BFT	Bio Floc Technology
CA	Continuous Aeration
FLR	Feed-Loading Rate
FV	Floc Volume
FCR	Feed Conversion Ratios
GIFT	Genetically Improved Farmed Tilapia
IMTA	Integrated Multitrophic Aquaculture
IA	Intermittent Aeration
RAS	Recirculating Aquaculture Systems
TSS	Total Suspended Solids
VSS	Volatile Suspended Solids

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ABSTRACT

Biofloc technology, known as the blue revolution in the field of aquaculture, lowers the concentration of nitrogen, removes harmful substances and converts them into food sources by the addition of carbon through an external source to maintain the C/N ratio in aquaculture water. The purpose of this research was to investigate the effect of different C/N ratios and feed loading rates on the growth of bioflocs and explore the impact of intermittent aeration on the growth performance of Oreochromis niloticus (GIFT) in BFT. This study was performed in two phases. In the first phase, the effect of different C/N ratios and feed loading rates (FLRs) on the growth of bioflocs was studied by measuring TSS, VSS and FV. Moreover, the concentration of ammonia nitrogen (NH_4^+-N), nitrite nitrogen (NO_2^--N) and nitrate nitrogen (NO_3^--N) in indoor tanks without fish were measured. The C/N ratio with the maximum concentration of TSS, VSS, and FV and minimum concentration of ammonia nitrogen (NH_4^+-N) was identified. Three feed loading rates 20 g/m^2, 40 g/m^2, 60 g/m^2 for each C/N ratio of 12:1, 16:1, and 20:1 was studied using 9 treatments in duplicates, set up in indoor tanks with a volume of 70.4 L. The results demonstrated that the highest concentration of TSS and FV was achieved in C/N 16:1 and the feed loading rate of 60 g/m^2. The variation patterns of the average levels of total ammonia nitrogen showed a decreasing trend and maximum removal in the tank with the maximum concentration of TSS, VSS and FV. Proximate composition results showed an increasing trend in the Crude Protein content with the increase in FLR and C/N. The highest crude protein content (25.06 ± 0.22%) was observed in C/N 16:1 and FLR 40 g/m^2. In the second phase, the impact of different rates of intermittent aeration on water quality and the growth performance of Oreochromis niloticus (GIFT) with a C/N ratio of 16:1 was studied in a 47-day trial. A total of 224 fish (124.79 ± 5.89), fed twice daily with a commercial diet at 2% of their body weight, cultured in 4 treatments, continuous aeration (CA) and intermittent aeration with different duration of aeration and non-aeration cycles (CA, IA 5 min OFF/55 min ON, IA 10 min OFF/ 50min ON), IA 15 min OFF,45 min ON), in 8 outdoor tanks with a volume of 1000 L and a stocking density of 28 fish/tank. The results indicated a difference in the values of DO, EC, TDS, TSS, VSS and FV between treatments, the concentration decreased with the increase in the off-period aeration duration. TAN and phosphates showed a decreasing trend with the increase in off-period duration while nitrites and nitrates were not different among treatments. The highest fish weight gain (107.3 ± 2.90 g), length gain (1.6 ± 0.03 cm), and protein efficiency ratio (PER 3.45 ± 0.09) were measured in CA. The lowest (1.77 ± 0.03) and highest

(0.97 ± 0.03) feed conversion ratio (FCR) and the highest (1.37 ± 0.02 %/day) and lowest (0.83 ± 0.03 %/day) specific growth rate (SGR) were observed in IA (15 mins OFF/ 45 mins ON) and CA, respectively ($P < 0.05$). The highest crude protein content (25.12 ± 0.65%) was observed in IA (15 min OFF/45 min ON). Overall, the biofloc system with continuous aeration showed better results for the water quality and growth performance of GIFT Tilapia as compared to intermittent aeration.

CHAPTER 1

INTRODUCTION

1.1Background

In recent times, global aquaculture production has increased while the capture of fishery production has declined (SARKER et al., 2019). Between 1990 and 2012, the overall output of fisheries through aquaculture rose from 13.4 to 42.2% (FAO, 2014). Animal husbandry and fisheries are the world's major sources of animal protein. Fish and related aquaculture products are the most suitable and secure sources of animal protein as they include all the required amino acids and are also suitable sources of unsaturated fatty acids (Ruxton et al., 2005).

Nutrients play an important role in aquaculture production, as they promote the fish's development and health. Maintaining a healthy balance of nutrients is critical, ultimately leading to increased production. Between 2007 and 2008, the prices of the components used in fish feed rose significantly, ranging from 20 to 92% (FAO, 2010). Aquafeed, which is the most crucial expense, accounts for around 50–70% of the production budget allocation. Additionally, the industrial sector is increasingly using seafood to produce fishmeal and fish oil products as the demand for seafood for human consumption rises (Péron et al., 2010). Aquaculture produces its feed from inexpensive sources, specifically waste fish, which amounts to approximately 5–6 million metric tons (FAO, 2010). Consequently, to minimize the initial costs, there has been a focus on researching methods to replace fishmeal and fish oil with more affordable plant protein sources (SARKER et al., 2019). Therefore, there has been a significant reduction in the cost of protein sources, which has led to an increase in the economic sustainability of animal production, including aquaculture (Albuquerque I Rabello CBV I Santos MJB I Lima MB II et al., n.d.).

Biofloc technology (BFT) emerges as a promising technology in this field and changes the perspective about clear water being suitable for aquaculture (Avnimelech, 1999). It involves converting recyclables into protein-rich bioflocs, which is used as a natural live food in the culture system. The biofloc technology heavily consists of the advancement of suspended clusters composed of phytoplankton, microorganisms, and aggregates of both live and deceased organic materials, as well as bacterial grazers (Hargreaves, 2013). The goal is to recycle leftover feed and enhance feed utilization. It improves the utilization of a group of microorganisms to produce high-protein bioflocs, an additional food source that filter-feeding species can consume. The cost-effective sources of carbohydrates promote both the growth of heterotrophic bacteria in the pond and the release of nitrogen through the creation of microbial proteins (Avnimelech, 1999). However, ensuring the floc remains in suspension and that the water quality remains unchanged requires adequate aeration and mixing (Hargreaves, 2013). The microbes in the water, mostly heterotrophic bacteria, work as a biofilter to lower the amount of ammonia (NH_4) and make it easier for nitrogen to be absorbed, which is better than nitrification. Heterotrophic bacteria, which grow rapidly and produce more microbial biomass per unit of substrate, can restrain ammonium growth more effectively than nitrifying bacteria (Hargreaves, 2006). Internally, bioflocs are prioritized as a replacement for a fish meal, primarily generating it from a microbial meal (Kuhn et al., 2009). The particles exhibit an irregular shape and an uneven distribution of size. They are fine, may easily compress, and have a high level of porosity (>99%). Additionally, they are highly absorbent (Chu & Lee, 2004). These systems exhibit minimal water exchange, resulting in reduced water requirements and little effluent water output, hence minimizing their influence on the surrounding environment (Avnimelech, 2007). The system minimizes environmental degradation by reducing the need for pelleted feed and efficiently utilizing nutrients from fish waste and uneaten feed (Hargreaves, 2013). The species of fish chosen is an important factor as the suitability of this technology depends on how well the selected species can eat the bacterial flocculates and digest and assimilate the microbial protein.

1.2 Problem Statement

Biofloc technology promotes the development of microbes that are beneficial for improving water quality. These microbes are highly useful for transforming toxic substances such as ammonia into less harmful forms. However, the main problem in biofloc technology is managing the ever-growing levels of ammonia that could accumulate throughout the culture's growth, and to counter this difficulty, external carbon sources such as jaggery and molasses can be utilized. This will facilitate the development of heterotrophic bacteria to break down the ammonia efficiently. Moreover, continuous aeration is also required to maintain microbial activity, which eventually increases the operational cost of the fish farm. Essentially, the main objective of this effort is to find a way to manage the costs of aeration and to incorporate carbon to reduce ammonia levels efficiently. Therefore, it is a matter of concern to find innovative methods and implement effective strategies to establish cost-effective and environmentally suitable BFT systems. By doing so, aquaculture activities can be made economically feasible, and water quality standards for cultivating aquatic species can be fulfilled.

1.3Objectives of the Study

This study was carried out at a lab scale as well as on a pilot scale, and the objectives were:

1. To study the impact of C/N and feed loading rates (FLR) on biofloc growth.
2. To explore the use of intermittent aeration for fish/shrimp culture.

CHAPTER 2

LITERATURE REVIEW

This chapter provides an in-depth analysis of prior research undertaken by other scholars that is relevant to the current research subject. This chapter seeks to identify gaps, establish the context for the current research, and illustrate its significance through critically evaluating the existing literature. The discussion recognizes the contributions of past authors and provides a rationale for how the current study expands upon, differs from, or addresses the gaps left by these earlier works.

2.1Background

Future food production from our aquatic surroundings will primarily come from aquaculture. Aquaculture badly hurts biodiversity as it uses resources including land, water, and feed. These resources are then converted into products useful for society and after usage released into greenhouse gases and wastes from uneaten food, feces, and urine products, chemotherapeutics, microorganisms, parasites, and feral animals into the environment. Alterations in food webs and habitat loss are examples of indirect negative effects. Direct negative consequences can arise from introducing genetic material and exotic materials and from the discharge of poisonous chemicals, eutrophicating substances, illnesses, and parasites into natural populations (Troell et al., 2017a)

Aquaculture farming is widely practiced worldwide, and in many underdeveloped nations, it is primarily seen as a means of generating revenue and food. Aquaculture often uses freshwater and saltwater water as its primary supplies. Particularly in Pakistan, an agricultural nation with plenty of water resources. Pakistan has a lot of space in rivers, lakes, and ponds (8563820 km^2), making it an ideal place for aquaculture cultivation. Moreover, the Pakistani government controls an Exclusive Economic Zone spanning roughly 350 nautical miles. Furthermore, aquaculture as a whole accounts for nearly 1% of the nation's GDP (Laghari & Correspondence, 2018).

2.2BFT Mechanism

BFT is a water purification process that eliminates ammonia released by aquaculture organisms through the use of heterotrophs and nitrifying bacteria. It can be categorized into algae-based photoautotrophic, nitrifying bacteria-based chemoautotrophic, heterotrophic bacteria-based heterotrophic, and mixed trophic systems (Kim et al., 2020). The basis of Biofloc Technology

(BFT) is based on the decomposition of ammonia, which is produced by excrement and leftover feed, by efficient biofloc microorganisms. This ammonia serves as a nutrition for heterotrophs. BFT employs a cyclical framework in which efficient microorganisms thrive by consuming ammonia and creating bioflocs by bacterial aggregation. These flocs are then used as a food source for aquaculture animals, with little to no water replacement (Pinho et al., 2022). The pictorial view of the mechanism of biofloc technology is shown in Fig. 2.1, the same is also explained by (Yu et al., 2023).

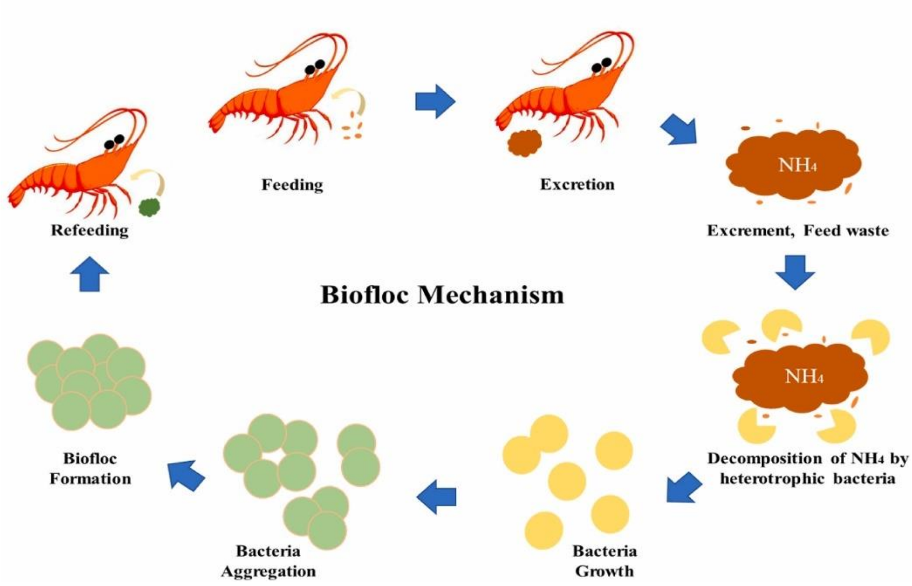


Figure 2.1*:* Mechanism of Biofloc Technology

2.3 Waste in Aquaculture System

Aquaculture is also a production enterprise, just like other enterprises worldwide. As it is a production enterprise wastes must be associated with it, either of unused inputs or by-products. Often such waste has very little or no economic value to the environment. In conventional methods of aquaculture systems, the amount of waste production from aqua culture irrespective of the fish culture, is very high, like one fish can generate nitrogen and phosphorous having weight of 0.8 and 0.1 kg respectively. This much production of waste by fish is comparable to the waste production of 73 people per day. Further, there is the generation of metabolic waste discharged by 63000 tons of fish produced which is equal to the waste generated by 5 million people in Japan in 1999 (Dauda et al., 2019). This concern shows that there is a need for a proper method to ensure sustainable identification of aquaculture. Godfray along with his fellows in 2010 introduced a production system i.e., sustainable intensification, and explained it as a system where more food is produced from the same area of land without increasing the environmental impacts (Charles et al., 2010).

Several methods are available to manage waste in aquaculture systems, such as Recirculating Aquaculture systems (RAS), Integrated Multitrophic aquaculture (IMTA), constructed wetlands, fishponds, and aquaponics. All these methods help manage water usage, waste, and dissolved nutrients by managing harmful by-products (Dauda et al., 2019). Among these, biofloc technology emerged as a promising solution for managing waste in aquaculture systems.

2.4 Biofloc Technology System

Biofloc technology has a minimal environmental impact when employed in closed systems since spent water is not dumped into natural water sources. BFT was first created as an affordable way to lessen waste and help the environment, particularly in places with limited water supplies. It is also a substitute for sustainable aquaculture; in this method, fish waste is converted into biomass using bacteria (called bioflocs). This system's adjusted CN ratio converts inorganic nitrogen into bioflocs, or microbial waste, which reduces inorganic nitrogen. BFT can serve as a sustainable and promising solution in wastewater reduction, and aquaculture production which uses minimum land use and water resources (Zafar & Rana, 2022). Since they lower feed conversion ratios (FCR), the bioflocs produced by this procedure are the best replacement for fishmeal in a diet. Protein-rich and devoid of organic polyhydroxybutyrate polymer, which is added to commercial diets to assist in constructing a full and healthy food chain and enhance growth performance, are two desirable characteristics of biofloc. Better biosecurity, reduced feed consumption, less disease introduction, increased growth and survival, decreased water exchange, and consequently higher production are all benefits of BFT.

2.4.1 History of BFT

Early in the 1970s, the French Research Institute for Exploitation of the Sea in Tahiti developed the bio floc technique. Prominent investigator Gerand Cuzon was a key innovator in this field. This led to the expansion of shrimp farming into the commercial sector. To begin research on BFT shrimp and fish farming, active suspension systems of microorganisms, or "microbial soup," were used (Raza et al., 2024a). To reduce pollutants, the most recent version of BFT strongly emphasizes the utilization of carbon substrates and zero-water-exchange technology.

The use of BFT broadened and tended to encompass a wide range of aquaculture species in the late 2000s. These species include bocachico (trochilus magdalalenae), golden crucian carp (Carassius auratus), giant freshwater shrimp (M. rosenbergii), pink shrimp (F. merguiensis), tiger shrimp (P. Monodon), white leg shrimp (L. vannamei), tilapia (Oreochromis niloticus), channel catfish (I. punctatus), and African cichlid (pseudotropheus saulosi). Because they efficiently consume bioflocs as a source of protein, shrimp and tilapia stand out among all these animal species as being suited for use in biofloc technology.

2.4.2 Applications of BFT

The BFT method has been effectively utilized for grow-out purposes, however, there is limited understanding of the advantages of bio-flocs in breeding. As an illustration, in the prawn business, the adoption of closed-cycle broodstock has emerged as a crucial measure to ensure biosecurity and prevent vertical transmissions of viruses, which have become widespread globally. Furthermore, this sector lays significant emphasis on penaeid breeding programs, which are typically carried out in enclosed facilities. These programs meticulously manage the production process throughout multiple generations (Marlowe et al., 2019a).

Bio-floc is an innovative method for managing diseases that differs from traditional procedures including antibiotics, antifungal treatments, and the use of probiotics and prebiotics. The inherent probiotic impact of BFT might manifest either internally or externally. This phenomenon is facilitated by a substantial assemblage of microorganisms, primarily bacteria, which are regarded as the initial trophic level inside the system (Emerenciano et al., 2013).

These days, aquaponics has successfully incorporated BFT. Rich biota, or biofloc microorganisms, and a range of minerals, including macro- and micronutrients derived from unconsumed or partially digested feed, appear to have a role in plant nutrition. UVI also developed a well-known example of the connection between aquaponics and bio-floc (Battisti et al., 2024). Nonetheless, special consideration must be given to the use of BFT in aquaponics, particularly concerning the control of solid levels in the water. Excessive adhesion of microorganisms to plant roots (biofilm) might result in injury to the roots due to high concentration of solids. reduced oxygenation and stunted development. It's common to need settling and filtering devices.

2.5 Microorganisms in BFT

Microbes are essential to aquaculture systems. While many creatures benefit the environment, such as removing pollutants, providing food for other species, and recycling organic waste, certain microbes can have negative effects. There are 24.6% phytoplankton, 3% bacteria, and a tiny percentage of protozoans (98% flagellates, 1.5% rotifers, and 0.5% amoebae) together with 32.2% dead material and 39.25% ash among the microorganisms found in bio-flocs (Khanjani et al., 2022). Although rotifers, algae, fungi, and ciliated creatures are all vital species, heterotrophic bacteria are considered the dominating species among all other organisms (Tepaamorndech et al., 2020). The author explained the importance of microorganisms and emphasized the functions of Biofloc organisms (BFOs) in BFT technology, including pathogen competition, nutritional content support, and water quality maintenance.

2.6 Nutrient Cycle

Nitrogen and carbon are crucial constituents in the existence of all living beings. Aquatic creatures obtain protein in the form of amino acids, nucleic acids, and other macromolecules, which make up 5% of their dry weight. Aquatic systems contain a diverse array of organic chemicals (mostly urea and amino acids) and inorganic forms, including gaseous compounds (such as N_2) as well as anions and cations. The microorganism plays a crucial role in the alteration and movement of nutrients in the aquatic environment. The process of transformation is referred to as the nitrogen cycle. The nitrogen cycle encompasses four well-established processes: nitrogen fixation, mineralization, nitrification, and denitrification. These processes are dependent on environmental conditions and certain types of microorganisms. The environmental conditions include oxygen availability, pH levels, temperature, nutrient availability, and organic matter. Some of the microorganisms crucial for these processes are cyanobacteria, Nitrosomonas, and paracoccus. The regulation of these processes is determined by the quantity of nitrogen components being released into an environment (Minaz & Kubilay, 2021).

The BFT mechanism is very reliant on the carbon-to-nitrogen ratio. It enables the effective regulation of the growth of heterotrophic bacteria, which depends on the presence of organic carbon. The composition and characteristics of the organic carbon sources utilized in the production of bio-flocs play a crucial role in determining the threshold ratio at which heterotrophic bacteria will surpass nitrifying bacteria (Marlowe et al., 2019b).

BFT relies on the optimization of the C/N ratio to promote the proliferation of heterotrophic bacteria, algae, and other microorganisms, hence facilitating nutrient recycling. The three primary functions of bio-floc are water quality regulation, provision of nutritional supplementation for cultured species, and engagement in microbial competition against pathogens. The cohesive polysaccharide framework that binds bio-flocs is referred to as mucus and is produced by bacteria. The cohesion of the bio-flocs is further facilitated by the filamentous bacteria and the electrostatic interactions among the constituent particles (Raza et al., 2024b).

2.7 Species Cultured in BFT

The first stage in designing a bio-floc technology system is to choose the species. Optimal outcomes are obtained when species can extract the highest amount of nutrients from the flocs present in the cultured water. The most optimal species are those that possess a high tolerance for elevated sedimentation levels in water and can sustain their resilience in conditions of poor water quality. Tilapia and shrimps, for example, possess physiological adaptations that allow them to successfully absorb bio-floc and metabolize microbial protein. This enables them to effectively utilize bio-floc as a source of food (Raza et al., 2024b).

It's normal practice to raise shrimp, tilapia, and crabs in various kinds of bio-floc systems. As the fish of the twenty-first century, tilapia has become more and more popular, accounting for a growing portion of the world's fish production. With ongoing production taking place in about 135 nations, it is currently the species that is cultivated worldwide most extensively. Tilapia has shown tremendous promise in aquaculture in many poor nations that are situated in warmer climates since this type of fish prefers warm water and is easy to raise. The vast range of climatic conditions that tilapia can withstand is indicative of the tougher nature of fish (Marlowe et al., 2019c).

2.8 Factors Affecting BFT

2.8.1 Carbon Source

Several studies have investigated to determine how carbon source affects the microbial diversity of biomass and fish growth performance. Deng et al 2018 utilized tapioca starch, plant cellulose, and a blend of both provided by Aquaculture International. Upon conducting trials in 300 L tanks for 42 days, it was noted that the tapioca starch group exhibited a more substantial weight gain in comparison to both the control group and the other two groups. It was also found that different organic carbon sources in the BFT system can reduce the concentration of ammonia-nitrogen (Deng et al., 2018). The abundance of heterotrophic bacteria in the culture water is crucial for the effectiveness of BFT. Therefore, additional carbon sources are typically introduced to achieve a proper carbon-nitrogen ratio. The major objective of adding carbon should be to create the best circumstances for aquaculture while keeping costs as low as possible (Li et al., 2023).

2.8.2 C/N Ratio

The C/N ratio is the primary determinant of BFT's applicability. The primary goal of adjusting the C/N ratio is to keep it at the right levels, giving the heterotrophic bacteria in the culture water the best possible living space and regulating the number of organic pollutants in the water (Lal et al., 2024). As a result, the BFT system converts ammonium and other nitrogenous organic waste components into bacterial biomass, which serves as a food supply for aquatic life. In this way, BFT serves as an extra nutrition source for fish while simultaneously enhancing the quality of the water used in aquaculture and requiring less water (Li et al., 2023).

2.8.3 The Effect of Feeding Regimes

One effective approach to enhance aquaculture output and sustainability is focusing on augmenting the nutrient composition of the diet. The provision of feed and the method of feeding, which are crucial components in aquaculture, will motivate the producer to adopt BFT technology. Therefore, BFT must contribute significant value to the country's economy (Lal et al., 2024). Elevating the dietary nutritional content is viable for enhancing aquaculture productivity and sustainability. In a study conducted in 2019, Durigon used BFT to assess the quantities of digestible calories and digestible protein in brackish waters (70% tap water and 30% sea water). The study investigated the growth and health of Nile tilapia when fed diets with different levels of digestible protein (22%, 26%, and 30%) and digestible energy (3000, 3150, and 3300 kcal/kg). The results showed that the feed composition consisting of 26% digestible protein and 3150 kcal/kg digestible energy levels is the optimal choice for fish (Durigon et al., 2020).

2.8.4 Stoking Density, Aeration, and Salinity

One of the most crucial factors in the aquaculture system is stocking density. Studies have linked high stocking densities in aquaculture to variable growth rates, poor ultimate weights, feed intakes, and even mortality. Alternative research is required to determine how stock intensity affects growth performance and disease resistance, particularly in traditional aquaculture (Troell et al., 2017b).

Fish health is improved by the abundance of bioactive substances in biofloc produced in culture water, including taurine, carotenoids, and fat-soluble vitamins. On the other hand, a mixed composition in an aquaculture setting will result in more turbid water. This turbidity of water is due to the high density of organic matter, microorganisms, and suspended particles which leads to the formation of bioflocs. Therefore, the water of fish in BFT-based cultures must be very turbidity-resistant (Lal et al., 2024).

2.9 Optimization Techniques in BFT

The cultivation of marine shrimp typically takes place in coastal cities. However, in landlocked cities without access to the coast or ocean, the use of seawater simulation culture facilities is crucial as a substitute for importing marine shrimp. Hence, it is imperative to research to minimize the expenses associated with salt in the construction of these systems that rely on BFT. A BFT-based shrimp culture was studied in raceway pools using three different salinity ratios (10%, 20%, and 30%) (Ray & Lotz, 2017). There were no appreciable differences in growth performances or FCR between treatments. The treatment refers to salinity levels in shrimp culture. Shrimp growth was unaffected by the variation in salinity rate. As a result, it demonstrated cheaper output at a salinity of 10% (Troell et al., 2017b) Based on the results of this study, it is evident that doing feasibility studies in seawater simulation projects is crucial for mitigating future concerns over the facilities. Optimization Techniques in BFT

Concerning the BFT optimization strategies, the main principles are focused on enhancing system efficiency by altering one or more parameters. Thus, the major practices include altering the C/N ratio, which forms one of the key strategies for maintaining the necessary balance between nutrient cycling and microbial growth. In a similar breadth, some studies have indicated that increasing the C/N ratio facilitates the aquaculture species' immune status and the water quality in the pond but also increases the amount of carbon being lost. Thus, it is possible to conclude that the key to achieving the optimal C/N ratio is to find a balance that will bring maximum benefits and minimum losses. Also, there is a way of adding carbon; what kind of carbon is used is important to get the best results in biofloc systems. While some carbon sources like glucose stimulate the system immediately, they cause unsuitable fluctuations in DO levels since they include both advantages and disadvantages of the corresponding technological process. On the other hand, few findings have been established concerning slow-release carbon sources like polyhydroxyalkanoate (PHA's) that mimic steady carbohydrate feedings such as polyhydroxybutyrate (PHB). The integration of BFT with other technologies such as denitrification systems or aquaculture, it is possible to enhance the usage of resources and reduce CO_2 emissions; to achieve environmentally friendly aquaculture (Li et al., 2023).

2.10 Benefits of the BFT system

Existing research demonstrates that monitoring nitrogenous chemicals is essential in investigations on biofloc technology (BFT). It has been discovered that when these systems are effectively maintained, they can significantly improve water quality. The BFT system achieves good water quality under zero water discharge circumstances by efficiently converting ammonium to microbial biomass and nitrate, surpassing the rate of nitrification processes (Emerenciano et al., 2013).

It can be seen that animals cultured in the BFT system generally show higher growth performance than traditional methods. The traditional technology of aquaculture carries the risk of contamination by pathogenic microorganisms due to the inflow and outflow of water from outside. This risk is at the minimum level in the BFT technology (Li et al., 2023).

Aquaculture species must possess resilient immune systems and maintain optimal physical conditions to ensure rapid and healthy growth. Several studies on BFT systems have investigated the immunological response, antioxidant activity, stress parameters, and enzyme activities of the generated species for this purpose (Emerenciano et al., 2013).

There is a restricted range of species that can be cultivated in BFT systems. Nonetheless, omnivorous and herbivorous species are often encouraged to be farmed rather than carnivorous species, according to FAO and national development predictions. Species fed at low trophic levels are often the ones that are raised in BFT systems most frequently. Although this problem appears to be a drawback at first, it plays a significant role in fulfilling the demand for protein (Minaz et al., 2024).

For the system's micro and macro-organisms, the BFT needs high and constant aeration. There aren't many optimization studies in the literature since these raise running costs. To reduce the cost of aeration in this situation, more research on aeration optimization is required (Mugwanya et al., 2021).

The mentioned operational and technological challenges explain the need for further research and imagination to increase the applicability and scalability of BFT in various aquaculture contexts.

2.11 Limitations of BFT

The authors noted several drawbacks to this strategy and challenges in utilizing bio-floc technology (BFT) to improve sustainable aquaculture (Khanjani et al., 2024). Temperature, pH level, dissolved oxygen, and C/N ratio are among the variables that must be carefully regulated to maintain the stability of the microbial population and the system's overall effectiveness. This is one of the main drawbacks. If these parameters are not properly regulated, harmful compounds like ammonia and nitrites might build up and affect some of the aquatic species that will be farmed (Markou et al., 2024). The requirement to continuously pump water and saturate it with oxygen is another drawback of BFT systems, increasing energy consumption and operational expenses. Owing to their intricacy, professional personnel are frequently required, which might hinder their broader implementation, especially in settings with limited resources. Moreover, the concentration of bio floc particles and suspended solids may impede water purity and system cleanliness, resulting in clogged filters and decreased system efficiency (Lal et al., 2024).

CHAPTER 3

MATERIALS & METHODS

This chapter discusses in detail the materials and methods used in this study. The study was divided into two phases: a lab-scale experiment and a pilot-scale experiment. The details of these phases are given in the sections below.

3.1 Lab Scale Experiment

For this study, the lab scale experiment was conducted at the Environmental Toxicology Lab based in IESE, NUST. A total of 9 tanks were prepared for this experiment. Initially, a mixture of 0.01 g/L probiotic, 1 g/L sea salt, 0.1 g/L jaggery, 0.05 g/L calcium carbonate ($CaCO_3$), and 0.005 to 0.008 g/L ammonium sulfate $(NH_4)_2SO_4$ was prepared and added into the tank. Continuous aeration was provided in the tanks, and the temperature of the tanks was maintained at 30ºC using submersible heaters with 300 W thermostats. The systematic diagram of the lab scale setup is shown in Fig.3.1.

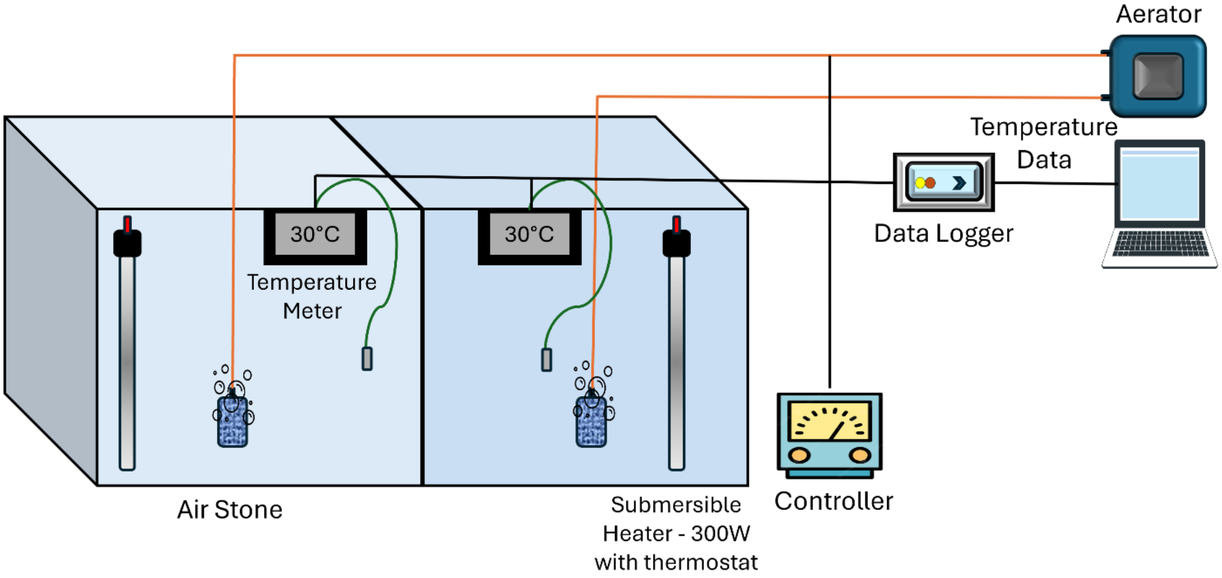
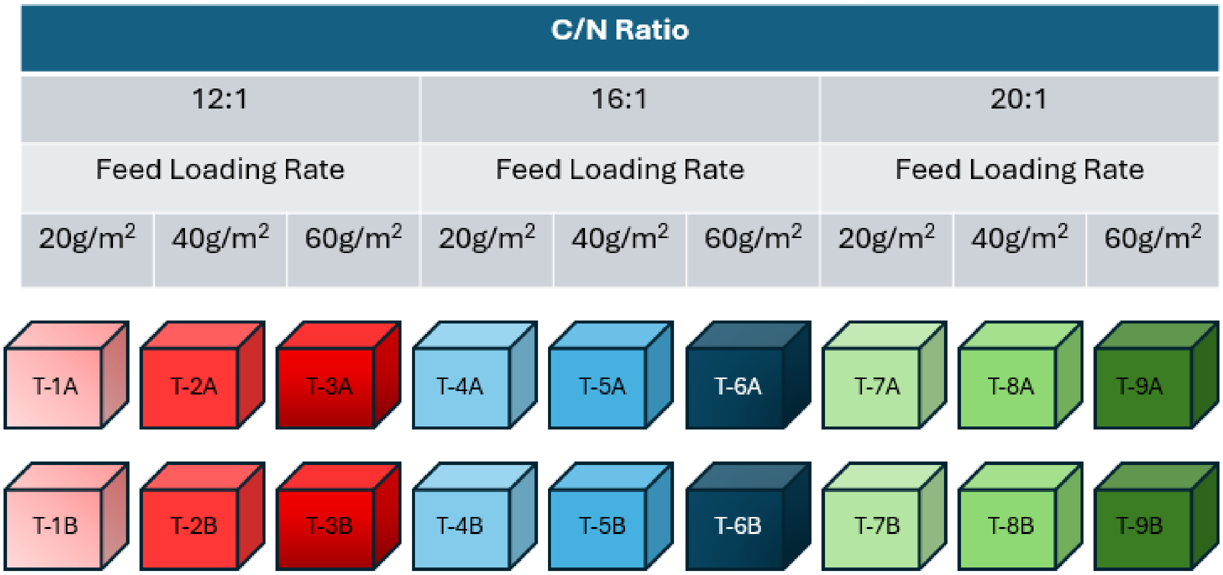


Figure 3.1 Systemic diagram of lab scale setup

Three different C/N ratios (12:1, 16:1, 20:1) were used, and on each C/N ratio, three different feed loading rates (20 g/m^2, 40 g/m^2, 60 g/m^2) were tested. This was to optimize the C/N ratio and analyze the bacterial growth and ammonia removal (Wang et al., 2015). The total duration of the experiment was 17 days. All the experiments were performed in duplicates. The operational matrix of the lab scale setup in shown in Fig. 3.2.



C/N Ratio								
12:1			16:1			20:1		
Feed Loading Rate			Feed Loading Rate			Feed Loading Rate		
20g/m^2	40g/m^2	60g/m^2	20g/m^2	40g/m^2	60g/m^2	20g/m^2	40g/m^2	60g/m^2
T-1A	T-2A	T-3A	T-4A	T-5A	T-6A	T-7A	T-8A	T-9A
T-1B	T-2B	T-3B	T-4B	T-5B	T-6B	T-7B	T-8B	T-9B

Figure 3.1: Experimental matrix of lab-scale setup

3.1.1 Parameters Tested

The parameters tested in the lab scale experiment, along with the methods used, are given in Table 3.1.

Table 3.1: Parameters tested in the lab scale experiment

Parameters	Unit	Methods
pH	-	pH meter
Electrical Conductivity	mS/cm	Conductivity meter
Temperature	°C	Temperature sensor
Dissolved Oxygen	mg/L	DO meter
Total Dissolved Solids		APHA, 2017
Total Suspended Solids		
Volatile Suspended Solids		

Suspended Solids/ Floc Volume	ml/L	Imhoff Cone
Alkalinity	mg/L	APHA, 2017
Phosphates		
Nitrates		
Nitrites		
Ammonium-Nitrogen		

Proximate composition parameters such as moisture content, lipids, protein, and ash were also analyzed. Total Kjeldahl assembly was used for crude protein, while a solvent extractor was used for lipid content.

3.2 Pilot Scale Experiment

The pilot scale study was conducted outside the IESE building. Similar to the lab scale, nine tanks were prepared, each with a capacity of 1000 liters. Initially, the area where tanks were constructed was cleared and leveled by filling mud. Proper channels were constructed to carry wastewater from the tanks to the drainage. Polyethylene sheets and steel grills were used in the construction of tanks. The systematic diagram of a tank of the pilot-scale system is shown in Fig.3.2.

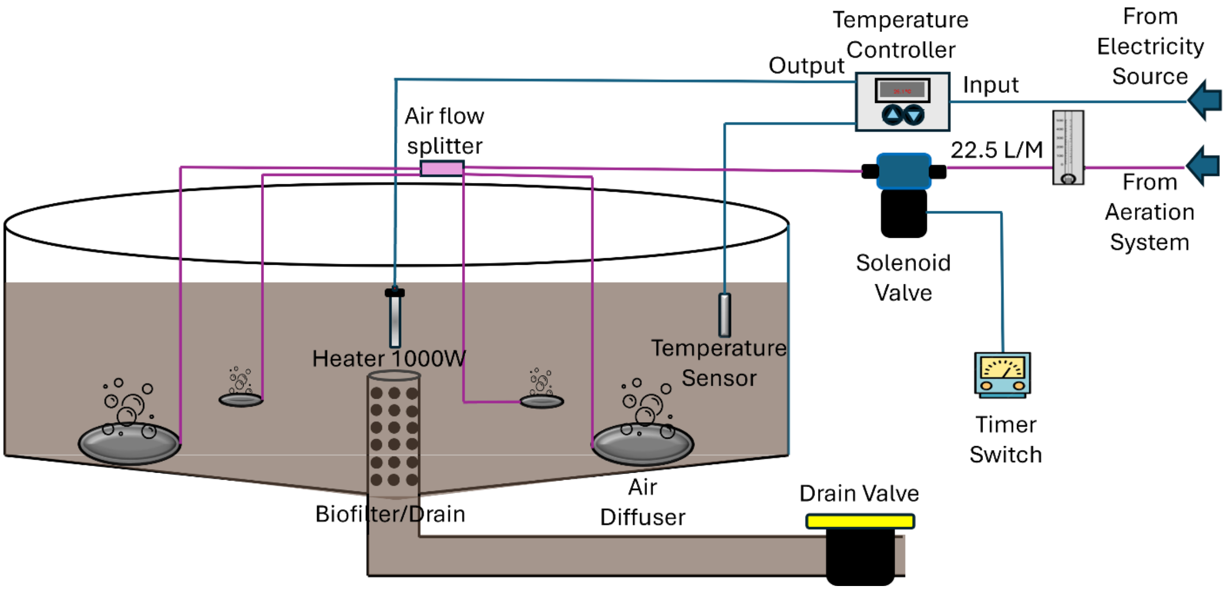


Figure 3.2: The systematic diagram of the Pilot Scale Setup

Aeration is provided in each tank using aerators, and an airflow splitter is installed to control the direction and flow of air in each tank. 1000 W heaters, along with a temperature sensor and controller, were installed in each tank to maintain the temperature, and a controlled temperature environment was necessary to produce fish. Solenoid valves were also installed with every tank, and a timer was set to regulate the airflow in the tanks. The detailed construction and fabrication of the pilot scale system are shown in Fig.3.3.

For fish to grow, commercially available feed pellets are used. This feed was purchased from a local company namely Hi-Tech Feeds based in Lahore, Pakistan. The pellet diet utilized in this study had a moisture content of 11%, crude protein content of 30%, crude lipid content of 4%, phosphorus content of 2%, lysine content of 13%, crude ash content of 13%, and crude fiber content of 12%. The feeding rate was set at 2% of the total stored biomass and was modified every 7 days. The feed of a similar composition was also used in previous studies (Saha et al., 2022).

(a) (b)


(c)


(d)


(e)


(f)

Figure 3.3: Detailed construction and fabrication phase of Pilot-Scale Study (a) Research site (b) Water level (c) Civil works (d) Base curing (e) Drainage system (f) Wire cage installation

(g) Aeration System (h) Complete research facility

3.2.1 Different Phases of Experiment

In the first phase of the experiment, tanks were installed. Similar to the lab-scale experiment, a mixture of 0.01 g/L probiotic, 1 g/L sea salt, 0.1 g/L jaggery, 0.05 g/L calcium carbonate, and 0.0416 g/L ammonium sulfate was prepared and added to each tank. The C/N ratio of 16:1 was maintained in the second phase, and bioflocs were given a certain time for growth. In the third phase of this experiment, Genetically Improved Farmed Tilapia (GIFT) were added to each tank. Total 28 Tilapia fishes were added to each tank. The calculation on stocking density were based on the optimum final stocking density suggested by Hargreaves for Tilapia (Hargreaves, 2013). Another study conducted on GIFT Tilapia stocking density found maximum survivability in tanks with the stocking density of 25 fish/m^3 (WIDANARNI et al., 2012). Fish were kept in the tanks to acclimate to the environment before the start of the study period. Feed in each tank was maintained as per the C/N ratio. In the last phase of the experiment, the nutrients (jaggery) and feed of the fish were maintained in each tank, and the sampling was performed to analyze selected parameters. The operational conditions used in each tank are shown in Fig.3.4.

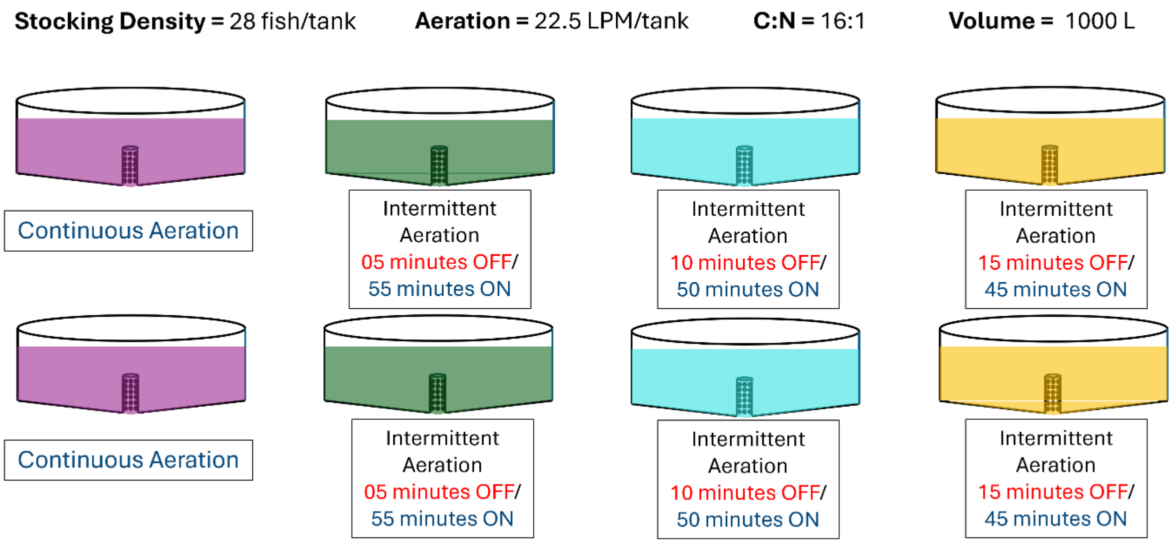


Figure 3.4: Operational Conditions for Each Tank

3.2.2 Water Quality and Fish Growth Parameters

Water quality parameters were monitored separately in each tank and the readings were recorded. The parameters tested are the same as the lab-scale study and are mentioned in Table 3.1.

Fish growth parameters monitored during the study period are summarized in Table 3.2.

Table 3.2: Different fish growth parameters.

Parameters	**Formula**	**References**
Length gain (cm)	Total length gain (cm/fish): LG = L2 – L1	(Wang et al., 2015)
Weight gain (g)	Total weight gain (g/fish): WG = W2 – W1	
Feed conversion ratio (FCR)	FCR = dry matter intake (g)/weight gain (g)	
Protein efficiency ratio (PER)	PER = weight gain (g)/protein ingested (g)	
Feed conversion efficiency (FCE)	FCE = 100 × weight gain (g)/ feed consumed (g)	
Survival rate (%)	Survival rate (%) = 100 × (final number of fish/ initial number of fish)	

3.3 Statistical Analysis

All the fish growth parameters data from the pilot scale experiment was statistically analyzed by using SPSS software 27.0. The same was used in the literature (Zhang et al., 2022). Data was exhibited as mean ± standard deviation and analyzed by using one-way ANOVA after assessment of homogeneity of variance. When significant differences among treatments were found, Duncan's multiple range test was used to identify differences among all the treatments. Results were considered statistically significant at the level of $p < 0.05$.

CHAPTER 4

RESULTS & DISCUSSION

This chapter discusses the complete study's results in detail. The first phase details the results of lab-scale experiments, followed by the results of pilot experiments.

4.1 Results of Lab-Scale Experiments

The results of lab-scale experiments are presented in the following sections.

4.1.1 Dissolved Oxygen

The three different feed-loading rates (FLR) and C/N ratios were used, and the DO value was checked and reported during the whole experiment. The average DO values for FLR 20 at three different C/N ratios (12:1, 16:1, 20:1) were 6.60 ± 0.09 mg/L, 6.37 ± 0.14 mg/L, and 6.40 ± 0.27 mg/L respectively. Similarly, for FLR 40 and 60, the DO values at three different C/N ratios were 6.33 ± 0.11, 6.13 ± 0.18, 6.10 ± 0.19 mg/L and 6.29 ± 0.21, 6.12 ± 0.22, and 6.13 ± 0.15 mg/L respectively. It may be observed from Fig.5 that the DO value decreased as the number of days increased. Tank 1 with FLR 20 and C/N 12/1 had the highest DO values, and Tank 6 with FLR 60, and C/N 20:1 showed the lowest value of DO, indicating high microbial activity. The results showed that the C/N ratios and FLR in the biofloc technology significantly influence the DO concentrations. Moreover, adding carbohydrates from external sources, such as jaggery, decreased DO levels; Do levels also decreased as the density of flocs increased. These results align with previous studies of Luo (Luo et al., 2017) and Widanarni (WIDANARNI et al., 2012), respectively.

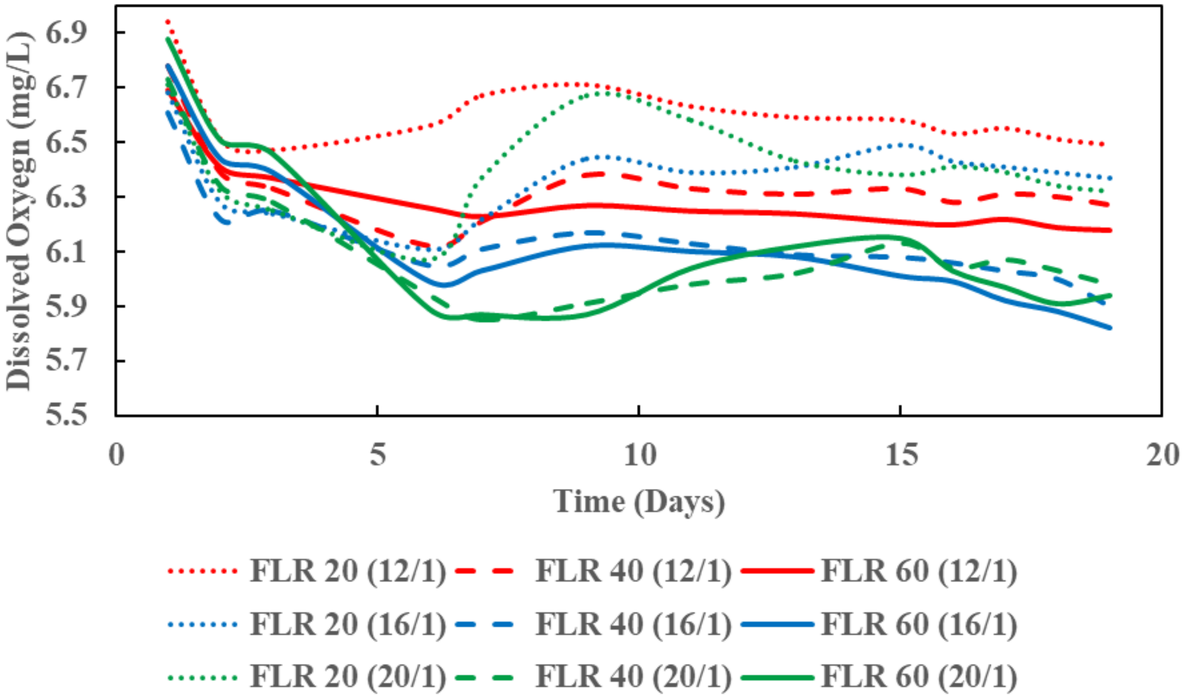


Figure 4.1: Dissolved Oxygen of Lab-Scale Experiment.

4.1.2 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

The total suspended solids (TSS) and Volatile suspended solids (VSS) experiments were performed for three different FLR and C/N values and the results are shown in Fig.6 and 7. The TSS and VSS at each C/N ratio increases with time and with an increase in feed-loading rate. Moreover, the C/N ratio is also responsible for the formation of biofloc and enhanced microbial activity in the tanks. The results showed that the tank with the highest feed loading rate (FLR 60) and C/N ratio of 16:1 has the highest level of biofloc formation and hence has the higher TSS. Conversely, lower feed loading rates (FLR 20) with a lower C/N ratio (12:1) result in lower TSS levels, indicating less biofloc formation. Similarly, the VSS value also increased and as depicted in Fig.7 showed a maximum value of 37 mg/L for FLR 60 at a C/N ratio of 16:1. These results of TSS and VSS can also be justified by one of the literature, where Haghparast (Haghparast et al., 2020) suggested that the levels of TSS, VSS and SS were increased in the biofloc system with the increase in the C/N ratio.

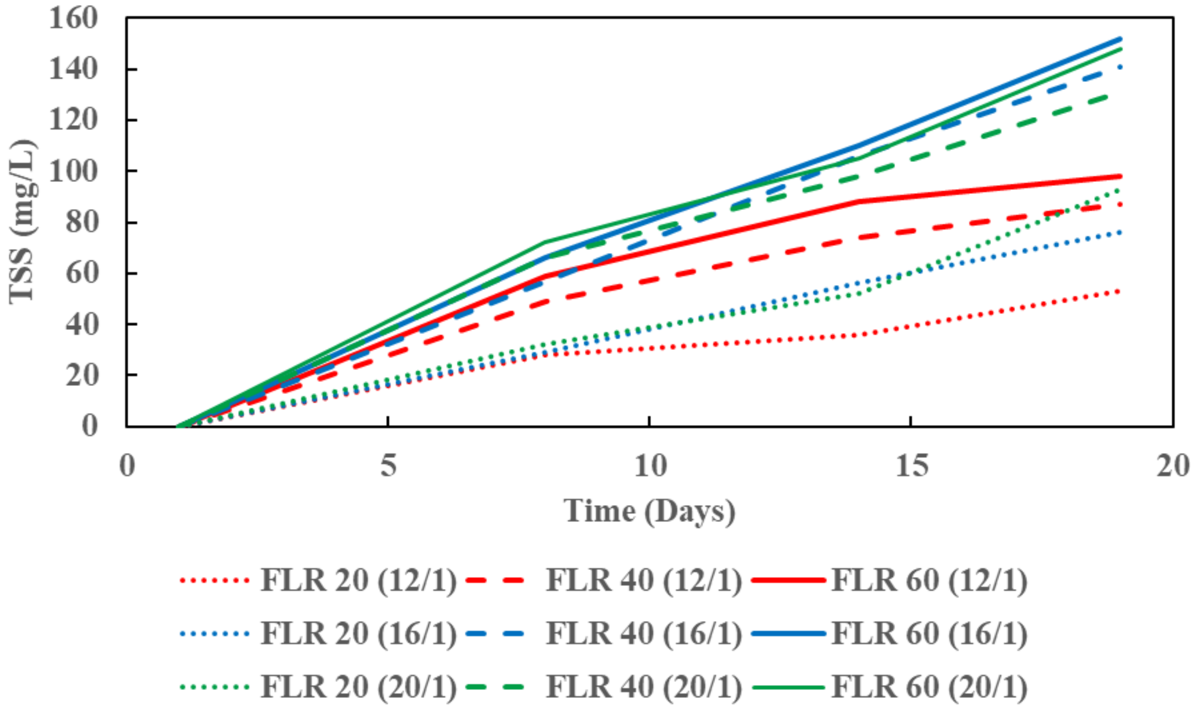


Figure 4.2: TSS of Lab-Scale Experiment

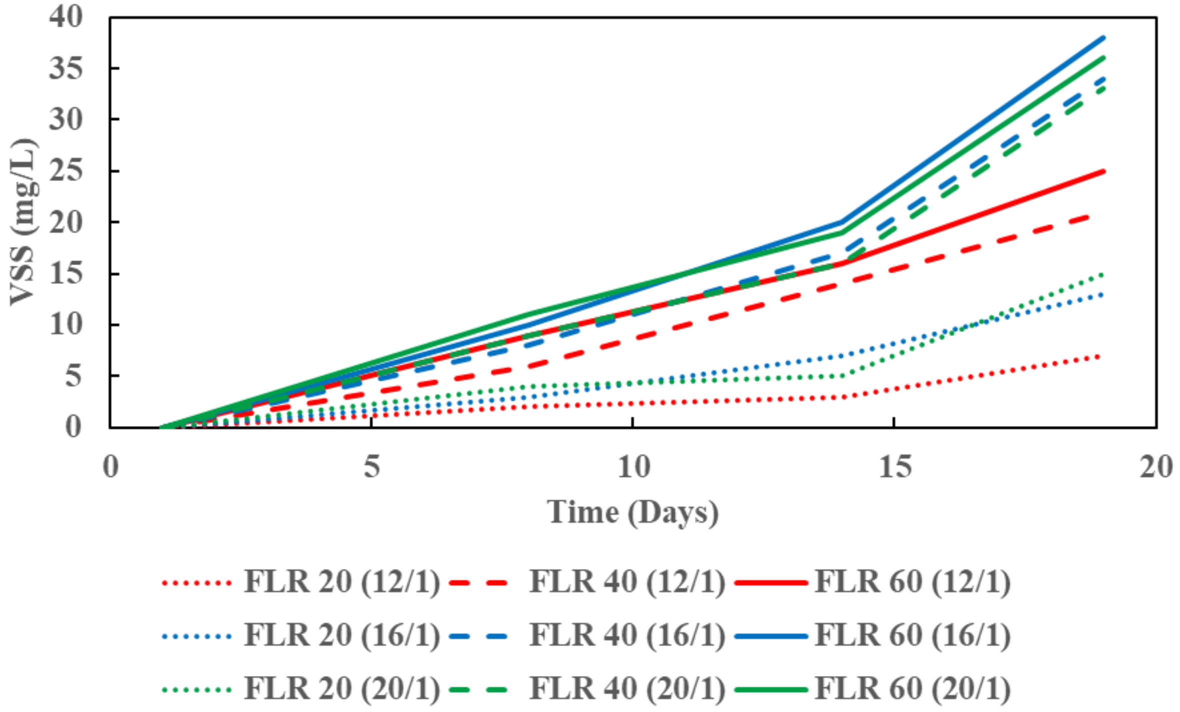


Figure 4.3: VSS of Lab-Scale Experiment

4.1.3 Floc Volume (FV)

The results of floc volume (FV) showed increasing trends similar to TSS and VSS. The floc volume increased with an increase in the C/N ratio and this change in floc volume level during the experiment is depicted in Fig. 8. The highest FV was observed in the case of C/N ratio 16/1 (FLR 60) whereas, the lowest FV was observed when the C/N ratio was 12:1 (FLR20). The variations in the C/N ratio had a direct impact on the density of heterotrophic bacteria, as previously mentioned. Specifically, when the ratio increased, the density of heterotrophic bacteria also increased.

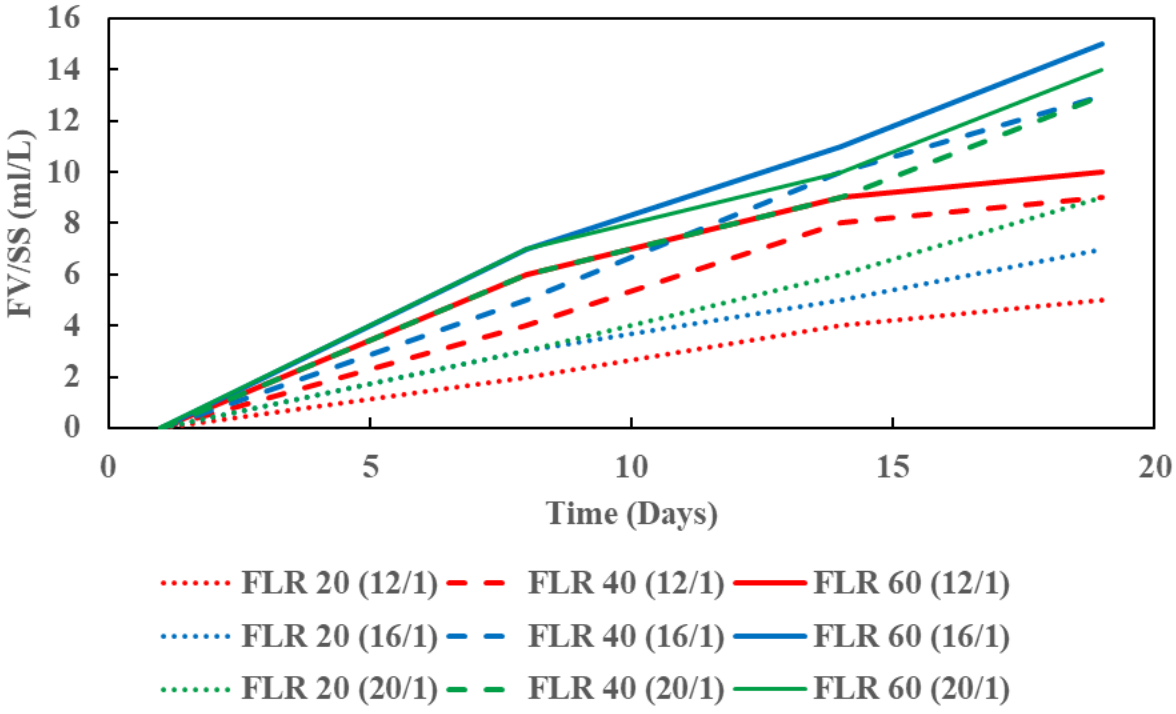


Figure 4.4: Floc Volume Analysis of Lab-Scale Experiment

4.1.4 Total Ammonia Nitrogen (TAN)

In this study, the average levels of Total ammonia nitrogen (TAN) at three FLR and C/N ratios were examined. The results indicated an initial upward trend during the experimental period. Nevertheless, there was a decline in the trend when the C/N ratio increased, particularly during the period between Day 6 and 14, followed by a subsequent stabilization by Day 17 (Minabi et al., 2020). The minimum and maximum levels of total ammonia nitrogen (TAN) were recorded when the carbon-to-nitrogen (C/N) ratios were 16:1 and 12:1, respectively. FLR 60 (16/1) exhibited the highest ammonia conversion rate and the lowest concentration of total ammonia nitrogen (TAN) at the end of the process. Conversely, FLR 40 (12/1) exhibited the least efficient conversion of ammonia and the highest concentration of total ammonia nitrogen (TAN) at the end, suggesting a lower level of microbial conversion. This could be attributed to the low activity of AOB due to less availability of organic carbon for bio flocculation and expansion of heterotrophic culture mass (Minabi et al., 2020). Figure 4.5 displays the variability in the TAN measurements throughout the experiment.

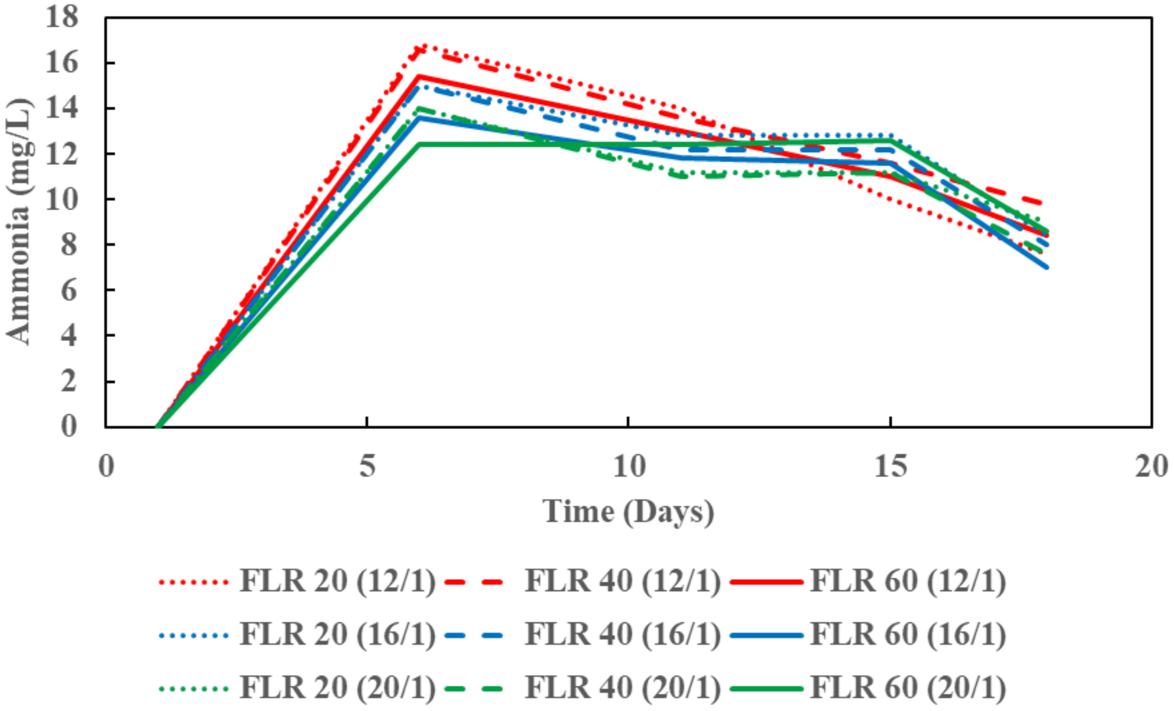


Figure 4.5: Total Ammonium Nitrogen Analysis of Lab-Scale Experiment

4.1.5 Other Water Quality Parameters

Table 4.1 presents the average values of water quality metrics in several treatments, which have diverse C/N ratios and feed loading rates. In general, there were no notable variations in pH, water temperature, nitrite nitrogen, and nitrate nitrogen across the different treatments. However, differences were observed in other qualitative parameters, including total dissolved solids (TDS), total suspended solids (TSS), volatile suspended solids (VSS), fixed volatile solids (FV), total ammonia nitrogen (TAN), alkalinity, and phosphates. These differences were observed when comparing treatments with different carbon-to-nitrogen (C/N) ratios and feed loading rates. The pH readings did not show any differences across the treatments; however, the tank with a C/N ratio of 12:1 and FLR of 20 g/m^2 had the highest recorded values.

The values of EC and TDS showed a clear increasing trend during the culture duration. The difference among treatments was not dependent on the change in C/N, though it showed an increasing trend with the increase in FLR within each C/N ratio [51]

Nitrites and nitrates did not show differences among treatments. Nitrites increased at the start but then showed a decreasing trend across all variations while nitrates showed an increasing trend throughout the study duration. The concentration of phosphates increased with an increase in the C/N ratio and feed loading rate. The highest concentration of phosphates (2.52 ± 0.25 mg/l) was observed in C/N 20:1 and FLR 60 g/m^2 and the lowest concentration (0.08 ± 0.01 mg/l) was observed in C/N 12:1 and FLR 20 g/m^2. Alkalinity displayed an increasing trend throughout the study period, the highest and lowest mean values were recorded in C/N 20:1 and FLR 60 g/m^2 and C/N 12:1 and FLR 20 g/m^2 respectively.

Table 4.1: Water quality parameters performed in the Lab-Scale Experiment

	C/N ratio								
	12:1			16:1			20:1		
	Feed Loading Rate			**Feed Loading Rate**			**Feed Loading Rate**		
Parameters	20g	40g	60g	20g	40g	60g	20g	40g	60g
Temp (am) (°C)	29.22 ± 0.17	29.49 ± 0.12	29.32 ± 0.09	29.61± 0.08	29.58 ± 0.21	29.35 ± 0.06	29.03 ± 0.18	29.59 ± 0.22	29.58 + 0.13
Temp (pm) (°C)	29.41 ± 0.06	29.67 ± 0.11	29.54 ± 0.05	29.74 ± 0.11	29.71 ± 0.14	29.49 ± 0.05	29.11 ± 0.12	29.71 ± 0.11	29.66 ± 0.16
pH	8.33 ± 0.03	8.19 ± 0.04	8.27 ± 0.02	8.28 ± 0.04	8.26 ± 0.02	8.22 ± 0.03	8.27 ± 0.02	8.23 ± 0.01	8.20 + 0.02
TDS (mg/L)	1240.70 ± 0.06	1293.50 ± 0.04	1276.70 ± 0.07	1278.60 ± 0.02	1232.44 ± 0.03	1266.5 ± 0.08	1228.0 ± 0.01	1220.1 ± 0.02	1278.60 ± 0.05
EC (µ mho/cm)	2321.29 ± 8.25	2435.14 ± 10.36	2420.14 ± 11.14	2323.71 ± 14.26	2340.86 ± 17.18	2370.00 ± 21.67	2298.86 ± 18.21	2362.29 ± 22.21	2377.14 ± 24.41
NO_2^- (mg/L)	0.63 ± 0.11	0.29 ± 0.05	0.86 ± 0.14	0.60 ± 0.09	0.68 ± 0.13	0.53 ± 0.10	0.63 ± 0.12	0.49 ± 0.08	0.57 ± 0.11
NO_3^- (mg/L)	3.71 ± 0.32	0.89 ± 0.18	1.72 ± 0.21	2.59 ± 0.24	3.40 ± 0.35	3.78 ± 0.37	2.18 ± 0.22	2.35 ± 0.23	2.94 ± 0.26
Alkalinity (mg/L)	105 ± 7.00	113.75 ± 8.00	116.25 ± 5.00	116.17 ± 6.13	118.75 ± 5.00	126.5 ± 5.00	121.25 ± 5	125 ± 2.5	131.5 ± 1.50
PO_4^{-3} (mg/L)	0.08 ± 0.01	0.88 ± 0.12	1.00 ± 0.16	0.09 ± 0.01	0.69 ± 0.05	2.24 ± 0.19	0.09 ± 0.01	0.88 ± 0.13	2.52 ± 0.25

4.1.6 Proximate Composition of Feed and Flocs

The results of the proximate composition analysis of feed and bioflocs among different variations of C/N ratios and FLRs are recorded in Table 4.2. The results indicate that the Crude Protein content is impacted by the increase in C/N ratio and feed loading rate. Results in another study exhibited a similar trend (Solanki et al., 2023) .The highest value of crude protein among flocs is recorded in C/N 20:1 and FLR 40 g/m^2 and the lowest value in C/N 12:1 and FLR 20 g/m^2. For Ash content, the highest values were recorded in C/N 20:1 and FLR 60 g/m^2 indicating that the increase in C/N and FLR had some impact on it. Crude Lipid content was similar among the treatments.

Table 4.2: Proximate Analysis (% dry weight) of Lab-Scale Experiment.

	Feed	**C/N ratio**								
		12:1			16:1			20:1		
		Feed Loading Rate			**Feed Loading Rate**			**Feed Loading Rate**		
Parameters		20g/m^2	40g/m^2	60g/m^2	20g/m^2	40g/m^2	60g/m^2	20g/m^2	40g/m^2	60g/m^2
Crude Protein %	29.04 ± 0.58	19.68 ± 0.52	20.27 ± 0.61	20.75 ± 0.44	23.08 ± 0.17	23.65 ± 0.22	24.43 ± 0.14	24.16 ± 0.31	25.06 ± 0.22	24.87 ± 0.55
Crude Lipids %	3.11 ± 0.27	2.21 ± 0.13	2.25 ± 0.15	2.29 ± 0.22	2.37 ± 0.32	2.51 ± 0.24	2.59 ± 0.15	2.48 ± 0.44	2.57 ± 0.23	2.53 ± 0.22
Ash %	37.17 ± 1.21	31.28 ± 0.32	31.49 ± 0.21	31.87 ± 0.24	31.51 ± 0.31	31.92 ± 0.18	32.15 ± 0.22	33.32 ± 0.45	34.08 ± 0.31	34.43 ± 0.08
Moisutre %	4.35 ± 0.04	71.03 ± 0.55	71.26 ± 0.45	70.52 ± 0.87	70.23 ± 1.28	71.08 ± 0.35	70.88 ± 0.76	71.22 ± 0.49	70.66 ± 0.58	70.54 ± 0.44

4.2 Pilot-Scale Experiments

The results of Pilot-scale experiments are presented in the following sections. The C/N ratio of 16/1 selected from the lab-scale experiment was used for this experiment along with other variable parameter being the aeration.

4.2.1 Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS)

The total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed in all the tanks. The C/N ratio of 16:1 was fixed in each tank with variation in aeration. Three different intermediate aerations (IA) were used i.e., IA (5/55 min), IA (10/50 min), and IA (15/45 min). Moreover, continuous aeration was also used in one of the tanks. It may be observed from the results (Fig. 4.6 and 4.7) that TSS and VSS values increased over time, and in the case of continuous aeration, their values are higher as compared to intermediate aeration. The continuous aeration enhances the production of TSS and VSS in the tank by promoting the circulation and mixing of water. Due to this circulation and mixing of water, the organic matter and microbial communities combine to form flocs which eventually increase the formation of TSS (Minaz & Kubilay, 2021). Additionally, the continuous aeration is also the source of a constant supply of oxygen in the tank and helps heterotrophic bacteria to metabolize. This metabolizing activity contributes to the breakdown of organic matter leading to an increase in VSS (Brandão et al., 2024).

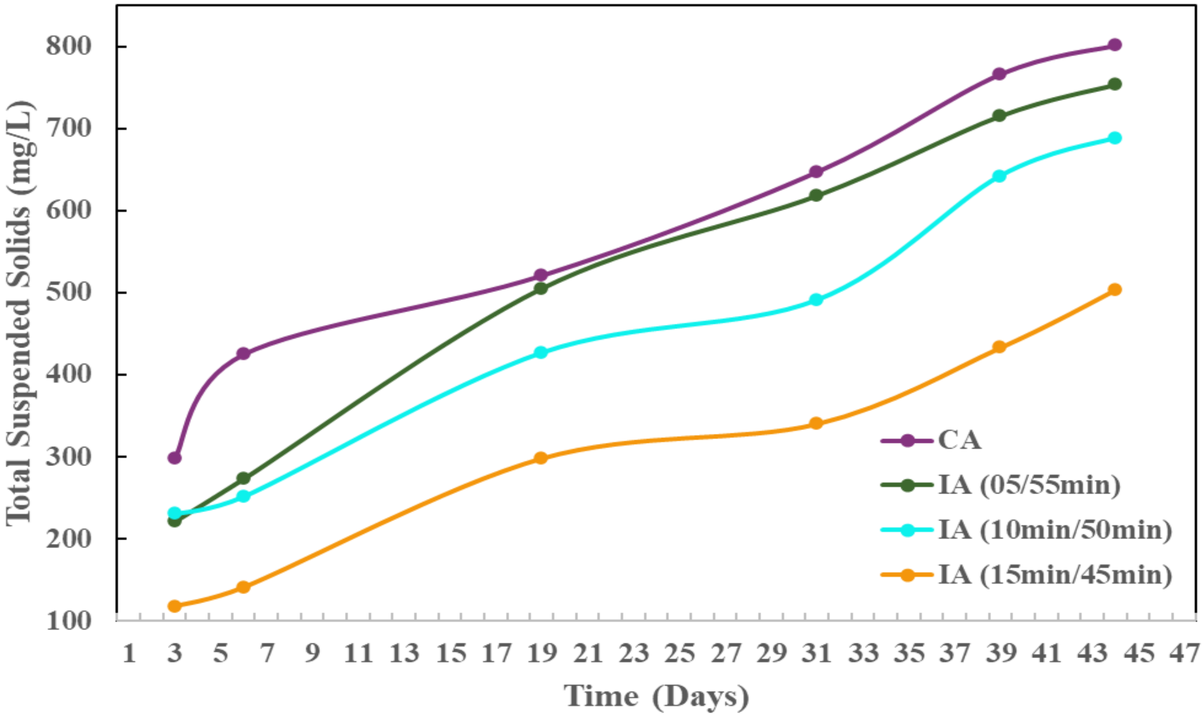


Figure 4.6: TSS Analysis of Pilot-Scale Experiment

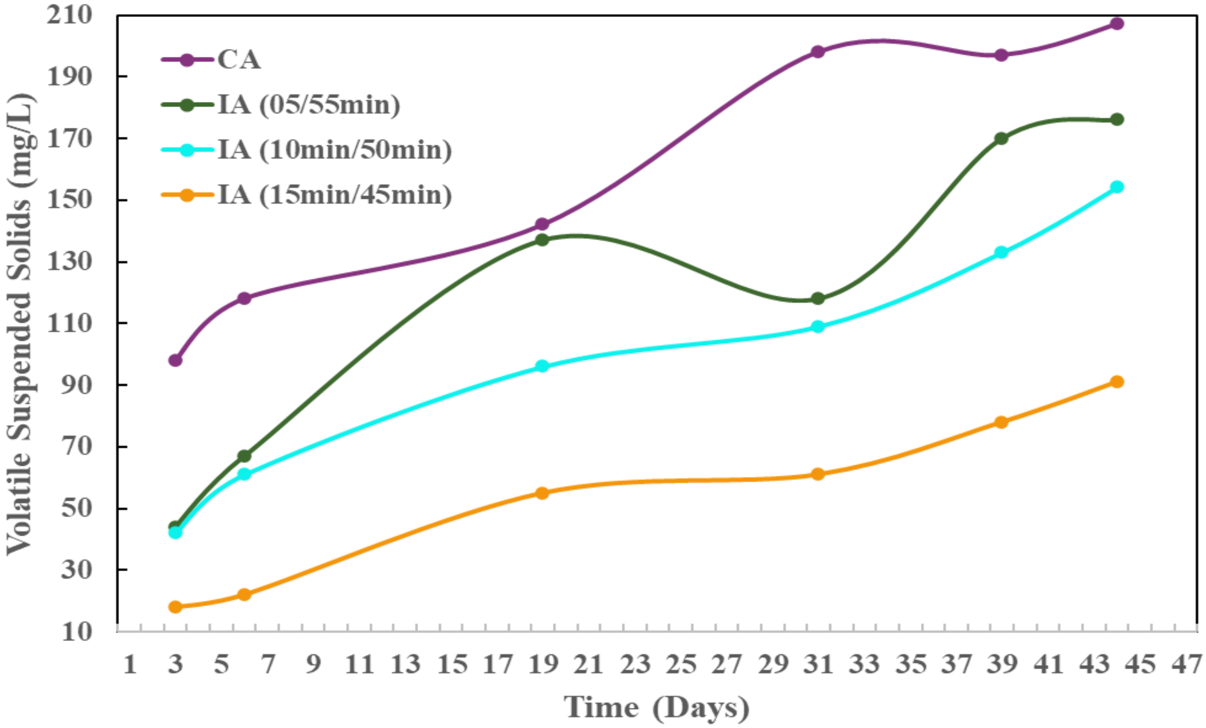


Figure 4.7: VSS Analysis of Pilot-Scale Experiment

4.2.2 Floc Volume

The effect of continuous aeration and intermediate aeration on the floc volume is shown in Fig. 12. The floc volume increases in ml/L and was higher in the case of continuous aeration throughout the experimental timeframe. However, in the case of intermediate aeration, floc volume was high when IA was 05/55 min and lower floc volume was recorded when IA was 15/45 min. The higher floc volume during continuous aeration could be due to the enhanced mixing and suspension of particulate matter. This would help microorganisms to form flocs. Moreover, continuous aeration contributes to maintaining the DO level in the tank and helps grow bacteria crucial for flocs. The floc volume increases as these microorganisms get attached to the particulate matter. These findings are in line with the previous studies (Minabi et al., 2020).

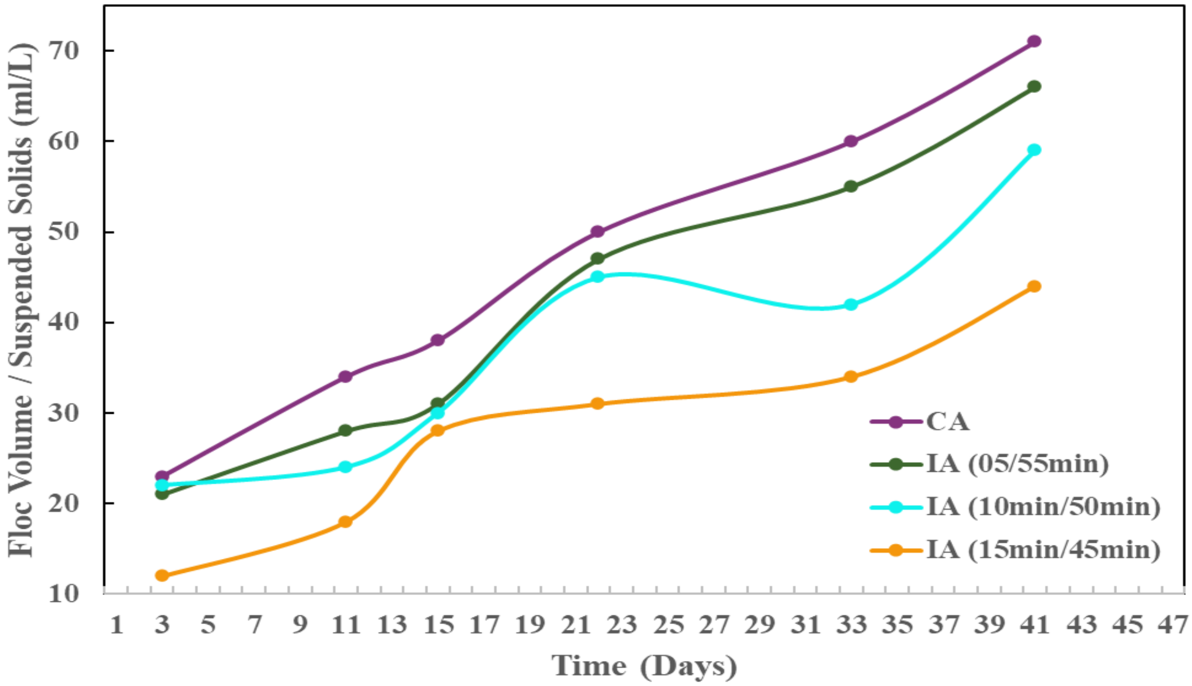


Figure 4.8: Floc Volume Analysis of Pilot-Scale Experiment

4.2.3 Total Ammonia Nitrogen (TAN)

The concentration of total ammonia nitrogen (TAN) was studied, and the results showed a decreasing trend throughout the experimental period for continuous and intermediate aeration tanks (Minabi et al., 2020). IA (10/50 min) and IA (15/45 min) showed some variations at the start of the experiment however after day 7, values of TAN started to reduce. Similar trends are also observed in the case of CA, however, the ammonia conversion rate in the case of CA was constant and higher as compared to variable intermediate aerations. The reason for this could be the continued supply of oxygen (in the case of CA and variable IA) which accelerates the nitrification process (Lu et al., 2022a). This process allows the conversion of ammonia to nitrite with the help of ammonia-oxidizing bacteria (AOB) and then the conversion of nitrite into nitrate with the help of nitrite-oxidizing bacteria (NOB) (Kornaros et al., 2010). The variation in the TAN results during the experiment is presented in Fig. 13. It is clear from earlier studies that intermediate aeration can significantly improve nitrogen removal once it is optimized. The reason for that could be the time provided to the microorganisms to be able to bear the variable loads (Lu et al., 2022b).

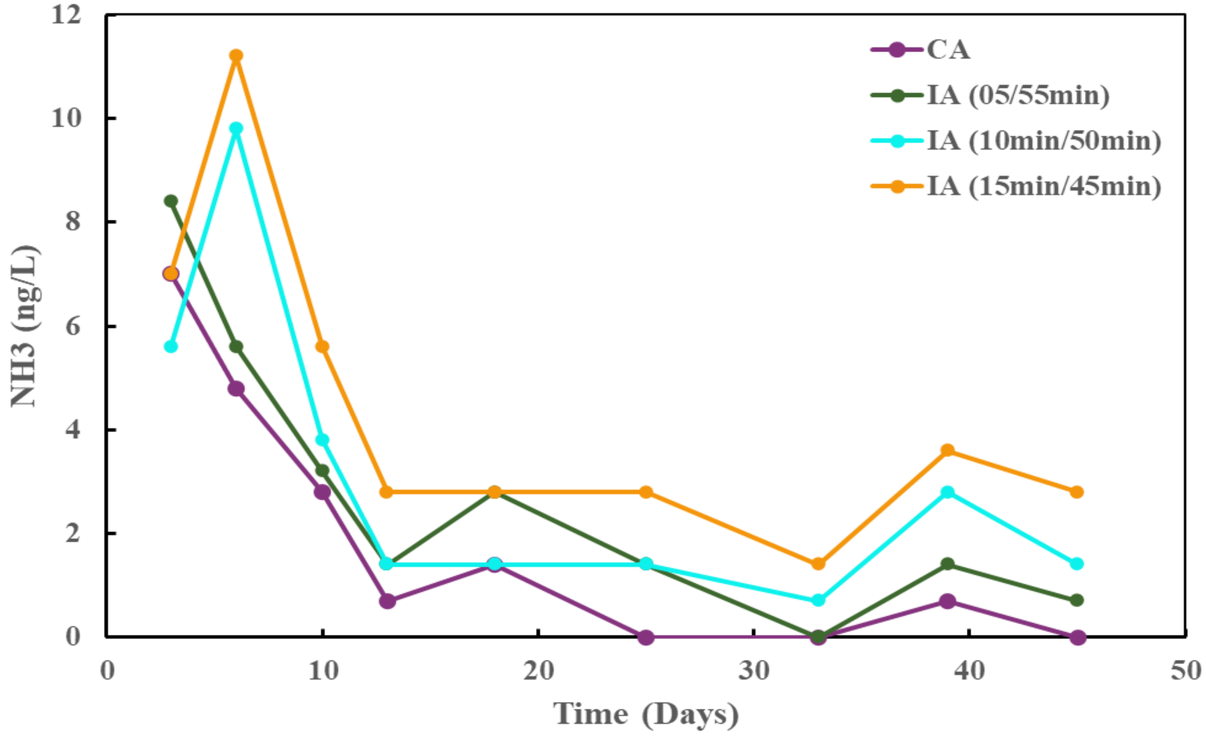


Figure 4.9: Total Ammonium Nitrogen Analysis of Pilot-Scale Experiment

4.2.4 Other Water Quality Parameters

The mean values of water quality parameters among different treatments of continuous aeration and varying rates of intermittent aeration are reported in Table 4.3. Generally, pH and water temperature remained in the similar range throughout the experiment in all treatments though the highest values of pH were recorded in the tank with CA., Water temperature throughout the culture period remained (23.67 ± 1.35 °C) which was below the optimum temperature range for GIFT tilapia (25 - 32 °C) as suggested by another study by Pandit N, et. al (Pandit & Nakamura, 2010).

The values of EC and TDS showed a clear increasing trend during the culture duration, recording highest values for EC and TDS in CA and the lowest values in IA (15min OFF/45min ON). Nitrates showed an increasing trend throughout the experiment. The highest and the lowest values were recorded in CA and IA (15min OFF/45min ON) respectively. Nitrites and phosphates did not show differences among treatments. Nitrites increased at the start but then showed a decreasing trend across all variations of aeration. Phosphate concentration showed an increasing trend during the culture period.

Table 4.3: Summary of various monitored parameters for Pilot-Scale Experiment

Parameters	**CA(A)**	**IA (05min/55min)**	**IA (10min/50min)**	**IA (15min/45min)**
Temp (°C)	23.08 + 1.56	24.20 + 1.20	23.69 + 1.35	23.68 + 1.30
pH	8.40 + 0.09	8.39 + 0.11	8.38 + 0.08	8.38 + 0.09
DO (mg L^{-1})	7.33 + 0.24	6.72 + 0.28	6.45 + 0.25	6.33 + 0.35
TDS (mg L^{-1})	1315.00 + 0.04	1382.47 + 0.02	1206.07 + 0.03	1058.93 + 0.01
EC (µ mho/cm)	2538.33 + 32.45	2685.83 + 42.85	2314.17 + 22.75	2083.50 + 28.22
NO2 (mg L^{-1})	0.43 + 0.07	0.13 + 0.05	0.29 + 0.04	0.12 + 0.02
NO3 (mg L^{-1})	12.69 + 0.12	11.65 + 0.17	11.25 + 0.22	10.18 + 0.15
PO4 (mg L^{-1})	2.08 + 0.04	2.24 + 0.07	2.17 0.11	1.08 + 0.09

4.2.5 Proximate Composition

The results of the proximate composition analysis of feed and bioflocs among different variations of aeration on and off durations were analyzed and reported in Table 4.4. The results indicate that the Crude Protein content is impacted by the aeration strategies. The highest value of crude protein content among different variations of aeration is recorded in IA (15min ON/45min OFF) and the lowest value is in CA. For Ash content, the concentration decreased with an increase in the non-aeration duration. The highest and the lowest mean values were recorded in CA and IA (15min OFF/ 45min ON). This could be attributed to more dead fecal matter present in the tank due to lesser DO present to facilitate decomposition by heterotrophic bacteria and more fecal matter present as identified by another study (Lara et al., 2017), Crude Lipid and Moisture content showed no difference among the treatments ($P < 0.05$).

Table 4.4: Proximate Analysis (% dry weight) of Pilot-Scale Experiment.

Parameters	**CA(A)**	**IA (05min/55min)**	**IA (10min/50min)**	**IA (15min/45min)**
Crude Protein %	22.17 ± 0.21	23.72 ± 0.24	23.04 ± 0.14	25.12 ± 0.65
Crude Lipids %	2.55 ± 0.45	2.59 ± 0.28	2.61 ± 0.34	2.62 ± 0.32
Ash %	32.22 ± 0.13	32.67 ± 0.27	33.08 ± 0.44	33.68 ± 0.67
Moisture %	69.26 ± 0.32	68.58 ± 0.87	68.89 ± 0.91	69.87 ± 0.36

4.2.6 Fish Growth Parameters

The Statistical results of all the fish growth parameters of the pilot-scale experiment are explained in the below sub-sections and are also tabulated in table 4.7. The mean values of fish growth parameters reared in the tanks with variation in aeration duration are analyzed by one-way ANOVA using SPSS 27.0 software and recorded in Table 4.5. The difference in body weight gain and body length gain were observed to be significantly different among all treatments ($P < 0.05$). The highest values of body weight gain (107.3 ± 2.90) and body length gain (1.6 ± 0.01) were recorded in the study treatment with continuous aeration (CA). The values for body weight gain and length gain decreased as the off duration of aeration were increased and the lowest values for weight gain (58.5 ± 1.08) and length gain (0.9 ± 0.02) were recorded in IA (15min OFF/ 45min ON) respectively.

The FCR, SGR, and PER results showed significant variations across the treatments of the biofloc system, which varied in aeration durations ($P < 0.05$). The treatment CA had the lowest value for FCR. As the off duration of aeration increased, the values fell, reaching the maximum value in IA (15 min OFF/ 45 min ON). The SGR exhibited a contrasting pattern compared to the FCR, with the most elevated and lowest values observed in the CA and IA (15 min OFF/ 45 min ON) treatments, respectively. The CA treatment exhibited the highest values for PER, which displayed a similar fluctuation trend as SGR.

Table 4.5: Growth performance and feeding parameters of Tilapia.

Parameters	**CA(A)**	**IA (05min/55min)**	**IA (10min/50min)**	**IA (15min/45min)**
Length gain (cm)	1.6 ± 0.3^{b}	1.3 ± 0.2^{ab}	1.1 ± 0.3^{ab}	0.9 ± 0.2^{a}
Weight gain (cm	107.3 ± 18.15^{b}	79.4 ± 15.29^{a}	66.5 ± 13.13^{a}	58.5 ± 10.08^{a}
FCR	0.97 ± 0.13^{a}	1.38 ± 0.17^{b}	1.59 ± 0.13^{bc}	1.77 ± 0.12^{d}
SGR	1.37 ± 0.12^{b}	1.05 ± 0.15^{a}	0.93 ± 0.16^{a}	0.83 ± 0.13^{a}
FCE	1.04 ± 0.13^{b}	0.74 ± 0.16^{a}	0.63 ± 0.12^{a}	0.57 ± 0.11^{a}
PER	3.45 ± 0.19^{c}	2.43 ± 0.13^{b}	2.11 ± 0.14^{a}	1.89 ± 0.13^{a}
Survival rate (%)	100 ± 0	100 ± 0	98.08 ± 2.72	92.31 ± 5.44

Values with different letters are significantly different ($P < 0.05$).

CHAPTER 5

CONCLUSIONS & RECOMMENDATIONS

Biofloc technology (BFT) demonstrates significant potential as a sustainable method for fish farming, particularly under optimal conditions with a C/N ratio of 16:1 and a feed loading rate of 60g/m². This setup facilitates maximum biofloc growth and effective ammonia conversion, improving feed efficiency and yield. However, lower C/N ratios (12:1) and feed loading rates (20g/m²) lead to reduced BFT performance in ammonia conversion and protein retention, highlighting the critical role of heterotrophic bacteria in synthesizing a bacterial protein from carbohydrates, residual feeds, and feces.

Continuous aeration has proven more effective than intermittent aeration, resulting in greater biomass gain, lower feed conversion ratios (FCR), and higher fish survivability rates. Nonetheless, intermittent aeration with short off periods (less than 10 minutes per hour) can still maintain essential oxygen levels, water quality, and microbial activity in BFT systems.

There remains a significant research gap in understanding optimal floc morphology, composition, and nutritional value for BFT operations, as well as the effects of temperature control and passive heating. Further studies on biofloc technology across different fish and shrimp species, as well as its application in brackish and seawater cultures, are necessary to enhance aquaculture diversity, farmer profitability, and overall system sustainability. Addressing the research needs in aeration modes, particularly intermittent aeration, could optimize efficiency and reduce operational costs in BFT systems.

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