CENTRO DE INVESTIGACIONES BIOLÓGICAS DEL NOROESTE, S.C.

Graduate Studies Program

Oreochromis niloticus IN RAS AND BFT INTEGRATING AQUACULTURE WITH HYDROPONICS HORTICULTURE IN NON-RECIRCULATED SYSTEM

THESIS

To obtain the degree of

Doctor of Science

Use, Management and Conservation of Natural Resources
(Special field: Aquaculture)

Submitted by

Yenitze Elizabeth Fimbres Acedo.

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ACTA DE LIBERACIÓN DE TESIS

En la Ciudad de La Paz, B. C. S., siendo las 12:00 horas del día 20 del Mes de Julio del 2019, se procedió por los abajo firmantes, miembros de la Comisión Revisora de Tesis avalada por la Dirección de Estudios de Posgrado y Formación de Recursos Humanos del Centro de Investigaciones Biológicas del Noroeste, S.C., a liberar la Tesis de Grado titulada:

Acuicultura de *Oreochromis niloticus* en SAR y TBF integrada con horticultura hidropónica en sistemas sin recirculación.

Presentada por el alumno:

Yenitze Elizabeth Fimbres Acedo

Aspirante al Grado de DOCTOR EN CIENCIAS EN EL USO, MANEJO Y PRESERVACIÓN DE LOS RECURSOS NATURALES CON ORIENTACIÓN EN **ACUÍCULTURA**

Después de intercambiar opiniones los miembros de la Comisión manifestaron su **APROBACIÓN DE LA TESIS**, en virtud de que satisface los requisitos señalados por las disposiciones reglamentarias vigentes.

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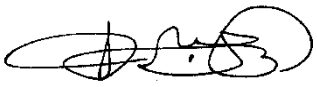
Summary

The accelerated growth of aquaculture has enabled the development of different production models, including recirculation aquaculture system (RAS) and Biofloc Technology (BFT). The search for systems that enable sustainable intensive production has led to the generation of integrated systems, but generation and release of particulate waste remains a challenge. To integrate hyper-intensive aquaculture production systems, this research is divided into four phases: (I) hyper-intensive *Oreochromis niloticus* tilapia rearing in RAS; (II) hyper-intensive *O. niloticus* rearing in BFT; (III) characterizing liquid and particulate residuals, particulate fraction mineralization; and (IV) incorporating residuals into hydroponic horticulture crops in NFT. For RAS, a mathematical model was designed to administer daily protein intake (DPI), which was applied for 34 weeks with three treatments T1:1.4, T2: 1.2 and T3: 1. adjusted weekly using biometrics. Additionally, nutrient flow in the liquid and solid fractions, water quality of the crop, growth parameters, nutritional composition of the organisms were characterized. The final growth of the organisms was 908 ± 57.9 g (T1), 887 ± 113.5 g (T2) and 702.2 ± 38.1 g (T3). The DPI conditioned growth, food conversion ratio (FCR), amount of lipids and protein the whole animal contained ($p < 0.05$) but not the condition of the fillet. DPI can be used to provide the necessary protein at every growth stage, favoring food rationalization, organism growth and avoiding waste and waste generation. For BFT (Phase II), five treatments with three replicates were applied for 40 weeks, implementing three trophic levels: T1 = chemotrophic; T2 = heterotrophic; T3 and T4 = photoautotrophic (*Chlorella sorokiniana*-2805 and *sorokiniana*-2714, respectively) and T5 = *C.* spp). All growth factors (weekly biometrics), crop quality (NO_2, NO_3, PO_4, NH_4) were monitored. Organisms (n = 9) and biofloc samples were taken at the initial time, week 10, 20, 30, 40 for elemental, proximal and amino acid analyses. The contribution of microalgae favored growth and nutritional profile of the organisms ($p < 0.05$), in addition to generating a system with more stable parameters ($p < 0.05$) and allowing a recovery of higher amounts of Ca, Mg and P ($p > 0.05$). In phase III the recovered particulate fraction generated in RAS and BFT (dried, sprayed and analyzed (16 elements) were processed through five mineralization methods: (a) aerobic, (b) anaerobic, (c) acid with H_2SO_4, (d) acid with HNO_3, and (e) incineration. The best method was acid mineralization with H_2SO_4, which allowed greater recovery of P, Ca, S ($p < 0.05$) even at levels higher than those contained in Steiner and Hoagland commercial hydroponic solutions. In phase IV, the residuals of the BFT liquid fraction were reused, using lettuce (*Lactuca sativa*), basil (*Ocimum basilicum*), arugula (*Eruca sativa*), spinach (*Spinacia olearacea*) and pak choi (*Brassica rapa* susp. *chinensis*) in nutrient film technique (NFT) hydroponics. This research found that BFT effluents can be applied for hydroponic crops, but the

complementation of phosphorus and iron is necessary, applying methodologies for microalga flocculation and filtration processes. The results generated during the four stages of this study have allowed us to design models of integrated systems to be applied in arid areas. The characterization of the residuals also allowed us to design the methodologies needed for implementing liquid and particulate fractions in attached hydroponics crops by NFT, as well as detecting the challenges and factors that have to be evaluated for the implementation of integrated intensive production systems with full use of the waste generated.

Keywords: *Oreochromis niloticus*, Daily Protein Intake, RAS, BFT, photoautotrophic phase, hydroponics.

Approval



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Resumen

El crecimiento acelerado de la acuicultura ha permitido el desarrollo de distintos modelos de producción, donde destacan los sistemas de recirculación (SAR) y los sistemas de Tecnología de Biofloc (TBF). La búsqueda de sistemas que permitan producciones intensivas sustentables ha llevado a la generación de sistemas integrados, sin embargo, la generación y liberación de residuales particulados sigue siendo un reto. Con el propósito de integrar sistemas acuícolas de producción hiperintensivos, la presente investigación se divide en cuatro fases: I) cultivo de tilapia *Oreochromis niloticus* hiperintensivo en SAR, II) cultivo de *O. niloticus* hiperintensivo en TBF, III) caracterización de los residuales líquidos y particulados, mineralización de la fracción particulada, IV) incorporación de los residuales a cultivos en horticultura hidropónica en NFT. Para SAR se diseñó un modelo matemático para administrar la ingesta diaria de proteína (IDP), el cual se aplicó durante 34 semanas con tres tratamientos T1: 1.4, T2: 1.2 y T3: 1. ajustándose semanalmente mediante biometrías. Se caracterizó el flujo de nutrientes en la fracción liquida y sólida, la calidad de agua del cultivo, los parámetros de crecimiento, la composición nutricional de los organismos. El crecimiento final fue de 908 ± 57.9 g (T1), 887 ± 113.5 g (T2) y 702.2 ± 38.1 g (T3). El IDP condiciono el crecimiento, el FCA, la cantidad de lípidos y proteína que contenía el animal completo ($p < 0.05$), pero no la condición del filete. El IDP puede ser usado para proveer de la proteína necesaria en cada etapa del crecimiento, favoreciendo la racionalización de alimento, el crecimiento de los organismos y evitando el desperdicio y la generación de residuales. Para TBF (fase II) se utilizaron 5 tratamientos con tres replicas, implementando tres niveles tróficos (T1 = quimioautotrófico, T2 = heterotrófico, y fotoautotrófico (T3=*Chlorella sorokiniana*-2805, T4= *C. sorokiniana*-2714, T5=*C*. spp.) durante cuarenta semanas. Se monitoreo todos los factores de crecimiento (biometrías semanales), la calidad del cultivo (NO_2, NO_3, PO_4, NH_4). Se tomaron muestras organismos (n 9) y de biofloc en el tiempo inicial, semana 10, 20, 30, 40 para análisis elementales, proximales y aminoácidos. La aportación de microalga favoreció el crecimiento y el perfil nutricional de los organismos (p <0.05), además genero un sistema con parámetros más estables ($p < 0.05$), permitió recuperar mayor cantidad de Ca, Mg y P (p >0.05). En la fase III se recuperó fracción particulada generada en SAR y TBF, (secada, pulverizada y analizada (16 elementales) y se procesó a través de cinco métodos de mineralización: a) aeróbica, b) anaeróbica, c) ácido con H_2SO_4, d) ácido con HNO_3, y e) incineración. El mejor método fue la mineralización ácida con H2SO4, la que permitió mayor recuperación de P, Ca, S (p <0.05), incluso a niveles mayores que los contenidos en las soluciones hidropónicas comerciales de Steiner y Hoagland. En la fase IV se reutilizaron los residuales de la fracción liquida del cultivo de TBF, utilizando lechuga (*Lactuca*

sativa), albahaca (*Ocimum basilicum*), arugula (*Eruca sativa*), espinaca (*Spinacia olearacea*) y pak choi (*Brassica rapa* susp. *chinensis*) en hidroponía de NFT. Encontrando que los efluentes del TBF pueden ser aplicados para los cultivos hidropónicos, pero es necesario llevar a cabo la complementación de fosforo y hierro, aplicar metodologías para la floculación de la microalgas y procesos de filtración. Los resultados generados durante las cuatro etapas de esta investigación nos han permitido diseñar modelos de sistemas integrados para ser aplicados en zonas áridas. La caracterización de los residuales nos ha permitido diseñar las metodologías necearías para la implementación de la fracción liquida y particulada en cultivos adjuntos de hidroponía por NFT, así como detectar los retos y los factores que tienen que ser evaluados para la implementación de sistemas integrados de producción intensiva con aprovechamiento completo de los residuales generados.

Palabras clave: *Oreochromis niloticus*, Ingesta Diaria de Proteína, SAR, TBF, fase fotoautotrófica.

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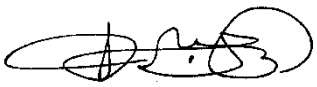

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Dedication

I would like to dedicate this thesis work to my family and to all those who made it possible for me to achieve each one of the goals and overcome each obstacle.

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Content

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Abbreviations

°C, degrees Celsius
µm, Micrometer
AA, Amino acids
AMT, aquatic multi trophic system
CF, Consumed food
cm, centimeter
FCR, Food Conversion Ratio
LF, Liquid Fraction
PF, Particulate Fraction
TP, Total Phosphorus
g gram
h time
H_2SO_4, Sulfuric Acid
HNO_3 Nitric Acid
ICP-AES, Optical spectrophotometry
DPI, Daily Protein Intake
In, Natural Logarithm
Kg, kilogram
KOH, Potassium Chloride
L, liter
TL, Total Length
m, Meter
mg, milligram
min, minute
MJ, Mega Joule
mL, milliliter
MR, Registered Trademark
NFT, Nutrient Film Technique (English acronym)
TN, Total Nitrogen
DO, dissolved oxygen
Org., Organisms
P40, 40% protein food
S, Survival
RAS, Recirculating Aquaculture System
Spp., Species
TAN, Total Ammoniacal Nitrogen
BFT, Biofloc Technology
PER, Protein Efficiency Ratio

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1. INTRODUCTION

1.1 Production systems

Food security plays an important role because of population growth, which exceeds 7.2 billion, and it continues to grow (FAO, 2016). Expectations indicate that by 2050-world population will have increased to more than 9 billion people (Godfray *et al*., 2010a). Thus, conventional food production systems, including intensive animal production, have to change (Godfray *et al*., 2010b) due to the increase of inputs, such as energy, pollution, and climate change, among other factors that limit these systems (Ahmed *et al*., 2018).

By 2050 more than 70% of the world's population is expected to be urban areas (FAO, 2009a). Urbanization will bring with it changes in life styles and consumption patterns: overconsumption, contamination, more competition for space, necessity of more space in urban areas (Hubacek *et al*., 2007). In combination with income growth, it may accelerate the ongoing diversification of diets in developing countries (FAO, 2009a). While the shares of grains and other staple crops will be declining, those of vegetables, fruits, meat, dairy, and fish will increase. While the share of the urban population is growing, though, rural areas will still be home to the majority of the poor and hungry for quite some time. Living in hunger hot spots, often ecologically fragile areas, many inhabitants have to cope with conditions of high population pressure and deteriorating ecosystems (FAO, 2009b, 2009a), but sustainable production activities as inland aquaculture could be a solution for these areas; thus, investing in aquaculture production allows strengthening these communities (Budzich-Tabor *et al*., 2018).

The fastest growing productive activity in recent decades has been aquaculture (Froehlich *et al*., 2018) with an annual growth of 5.8% during the period from 2000 – 2018 (FAO, 2018), faster than other segments of animal production (Ganguly *et al*., 2013) with more than 598 farming species from freshwater, brackish water and

marine environments (fish, crustaceans, mollusks, echinoderms, amphibians). This activity represents one of the most viable alternatives for food production (Brooks and Conkle, 2019). Consequently, various production models have developed to increase production and minimize time besides the negative factors in other productive areas, such as limitation of freshwater supplies, loss of arable land, degradation and loss of soil nutrients (Goddek *et al*., 2015), collapse in fisheries (Hilborn *et al*., 2005), among others.

Aquaculture currently supplies around 68% of the fish consumed globally and plays a key role in efforts to eliminate hunger and malnutrition in the world (Béné *et al*., 2016; FAO, 2018). Therefore, the market has had a great influence on production systems, generating a wide diversification in farmed species and with the quality, they are produced (Natale *et al*., 2013).

Among the relevant species in freshwater aquaculture, cichlids and cyprinids (tilapias and carps) represent the largest fish production group in the world (FAO, 2014). Tilapia is the common name given to three genera of the Cichlidae family: *Oreochromis*, *Sarotherodon* and *Tilapia* (Wang and Lu, 2016), of which the most important species for aquaculture are in the genus *Oreochromis* (*O. mossambicus, O. aureus*, *O. niloticus*). *Oreochromis niloticus* is the most cultivated (Moura *et al*., 2016), and it represents 80% of the tilapia farmed worldwide (Santos *et al*., 2019).

The expansion of aquaculture production, especially for species as tilapia, is evident in the relative growth rates per capita consumption of different species groups in recent years (FAO, 2018). The interest for this species began in the 1980s. Kumar & Engle (2016), described three important periods for tilapia production growth: (1) initial phase (1981–1990), (2) early growth phase (1991–2000), and (3) rapid growth phase (2001–2012).

O. niloticus is the sixth most important species worldwide in terms of value, but the third most important of those traded internationally, following white-legged shrimp

and Atlantic salmon. In terms of quantity produced, tilapia is more important than salmon and shrimp (Kumar and Engle, 2016). Tilapia differs from shrimp and salmon in that they are grown as a subsistence crop in many developing countries across the world (Kumar and Engle, 2016), but they are also grown as a high value crop for domestic urban markets (as in Egypt and Brazil) and for export; its demand exists within a highly diverse market, highly developed western markets, the poorest communities in developing countries (Norman-López and Bjørndal, 2009), and each time in more diverse production systems, making it a model species for new systems of production.

The success of farming this species is related to the good performance of its genetic lines on its growth (Mamun *et al*., 2007), which derives from higher yield in feeding and adaptation to various protein sources (El-Sayed and Tacon, 1997), cultivation techniques (extensive, semi-intensive, intensive), as well as to various production models (Santos *et al*., 2019); it has also shown greater tolerance at wide ranges of pH, salinity, temperature and dissolved oxygen (Mjoun Kamal *et al*., 2010). Its high production and acceptance in the market (Wang and Lu, 2016) are factors that have played in its favor.

Production of tilapia is widely distributed around the world (Fitzsimmons, 2000). It is farmed in more than 85 countries worldwide with production methods ranging from artisanal to intensive operations (Norman-López and Bjørndal, 2009). Tilapia farming ranges from a rural subsistence (extensive and low input practices for non-commercial and household consumption) to a largescale (intensive, commercial purpose and market driven capital) level, depending on the intensity of the management used (Gupta and Acosta, 2014). In Asia, the Philippines was the pioneer for cage rearing in lakes and reservoirs in the region and semi-intensive and intensive farming practices (Guerrero 2002). In Mexico, cage culture systems include floating cages, net pens that use staked sides and the rest on the bottom, and wooden corrals that enclose portions of a lagoon (Fitzsimmons, 2000).

Semi-intensive pond tilapia rearing is typically integrated with agricultural or animal husbandry activities because pond fertilization with organic (e.g., crop residues or manures) fertilizers can promote natural pond productivity in addition to being directly consumed by tilapia (Watanabe *et al*., 2002). Polyculture of tilapia with other native fishes in freshwater ponds is also widely integrated with agriculture and animal farming in southeast Asia; particularly in Indonesia, Thailand, Vietnam, Cambodia and Myanmar (Gupta and Acosta, 2014). Most of the pond-based tilapia farmers grow tilapias under the monoculture system. Culture methods followed depending on nature of farmland and farmers' capacity to investment (Gupta and Acosta, 2014).

In America extensive pond rearing is still practiced in some areas, such as Mexico, the Dominican Republic, and Jamaica, where families typically consume most of the product and sell a small portion (Watanabe *et al*., 2002).Intensive rearing in raceways and round tanks with recirculating systems within green houses or insulated buildings to maintain warmth has been developed in the United States, Canada, Brazil and Mexico (Watanabe *et al*., 2002). In Canada and the United States, tilapia rearing in raceways using aquaponics system has been shown to be technically feasible and economically possible where fresh fish and vegetables receive a premium price (Fitzsimmons, 2000), but Countries of the Americas are relatively small markets and producers compared with China and other Asian countries (Watanabe *et al*., 2002).

1.2 Aquaculture production systems: Recirculating Aquaculture System (RAS) and Biofloc Technology (BFT)

Most recent aquaculture systems have been intensive (Subasinghe *et al*., 2009) because of the need to increase world food production from 25 to 70% (Hunter *et al*., 2017), so they need to be more efficient and sustainable (Campbell *et al*., 2014; Diana *et al*., 2013; Edwards, 2015).

To meet the needs of sustainable production, more efficient production systems have been implemented in recent decades, such as the Recirculating Aquaculture System (RAS) and Bioflocculation Technology (BFT) that can be integrated into plant production systems (hydroponics).

The main objective of these aquaculture systems is high fish production with a significant reduction in the generation of residuals, improvement in the nutrient cycle, energy flow, water use and management, raw materials, space, organic matter, and nutrient management, avoiding eutrophication (Choo and Caipang, 2015; Crab *et al*., 2012; Emerenciano *et al*., 2017; Endo and Takeuchi, 2009; Fleckenstein *et al*., 2018; Gallardo-Collí *et al*., 2019; Liu *et al*., 2014; Losordo *et al*., 1998; Riche and Garling, 2003; Zhang *et al*., 2011). Residuals can be used for the production of several plant species, which allow obtaining a greater benefit for food production.

1.2.1 Recirculating Aquaculture System (RAS)

Recirculating aquaculture system (RAS) emerge as a demand for more efficient systems (FAO, 2006; Goddek *et al*., 2019) that allow the production of lower residual matter (Subasinghe *et al*., 2009). In the last decade, two production models have been established. The first one is found in intensive and semi-intensive farms with medium to high yields and the second one in family farms or cooperatives with medium to low yields (Naylor *et al*., 2000). However, expansion and development depend on the application of technologies to maximize resources, intensify production, maximize the use of water, nutrients, and minimize environmental impacts, so implementing enclosed or less water exchange systems is a prevailing necessity (Béné *et al*., 2016; Deutsch *et al*., 2007; Sapkota *et al*., 2008; Turcios and Papenbrock, 2014; Verdegem *et al*., 2006) (Fig. 1).

The RAS has allowed the development of sustainable models by laying the foundations for achieving production with lower water requirements (null

replacement) with intensive crops, also by promoting the development of aquaculture activities in areas where water is scarce (Piedrahita, 2003; Rurangwa and Verdegem, 2015). By requiring 250-1000 L of water per Kg of fish (Shnel *et al*., 2002), the expansion of productive systems in non-coastal areas was favored (Klinger and Naylor, 2012; Martins *et al*., 2010). While the implementation of RAS requires a great investment due to the high costs of the system components, it is compensated by the cultivation densities it manages (Rurangwa and Verdegem, 2015); moreover, it also eliminates residual discharge in the environment (Martins *et al*., 2010); mitigates pollution of the areas surrounding the crops (Zhang *et al*., 2011); allows nutrient recycling, more hygienic crops, better disease management (FAO, 2017; Piedrahita, 2003; van Rijn, 1996b) and favors maintenance of stable conditions of the cultivation tanks (Masser *et al*., 1999) (Fig. 2).

The interest in the use of these systems has increased (Losordo *et al*., 1999) since modernization of this model was used for cultivation of diverse species, generally of high value, such as salmon (Midilli *et al*., 2014; Sun *et al*., 2016) and trout. However, tilapia rearing in RAS began more than two decades ago successfully (Chen *et al*., 1994; Losordo *et al*., 1998; Masser *et al*., 1999; Riche and Garling, 2003; Suresh and Lin, 1992) because of the high density obtained and its high consumption in the productive areas of this species, which allowed having the product within market reach (Shnel *et al*., 2002). It has not only been used for farming and grow-out of organisms but also for breeding and rearing larvae and fingerlings (Gullian-Klanian and Aramburu-Adame, 2013; Gullian-Klanian *et al*., 2013; Martins *et al*., 2010).

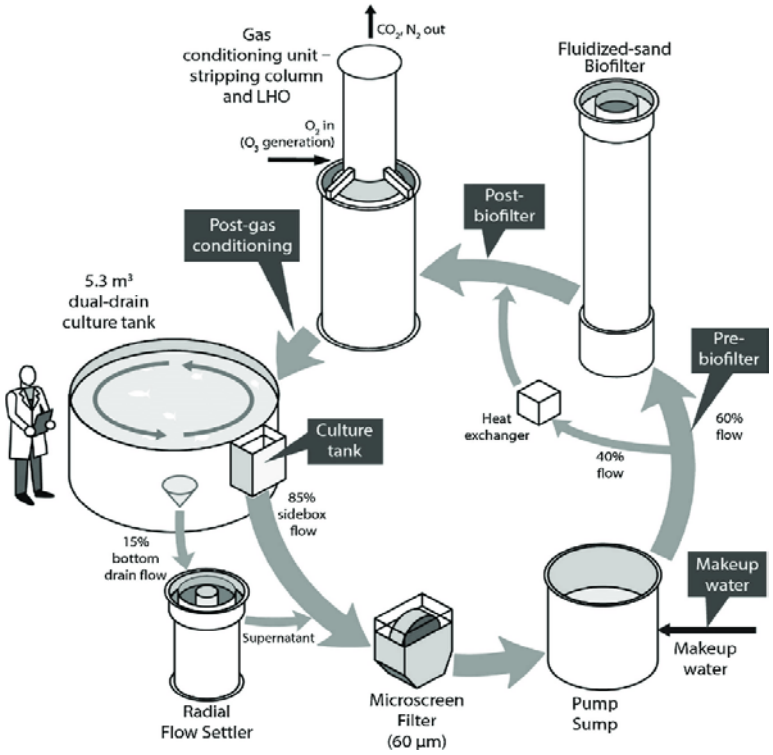

Figure 1. Exemplification of the commercial recirculating aquaculture system (RAS). The arrows indicate the process flow diagram of an individual experimental-scale RAS, (figure obtained from Goddek *et al*., 2019). The component in RAS could be changed, depending on density, size, in general, the necessity of the species in culture.

Because RAS has been considered a more sustainable method for fish production (Badiola *et al*., 2012), several countries have tried to develop this system but at a lower cost (Fig. 2) (Watanabe *et al*., 2002), and Mexico has been no exception (Soto-Zarazua *et al*., 2010). One of the challenges to overcome is the production of organic matter within the system (Shnel *et al*., 2002; van Rijn, 1996; 2013) as it happens in Mexico (Soto-Zarazua *et al*., 2010).

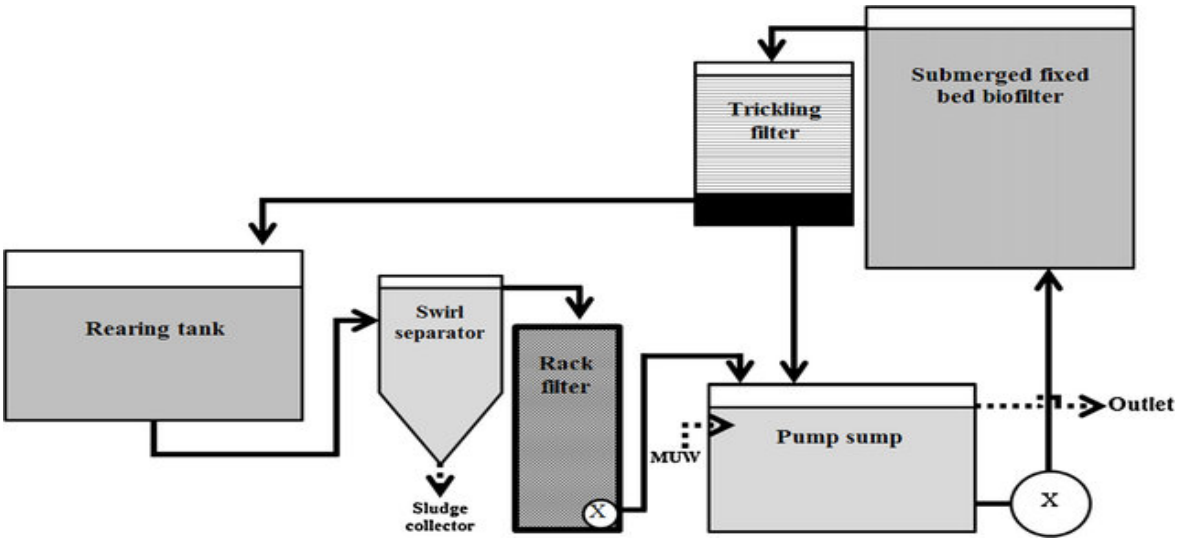

Figure 2. Recirculating aquaculture system (RAS). This scheme explains in a general manner the most common RAS components. Black arrows indicate water flow (Photo obtained from Fernandes, 2015).

1.2.2 Bioflocculation Technology (BFT)

Bioflocculation technology (BFT) is a production system with low or zero water exchange (Azim and Little, 2008) that allows recycling unconsumed food and waste produced by organisms in flocs, which are available for rearing organisms (Fig. 3a) (Avnimelech, 2003). The basis are the microorganisms that inhabit the system and colonize these flocs, generating important protein contribution and forming part of the processes for recycling nutrients (nitrogen cycle) through microbiological autotrophic and heterotrophic communities, including bacteria, fungi, microalgae and zooplankton (a microeukaryotic community) (Fig. 4) (Ebeling *et al*., 2006; Gallardo-Collí *et al*., 2019b, 2019a; Martínez-Córdova *et al*., 2015). These microorganisms play three important roles within the system (a) maintaining water quality by taking nitrogen compounds and generating proteins; (b) increasing nutrition by extra contribution; and (c) reducing pathogens within the system (Emerenciano *et al*., 2017; Martínez-Córdova *et al*., 2015). Another important basis is the C/N relationship (Avnimelech, 2003) where a carbon source is necessary to maintain the heterotrophic communities. Some of the most common carbon

sources are molasses, sugar or glycerol (Ekasari *et al*., 2010) that can maintain a relationship of C/N = 12-20:1 (Emerenciano *et al*., 2017). This type of crop is favorable for recycling protein, reducing the food conversion ratio (FCR) and increasing growth in high density crops (De Schryver *et al*., 2008).

BFT emerged as an alternative system for residual treatment and by restriction of water exchange due to environmental costs and regulations. Its beginnings date from the 1970s with different shrimp species. Then, in the 1980s an Ecotron program was developed; in the 1990s, countries, such as the United States of America (USA) and Israel, began to use BFT with tilapia and white shrimp. Since then several countries have implemented this technology (Emerenciano *et al*., 2013). One of the forerunners of BFT is Avnimelech (2009), who developed a practical guide for its use and implementation.

This technology has been very successful in farming tilapia with increases from 1128 to 128% in weight gained and specific growth compared to RAS (Luo *et al*., 2014); hence, it has been applied in the various tilapia stages from fingerlings and calves to their grow-out and reproduction (Crab *et al*., 2009; Day *et al*., 2016; Ekasari *et al*., 2015a, 2015b; Fleckenstein *et al*., 2018; García-Ríos *et al*., 2019; Luo *et al*., 2014). BFT favors *O. niloticus* growth, enzymatic activity, and immune response (Day *et al*., 2016; Long *et al*., 2015; Luo *et al*., 2014; Perez-Fuentes *et al*., 2018); it also reduces protein intake, tolerable levels of stress in reared organisms and improves growth (Azim and Little, 2008; Luo *et al*., 2014).

The use of BFT has been proposed as a substitute for fish meal (Ogello *et al*., 2014). Now this production system has been highly recognized because of the scope it has had in reducing the FCR, increasing growth, taking advantage of the residuals, supplying lower protein food and its recycling and constant food to the organisms (Becerril-Cortés *et al*., 2018; Crab *et al*., 2012; De Schryver *et al*., 2008; Gallardo-Collí *et al*., 2019a, 2019b; Hargreaves, 2013; Pinho *et al*., 2017).

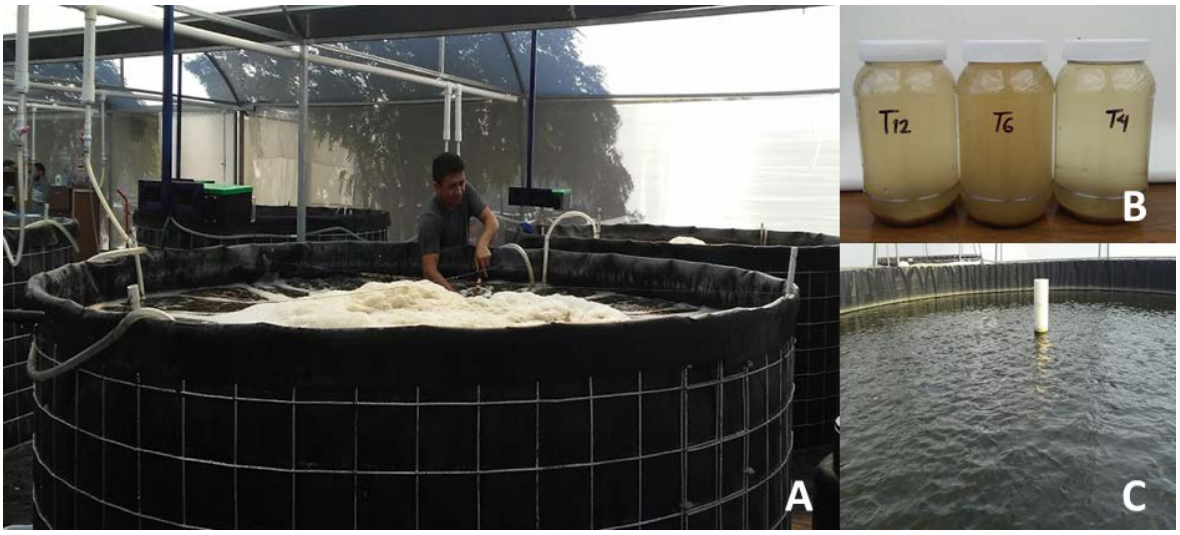

Figure 3. Bioflocculation system (A) Tilapia biofloc rearing, (B) water samples from heterotrophic biofloc, C) Biofloc tank (Photos from the author).

One of the important characteristics of BFT is the interaction carried out by the microorganisms of the cultivation tank by allowing the stable conditions to be maintained (Fig. 3b, c) (Avnimelech, 2007; Martínez-Córdova *et al*., 2017; Martínez-Porchas and Vargas-Albores, 2017). The microorganisms that inhabit the system fulfill three important functions: (i) water-quality maintenance by nitrogen-compound absorption that generates microbial protein "*in situ* "; (ii) nutrition that increases cultivation viability by reducing the FCR and decreasing costs by using less feed; and (iii) pathogen competition (Emerenciano *et al*., 2017): BFT, seen from an environmental point of view, has the primary advantage of minimizing the release of water containing residual organic matter in rivers, lakes and estuaries (Emerenciano *et al*., 2013).

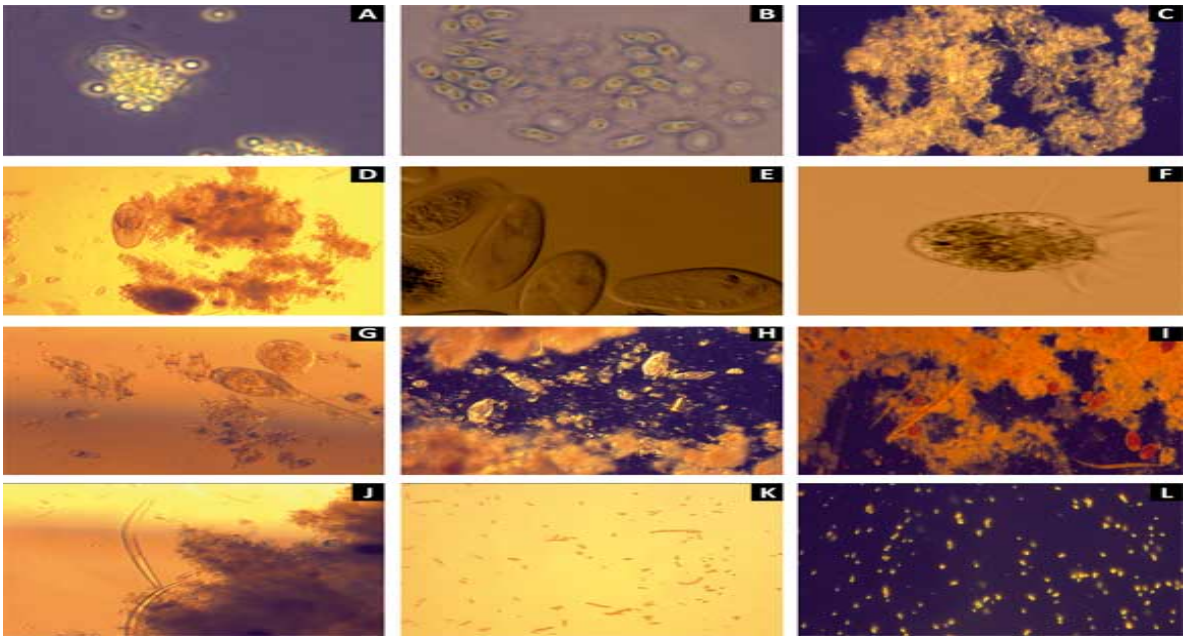

Figure 4. Diversity of the community that cohabit floc. (A) Chlorophyte algae (40x); (B) Cyanobacteria (40x); (C) Diatoms (10x); (D) Ciliate *Paramecium* genus (40x); (E) Ciliate *Colpidium* genus (100x); (F) Ciliate *Halteria* genus (100x); (G) Rotifers and ciliates (40x); (H) Rotifers *Philodina* genus (40x); (I) Rotifers and nematodes attached to the bacterial flocs (40x); (J) Nematodes (40x); (K) *Vibrio* sp. (40x); (L) Yeast *Rhodotorula* sp. (40x. (Photo and data obtained from (Monroy-Dosta *et al*., 2013).

1.3 Feeding and residual nutrients

Food accounts for approximately 50-70% of production costs (Valente *et al*., 2011). Depending on the type of food, its waste may oscillate 1-38%, as well as on feeding practices, rearing methods and the species used. It is also one of the most important sources contributing to organic and nutrient loads (responsible for environmental impacts) (Cho and Bureau, 2001; Wu *et al*., 1995).

Protein is the largest organic component in fish tissue; it constitutes 45-75% of the whole body based on dry matter (El-dahhar, 2007), so it is the first limiting factor to formulate cost-effective diets due to its high price in the market (Guimarães *et al*., 2008). In this sense, intensive tilapia rearing requires efficient food formulation with the ability to meet protein requirements during its growth period (Ahmad *et al*.,

2004). For juveniles, protein requirement is 30-40%, 20-30% for adults, and 35-45% for spawners; with these values optimal reproduction, spawning efficiency, larval growth and survival can be achieved (El-Sayed, 2004). Nonetheless, traditionally the feeding level has been adjusted according to the standard feeding tables developed for each species (Cho and Bureau, 1997), and it must be stopped near satiety (under close supervision).

Therefore, determining protein, energy, and availability of reliable data to achieve the right feeding level of fish in an integrated system could be a key factor in reducing production costs, increasing crop profitability (El-dahhar, 2007) and ensuring successful aquaculture (Ahmad *et al*., 2004; Koch *et al*., 2016). Nowadays, fish farming had a crucial necessity, which was improving the foods used and reducing the nutrients excreted in the water (Fournier *et al*., 2003).

Nitrogen (N) and phosphorus (P) are the main end-products of fish loading, and could affect not only rearing water, but also the environment as a whole (Lazzari and Baldisserotto, 2008). In *O. niloticus* rearing, Osti, *et al*. (2018) reported that 26% TN (Total nitrogen) and 45% TP (Total phosphorous) were reversed into fish biomass, 62% TN and 40% TP were retained in the fishpond, and 12% TN and 15% TP were exported via effluent. The output of N and P metabolic wastes by fish was determined by numerous endogenous and exogenous factors, such as genetics, life stage, size, rearing system, diet (Lazzari and Baldisserotto, 2008). Understanding the relationship with the balance of components in diets and the residual compounds in water is very significant for residual management in aquaculture; recognizing that several factors contribute to this relationship, such as, rearing size and practices, fish species, feed handling and characteristics are also important.

Reducing the outputs of these dissolved wastes is considered to be a key element for the long-term sustainability of aquaculture around the world (Cho and Bureau,

1997). One of the challenges for aquaculture expansion is implementing sustainable practices that ensure the good use of resources, residual treatment and management (Brooks and Conkle, 2019), food quality and management, food types, which affect the sedimentation processes of organic matter, resuspension, nitrification, and ammonification. In this sense, the quality and quantity of the residuals depends on the cultured and farmed species, and the amount and quality of feed (Wang *et al*., 2005).

The residuals produced by aquaculture can be divided into solid and dissolved (liquid) fractions (Cripps and Bergheim, 2000). Solid residuals can be classified into settleable and suspended, which come from unconsumed food or feces (Chávez-Crooker and Obreque-Contreras, 2010; Cripps and Bergheim, 2000). Dissolved residuals come from fish-excreted metabolites (through the gills or urine). Another part of the dissolved waste originates from the disintegration/suspension of residual nutrients from the solids found in the system (Amirkolaie, 2011).

Nitrogen is associated with protein, which is the main source of this element for fish rearing (Valente *et al*., 2011). In aquaculture systems, only 25% of the nitrogen entering the system is harvested through fish biomass and more than 70% is excreted in the form of ammonium (NH_4 +), which can be toxic when it accumulates (Hargreaves, 1998; Milstein *et al*., 2002). Within the productive systems, three ways have been used for processing ammonium: (a) consortium of autotrophic nitrifying bacteria; (b) consortium of heterotrophic bacteria where a source of carbon is required to convert the ammonium directly to microbial biomass; and (c) removal by algae through photoautotrophic processes (Emerenciano *et al*., 2017). In the first case, ammonium becomes nitrite within the system (NO_2-N) due to the activity of nitrous-bacteria (*Nitrosomonas, Nitrosococcus, Nitrosospira, Nitrosolobus,* and *Nitrosovibrio*) before being transformed into nitrate (NO_3-N) by the Nitro-Bacteria (*Nitrobacter, Nitrococcus, Nitrospira*, and *Nitrospina*) (Ahn, 2006; Hagopian and Riley, 1998). The final

product of this bacterial conversion is nitrate, which is the main source of nitrogen for plant growth in aquaponic systems (Bergheim *et al*., 1993; 1998).

Approximately 7-32% of the total nitrogen (TN) and 30-84% of the total phosphorus (TP) are in the particulate fraction (PF) (Bergheim *et al*., 1993), where the main components are unconsumed food and feces. This fraction requires separation for efficient management of crop water quality, so solid waste management is the most critical process (van Rijn, 2013). The decomposition of solids can degrade water quality of the system and effluents, which directly and indirectly affects the organisms' health, as well as the quality and traceability of the water discharged from the system (Summerfelt *et al*., 1999; 2001).

Solid decomposition is also the primary objective of aquaculture effluent treatment since it can affect the aquatic environment, so it must be intercepted and withdrawn as thoroughly as possible before it is released (Bergheim *et al*., 1998; Chen *et al*., 1997; Summerfelt *et al*., 1999; Cripps and Bergheim, 2000).

Wastewater treatments are usually physical processes, including sand filters and mechanical systems. Moreover, biological processes use submerged biofilters, drip filters, rotating biological contactors and fluidized bed reactors that are used for organic matter oxidation, nitrification or denitrification (van Rijn, 1996). Nonetheless, the disadvantages of these treatments are that they produce solids, require much more energy and depend on frequent maintenance. Therefore, the development of an effective and low-cost treatment is essential for the current expansion of aquaculture (Muga and Mihelcic, 2008).

One of the most intriguing strategies to intensify production and at the same time reduce waste is the development of RAS (Badiola *et al*., 2012). This system is designed to collect and eliminate waste products, unconsumed food, and residual organic matter through microbiological and/or mechanical processes in biofilters, allowing effluents to be treated and then recirculated through the system (Fig. 2)

(Hutchinson *et al*., 2004; Summerfelt *et al*., 1999). It also offers the possibility of a large-scale sustainable production by greatly reducing the use of space, water (10% replacement) (Verdegem *et al*., 2006) and increasing disease control (Midilli *et al*., 2012). Moreover, it is relevant in areas where water supply and/or the effects of nutritional burdens on surrounding aquatic systems limit the current scope of aquaculture production (Martins *et al*., 2010; Piedrahita, 2003).

1.4 Integrating aquaculture and plant production

Several models of integrated systems incorporate RAS or BFT with plant crops (floating bed hydroponics) or NFT (Nutrient Film Technique) (Okemwa, 2015; Rupasinghe and Kennedy, 2010; Trang *et al*., 2010); for example, aquaponics (aquaculture production + plant production) (Bosma *et al*., 2017; Diver and Rinehart, 2010; Losordo *et al*., 1998; Rakocy, 2007; Somerville *et al*., 2014; Wardlow *et al*., 2002) and several multitrophic crops (AMT) (Barrington *et al*., 2010; Chopin *et al*., 2012; Robinson *et al*., 2008). Some of the advantages of these systems are using less surface, generating two or more products with high yield in the same system, recycling waste, water and raw materials, and lower or null replacement rate (Schneider *et al*., 2005; Zhang *et al*., 2011).

Additionally, the most important characteristic is reusing the residuals that the common aquaculture systems generate in the liquid and solid fractions. In a general manner aquaponics could be classified in coupled (close) or decoupled (open) culture. Coupled aquaponics combines three classes of organism (1) aquatic, (2) bacteria, (3) symbiotic or complementary plants that benefit from each other in a closed recirculated water body (Fig. 5). Water serves as a medium of nutrient transport, mainly from dissolved fish waste, which is converted into nutrients for plant growth by bacteria. In a coupled aquaponics system, volumes are critically important (i) aquaculture unit following the principles of recirculating aquaculture systems (RAS), (ii) bacterial growth substrate and (iii) space for plant

units and amount of plants to be cultivated. Together, they all form the aquaponics unit (Fig. 5) (Goddek *et al*., 2019).

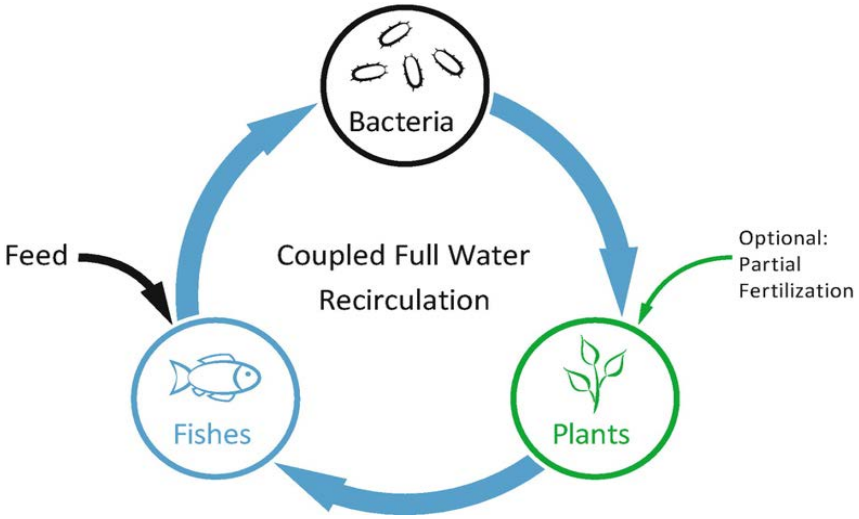

Figure 5. Coupling aquaponics systems with three principal components; (1) aquatic organism, (b) residual water (bacterial community), (c) plants (Figure obtained from: Goddek *et al*., 2019).

Decoupled systems differ from coupled systems insomuch as they separate the water and nutrient loops of both the aquaculture and hydroponics unit from each other and thus provide water chemistry control in both systems (Fig. 6) (Goddek *et al*., 2019). The most common system is the incorporation of RAS and hydroponics unit dispensable in two individual water cycles (Goddek *et al*., 2016), which allow manipulating individual parameters from aquaculture and hydroponics and maintaining individual characteristics (Fig. 6). Mineral transfer from aquaculture to hydroponics support efficient nutrient recycling (Graber and Junge, 2009; Turcios and Papenbrock, 2014), reducing pollution by contributing effluents from aquaculture to eutrophication of aquatic environments through the addition of organic matter (Brown *et al*., 1999; Endo and Takeuchi, 2009).

This model allows incorporating two or more components in addition to the system; for example, the mineralization process or recovery of the liquid fraction

from aquaculture, maintained until needed by hydroponics, also allows recovery of the solid fraction (Fig. 6). This solid fraction can be processed by mineralization in a special component. In non-recirculating systems, the intermittent elimination of considerable quantities of nutrient-rich water leads to high consumption, as well as to contamination of surface water and groundwater (Goddek *et al*., 2015). Thus, unifying and implementing techniques that allow reusing both liquid and solid fractions is one of the compelling ways to develop more eco-efficient integrated systems. Several possible ways have been applied for the beneficial elimination of organic waste from aquaculture, especially the solid fraction, such as application in agricultural land, composting and integrated systems as aquaponics or hydroponics (Yeo *et al*., 2004). Nevertheless, they have not been permanently or constantly applied in aquaculture systems.

Nutrient removal and water reuse rates in hydroponic systems (a kind of decoupled aquaponics) vary widely, depending on the medium used for plant cultivation, flow and type of plants. For example, nitrate and phosphorus elimination rates range from 9-93% and 0-53%, respectively (Graber and Junge, 2009; Endut *et al*., 2010). The incorporation of the solid fraction, which is rich in nutrients, such as phosphorus, potassium, and calcium that are necessary in plant cultivation (Fimbres, 2015), is currently a problem. Hence, in recent years, efficient methods of mineralization have been implemented in aquaponics systems (da Silva Cerozi, 2016) (processes used in the treatment of municipal waste) with a simple methodology that allows treating solid residuals; however, their incorporation into horticultural production systems is not common.

At present, a globalized trend has emerged to empower the most vulnerable sectors in the use of integrated systems (coupled or decoupled systems) (Naylor *et al*., 2009) to improve intake of protein-rich foods. Therefore, the implementation and development of integrated systems promises to be a growing sector in the

following decades (Barrington *et al*., 2010; Béné *et al*., 2016; Jhansi and Mishra, 2013; FAO, 2018).

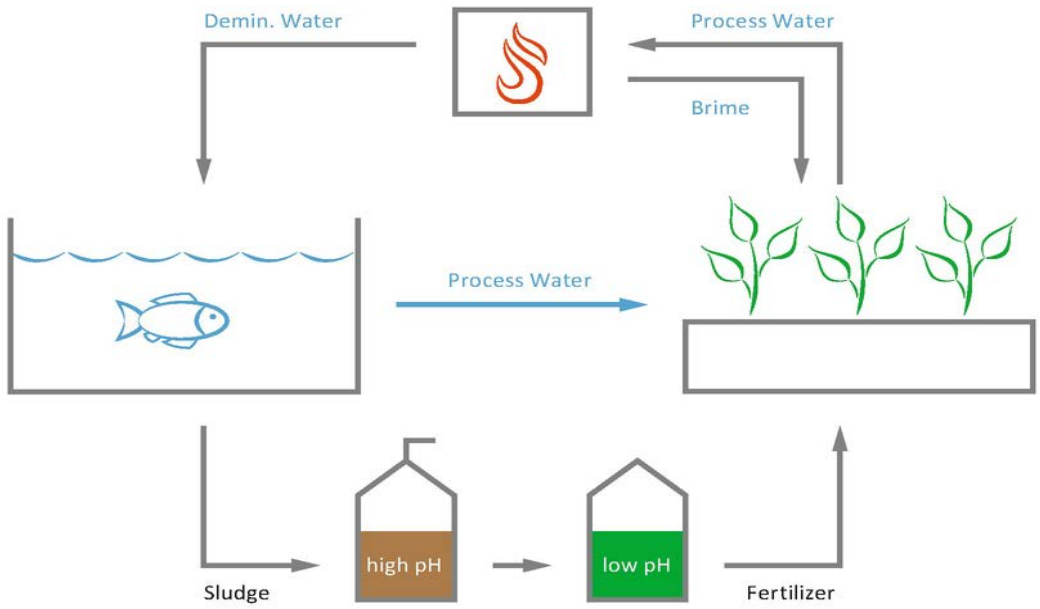

Figure 6. Decoupling aquaponics systems with three principal components; (1) aquatic organism, (b) water residual (bacteria community), (c) plants (Figure obtained from: Goddek *et al*., 2019).

2. BACKGROUND

2.1 Integrated Systems: Implementation of RAS and BFT with Hydroponics

In the last few years, a revolution started in the design and implementation of a new model of aquaculture production. The conventional models (RAS, BFT, hydroponics), were modified to link their advantages and strengthen productions. Goddek *et al*., (2019) described several new systems: algaponics, aeroponics aeroaquaponics, maraponics, haloponics, BFT, and vertical aquaponics. For the interests of this research, it focuses on the development of RAS-Hydroponic (Aquaponics), and BFT+Hydroponics.

In the 1950s, RAS was implemented in arid regions to get better water use with complex aquatic production systems that involve a range of physical, chemical and biological interactions (Goddek *et al*., 2019). Water reutilization rate may range from 80 to 99% reducing the environmental impact of aquaculture and water requirement (Tidwell, 2012), but sludge disposal from such systems remained problematic; this situation lead to the advent of aquaponics that consists of a combination of RAS and a hydroponics system (Rakocy, 2007) wherein the recycling of nutrients produced by fish as fertilizer for plants proved to be an innovative solution to waste discharge that also had economic advantages by producing a second marketable product (Fig. 7) (Goddek *et al*., 2019).

In the early 1990s, two groups working independently in Israel at the Technion University and in the United States at Waddell Mariculture Center (WMC) began to publish a series of papers on the application of reduced and then zero exchange production technologies for tilapia and shrimp, respectively; here is where BFT came to light (Ray, Leffler, and Avnimelech in Tidwell, 2012).

BFT has grown over the past twenty years; it can be applied in ponds, tanks, or raceways of various scales (Ray, Leffler, and Avnimelech in Tidwell, 2012). In

general it has successfully expanded in large-scale shrimp farming in Asia, in South and Central America, as well as in small- scale greenhouses in the USA, Europe, and in Mexico, Brazil, Ecuador and Peru with the nursery phase for commercial-scale tilapia rearing farms in Mexico, Colombia and Israel. Tilapia and shrimp are well suited to take advantage of natural productivity in aquaculture systems (Ray *et al*., 2010).

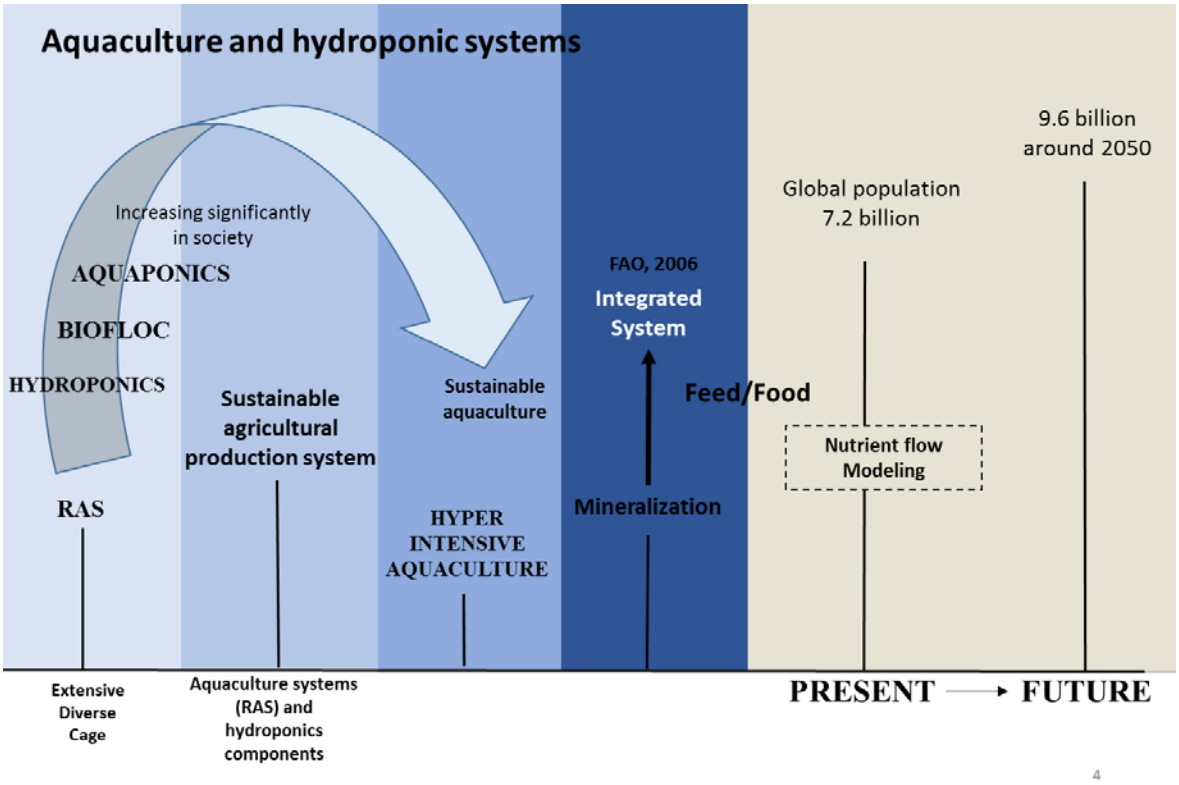

Figure 7. General scheme from the evolution of the aquaculture system, including the mineralization methods (Scheme from the author).

Bioflocs are nutrient rich microenvironments embedded within nutrient-poor water. These systems can provide comparatively biosecure, more environmentally benign, and financially sustainable aquaculture production (Ray, Leffler, and Avnimelech in Tidwell, 2012). A new tendency is the unification BFT+hydroponics where two important processes that occur into BFT could be the key for the integration with hydroponics: assimilation of excess nitrogen into microbial biomass

(Nitrifying bacteria ultimately transform toxic ammonia to the relatively nontoxic nitrate compound) (Crab *et al*., 2007), nutrient that has a high affinity with plants and its mineralization through nitrification and denitrification processes (Goddek *et al*., 2019).

Early studies implementing the effluents from biofloc are: Pinho *et al*. (2017) worked with tilapia and lettuce; da Rocha *et al*. (2017) compared the production of lettuce between aquaponics and biofloc with silver catfish; Lenz *et al*. (2017) described the production of lettuce with effluent from BFT with low salinity; and Gallardo-Collí *et al*. (2019a) reused the effluents from biofloc twice.

Something needs to be done to stop increasing this tendency in concentration of solids, which could affect plant roots and impact nutrient absorption and oxygen availability. An important subject for further studies is managing solids where their impact (particulate fraction and also dissolved fraction) in aquaponics systems should be considered when applying BFT (Goddek *et al*., 2019) because it can also conduce to anaerobic processes into the system (Avnimelech, 2003).

2.2 Flow of macro and micro nutrients

An important technique for the success of integrated systems is nutrient flow and the balance between nutrient production and consumption (Love *et al*., 2015b); if any of these components fails in the system, an imbalance may occur preventing production to be maintained; another important factor is the age of the crop, density and biological characteristics of the organisms along with physical parameters (temperature, DO, pH) (Love *et al*., 2015a).

Nutrient flow is a useful tool, but only the flow of N and P nutrients has been described in aquaponics (Cerozi and Fitzsimmons, 2017; Yogev *et al*., 2017, 2016) with less emphasis on macro and micronutrients of great interest, such as K, Ca, Mg, Mn, Co, as reported in Ru *et al*. (2017). Among the few studies that described

the flow of macro and micronutrients, Seawright *et al*. (1997) detailed nutrient flow in an integrated system by aquaculture and hydroponics and analyzed the concentrations of Ca, Cu, Fe, K, Mg, Mn, NO-N, Na, P, and Zn in relation to biomass increase in a culture of tilapia and lettuce (*Lactuca sativa longifolia*) cultivation. The authors highlighted the deficiencies in the nutrients for hydroponic crops; later, Buzby and Lin (2014) indicated the importance of nutrient balance in a system of aquaponics with lettuce and flowers of the genus *Nasturtium sp*., models that allowed them to evaluate the removal of TAN and NO_3.

For the description of nutrient flow, its concentration, growth and effects on crops have been analyzed depending on the medium used and how they were used (Roosta and Afsharipoor, 2012; Roosta and Hamidpour, 2011). Endut *et al*. (2016), described nutrient balance in an aquaponics system with recirculation in spinach (*Iponema aquatica*), mustard (*Brassica juncea*) and African catfish (*Clarias Garierpinus*) and observed that mustard absorbed greater N but spinach maintained better water quality. Rafiee and Saad (2005) analyzed nutrient flow and organic matter production with five red tilapia groups of different sizes and found a correlation between the nutrients obtained in relation to size with an average absorption of 11.46% Fe, 13.43% Zn, 6.81% Mn, 3.55% Cu, 26.81% Ca, 20.29% Mg, 32.53% N, 7.16% K, and 15.98% P. However, in these descriptions the implementation of PF in aquaponics systems has been null. Prior to this study, the nutritional content of the matter has been described finding rich P, Mg, K, Fimbres (2015), however, its application has been used only in wetlands, excluding integrated systems (aquaponics, hydroponics).

2.3 Feeding efficiency

A large amount of data regarding feeding crop species is available because this activity generates the highest production costs (El-Saidy and Gaber, 2005; Koch *et al*., 2016; Ng and Romano, 2013) since it is not only the diet supplied but also the amount of protein that it must contain. Abdel-Tawwab *et al*. (2010) analyzed

different development stages with food of 25, 35 and 45% of protein in tilapia larvae (0.4-0.5 g), fry (17-22 g) and juveniles (37-43 g) and found a relationship in growth, FCR and specific weight gained; for larvae the best food was 45% of protein and 35% for fry and juveniles. Other elements as the ideal protein concept (Furuya *et al*., 2004) have been studied, and various feeding methods have been detected in tilapia farming, such as satiety (Kasper and Brown, 2003; Koch *et al*., 2016; Lin and Luo, 2011; Trosvik *et al*., 2013), alternating feeding (Bolivar and Jimenez, 2006), feeding based on fixed weight percentage during the experiment (Abdelghany and Ahmad, 2002; Azim and Little, 2008; Liu *et al*., 2018; Nguyen *et al*., 2009; Ogunji and Wirth, 2000; Sun *et al*., 2016; Thompson *et al*., 2012; Tran-Duy *et al*., 2008) with variation from 2% to 16% depending on the organism weight (Fasakin *et al*., 2005; Loum *et al*., 2013), feeding through compensatory growth (Mohanta *et al*., 2016) and feeding using g/kg (5 g of food for every 100 g of weight of the organism) (Richter *et al*., 2003).

At the end of the 1990s, Japan Fisheries Agency designed a food model that was applied by Tokyo University of Marine Sciences and Technology (TUMSAT); this model provides an equation that allows rationing food according to the daily protein requirement, which avoided its waste and provided crop organisms with the right amount of protein regardless of its percentage in food (Fisheries Agency of Japan, 1995). In recent years, models have been developed to define the amount of protein and energy (Belal, 2005). Van Trung *et al*. (2011) designed a bioenergetic factorial model where they analyzed a great number of parameters that allowed determining the optimal specifications of protein and dietary energy for *O. niloticus*.

2.4 Effluents from RAS and BFT

Residual organic matter has been a problem in aquaculture production systems due to the environmental impact generated by unloading important concentrations of N and P (eutrophication, coastal zone pollution, mortality of the surrounding fauna, contamination by antibiotics and chemical residues, algal blooms, among

others) (Endo and Takeuchi, 2009; Michael-Kordatou *et al*., 2015; Turcios and Papenbrock, 2014). In response, integrated production systems have been developed either by aquaponics, agriculture, fertigation or in RAS where the liquid fraction is used for its richness in nitrogenous components; 25% of the N content in the food is retained by fish with a release of 77%, of which 62% is in the liquid fraction (LF) and 13% in the particulate fraction (PF) (Hargreaves, 1998). The most common system to treat LF is implementing biofilters that allow toxic substances, such as ammonium to be converted into nitrates. However, for an optimal functioning if the appropriate temperature, pH and DO conditions exist, the nitrifying bacterial communities will prevail (Schneider *et al*., 2005).

Given the importance of the PF, various techniques have been implemented for eliminating or reducing residual organic matter, such as digestion, dehydration, use of bio-bags, belt filters, membrane reactors, either individually or jointly, to achieve coagulation or flocculation of suspended solids (van Rijn, 2013). The easiest method is sedimentation, but it does not remove dissolved organic matter; hence, implementing and designing models to treat this waste have been topics of discussion for more than two decades. Therefore, systems, such as BFT and wetlands (Lin *et al*., 2002; Sindilariu *et al*., 2007; Uggetti *et al*., 2010) are available now or have been used as fertilizer for terrestrial plants or reduced to inorganic fertilizer for farmland (Rafiee and Saad, 2005).

Another important process implemented in plant wastewater treatment is mineralization (Lovley and Phillips, 1986; Parameswaran and Anderson, 2007; Tampio *et al*., 2016; Trzcinski and Stuckey, 2009; Zech *et al*., 1997), a technique that in recent years has been incorporated into integrated systems (Cerozi and Fitzsimmons, 2017). Among the processes that have been described, one is anaerobic mineralization, which is carried out in the absence of oxygen where bacteria mineralize the compounds and release methane and carbon dioxide (McKennedy and Sherlock, 2015); it is a widely used method to process residual organic matter, produce energy in the form of biogas and nutrient-rich residuals

"product of bio-digester" (Garfí *et al*., 2016; Lovley and Phillips, 1986). These residuals have been used in agriculture to increase soil quality and germination, water retention by using the bio-digester product with the same or better results than with chemical fertilizers (Sattari *et al*., 2012; Saveyn and Eder, 2014). Although aerobic digesters have been little implemented for the treatment of the PF, their function is to mineralize this fraction through aeration, which must be continuous (Fimbres, 2015). Anaerobic and aerobic bioreactors have been implemented to counteract the negative factors of both systems when they work on an individual basis. Currently, four types of bioreactors are available (1) integrated with physical separation of aerobic-anaerobic zone; (2) integrated without physical separation of aerobic-anaerobic zone; (3) sequenced of anaerobic-aerobics reactors; (4) combined with aerobic-anaerobic crops (Chan *et al*., 2009; Novak *et al*., 2011; Parameswaran and Anderson, 2007).

Another technique is dehydration of organic matter that allows its manipulation, transfer and processing, a technology used prior to composting, incineration or land filling (Uggetti *et al*., 2010; Zhang *et al*., 2004), but this process involves previous mechanical techniques, such as centrifugation, filtration or other processes, such as evaporation, evapotranspiration and percolation. The implementation of wetlands contiguous to production areas is a technique that has been implemented for residual PF treatment of aquaculture crops, which has been used in Europe since the 1980s (Lin *et al*., 2002; Sindilariu *et al*., 2007; Uggetti *et al*., 2010). In spite of the advances in this matter, the concern for the increase in crop intensification, which is linked to the increase in the production of residual organic matter (Buhmann and Papenbrock, 2013; Lin *et al*., 2002; Zhang *et al*., 2011), has led to open other lines of research to reduce the production of organic matter in crops by increasing digestibility in diets, implementing more rigorous feeding plans, more diets attached to species needs and more sophisticated mechanisms to remove and treat residual organic matter (Essa *et al*., 2010; Santos *et al*., 2019, 2016; Standen *et al*., 2016). Other methods that have been applied

are enzymatic digestion and acid hydrolysis, using sulfuric or nitric acid as a precursor for mineralization (Endo, 2012; Tahan *et al*., 1993; Tampio *et al*., 2016). Therefore, according to the foregoing reasons, this study proposes the use of the PF for hydroponic crops.

2.5 Mass balance and nutrient flow modeling

Mass balance is a tool used to theoretically quantify the production of organic waste matter in the cultivation systems (Heinsbroek and Kamstra, 1990), which may include multiple factors involved in production. An important aspect is to determine water quality at the beginning of the crop for a suitable mass balance. Mass balance has been applied in RAS as a tool to quantify the operation, measure the volume of the system components and know what type of nutrients are generated (Hermoso *et al*., 2016).

Furthermore, mass balance has been applied to N and P because they are the nutrients that the crop system generates the most. Not only has it been analyzed in RAS, Paéz-Osuna *et al*. (1997) characterized nutrient flow and mass balance in a semi-intensive shrimp crop; they described the system N and P inputs and outputs and highlighted that this type of studies allows understanding the processes associated with the environment caused by the entry of nutrients, which in turn has generated important information for decision making. Casillas-Hernández *et al*. (2006) described nutrient flow and mass balance of a shrimp crop with two feeding methods, in which they managed to quantify gains and losses of N and P of the system used.

This type of tools has made it possible to elucidate food utilization and proper use, efficiency in its consumption and water quality, and thus understand the biogeochemical processes performed in the cultivation system with greater precision (Casillas-Hernández *et al*., 2006). The N and P flows and their retentions are based on the concept: output = input-retention. This retention can be

expressed as g/kg of food (wet weight) or as a fraction of the total nutrient administered with the food (% of nutrients in the feed). The retention of N and P is estimated based on the proximal composition of crop organisms, food conversion ratio (FCR) and production rates (Schneider *et al*., 2005). Modern RAS systems implement computer programs for mass balance to determine outputs and inputs, internal changes (conversions and consumptions) generated in the production system as it allows visualizing the changes that could take place and thus maintain adequate values of TAN, NO_2, NO_3 and others nutrients (Fig. 8) (Hutchinson *et al*., 2004).

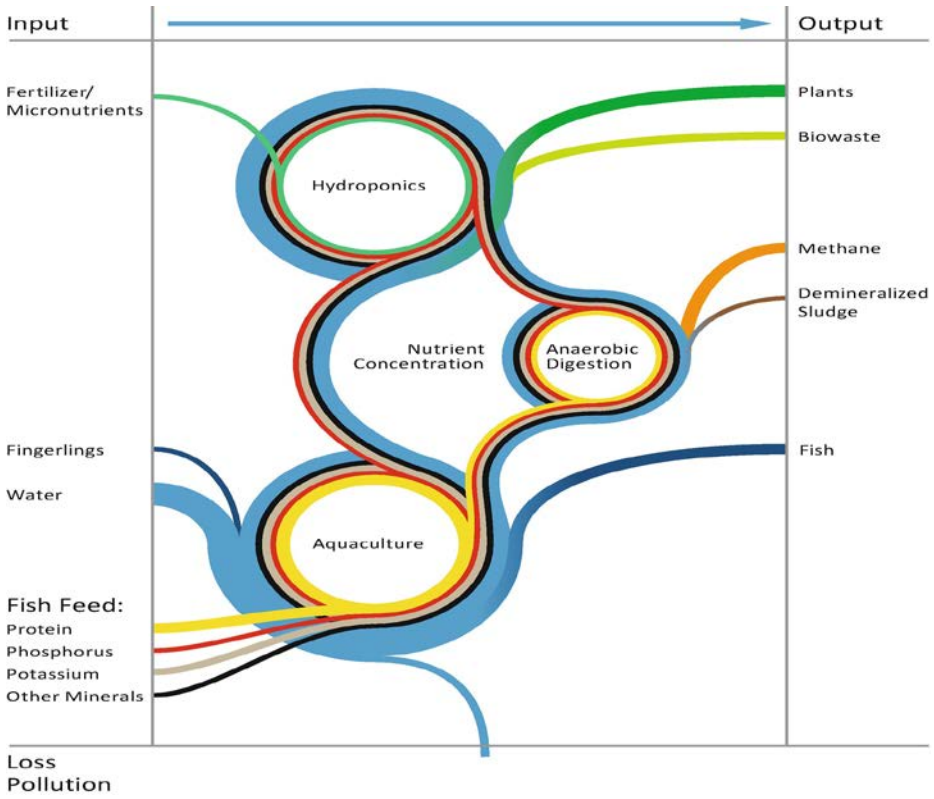

Figure 8. General scheme that shows all the points that participated in the nutrient flow into a decoupled system; figure from Goddek *et al*., (2019).

3. JUSTIFICATION

3.1 Scientific importance

Sustainable intensive aquaculture is the modality that aquaculture production systems aim to be. Therefore, for the development of more efficient systems, it is necessary to generate strategies for the description, management and implementation of the residuals in associated crops to increase production with the reuse of the raw matter and water.

3.2 Technological importance

Developing and implementing methodologies favor reduction of the amount of discarded organic matter while nutrient flow and modeling are optimized according to the needs of the intensive production system.

3.3 Development importance

The transfer of production models favors the establishment of two intensive crops in the same system with the use of methodologies that can be implemented for the recovery and management of residuals within the RAS or BFT systems and a horticultural phase.

3.3.1. Economic relevance

By implementing an intensive production system where the residuals generated in a horticultural phase are used, the quantity of required chemical inputs can be reduced with a lower environmental impact than conventional production systems.

3.3.2. Environmental relevance

When the particulate fraction of the residual organic matter is released into the environment, it may cause pollution problems, eutrophication of the coastal zone and waste of nutrients with high economic and nutritional value, such as

phosphorus (high value finite nutrient). The system developed with the methodology used will allow recovering the organic matter that is currently wasted to use it in an intensive alternative crop that enables two productions and therefore greater biomass.

3.3.3. Social relevance

The final objective of the project is to generate a prototype of integrated production system that allows having intensive crops in both phases (aquaculture and hydroponics), in addition to taking advantage of all the nutrients that the system generates.

4. HYPOTHESIS

1. If the protein intake in RAS culture is related with growth, FCR, and residual (N and P) production in *O. niloticus* rearing, then implementing the Daily Protein Intake (DPI) (mathematical model) for controlling the amount of protein that *O. niloticus* receives during rearing will improve production performance, FCR and the amount of residual (N and P) that the system produces.

2. If the heterotrophic and chemotrophic community in the BFT system promotes efficiency in the nitrogen cycle and improves nutrition in *O. niloticus* rearing, implementing an additional source with microalgae *Chlorella* spp., *C. sorokiniana*-2085 and *C. sorokiniana*-2714 will increase production, survival and nutrition in nursery and grow-out phases in *O. niloticus.*

3. If the particulate and liquid residuals of the aquaculture systems (RAS and BFT) are rich in nutrients, then their recovery, mineralization and implementation will provide the necessary nutrients for an integrated system (NFT) with a horticultural phase.

5. OBJECTIVES

5.1 General Objective

To characterize the macro and micro-nutrient flow in an integrated system by an aquaculture phase in RAS and BFT environments and a hydroponic horticultural phase in NFT.

5.2 Particular Objectives

1. Calculating the flow of macro and micro nutrients in a system integrated by an aquaculture phase in RAS and BFT environments, a bioprocess phase and a hydroponic horticultural phase in NFT.

2. Modeling the flow of macro and micro nutrients in an integrated system, depending on the daily protein intake (DPI), bioflocculation factors (chemotrophic, heterotrophic and phototrophic phases), biomineralization of residuals and complementation of nutrients in hydroponic horticulture.

3. Estimating plant production in the horticultural phase through the implementation of effluents produced by the bioflocculation processes.

6. MATERIAL AND METHODS

The experiments were divided into four areas (I) aquaculture phase in RAS; (II) aquaculture phase in BFT; (III) mineralization processes; (IV) horticultural phase in NFT (nutrient film technique) (Fig. 9).

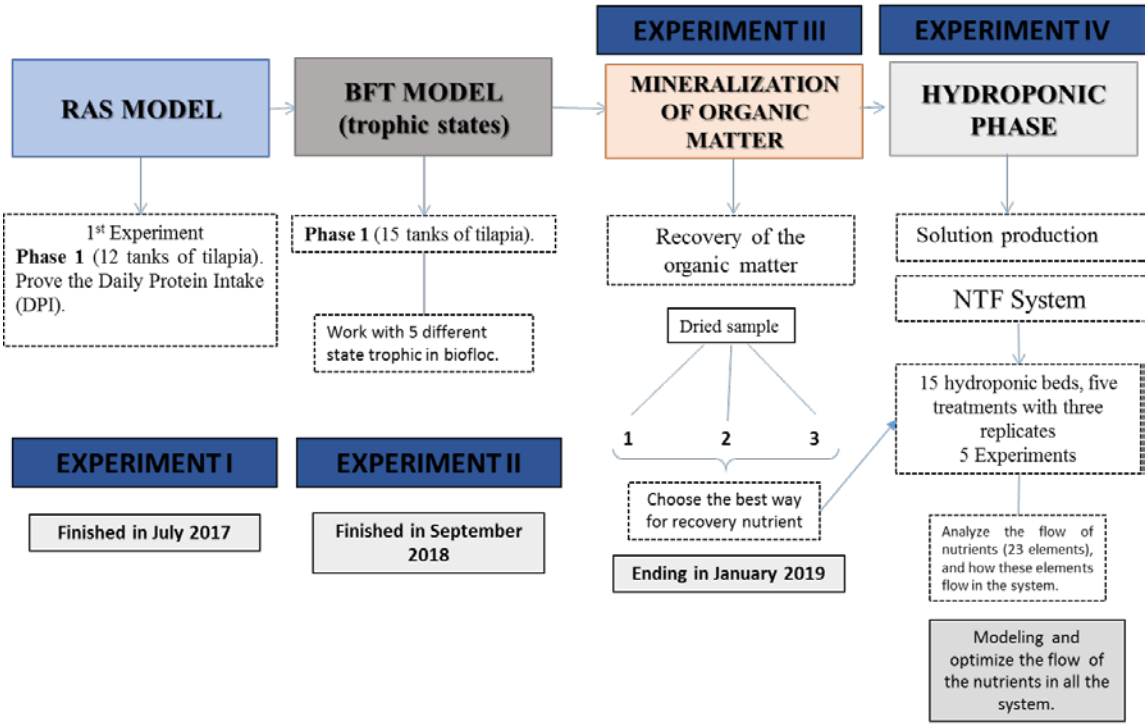

Figure 9. Diagram with the general experiment characteristics. (I) RAS model (Experiment I), II) BFT mode (Experiment II), III) Mineralization process with the solid fraction from RAS and BFT (Experiment III), IV) Hydroponic NFT (Experiment IV). In time line started in 2016 ended in 2019.

EXPERIMENT I – RAS

6.1 Biological system for RAS

Masculinized *Oreochromis niloticus* obtained from the UNCIBNOR+ Unit located in Tepic, Nayarit, Mexico with sizes of 2.3 ± 0.8 g (n = 90) were used. After their landing, they underwent acclimatization and growth processes (15 weeks) until they reached the size for the first experiment (70 g). During this time, Nutripec feed

was supplied, purine-Cargill® (Vevey, CH) with 44% protein (IDP 1.0), six times a day.

Figure 10. The greenhouse was part of the Project Science and Technology Research Partnership for Sustainable Development (SATREPS), Japan International Cooperation Agency (JICA), Japan Sciences and Technology Agency (JST); covered with plastic, anti-aphid mesh and Luminet®, MX with 20% reduction, divided into three experimental areas: Phase 1 Aquaculture in RAS; phase 2 hydroponics in floating bed; and phase 3 for cultivation in soil by fertirrigation. Experiment I was performed in the aquaculture phase of the greenhouse.

6.1.1 Arrangement to the RAS system before Experiment I

The system was delivered by the construction company with a series of irregularities that prevented the optima system operation. The main omissions were in the settling tank. For operating the RAS system, it was necessary to work in three lines for one month: (1) implementing supplements for settling tank (clarifiers, attachment for particle addition, pH buffer; (2) implementing a flute to optimize flow and fall of water from the nitrification tank to the fish tank; (3) Installing aeration in the mineralization tank (Fig. 13).

6.1.2 Preliminary conditions RAS experiment

The study was conducted at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR-Biohelis) in La Paz, Baja California Sur, Mexico where a system was

established consisting of 12 tanks with a capacity of 1 m^3 (Fig. 12). Each unit was formed by a conical sedimenter (156 L), a foam fractioner (5 L), a bio-nitrificator (339 L, with 0.1 m^3 of biomedia (35 mm x 18 mm), a submersible pump (60 W, capacity 500 GPH, EVANS®, Jalisco, MX) and a mineralizer (89 L) (Fig. 13). The system was maintained with aeration through a 1-Hp blower, and in each unit a density of 100 org/m^3 was handled. Three treatments with four replicates were applied.

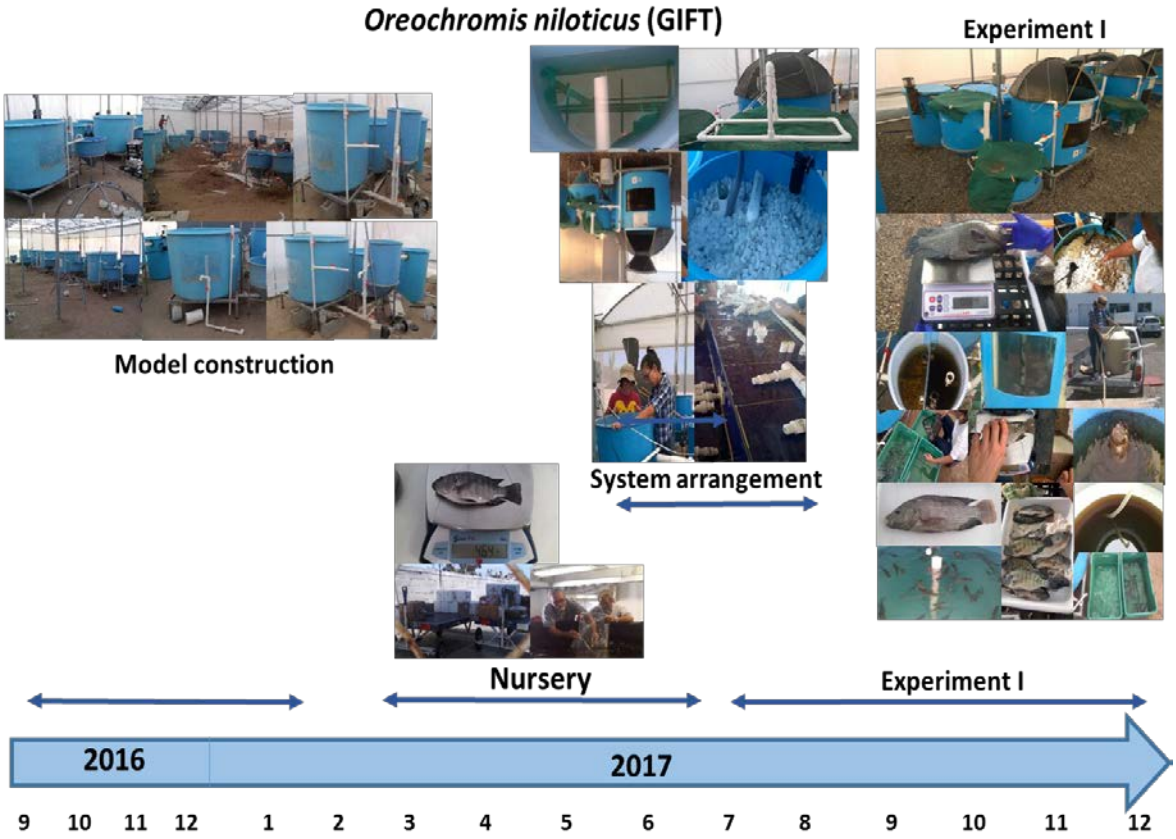

Figure 11. Timeline for construction model, arrangements, nursery and experiment I. 2016 to 2017.

Water circulation in the system went from the fish tank from 1 m^3 to the settler (156 L); then, it was separated and the particulate fraction (PF) was contained. The liquid fraction (LF) was driven to the skimmer to remove fine sediments and

directed to the bionitrificator (339 L) from where it subsequently returned to the fish tank. The spare part in the system was every 47.6 min (Fig. 13).

Figure 12. Recirculating Aquaculture System conformed by 12 individual units with recirculation. The tanks were covered with a black mesh dome (60% light retention). The tanks, settlers, bionitrificators and mineralizers were manufactured with fiberglass, coated with high quality epoxy paint blue color.

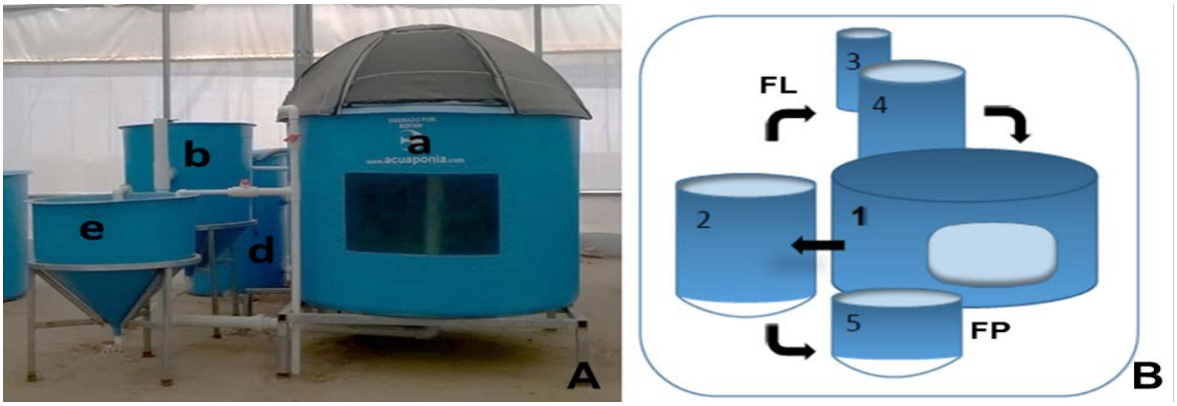

Figure 13. Components and water flow in RAS; (A) Individual system conformed by (a) one tank of 1 m^3; (b) A 156-L sedimenter; (c) a 5-L foam fractioner; (d) a 339-L bionitrificator; (e) a conical 89-L mineralization tank; and (f) a 60-W pump. B) Black arrows indicate water flow within the system: 1-4 indicate the liquid fraction (LF) flow and 1, 2, 5 indicate the particulate fraction (PF) flow.

6.1.3 Experimental RAS Design

Before the experiment, initial weight of the organisms was 76.9 ± 3.94 g (n = 90). Accordingly, the daily protein intake (DPI) was assessed at 140, 120 and 100% with commercial feeds (Nutripec, purine-Cargill®, Vevey, CH), containing 40% protein (humidity ≥ 8.35 ± 0.09%, lipids 7.58 ± 0.15%, crude fiber 1.8 ± 0.27%, ashes 8.87 ± 0.12%, free nitrogen energy 41.12 and, energy 4,698 ± 5.83 cal/g) (Fig. 14). Feeding was provided every three hours from 06:00 to 21:00 h (six servings a day) seven days a week for 34 weeks, taking the equation developed by Japan Fishery Agency (1995) as reference, which calculated the DPI adjusted by linear regression. The function result was DPI = -3 818 in (weight) + 30 158 where DPI is expressed in grams per protein/Kg of biomass with the correlation of R^2 = 0.9914 (Fig. 15). According to these values, three treatments were implemented (DPI 1.4, IRDP 1.2, DPI 1.0), corresponding to 140, 120 and 100% with four replicates. The tanks within the treatments were distributed by randomization. Intake was adjusted weekly through biometry to update weight of the fish of each treatment (30 fish/tank).

6.1.4 Feeding strategy analysis

To define the feeding strategy more accurately, more than 50 articles were consulted, including topics related to feeding strategies; thus, general data of reared tilapia were obtained, such as density, feed protein level, initial weight, initial biomass, final weight, final biomass, feeding strategy used (satiety, satiety with time, fixed biomass percentage, variable biomass percentage, specific weight g/Kg), food conversion ratio, given rations and growth period. These data from the DPI were used and compared with the DPI for temperatures (24 °C, 26 °C, 28 °C).

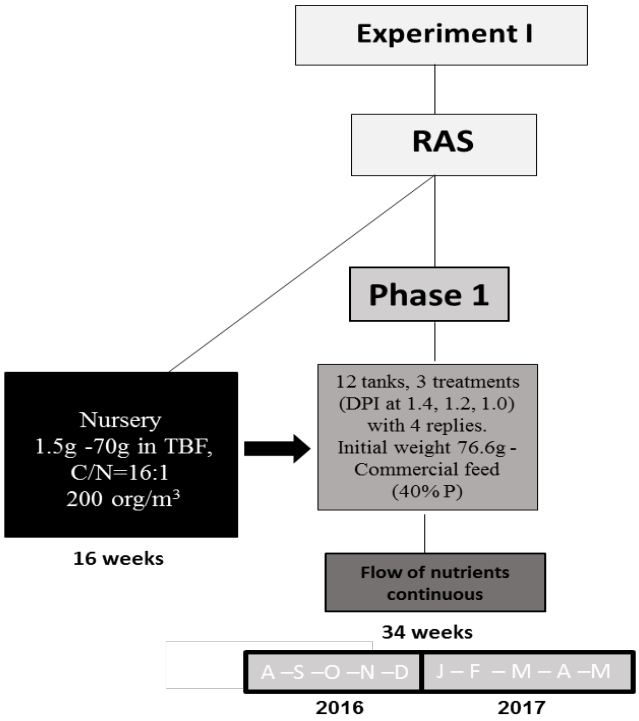

Figure 14. Timeline of experiment I. Developed during 34 weeks, to prove the implementation of the Daily Protein Intake with three treatments DPI 1.4, DPI 1.2, DPI 1. 0. Two stages: nursery and organism growth.

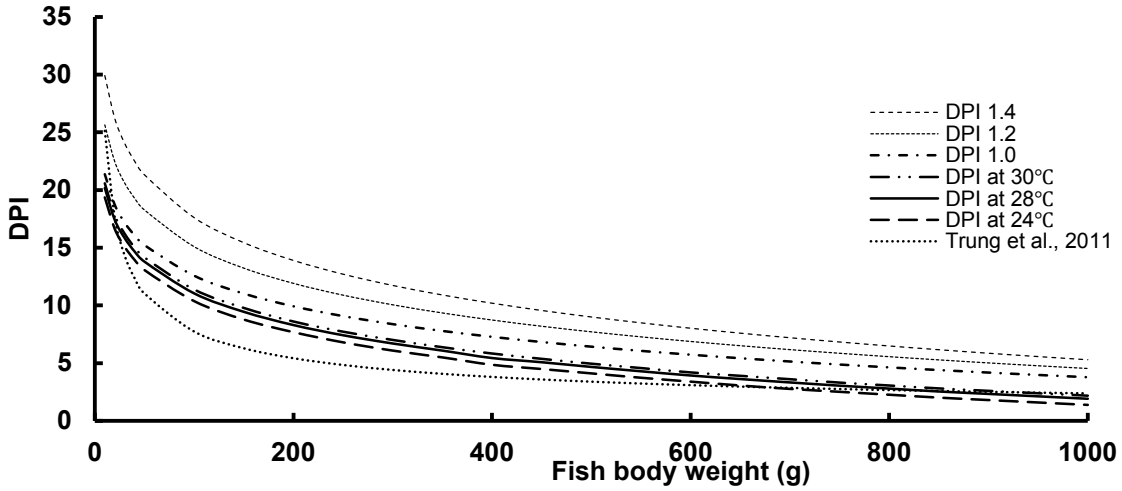

Figure 15. Implementation of the daily protein intake (DPI). DPI 1.4 = 140%, DPI 1.2 = 120%, DPI 1.0 = 100% at different sizes (DPI = -3.818 LN (BW) + 30.158 equation estimated for this study); estimated equations for different temperatures (DPI =-3.997 LN (BW) + 29.79, for 30 °C, DPI = -3.965 LN (BWWT) + 29.305 for 28°C, and DPI = -3.902 LN (BW) + 28.334 for 24 °C.

EXPERIMENT II- BFT

6.2 Construction of the BFT system

For developing a BFT and hydroponic experiment, it was necessary to design and build the greenhouse with the nursery and grow-out areas for *O. niloticus*, management, collection areas for particulate waste and for hydroponics cultivation. By the end of 2016 until 2017 work in design and construction had been performed, and by 2017 the nursery phase had started (Figs. 16, 17).

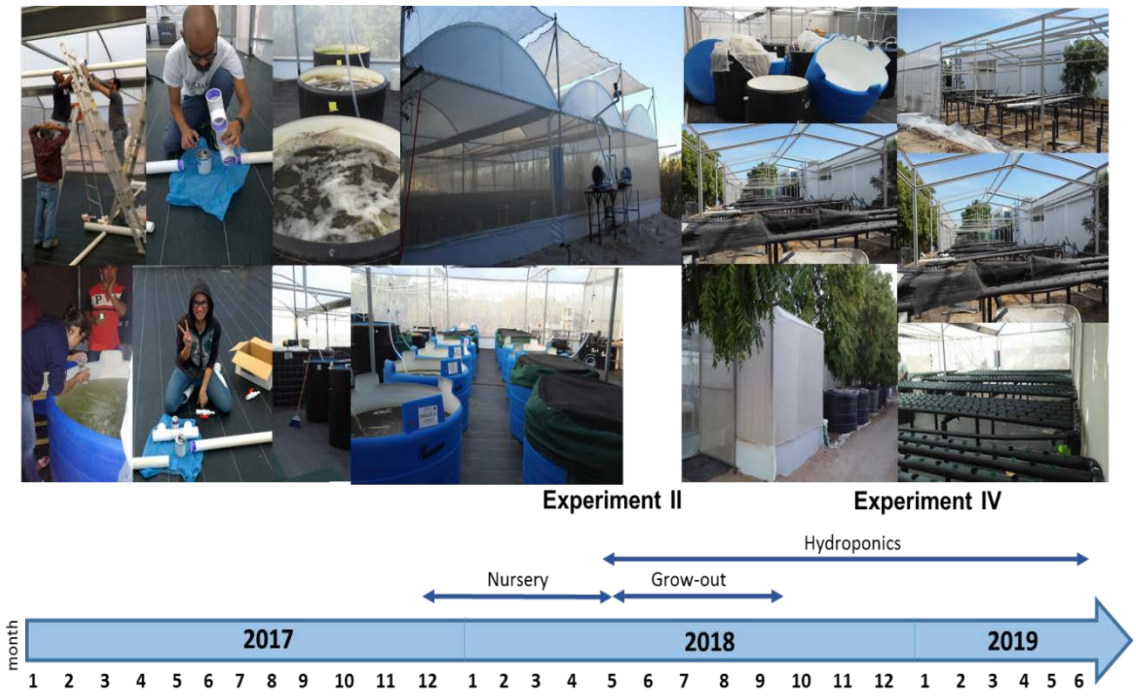

Figure 16. Timeline of BFT and Hydroponic greenhouse construction. It started into 2017 ended into 2019 (Photos from the author).

6.3 Biological system

Approximately 3 000 masculinized tilapia (*O. niloticus*) with an initial weight of 0.33 ± 0.14 g (n = 90) arrived from the UNCIBNOR+ Unit. Experiment II was divided into two stages: Stage I (weeks 1-20): Maternity with organisms from 0.33 ± 0.14 g to 60 g with a density of 180 fish/m^3, Stage II growth (weeks 21-40) with fish from 60 g to 500 g with low density (55 fish/m^3). The rearing method was BFT with five

treatments distributed randomly with three replicates each. Q = chemotrophic, H = heterotrophic, and three photoautotrophic treatments that correspond to: CV = *Chlorella sorokiniana*-2714, CS = *C. sorokiniana*-2805, CN = *Chlorella* spp. Fish were fed with Nutripec commercial feed, purine-Cargill® (Vevey, CH) with 44% protein, according to the DPI 1.2 (Fimbres *et al*., 2019) function (Fig. 15), five times a day, every three hours from 08:00 to 20:00 h. To monitor growth and adjust DPI, weekly biometry (30 fish/tank) was performed.

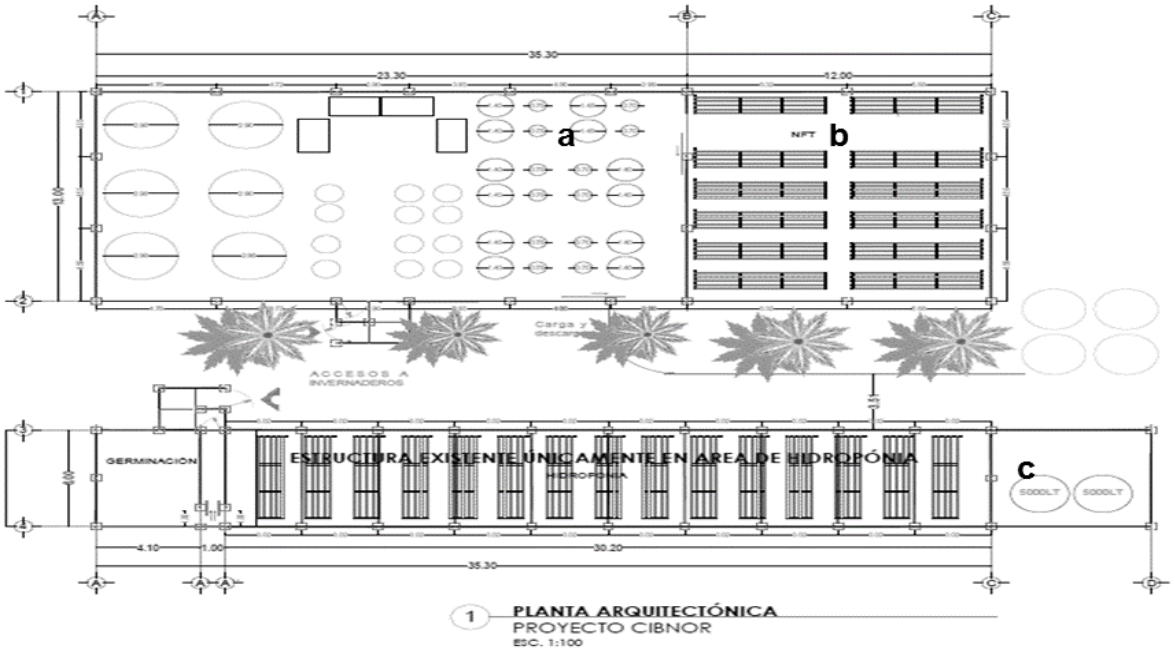

Figure 17. BFT-Hydroponics greenhouse design plans. Biofloc Technology (BFT) and Hydroponics greenhouse distribution; (a) section BFT (15 tanks of 1 m^3) especial area for trophic level experiment (Experiment II); (b) Hydroponics section A (12 hydroponic beds of NFT); (c) Hydroponics section B (15 hydroponic beds in NFT).

6.3.1 Preliminary Bioflocculation Technology conditions

The study was conducted at BioHelis Innovation and Technology Park at Centro de Investigaciones Biológicas del Noroeste (CIBNOR) in La Paz, Baja California Sur, Mexico in a greenhouse (35.3 m x 13 m) covered in plastic and shade mesh with 20% light retention; 15 cultivation tanks of 1 m^3 (1.39 m x 0.74 m) were

established, provided with aeration by a 1-Hp blower. The base of these tanks was covered with a ring of a diffuser hose with diameter of 2.4 cm to provide constant aeration, which allowed maintaining the solid fraction suspended and generating sufficient aeration in the system (> 6 mg/L, O_2). The experiment lasted 40 weeks.

6.3.2 Experimental design

6.3.2.1 Chemotrophic treatment

Inoculation of the treatment Q = chemotrophic was obtained from the RAS bionitrificators; 3 Kg of microbeads (biomedium) were obtained from each bionitrificator (12 bionitrificators with 339 L and 0.1 m^3 of biomedium (35 mm x 18 mm); once mixed, they were placed in two 1-m^3 tanks in darkness and inoculated every other day with a solution of urea and phosphorus (Ebeling *et al*., 2006); the proportion used was replaced by clear water. This treatment uses inorganic compounds as a source of energy in which nitrifying bacteria grow in the absence of light (Veuger *et al*., 2013). The tanks of this treatment were covered by green color shade mesh (90% light retention) (Fig. 18b).

6.3.2.2 Heterotrophic treatment

The H = heterotrophic treatment was also covered with green shade mesh (90% light retention) (Fig. 18b). Each tank was inoculated with 100 L of water from the RAS bionitrificators, and a carbon source (commercial sugar) was added daily to maintain a C/N = 13:1 ratio. Sugar was added together with the first food ration in accordance with (Avnimelech, 1999).

6.3.2.3 Photoautotrophic treatment

Nine tanks without mesh cover were used for this treatment and inoculated at the time according to each of the species used (Fig. 18c-d).

6.3.2.3.1 Inoculation

Three species of Microalgae *Chlorella sorokiniana*-2714, *C. sorokiniana*-2805 and *Chlorella.* spp. from CIBNOR Environmental Microbiology group were used for inoculation at a concentration of 10^7 cells/L per treatment (Fig. 19b-d). The microalgae were kept in a synthetic mineral solution (C30) and under lighting conditions in Erlenmeyer flasks for 15 days; then, it was placed in 20-L cylinders for 15 days more at room temperature (23 ± 1 °C), under fluorescent lighting (60 µmol s^{-1} m^{-2}) conditions and aerated using a 12 V-3.20 LPM Commercial Aquarium pump (AQUA 12W, EVANS®, Jalisco, MX). After that, they were transported to the BFT greenhouse and placed in the tanks of their respective scaling (level of cultivation 200 L). Each treatment had its tank A and scaling tank B (alternating crops) (6-1 m^3 tanks). The nutrition of the microalgae was done with Fertiplus® (Coyoacan, MX). Each week, 200 L of microalgae were harvested and placed in the corresponding CN, CV, CS tanks (3 tanks per treatment).

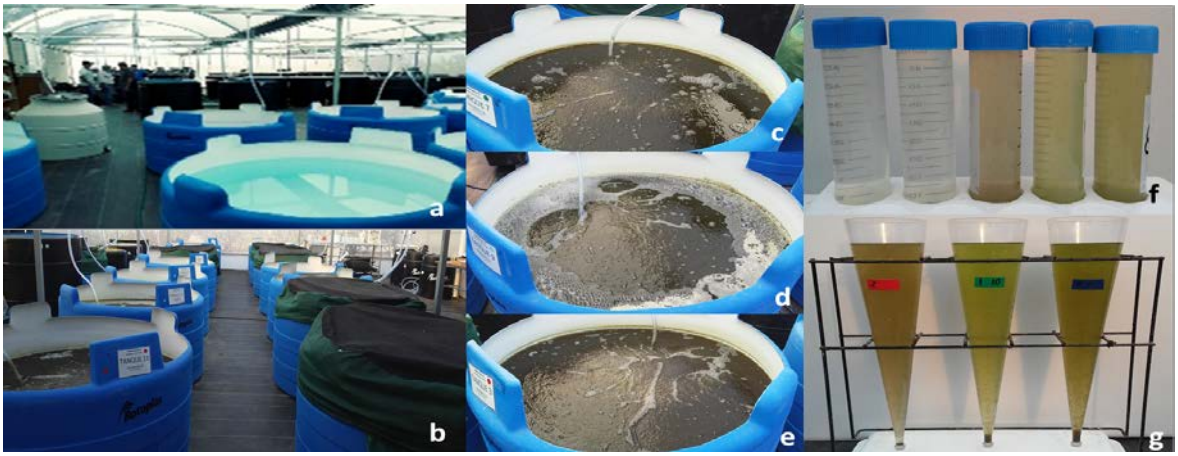

Figure 18. Experiment II Bioflocculation Technology; Inoculation of treatments initial time = t0; (b) prepared treatments; (c) *C. sorokiniana*-2714 (CV); (d) *C. sorokiniana*-2805 (CS); (e) *C.* spp. (CN); (f) Samples of the liquid fraction, from left to right Q, H, CN, CV, CS and (g) samples in Imhoff cones from left to right *C. sorokiniana*-2805, *Chlorella* spp., *C. sorokiniana*-2714.

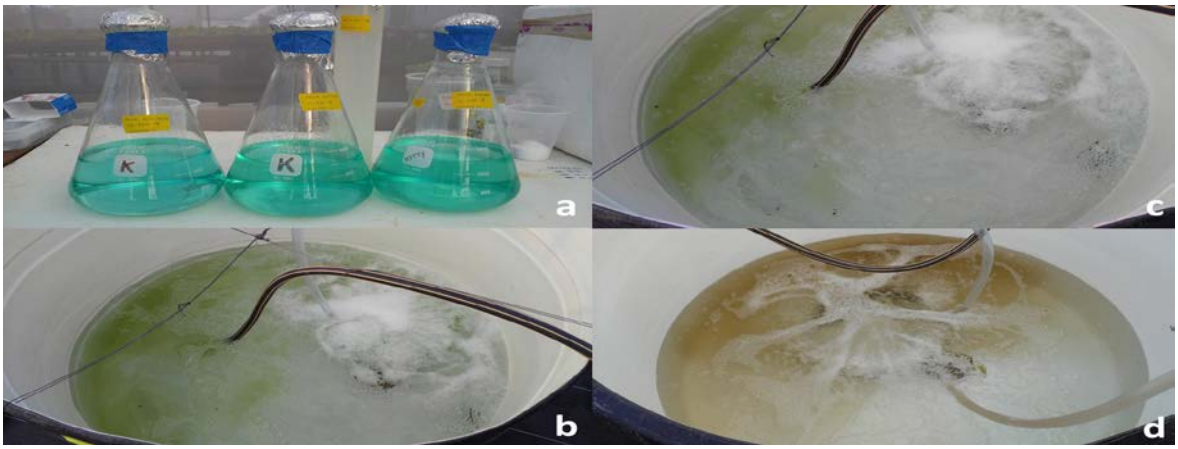

Figure 19. Microalgae inoculation process (a) Fertiplus® nutritional solution for microalgae growth; (b) *C. sorokiniana*-2714 (CV); (c) *C.* spp. (M); (d) *C. sorokiniana*-2805 (CS).

6.4 Evaluation of the agencies

6.4.1 Growth parameters

Weekly biometrics were performed to adjust the food ration intake in the organisms (RAS and BFT). For this purpose, they were first anesthetized with Eugenol at 0.2 ml/L in 40 L of clear water (NOM-051-ZOO-1995); (NOM-062-ZOO-1999); (NOM-046-ZOO-1995), then, total length (TL), partial length (PL), weight (W) were measured, which allowed obtaining data to calculate survival (S), food conversion ratio (FCR), specific growth (SG), percentage of daily weight (% DW), consumed feed (CF), and protein efficiency ratio (PER).

$$S = (\%) = (\text{final number of organisms}/\text{initial number of organisms}) * 100 \quad (1)$$

$$FCR = (\text{provided dry food (g)}/\text{organims wet weight (g)}) \quad (2)$$

$$SG = [(\ln \text{final weight} - \ln \text{initial weight})/\text{days}] * 100 \quad (3)$$

$$\%DW = [(\text{Final weight} - \text{initial weight}) / (\text{initial weight})] * 100 \quad (4)$$

$$CF = 100 * \text{Food consumed (g)} / (\text{average biomass (g)} * \text{days}) \quad (5)$$

$$PET = \text{Gained biomass (g)}/ \text{consumed protein (g)} \quad (6)$$

6.4.2 Sample preparation for nutritional analysis

Three organisms were obtained from each tank of each treatment (EXP. I) and of each tank (EXP. II) and sacrificed in deep anesthesia. In fresh they were dissected

and dried in a horizontal ventilation oven (VWR International Cornelius®, OR, USA) at 60 °C/24 hrs. The sample was crushed with an electric coffee mill (Krups®, DE, Model GX4100, 200 W), homogenized, and then used for proximal, elemental and amino acid analyses.

6.4.2.1 Proximal chemical analysis

The proximal composition was carried out according to the Association of Official Analytical Chemists (AOAC) procedures (Horwitz and Latimer, 2005). Humidity was determined by means of the weight loss method (100 °c/24hrs, according to Key: 930.15 AOAC-Horwitz and Latimer, 2005). Crude protein was carried out by the DUMAS Direct combustion method (key: 976.05 AOAC-Horwitz and Latimer, 2005) by using LECO Equipment® FP-528 and for the ethereal extract, the Foss Soxtec® Avanti method (Foss, Hogans, SE), using ether as solvent extractor. For crude fiber, the successive hydrolysis method (Acid/base) was used (key: 798.10 AOAC-Horwitz and Latimer, 2005). To obtain ash level, 2 g of sample (biological material) were incinerated in a Thermolyne 6000 combustion furnace (Barnstead Thermolyne, Dubuque, IA, USA) at 600 °c/5hrs. The nitrogen-free extract was calculated by difference: 100-(% protein +% lipid +% crude Fiber +% ashes), and free energy was calculated using an automatic adiabatic calorimeter (Parr Instruments, model 1261, Moline, IL, USA).

6.4.2.3 Elemental analysis

For the elemental analysis, 2.5 g of PF sample, fish carcass (3 per tank), were digested based on the APHA 3050 method; the solution was filtered with Whatman Filters® (Maidstone, Kent, UK) (5 µm), and the extract was analyzed using an optical spectrophotometer (ICP-AES VARIAN model Liberty II; Mulgrave, AU). Finally, N was determined by Kjeldahl method and the rest of the elements by direct reading in ICP-AES.

Table I. Macro- and microelements analyzed in diets in biological and residual components of the system, with which the nutrient flow in the RAS was analyzed.

ANALYZED ELEMENTS[1]		
Nitrogen (N)	Phosphorous (P)	Calcium (Ca)
Selenium(Se)	Potassium (K)	Magnesium (Mg)
Boron (B)	Cupper (Cu)	Iron (Fe)
Molybdenum (Mo)	Magnesium (Mn)	Zinc (Zn)
Cobalt (Co)	Sulfur (S)	Sodium (Na)

Notes: [1] Essential elements required for plants for an optimal development. Macronutrients (N, P, K, Ca, Mg, S) and micronutrients (B, Cu, Fe, Mn, Mo, Zn).

6.4.2.4 Amino acid analysis

For the analysis of amino acids, 2-g samples of PF and the carcass of the organisms of each tank (Exp. I and Exp. II) were taken; once the samples were dried and pulverized at CIBNOR, the analysis was carried out at Tokyo University of Marine Sciences and Technology (TUMSAT), where they were determined according to the method of (Simpson *et al*., 1976), used for the auto amino acid analyzer (JCL-500V; JEOL, Tokyo, JP).

6.5 Parameter evaluation

The parameters were monitored once a day (08:00 h), throughout the experiment with a YSI-550 equipment to obtain temperature (°C), dissolved oxygen (DO) (mg/L), pH (range), conductivity (m/s), and salinity (ppt). Temperature and light intensity HOBO Pendant® sensors (HOBO Pendant® temperature/Light, Bourne, MA, USA) were placed inside each fish tank and in the greenhouse (one at each corner) and programmed to obtain measurements every five minutes during the whole experiment I.

6.6 Chemical analysis of effluents

6.6.1 Liquid fraction

For the analysis of the liquid fraction (LF), samples were collected in duplicate in 50-mL Falcon Tubes® (USA) of each tank each week during experiments I and II (Fig. 18f). Ammonium ($N\text{-}NH_4$), nitrites ($N\text{-}NO_2$), nitrates ($N\text{-}NO_3$), phosphates (P-

PO_4) were analyzed with a self-analyzer (Lachats´s QuickChem® 8500, Serie 2 ISAF, Loveland, CO, USA) reported by Standard Methods for the Examination of Water and Wastewater (SMWW). Samples were taken in all cases before feeding.

6.6.2 Particle fraction

The particle fraction for Exp. I was obtained through the settler harvests of each tank each week (during the 34 week-period) (60 samples). For Exp. II samples were obtained by siphoning from weeks 10 to 40 (45 samples). Once they were obtained, the sediments were quantified. The total sample of each tank was divided into two equal parts. The first part was placed in aerobic mineralization (Exp. I), where all the system harvests were accumulated with constant aeration (aerobic mineralization), which was maintained at room temperature 8Fig. 13b). In Exp. II the crops were placed in a low density polyethylene 1 100-L reservoir with a screw cap (Rotoplas®, Expel, MX) without aeration (anaerobic mineral). The second part of both systems was dried in a ventilation furnace (Stabil-Therm) at 80 °C/24 h and pulverized with an electric coffee mill (Krups®, Model GX4100, 200 W, Maidstone, Kent, UK). The sample collection was used in the mineralization experiment (Exp. III) (Fig. 20).

EXPERIMENT III-Mineralization

6.7 Particulate fraction mineralization

The collection of the samples obtained in section 6.6.2, was divided into three equal parts to be processed by one of the following methods: incineration, acid digestion with H_2NO_3 or acid digestion with HSO_4 (Fig. 20). Proximal, elementary, fatty acid and amino acid analyses (6.4.2.1 to 6.4.2.4) were performed.

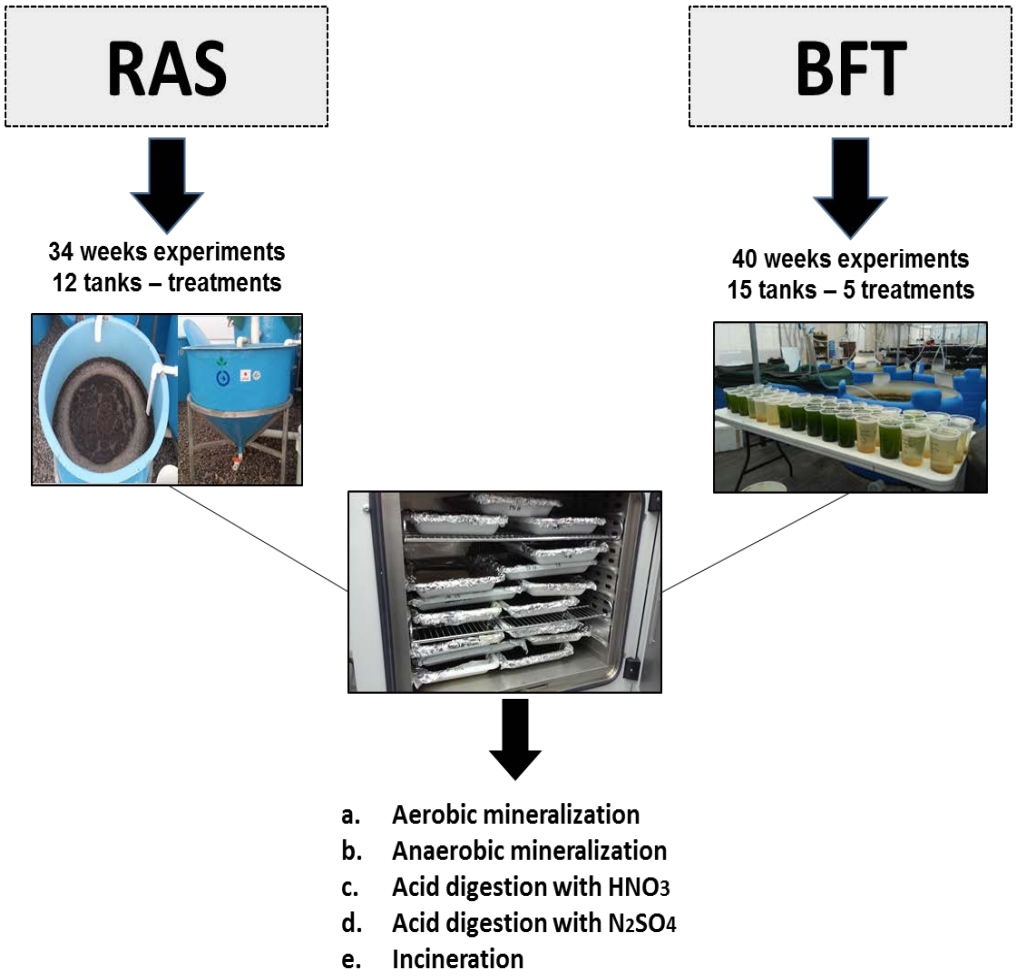

Figure 20. Diagram for the processing of PF obtained in EXP I and EXP II using five methods of mineralization (a) aerobic, (b) acid digestion with H_2SO_4, (c) incineration, d) acid digestion with H_2SO_4, (e) anaerobic mineralization.

6.7.1 Aerobic digestion

Experiment I PF crops, were placed in a conical mineralizer (89-L capacity) (Fig. 13e), with constant aeration (diffusing hose) (Delaide *et al*., 2018). The aeration was generated by a 1-Hp blower; the mineral was kept clogged with heavy-duty shady mesh for outdoors with 90% protection. For the PF crops, aeration was extinguished, the PF was mixed, and it was left to stand for two hours; then, the sample was taken, and PF storage was carried out during the 34-week period.

6.7.2 Acid digestion with HNO_3

The acid digestion with HNO_3 was performed following the process EPA-3050B where 2 g of dry specimen were placed in an Erlenmeyer flask; 5 ml of nitric acid (HNO_3-concentrate) were added, placed in an electric grid at 95 ± 5 °C until the digestion ended (change of color, evaporation of the substance) and allowed to cool; then 3 ml of H_2O_2 (30% hydrogen peroxide) were added and placed on an electric grid at 95 ± 5 °C until the effervescence ended. The 50 mL sample was mixed with deionized water, leaked with Whatman Filter®, USA of 50 µm, and the solution was recovered.

6.7.3 Incineration

For the incineration, 50 g of dry specimen were placed in a Thermolyne Combustion Furnace® 6000 (Barnstead Thermolyne, Dubuque, IA, USA) at 600 °c/5 h; then, the sample was cooled in a desiccator for 40 min; the ashes were recovered, resuspended and homogenized in deionized water, and finally, leaked with Whatman® 50-µm filter to recover the solution.

6.7.4 Acid digestion with H_2SO_4

The acid digestion with H_2SO_4 was carried out using the technique proposed by Endo and Takeuchi, 2009. In a Teflon container, 40 mmol H_2O_2 were added for each gram of PF and 6 ml of H_2SO_4 following the microwave technique at 175 °c for 15 min. This analysis was performed at TUMSAT.

6.7.5 Anaerobic Digestion

The PF obtained in EXP. II was accumulated in low density polyethylene 1 100-L reservoirs with screw cover (Rotoplas®, Expel, MX) (one for each treatment) without aeration, in darkness, at room temperature for 90 days (Fig. 18). At the end of this time period, the sediments were mixed, left to stand for two hours, and the samples were taken (Mirzoyan *et al*., 2012; Nguyen and Fricke, 2015).

EXPERIMENT IV – Hydroponic

6.8 Preliminary conditions of the hydroponic experiment

The hydroponics experiment was carried out in areas B and C of the greenhouses described in Figure 17, which were composed of a metal structure coated with white plastic polyethylene and anti-aphid mesh with 20% of light retention. Area B had 12 hydroponic beds with 34 holes per tube, six tubes per bed (204 holes in total); area C had 15 beds with 16 holes per tube and six tubes per bed (96 total) (tubes were high density polyethylene, 1-inch hole) (Fig. 17). Each bed had a polyethylene 250-L barrel (Tamboplas – Rotoplas®, MX), which contained the hydroponic solution. Recirculation was performed by an aquarium pump of 12 V-3.20 LPM (AQUA 12W, EVANS®, Jalisco, MX). The hydroponic system was designed based on the technique in NFT (Nutrient film technique) described by Azad *et al*. (2013).

6.8.1 Planting and germination

The seeds were placed on substrate of inert culture (SOGEMIX PG-M®, USA, 108 L), previously moistened and pasteurized to avoid phytosanitary problems; they were placed in ventilation oven (Stabil-Therm) at 80 °c/36 h planted in polypropylene germination trays with 200 cells (L x A x A = 54 x 28 x 4.3 cm-Tlalnepantla, MX). Approximately, three seeds per cell were placed and covered with black polyethylene to maintain the appropriate humidity and temperature; when well-defined seedlings were removed, the trays were taken out of the black bags, placed on the beds and daily irrigation was applied with Hoagland solution at 60% (Hoagland and Arnon, 1950).

6.9 Hydroponic experiments

Four hydroponic experiments were performed with *Lactuca sativa* (green lettuce), *Brassica rapa* subsp. *chinensis* (pak-choi), *Eruca sativa* (rucula), *Ocimum basilicum* (basil), *Beta vulgaris* subsp. *vulgaris* (Rocket) (Table II).

6.9.1 Treatments

Six treatments with two replicates were developed (section C). T1 = Control (Hoagland); T2 = Biofloc heterotrophic system (H); T3 = Biofloc chemotrophic system, T4 = Biofloc phototrophic system (*C. sorokiniana*-2805), T5 = *C. sorokiniana*-2714 and T6 = *Chlorella* spp. The water of each treatment was collected in a 1-m^3 tank (one tank/treatment), 300 L/tank of the Biofloc cultivation system, 900 L/treatment. When the water was harvested, it was left to settle for 24 h to remove the FP; then, it was filtered with a 5-µm nylon bag and supplemented with Hoagland micronutrients (Jones, 2004, see Hoagland solution description, Table III). Hoagland nutrients were prepared in three sets (A = Macronutrients (N, K, Ca, S), B = Magnesium (Mg) and phosphorus (P), C = Micronutrients (Fe, Mn, B, Zn, Cu, Mo). In each bed: line 1 and 6 *Lactuca sativa* (green lettuce) and *Eruca sativa* (rucula); line 2 and 5, *Lactuca sativa* (green lettuce); line 3 and 4, *Brassica rapa* subsp. *Chinensis* (pak-choi) and *Ocium Basilicum* (basil) (Fig. 21)

Table II. Code, common name and scientific name of the different species used in the hydroponic experiments.

Code	Common name[1]	Scientific name
AL	Basil	*Ocium basilicum.*
AR	Rucula	*Eruca sativa*
ES	Spinach	*Spinacea oleracea*
LO	Green letuche	*Lactuca sativa (green)*
PC	Pack choy	*Brassica rapa subsp. chinensis*

Notes: [1] common name could change depending on the regions.

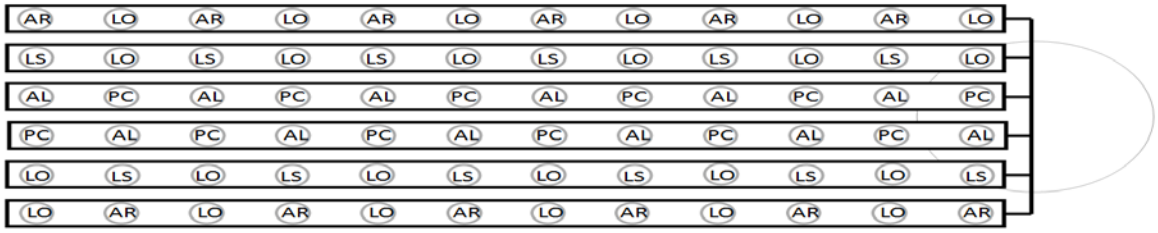

Figure 21. Plant distribution in hydroponic beds; the image illustrates the six rows of tubes and the holes with the corresponding plants; the bottom line indicates line 1, the upper one indicates line 6. Five different plant species were sown in each bed: line 1 and 6 *Lactuca sativa* (green lettuce) and *Eruca sativa* (rucula); line 2 and 5, *Lactuca sativa* (green lettuce); line 3 and 4, *Brassica rapa* subsp. *Chinensis* (pak-choi) and *Ocium Basilicum* (basil) (Figure design from David Vega).

Table III. Description of Hoagland and Steiner commercial nutritional solutions. Macro and micronutrients needed for plant nutrition.

	Nutrients (mg/L)	Hoagland (100%)[1]	Steiner (100%)[1]
(mg/L) Macronutrients	**N**	242	170
	P	31	50
	K	232	320
	Ca	224	183
	Mg	49	50
	S	113	148
(mg/L) Micronutrients	**Fe**	7	3 - 4
	Mn	0.5	1 – 2
	B	0.45	1 – 2
	Zn	0.48	0.2
	Cu	0.02	0.1 – 0.5
	Mo	0.01	0.1

Notes:[1] Values obtained from Jones (2003:83, 87 pp); Hoagland and Steiner solutions were prepared at 100%. Macronutrients required in graded proportions; micronutrients required in small proportion.

Tabla IV. Compendium of the five experiments performed in the hydroponics section (Experiment IV). A brief description of the treatments in each experiment is included.

T[1]	E[2]
1	Hoagland (Control)
2	Heterotrophic+ complementation
3	Chemotrophic+ complementation
4	*C. sorokiniana*-2714 +complementation
5	*C. sorokiniana*-2805 +complementation
6	*C.* spp + complementation

Notes*:* [1] T = treatments; [2] E1 – E5 = Experiments; Hoagland refer to the chemical hydroponic solution described in Table III. This solution used as control.

6.10 Biometrics

Biometrics was performed on the plants obtained (in each experiment) at initial, middle and final experimental time points. To estimate their initial weight, 12 specimens were taken at random per plant species. The entire plant was weighed, previously dried and cleaned, labeled and placed in paper bags (previously weighed). They were then dried in a ventilation oven (Stabil-Therm) at 70 ° C / 48 h; then, the bags were weighed to obtain the dry weight of each plant.

6.11 Parameter analysis and sampling

The parameters were taken once a day (08:00 am), temperature, OD, pH, REDOX, salinity, conductivity, alkalinity with a device (YSI 500). The liquid samples were placed in 50-ml tubes, to evaluate NO_2-N, NO_3-N, NH_4-N, PO_4-P (Fig.18f), according to the process described in section (6.6.1).

6.12 Plant analysis

The phenology of the plants and their phytosanitary status were visually checked daily, with emphasis on their color, based on Jones, 2004; Likewise, size, color, appearance of the leaves were recorded to detect possible pathologies, such as necrosis, chlorosis or any symptoms in plant leaves.

6.13 Statistical analyses

Once normality (Kolmogorov-Smirnov test) and homogeneity of variances (Levene test) were verified, one-way ANOVA and Tukey's tests (0.05) were performed to detect significant differences using Minitab 17 Statistical Software (2010). Physicochemical parameters and water quality were considered in the growth parameters of Exp I. For Exp. II two-way ANOVA was performed to define possible significant differences between treatments throughout the experiment; Sigma Stat 3.5 was used.

7. RESULTS

7.1 Preliminaries in RAS

Before the experiment started, several modifications were implemented into the system; the main one was the settling tank, the biofilter and the waterflow from the fish tank and settling tank (Fig. 22).

EXPERIMENT I

7.1.1 Data obtained during the RAS Experiment

The experiment period lasted 34 weeks implementing the different daily protein intake (DPI) values, DPI 1.0 = 100% (DPI = -3.818 ln (BW) + 30.158), DPI 1.2 = 120% (DPI = -4.582 ln (BW) + 36.19), and DPI 1.4 = 140% (DPI = -5.345 ln (BW) + 42.221).

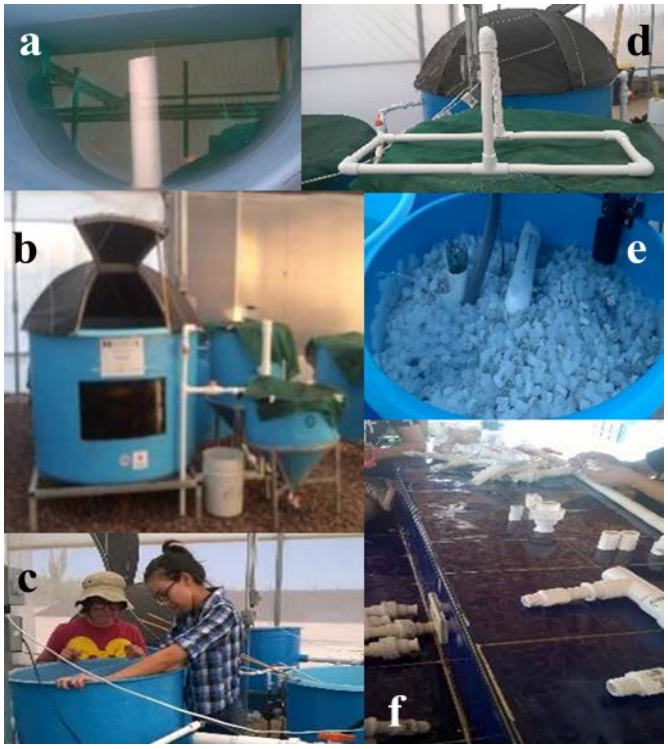

Figure 22. Arrangements in recirculating aquaculture systems (RAS) (a) settling tank; (b) recirculation in tank; (c) and (d) modification in the settling tank; (e) biofilter; (f) modification in the waterflow between tank and settling tank.

7.1.2 Growth from 2.4 to 70 g juveniles (Nursery period)

The organisms' growth did not show significant differences between tanks in the nursery period; the parameters were very stable throughout the experiment; the

biofloc was maintained at a density of < 30 ml/L (Fig. 23). The physical parameters did not show significant differences. Survival, final growth and SGR recorded significant differences where the best tank was T1. Body weight was measured from week 5; the average initial weight was 10.2 for T1 and 7.7 g for T2 (Table V).

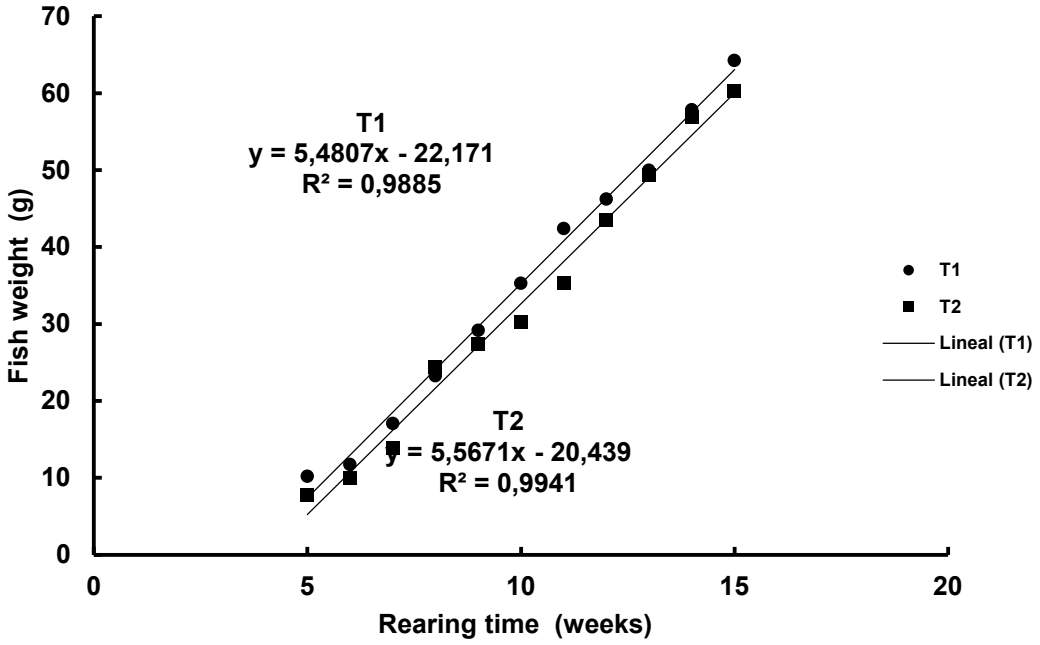

Figure 23. Growth of the *Oreochromis niloticus* during nursery implementing a Biofloc system C/N = 1:13.

7.1.3 Physical parameters in RAS

Table VI shows the seasonal average values of the physical parameters at week 34 of the experiment. The temperature fluctuated during the different seasons, but it did not record significant differences ($p > 0.05$) within the treatments during the experiment. The DO level decreased while salinity and conductivity gradually increased throughout the experimental period. The pH fluctuated with a trend toward acidification with increasing biomass in all three treatments (Table VI).

The greenhouse temperature fluctuated during all the experiments. Temperatures above 51°C were obtained in summer and autumn, with minimum temperatures from 21-22°C in summer and 15-16°C in autumn; the

winter temperature was above 46.7°C, with minimum temperatures from 13-14.3°C (Table VI).

Table V. Biological parameters obtained from *Oreochromis niloticus* with a density of 200 org/m^3 at week 15 of the nursery period.

Tank number	1	2
Temperature (° C)	23±0.3	25± 3.1
DO (mg/L)	7.5±1.0	7.6±1.0
pH	7.9±0.9	7.9±0.9
Biofloc C:N	13:1	13:1
Time (days)	105	105
(fish/m^3 density)	200	200
Initial weight average (g/fish)	2.3	2.3
Final weight average (g/fish)	60.2[b]	64.3[a]
Initial biomass (kg/m^3)	0.5	0.5
Final biomass (kg/m^3)	10.8[b]	12.4[a]
Total of weight (kg/m^3)	10.3[b]	11.9[a]
S (%)[1]	90[a]	88[b]
SGR (%/day)[2]	23.9[b]	25.7[a]
FCR[3]	0.4[b]	0.6[a]

Notes: [1] S = survival; [2] SGR = specific growth rate; [3] FCR = food conversion ratio. The average temperature is shown in the table, and the maximum and minimum values are also displayed. No letter in a row indicates no significant differences among the groups ($p > 0.05$).

The concentration of NH_4-N was greater than 30 mg/L after week four and greater than 50 mg/L after week 10 with significant difference in weeks 7, 9, 15 and 27. The NO_2-N concentration differences were significant in weeks 13, 21 and 25 ($p < 0.05$), and water transfer to horticulture helped lower the nitrogen residuals. The PO_4-P concentration did not show significant differences among treatments ($p > 0.05$), and the NO_3-N concentration recorded significant differences between treatments ($p < 0.05$) only in weeks 29 and 33 (Fig. 24).

7.1.4 Biological parameters in RAS

Higher growth was recorded in treatments DPI 1.4 and DPI 1.2 than in treatment DPI 1.0. The average fish sizes in the final week were 908.0 ± 57.9 g (DPI 1.4), 887.0 ± 113.5 g (DPI 1.2) and 702.2 ± 38.1 g (DPI 1.0). The highest biomass, SGR and average weight gain was obtained in DPI 1.4 and DPI 1.2. The FCR at final time and survival did not show significant differences ($p > 0.05$) at the end of experimental time among treatments (Table VII).

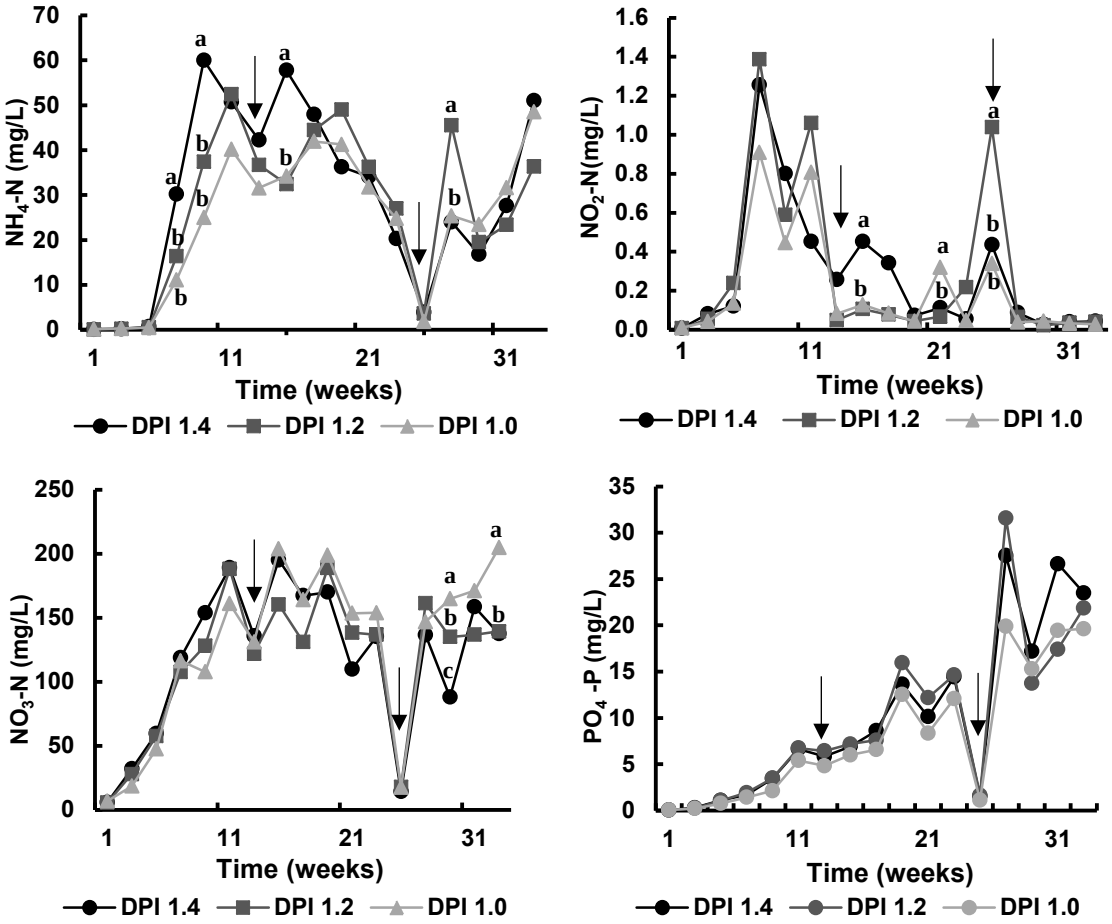

Figure 24. Samples for the water analyses (NH_4, NO_2, NO_3, and PO_4) were taken every two weeks during all experiments. No letter in a point indicates no significant differences among the groups ($p > 0.05$). The arrows in the graph indicate the water collected in weeks 18 and 25 (hydroponic experiment- data not shown).

The test applied to the treatments indicated that no significant differences ($p > 0.05$) were found in the growth parameters between DPI 1.4 and DPI 1.2; only was DPI 1.0 significantly lower ($p < 0.05$) (Table VII). The difference in average growth between the DPI 1.2 and DPI 1.0 treatments was 185.0 g, and growth in DPI 1.0 did not reach 800.0 g (Fig. 25). The standard length and average weight did not show differences among treatments in all the rearing period (Fig. 26).

Table VI. Average temperatures for each tank and season and the overall average of each treatment.

	INTERNAL PARAMETERS								
	SUMMER			AUTUMN			WINTER		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
Temperature (ºC) average	32.5±0.4	31.7±2.2	33.2±1.5	28.3±0.2	28.3±0.6	28.8±0.9	26.5±1.7	26.3±2.5	28.0±0.7
Maximum	39.3±6.2	41.6±3.0	37.5±2.9	32.4±1.0	32.7±1.0	33.2±1.9	29.7±1.5	29.5±3.6	31.0±0.7
Minimum	27.7±0.3	27.5±0.6	27.9±0.5	23.8±0.8	23.4±2.1	24.1±1.1	22.8±1.9	22.7±2.1	24.4±1.0
OD (mg/L)	6.1±0.7	6.2±0.9	6.1±0.4	5.6±0.8	5.5±0.8	5.5±0.5	5.2±0.8	5.3±0.9	5.2±0.8
Initial salinity (ppt)	0.9±0.2	0.9±0.1	0.8±0.1	2.4±0.2	2.4±0.3	2.0±0.3	4.5±0.2	4.5±0.0	4.2±0.1
Final salinity (ppt)	2.4±0.2	2.4±0.3	2.0±0.3	4.5±0.3	4.5±0.1	4.2±0.1	5.2±0.3	5.4±0.3	5.1±0.2
pH	6.7±0.2	6.7±0.2	6.6±0.1	6.07±0.1	6.0±0.0	6.0±0.1	5.8±0.5	5.6±0.4	5.7±0.5
Conductivity Initial (mS)	1.9±0.4	2.0±0.3	1.7±0.2	5.3±0.4	5.3±0.7	4.4±0.57	8.2±0.5	7.6±0.4	6.9±0.8
Conductivity final (mS)	5.3±0.2	5.2±0.6	4.3±0.6	8.1±0.6	7.5±0.4	6.9±0.9	10.5±0.3	10.3±0.2	9.5±0.2
	EXTERNAL PARAMETERS								
	SUMMER			AUTUMN			WINTER		
	T °C	max	Min	T °C	max	min	T °C	max	min
E1[1]	33.6±7.9	52.08	21.39	30.0±7.9	52.58	15.6	24.7±8.0	46.72	13.36
E2[1]	33.9±8.6	52.88	21.09	29.0±7.0	51.57	16.4	24.9±7.5	44.95	14.32
E3[1]	34.5±8.20	51.01	22.04	29.5±7.3	50.31	15.1	23.8±7.4	45.57	13.17
E4[1]	34.1±8.67	52.43	22.04	29.4±7.6	50.87	15.3	24.7±8.7	48.42	12.98

Notes: E= The average temperature is shown in the table, and the maximum and minimum values are also displayed. No letter in a row indicates no significant differences among the groups ($p > 0.05$

Table VII. Production performance of tilapia *Oreochromis niloticus*, using different DPI levels (1.4, 1.2, and 1.0) with a 100 fish/m^3 stock density for 34 weeks.

Parameter	DPI level		
	1.4	**1.2**	**1.0**
Density (fish/m^3)	100	100	100
Initial weight (g/fish)	79.2±5.1	76.5±3.9	74.9±1.7
Initial total length (cm)	15.9±0.2	15.8±0.3	15.6±0.1
Initial standard length (cm)	12.8±0.2	12.7±0.2	12.6±0.1
Final weight (g/fish)	908±57.9^a	887.7±113.5^a	702.2±38.1^b
Final total length (cm)	32.6±0.6^a	32.3±1.4^a	30.5±0.3^b
Final standard length (cm)	27.2±0.5^a	27.2±1.2^a	25.4±0.4^b
Biomass (kg)	74.61±4.9^a	73.0±10.2^a	56.45±3.5^b
Average weight gain (g fish/ week)	24.54±1.7^a	23.78±3.3^a	18.37±1.1^b
SGR (%/day)	0.43±0.01^a	0.43±0.02^a	0.39±0.01^b
S (%) (final time)	98.6±1.04^a	98.3±1.9^a	98.3±0.6^a
FCR (g feed/g fish) (final time)	2.4±0.2^a	2.4±0.3^a	2.1±0.1^a
% DW	3.98±0.2^a	4.04±0.6^a	3.01±0.2^b
Energy use (kW/kg fish)	4.44±0.3^b	4.60±0.6^b	5.76±0.3^a

Notes: [1]SGR= Specific growth rate, [2]S = survival, [3]FCR = food conversion ratio and [4]%DW = percentage of daily weight; energy use is kW/kg fish. Values in the same row with different superscript letters are significantly different ($p < 0.05$). No letter in a row indicates no significant differences among the groups ($p > 0.05$).

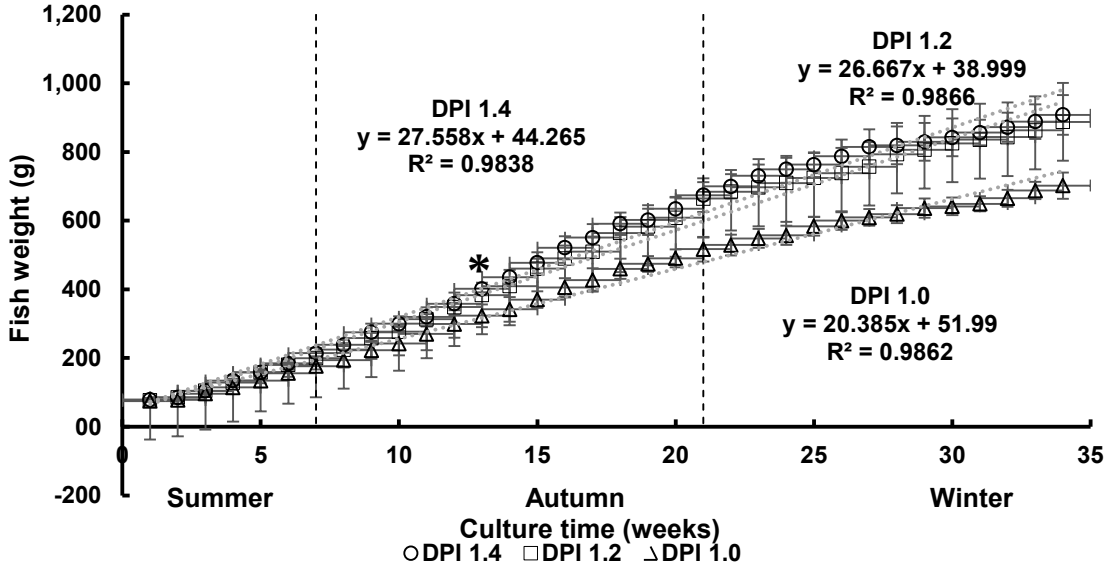

Figure 25. *Oreochromis niloticus* rearing time (weeks) to reach a certain weight (g) using different DPI levels (1.4, 1.2, 1.0) or 34 weeks in three seasons. After week 13 (asterisk), DPI 1.4 and 1.2 ($p > 0.05$) differed significantly from DPI 1.0 ($p < 0.05$). Implementing the different daily protein intake (DPI) values, DPI 1.0 = 100% (DPI = -3.818 ln (BW) + 30.158), DPI 1.2 = 120% (DPI = -4.582 ln (BW) + 36.19), and DPI 1.4 = 140% (DPI = -5.345 ln (BW) + 42.221).

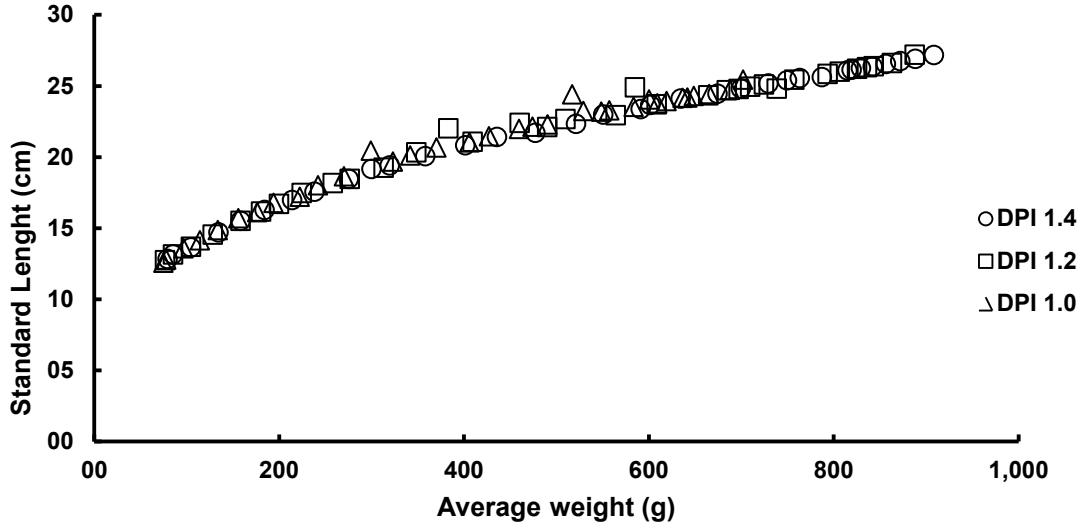

Figure 26. Relationship with the standard length and average weight during rearing time. Implementing the different daily protein intake (DPI) values, DPI 1.0 = 100% (DPI = -3.818 ln (BW) + 30.158), DPI 1.2 = 120% (DPI = -4.582 ln (BW) + 36.19), and DPI 1.4 = 140% (DPI = -5.345 ln (BW) + 42.221).

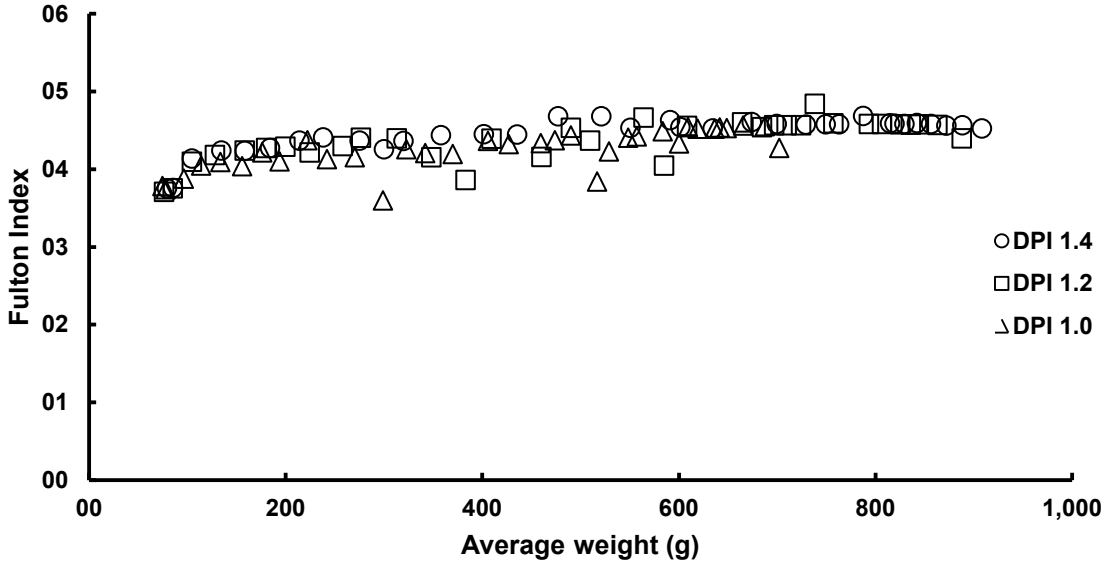

Figure 27. Relationship of the Fulton Index and the average weight (g) during all the culture period (34 weeks). Implementing the different daily protein intake (DPI) values, DPI 1.0 = 100% (DPI = -3.818 ln (BW) + 30.158), DPI 1.2 = 120% (DPI = -4.582 ln (BW) + 36.19), and DPI 1.4=140% (DPI = -5.345 ln (BW) + 42.221).

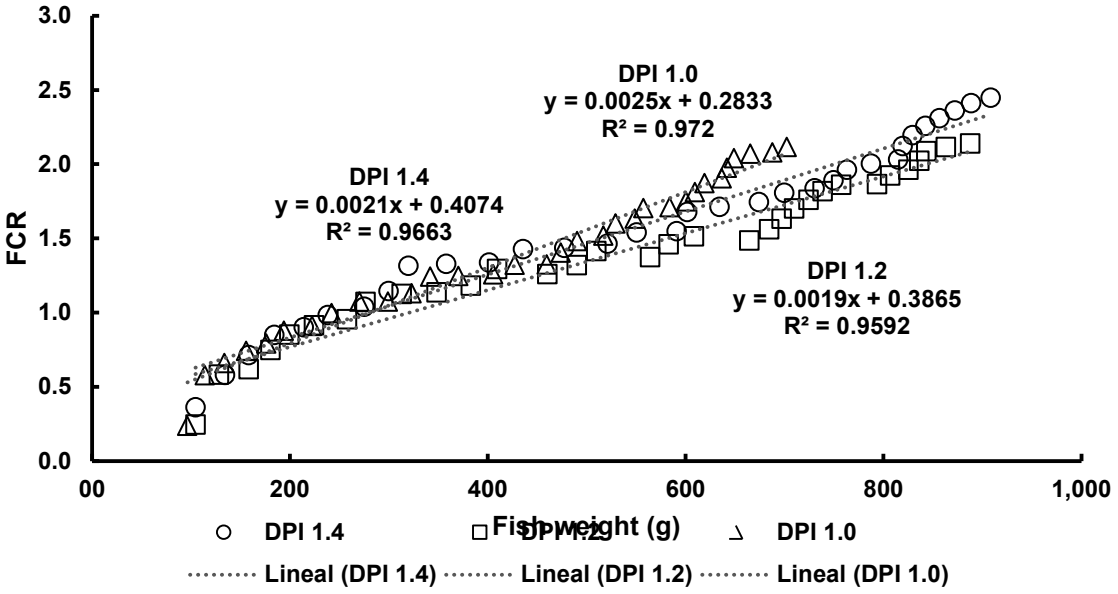

Figure 28. Relationship of the food conversion ratio FCR with weight (g) for 34 weeks at DPI 1.4, DPI 1.2 and DPI 1.0. Implementing the different daily protein intake (DPI) values, DPI 1.0 =100% (DPI = -3.818 ln (BW) + 30.158), DPI 1.2 = 120% (DPI = -4.582 ln (BW) + 36.19), and DPI 1.4 = 140% (DPI = -5.345 ln (BW) + 42.221).

The Fulton index showed variation among treatments without a difference between DPI 1.4 and DPI 1.2, only with DPI 1.0 (Fig 27). The FCR increased during the experiment (Fig. 28) and showed significant differences at weeks 13, 18 and 19 (p > 0.05) in DPI 1.4 DPI 1.2, and DPI 1.0 (b, ab, a, respectively). DPI 1.4 and DPI 1.2 reached the commercial size from 350 g to 900 g in less time (Table VIII).

The FCR got the same behavior. After the fish reached 700.0 g, no significant difference was found in treatments DPI 1.4 and DPI 1.2 Survival did not show significant differences among treatments in all the culture period (Table VIII).

7.1.5 Nutrient analyses in feed and *Oreochromis niloticus* in time zero.

The commercial feed with 40% of protein had a lower lipid level and a higher protein level. The same feed obtained the highest level of almost all elements except for Ni and S. Any feed diet contained Cu and P35, and P40 did not contain Na. The feed implemented in this research study had values of protein

40.6%, crude lipid 7.6% and crude fiber 1.8% (Table IX); the major amino acids were glutamate, aspartate and leucine. For carcass in time zero, the values were crude protein 59.8%, crude lipid 5.6% and ash 29.3% (Table IX). The major amino acids were arginine, leucine, lysine, alanine, aspartate, glutamate and glycine (Table X).

7.1.6 Nutrient analyses in fish

Carcass proximate analysis on week 17 indicated that moisture, crude lipid, ash, nitrogen-free energy and energy were different among treatments ($p < 0.05$); DPR 1.2 and DPR 1.0 had the highest protein levels with 53.79% and 53.86%, but in DPR 1.0 crude lipids decreased to the lowest level of 20.72%; DPR 1.4 had the highest level with 26.2%. At the final time point (34 weeks), all components were different among treatments ($p < 0.05$). DPR 1.2 showed a significantly higher level of nitrogen-free energy and energy, and DPR 1.0 showed the highest protein level but lower levels of crude lipids and energy and the highest ash level. In fillets, protein and nitrogen levels were higher in DPR 1.2, but the treatment had a significantly lower level of crude lipid and crude fiber but the highest energy content (Table XI).

In carcass the level of P and Ca increased at the final time point compared with initial time in DPI 1.4 and 1.0. S, Na, Fe increased in all treatments. At final time, K, Mg, B, Mn, Mo, Zn, Cu and Ni decreased in DPI 1.4; in DPI 1.2 P, K, Ca, Mg, B, Mn, Zn, Cu and Ni. In DPI 1.0 K, Mg, B, Mn, Mo, Zn and Ni. In fillet the elements that decreased when compared with carcass in initial time were P, Ca, Na, Mn, Zn and Cu in all treatments. DPI 1.0 showed a decrease in all microelements in fillet. Depurated fillet retained P, Mg, S, Fe and Co (Table XII). In the middle time point DPI 1.4 retained the highest level of P and Ni; DPI 1.2 K, Mg, S, Fe, Mn, Mo, Zn, Cu; and DPI 1.0 N, Ca, Mg, B, Mn, Mo. For final time in carcass DPI 1.4 retained the highest values of N and S; DPI 1.2 K, B, Fe; and DPI 1.0 retained P, Ca, Mg, Na, Mn, Mo, Zn, Cu and Ni. In the final time for fillet, the treatment that retained the highest level of the elements was depurate fillet with P, Ca, B, Fe, Mn and Zn (Table XIII).

Table VIII. Rearing time (weeks), food conversion ratio (FCR) and survival (S) required for *O. niloticus* to reach target weight (350 g to 900 g).

	Weeks to reach weight target (g)			FCR to reach weight target (g)			Survival (%)		
Weight (g)	DPI 1.4	DPI 1.2	DPI 1.0[1]	DPI 1.4	DPI 1.2	DPI 1.0	DPI 1.4	DPI 1.2	DPI 1.0
350	11.8 ± 0.5^{b}	12 ± 0.4^{ab}	14 ± 1.7^{a}	1.3 ± 0.1	1.1 ± 0	1.2 ± 0.2	99.7±0.5	99.7±0.5	99.2±1.0
500	15.8 ± 1.3^{b}	16.9 ± 1^{b}	20.5 ± 1.3^{a}	1.4 ± 0.2	1.3 ± 0.1	1.5 ± 0.2	99.4±0.6	99.7±0.5	98.9±0.8
600	18.8 ± 1.5^{b}	20 ± 1.4^{b}	26.6 ± 1.8^{a}	1.6 ± 0.3^{ab}	1.5 ± 0.2^{b}	1.8 ± 0.2^{a}	99.4±0.6	99.7±0.5	98.9±0.8
700	22 ± 1.4^{b}	24 ± 2.9^{b}	32 ± 0.0^{a}	1.8 ± 0.2^{b}	1.7 ± 0.2^{c}	2.1 ± 0.1^{a}	99.4±0.6	99.7±0.5	98.9±0.6
800	26.9 ± 2.5	28.2 ± 4.5		2 ± 0.3	1.9 ± 0.3		99.4±0.6	99.7±0.5	
900	31.5 ± 0.7	31 ± 2.8		2.3 ± 0.1	2.0 ± 0.2		99.4±0.6	99.7±0.5	

Notes: Each value represents the mean ± the standard deviation (SD). Values in the same row with different superscript letters are significantly different ($p < 0.05$). [1]*O. niloticus* in DPI 1.0 did not exceed 800 g. No letter in a row indicates no significant differences among the treatments ($p > 0.05$).

Table IX. Proximate and elemental analyses in dry weight of feed with different protein percentage (35%, 40% and 44%, implemented during the recirculating aquaculture system (RAS) and biofloc technology (BFT) experiment.

Code	Moisture (%)	Protein (%)	Nitrogen (%)	Lipid (%)	Crude fibre (5)	Ash (%)	ENF (%)	Energy (cal/g)
P40	8.50±0.05	44.47±0.05	7.12±0.01	4.88±0.03	1.93±0.06	9.16±0.03	39.56	4713.84±02.74

	Macronutrients mg/L[4]						
	N	**P**	**K**	**Ca**	**Mg**	**S**	**Na**
P35[1]	5.5	10434.6	9326.9	17470.3	4659.5	1505.1	nd
P40[2]	6.3	10867.4	9825.3	27240.9	4908.5	1747.3	nd
P44[3]	7.5	8965.3	7456.4	14451.5	3725.1	1946.8	1.5

	Micronutrients mg/L[4]							
	B	**Fe**	**Mn**	**Mo**	**Zn**	**Cu**	**Co**	**Ni**
P35[1]	31.2	172.9	2476.1	41.0	154.4	17.1	nd	2459.8
P40[2]	36.3	327.8	2864.2	48.4	255.4	23.1	nd	3704.0
P44[3]	26.8	306.9	1725.6	46.8	122.6	9.4	nd	4253.7

Notes: [1]P35 = 35% protein in feed, [2]P40 = 40% protein in feed and [3]P44 = 44% the protein in feed. [4]Elemental analyses of 16 elements; macro and micronutrients, obtained with ICP.

Table X. Amino acid analysis in *Oreochromis niloticus* in time zero and feed (40% protein) feed implemented during the recirculating aquaculture system (RAS) and biofloc technology (BFT) experiment.

	Amino acid (g/100 g)							
Essential AA			**Non-essential AA**			**Conditionally essential AA**		
	T	F		T	F		T	F
Arginine	3.4	2.0	**Alanine**	3.9	1.6	**Cysteine**	0.1	0.4
Histidine	0.8	0.7	**Asparagine**	-	-	**Glutamine**	-	-
Isoleucine	1.4	0.9	**Aspartate**	4.7	3.0	**Hydroxyproline**	0.8	0.3
Leucine	3.3	2.3	**Glutamate**	7.3	5.5	**Proline**	3.7	1.8
Lysine	3.5	1.7	**Glycine**	5.6	1.6	**Taurine**	1.3	-
Methionine	0.9	0.2	**Serine**	2.5	1.6			
Phenylalalnine	1.8	1.4	**Tyrosine**	1.5	1.1			
Threonine	2.3	1.2						
Tryptophan	-	-						
Valine	1.9	1.1						

Notes: [1]T0= *O. niloticus* in time zero, samples taken before that the Exp. II started. [2]F= commercial feed with 40% of protein.

Table XI. Proximate analysis on a dry weight basis for *Oreochromis niloticus* growth with different DPI levels at the middle (17 weeks) and final rearing time points (34 weeks).

	Middle time (includes carcasses)			**Final time (includes carcasses)**			**Final time (fillets only)**		
	DPI 1.4	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**
Moisture (%)	63.4±0.1^{c}	70.8±0.2^{b}	72.7±0.1^{a}	67.0±0.1^{c}	67.8±0.01^{b}	74.8±0.01^{a}	77.8±0.2^{b}	78.0±0.1^{b}	78.4±0.1^{a}
Crude protein (%)	48.5±0.3^{b}	53.8±0.3^{a}	53.9±0.1^{a}	51.9±0.3^{b}	51.0±0.2^{c}	60.6±0.2^{a}	80.6±0.1^{c}	87.0±0.1^{a}	82.4±0.2^{b}
Crude lipid (%)	26.2±0.1^{a}	22.8±0.1^{b}	20.7±0.1^{c}	28.2±0.01^{a}	27.6±0.2^{b}	8.8±0.1^{c}	3.6±0.1^{b}	3.3±0.1^{c}	3.9±0.1^{a}
Crude fiber (%)	0.2±0.0	0.3±0.1	0.2±0.1	0.1±0.01	0.1±0.1	0.2±0.1	0.4±0.1	0.2±0.1	0.3±0.1
Ash (%)	13.8±0.1^{b}	11.7±0.1^{c}	14.7±0.1^{a}	15.6±0.3^{b}	10.9±0.1^{c}	24.7±0.1^{a}	5.2±0.1^{b}	5.2±0.02^{b}	5.3±0.03^{a}
Nitrogen free energy (%)	11.4±0.01^{b}	11.4±0.01^{a}	10.5±0.01^{c}	4.2±0.01^{c}	10.3±0.01^{a}	5.8±0.01^{b}	10.3±0.01^{a}	4.3±0.01^{c}	8.1±0.001^{b}
Energy (cal/g)	5289.2±5.4^{a}	5139.3±7.8^{b}	4925.2±3.6^{c}	5338.5±12.3^{b}	5468.5±8.0^{a}	3999.2±7.8^{c}	4787.3±4.4^{c}	5010.5±1.4^{a}	4896.8±4.9^{b}
Lipid (mg/g protein)[1]	540.7±2.1^{a}	424.3±0.1^{b}	384.9±1.5^{c}	543.6±2.6^{a}	541.4±1.8^{a}	144.4±1.2^{b}	44.2±0.01^{b}	37.5±0.01^{c}	47.5±0.01^{a}
DP:DE (g/MJ)[2]	20.4±0.04^{c}	22.7±0.01^{b}	23.6±0.1^{a}	21.5±0.1^{c}	20.6±0.03^{b}	32.2±0.08^{a}	36.2±0.0^{c}	38.4±0.1^{a}	36.7±0.04^{b}

Notes: Means in each column followed by different letters are significantly different at $p < 0.05$. No letter in a row indicates no significant differences among the groups at $p > 0.05$. [1]There was 186.6 mg lipid/g protein in feed (commercial feed with 40% protein). [2]The DP:DE (g/MJ) was calculated with the following values: proteins 5.65 cal/g, lipids 9.45 cal/g, carbohydrates 4.11 and fiber 4.5 cal/g (NRC, 1993)

7.1.7 Nutrient flux in liquid in RAS

The amount of macro and micro elements retained in the liquid fraction did not show significant differences among treatments, only P in September and S in October and December (Table XIV). The accumulation of S and P started in September the other macro and micro nutrients accumulated throughout the experiment.

7.1.8 Comparison of the different strategies to feed *Oreochromis niloticus* and implementation of Daily Protein Intake (DPI).

The majority of the data analyses in the different feed strategies (e.g., satiation, satiation with time, fixed body weight percentage, variable body weight percentage, using a specific g/kg) showed sub-feeding, independently of the size or the strategy implemented. Moreover, few data indicated that the organism was overfeeding when compared with the data obtained with the DPI in the experiment and implemented at different temperatures (24°C, 26°C and 28°C) (Table XV).

Forty-nine research papers were analyzed and compared with the different feed strategies, which were almost all sub-feeding research. In general, the fry and fingerling phase was sub-feeding, compared with the high temperature 24°C, 28°C, and 32°C (Figure 29).

Table XII. Elemental analysis on a dry weight basis (carcass) for *Oreochromis niloticus* growth with different DPI levels in the middle (17 weeks) and final rearing times (carcass and fillet) (34 weeks).

		Middle time (includes carcass)			Final Time (includes carcass)			Final time (Fillet only)			
		DPI 1.4	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**Depurate[3]**
Macronutrients (g/L)[1]	**N, %**	6.7	8.3	9.5	9.7	8.4	9.2	11.6	12.5	13.9	12.7
	P	19816.6	20135.9	21126.6	25832.7	18826.3	42181.7	7816.2	7532.1	7673.0	8147.4
	K	8308.5	9481.8	8364.0	3725.8	4254.4	4838.1	5618.7	7967.6	6582.1	2890.0
	Ca	30957.3	30808.1	35294.4	47538.3	30662.9	79076.4	1579.1	1132.0	980.0	1444.2
	Mg	4150.8	4736.9	4178.5	1506.6	1305.6	2059.7	1258.2	1322.6	1400.9	1416.6
	S	1766.1	1973.3	1890.5	4895.2	5834.8	5643.1	8506.1	7865.0	8693.7	8566.7
	Na	nd	nd	nd	3596.2	3679.0	8171.8	2670.5	1327.7	1520.1	1330.3
Micronutrients (mg/L)[2]	**B**	27.0	23.3	24.0	14.9	19.6	21.2	17.6	17.2	14.6	15.5
	Fe	50.8	67.3	74.2	63.5	125.1	116.8	73.7	178.1	55.1	93.6
	Mn	1380.8	1359.9	1519.0	3.0	2.7	6.1	0.6	0.3	0.3	0.4
	Mo	4.2	3.0	4.1	nd	3.4	1.0	nd	nd	0.9	nd
	Zn	49.0	62.5	70.3	42.3	39.6	60.6	27.1	24.3	24.1	24.1
	Cu	8.2	10.4	8.5	7.6	4.4	11.2	0.3	1.5	0.7	0.9
*	**Co**	nd	nd	nd	nd	2.1	Nd	nd	1.4	nd	2.4
*	**Ni**	4305.6	3941.8	3891.2	nd	2.0	3.7	1.6	nd	2.1	nd

Notes: nd = Not detected, [1]Macroelements (N, Ca, K, Mg, Na, Si, P, S) and [2]microelements (B, Fe, Mn, Mo, Ni, Se, Zn). [3]Depurated = Before fish were scarified, they were depurated in clean water for 15 days.

Table XIII. Percentage of elements retained compared with the elemental content in feed for *Oreochromis niloticus* growth with different DPI levels at middle (17 weeks) and final (34 weeks) rearing time points.

		Feed	Middle time (carcass)			Final time(carcass)			Final time (fillet only)			
		mg/kg	DPI 1.4	DPI 1.2	DPI 1.0	DPI 1.4	DPI 1.2	DPI 1.0	DPI 1.4	DPI 1.2	DPI 1.0	Depurate[3]
Macronutrients (g/L)[1]	N, %	6.3	6.7	8.3	9.5	9.7	8.4	9.2	11.6	12.5	13.9	12.7
	P	10867.4	182.3	101.6	104.9	122.3	72.9	224	18.5	96.4	101.8	106.8
	K	9825.3	84.6	114.1	88.2	44.5	114.2	113.7	116.1	141.8	82.6	43.9
	Ca	27240.9	113.6	99.5	114.6	134.7	64.5	257.8	2	71.7	86.6	147.4
	Mg	4908.5	84.5	114.1	88.2	36	86.7	157.8	61	105.1	105.9	101.1
	S	1747.3	101	111.7	95.8	258.9	119.2	96.7	150.7	92.4	110.5	98.5
	Na	0	nd	nd	nd	nd	102.3	222.1	32.7	49.7	114.9	87.5
Micronutrients (mg/L)[2]	B	36.3	74.4	86.3	103	62	131.5	108.2	83	97.7	84.8	106.2
	Fe	327.8	15.5	132.4	110.3	85.6	197	93.4	63.1	241.7	30.9	169.9
	Mn	2864.2	48.2	98.5	111.7	0.2	90	226	9.8	50	100	133.3
	Mo	48.4	8.7	71.4	136.7	nd	nd	29.4	nd	nd	nd	nd
	Zn	255.4	19.2	127.5	112.5	60.2	93.6	153	44.7	89.7	99.2	100
	Cu	23.1	35.5	126.8	81.7	81.4	57.9	254.5	2.7	500	46.7	128.6
*	Co	nd	nd	nd	nd	nd	nd	Nd	nd	nd	nd	nd
*	Ni	3704	116.2	91.5	98.7	nd	nd	185	43.2	nd	nd	nd

Notes: nd = Not detected, [1]Macroelements (N, Ca, K, Mg, Na, Si, P, S) and [2]microelements (B, Fe, Mn, Mo, Ni, Se, Zn). [3]Depurated = Before fish was scarified, they were depurated in clean water for 15 days.

Table XIV. Analyses of macro and micro nutrient content in the liquid fraction obtained during RAS rearing, implementing DPI 1.4, DPI 1.2 and DPI 1.0 for five months.

Month	Treatment	N	P	K	Ca	Mg	S	Na
	Time zero	20.2	nd	1.9	63.5	30.9	nd	37.2
August	DPI 1.4	17.7±8.6	nd	4.8±2.5	71.4±21.9	35.6±14.1	nd	81.7±39.2
	DPI 1.2	14.7±9.4	nd	4.5±0.7	64.9±7.4	34.2±4.2	nd	76.4±6.5
	DPI 1.0	14±5.1	nd	5±1.9	72.5±19.3	36.0±11.0	1.3±1.0	95.5±28.5
September	DPI 1.4	132.1±39	1.9±0.3[a]	24.9±6.7	164.8±35.5	54.3±13.6	6.9±1.8	168.5±44.8
	DPI 1.2	114.0±23.5	1.9±0.3[a]	23.1±6.6	163.7±50.8	55.4±15.3	6.7±2.6	176.5±44.7
	DPI 1.0	107.8±19.5	1.2±0.2[b]	19.1±4.8	163.2±35.4	54.8±14.7	6.1±2.1	168.1±61.6
October	DPI 1.4	226.9±85.1	5.05±0.8	68.4±15.6	250.2±45.8	88.0±25.2	19.2±2.5[a]	293.9±97.0
	DPI 1.2	202.7±46.2	5.3±1.5	65.5±7	269.8±19.7	93.4±7.7	18.7±0.9[ab]	303.7±21.2
	DPI 1.0	207.1±65	3.7±0.3	46.8±12.7	249±67.3	77.8±24.5	14.4±3.0[b]	247.4±93.8
November	DPI 1.4	207.8±36.9	8.4±1.1	107.2±22.6	276.4±51.0	116.9±36.2	25.5±2.3	405.4±141.9
	DPI 1.2	232.3±96.1	7.8±1.9	81.9±28.4	248.3±78.5	95.3±31.9	19.1±6.1	326.1±113
	DPI 1.0	168.9±74.8	6.5±2.6	85.3±32	303.1±83.7	115.2±30.6	20.7±6.4	410.8±94
December	DPI 1.4	196.1±67.2	15.8±2.3	165.8±29.7	331.8±30.9	149.5±25.2	35.8±1.5[a]	487.5±89.6
	DPI 1.2	233.7±107.1	15.2±5	119.1±34.9	311.3±56.2	127.8±19.3	29.2±5.4[ab]	432.9±44
	DPI 1.0	239.1±141.2	12.3±5.3	108.3±37.6	321±67	121±23	24.8±5.7[b]	413.2±79.3

Month	Treatment	B	Fe	Mn	Mo	Zn	Co	Ni
	Time zero	0.1	nd	nd	nd	0.1	0.0	nd
August	DPI 1.4	0.2±0.1	nd	nd	0.04±0.03	0.1±0.04	0.01±0.01	0.02±0.01
	DPI 1.2	0.4±0.1	0.03±0.01	0.1±0.03	0.04±0.03	0.1±0.04	0.02±0.01	0.04±0.01
	DPI 1.0	0.6±0.1	0.1±0.02	0.2±0.09	0.1±0.05	0.1±0.03	0.01±0.01	0.03±0.01
September	DPI 1.4	0.9±0.2	0.1±0.01	0.2±0.06	0.1±0.03	0.1±0.03	0.02±0.01	0.02±0.01
	DPI 1.2	1.0±0.1	0.2±0.05	0.3±0.06	0.1±0.05	0.2±0.04	0.01±0.01	0.04±0.04
	DPI 1.0	0.2±0.03	nd	nd	nd	0.1±0.04	0.01±0.004	0.01±0.01
October	DPI 1.4	0.4±0.1	0.03±0.01	0.1±0.03	0.1±0.03	0.1±0.01	0.02±0.01	0.03±0.02
	DPI 1.2	0.6±0.02	0.1±0.02	0.2±0.02	0.1±0.02	0.1±0.04	0.02±0.02	0.02±0.02
	DPI 1.0	0.7±0.2	0.1±0.02	0.2±0.07	0.1±0.03	0.2±0.03	0.02±0.01	0.03±0.01
November	DPI 1.4	0.9±0.1	0.1±0.05	0.3±0.1	0.1±0.05	0.2±0.08	0.02±0.01	0.04±0.02
	DPI 1.2	0.2±0.1	nd	nd	0.006±0.003	0.1±0.03	nd	0.03±0.03
	DPI 1.0	0.4±0.1	0.04±0.02	0.05±0.02	0.05±0.02	0.1±0.02	0.01±0.01	0.03±0.01
December	DPI 1.4	0.5±0.1	0.06±0.03	0.2±0.04	0.1±0.1	0.1±0.03	0.02±0.004	0.03±0.02
	DPI 1.2	0.8±0.2	0.1±0.04	0.2±0.1	0.1±0.01	0.1±0.05	0.01±0.004	0.03±0.02
	DPI 1.0	0.8±0.1	0.1±0.07	0.3±0.1	0.04±0.01	0.2±0.06	0.02±0.006	0.03±0.01

Notes: nd = not detected, Macroelements (N, Ca, K, Mg, Na, Si, P, S) and microelements (B, Fe, Mn, Mo, Zn, Co, Ni).

Table XV. Results of the analyses of different research papers; these data show the different feeding strategies used in tilapia experiments. 1*. Oreochromis niloticus*, 2. *O. niloticus x O. aureus,* 3. *O. niloticus x O. mossambicus,* 4. *O. mossambicus,* and 5. *O. aureus*. The DPI (g protein/kg biomass) in this study was estimated using the information provided by different authors.

Species	Density (fish/ tank)	Protein level (%)	Initial weight (g)	Initial biomass (g)	Final weight (g)	Final biomass (g)	Percentage body weight (%)	DPI (g protein/kg biomass)	FCR	Feed ration	Culture period (Days)	Author
Strategies 1 and 2: Satiation feeding and satiation feeding with time												
1	25	30	1.21	30.25	5.32	133	•	6.7	2.01	nd	90	(Yigit and Olmez, 2009)
1	12	28	1.83	21.96	12.46	149.52	•	9.2	1.22	6	37	(Quadros *et al*., 2009)
1	30	30	13	390	273	8190	•	3.7	1.86	2	150	(El-Sayed, 1998)
4	20	30	1.07	21.4	34.75	695	•	4.2	1.18	2	84	(De Silva *et al*.,1991)
1	15	35	6.81	102.15	120.1	1801.5	•	5.5	0.95	3	60	(Koch *et al*., 2016)
1	30	30	4	120	20.5	615	•	9.3	1.74	3	56	(Lin and Luo, 2011)
1	30	36.3	0.7	21	35.8	1074	•	6.7	1.1	2	60	(Cao *et al*., 2008)
1	30	36.4	0.73	21.9	34.9	1047	•	6.9	1.14	3	60	(Cao *et al*., 2008)
1	25	29.7	3.5	87.5	23	575	•	5.0	1.4	nd	84	(Soltan, and Abdel-Moez, 2015)
1	10	40	10.61	106.1	29.91	299.1	•	5.1	1.44	3	112	(Ergün *et al*., 2009)
1	100	28.3	7.6	760	28.8	2880	•	8.3	1.5	2	51	(Ozório *et al*., 2012)
1	20	30	2.87	57.4	46.64	932.8	•	6.4	1.27	2	60	(Yangthong, Oncharoen, and Sripanomyom, 2014)
1	20	30	2.89	57.8	45.7	914	•	6.9	1.38	2	60	(Yangthong *et al*., 2014)
1	20	36	0.12	2.4	3.99	79.8	•	11.2	1.4	3	45	(Trosvik *et al*., 2013)
1	28	32	12.5	350	33.6	940.8	•	3.2	0.54	2	54	(Kasper and Brown, 2003)
1	10	37.91	0.154	1.54	6.164	61.64	•	8.6	1.43	nd	63	(Flores *et al*., 1995)
1	25	35	6.1	152.5	33	825	•	7.5	1.2	4	56	(Watanabe *et al*.,, 1980)
1	10	35	0.4	4	7.6	76	•	8.0	1.6	1	70	(Ahmad *et al*.,, 2004)
1	10	45	0.4	4	10.2	102	•	9.6	1.5	1	70	(Ahmad *et al*., 2004)
1	10	35	17	170	45.2	452	•	9.5	1.9	1	70	(Ahmad *et al*., 2004)
1	10	45	17	170	44.3	443	•	12.9	2	1	70	(Ahmad *et al*., 2004)
1	10	35	37	370	64.7	647	•	11.5	2.3	1	70	(Ahmad *et al*., 2004)
1	10	45	37	370	62.9	629	•	15.4	2.4	1	70	(Ahmad *et al*., 2004)
1	20	41.5	0.12	2.4	3.99	79.8	•	13.8	1.4	3	42	(Trosvik *et al*., 2013)
5	12	27.4	13.4	160.8	0.856	10.272	•	9.1	1.4	2	42	(Will *et al*., 2002)
2	28	30.1	2.7	75.6	65.8	1842.4	•	9.9	2.3	2	70	(Coyle *et al*.,, 2004)

2	20	33.2	1.93	38.6	12.3	246	•		7.6		1.61	2	70	(El-Saidy and Gaber, 2002)
Strategy 3: Fixed body weight percentage														
1	13	24	98.45	1279.9	138.58	1801.54	1.5		3.6		3.44	2	84	(Azim and Little, 2008a)
1	160	35	2.5	400	49.08	7852.8	3		10.5		2.03	1	180	(Bahnasawy, 2009)
1	14	30	1.37	19.18	4.42	61.88	5		15.0		1.78	2	56	(Chou and Shiau, 1996)
2	14	29.2	1.34	18.76	4.52	63.28	5		14.6		1.64	2	56	(Chou and Shiau, 1996)
1	30	30	24.5	735	79.5	2385	3		9.0		1.38	2	56	(Dato-Cajegas and Yakupitiyage, 1996)
1	20	27.5	1.18	23.6	21.6	432	4		11.0		1.623	3	nd	(Ghazalah *et al*., 2010)
1	30	35	4.35	130.5	19.79	593.7	3.5		12.3		2.27	3	54	(Khan, Siddique, and Zamal, 2013)
1	30	35	4.22	126.6	19.53	585.9	3.5		12.3		2.31	3	54	(Khan *et al*., 2013)
1	30	30	4.35	130.5	19.79	593.7	3.5		10.5		2.27	3	nd	(Khan *et al*., 2013)
1	15	35	15.87	238.05	80	1200	7		24.5		nd	nd	nd	(Liu, 2018)
1	30	46	50.61	1518.3	160.54	4816.2	3		13.8		0.83	2	56	(Long *et al*., 2015)
1	20	32	4.77	95.4	26.59	531.8	5.5		17.6		1.28	2	42	(Nguyen, Davis, and Saoud, 2009)
1	18	28	3.89	70.02	55.5	999	5.5		15.4		nd	2	70	(Nguyen *et al*., 2009)
1	3	35.45	1.1	3.3	3.12	9.36	10		35.5		nd	1	60	(Cavalheiro, Souza, and Bora, 2007)
1	10	30	0.34	3.4	2.4	24	6		18.0		1.9	3	nd	(Plascencia-Jatomea *et al*., 2002)
2	15	30	0.83	12.45	2.48	37.2	5		15.0		1.84	2	56	(Chou, Shiau, and Hung, 2001)
1	20	38.18	0.1	2	3.34	66.8	5		19.1		nd	4	35	(Thompson *et al*., 2012)
1	20	40.91	0.1	2	2.7	54	5		20.5		Nd	4	35	(Thompson *et al*., 2012)
1	15	30	21.3	319.5	60.15	902.25	3		9.0		Nd	2	70	(Twibell and Brown, 1998)
1	15	32	21	315	58.15	872.25	3		9.6		Nd	2	71	(Twibell and Brown, 1998)
1	19	33.7	0.8	15.2	3.41	64.79	5		16.9		1.49	2	56	(Shiau and Chuang, 1995)
1	25	30	1.21	30.25	5.32	133	10.0		30.0		2.01	nd	90	(Yigit and Olmez, 2009)
Strategy 4: Variable body weight percentage†														
							I%	F%	I	F				
1	25	45	0.33	8.25	10.05	251.25	10	5	45	22.5	1.34	2	84	(Abdel-Tawwab, 2012)
1	25	45	0.32	8	10	250	10	5	45	22.5	1.34	2	84	(Abdel-Tawwab, 2012)
1	10	34	1.43	14.3	6.22	62.2	10	4	34	13.6	1.69	2	42	(Diop *et al*., 2013)
1	50	40.2	0.02	1	1.87	93.5	20	6	80.4	24.12	1.01	4	77	(Hussein *et al*., 2013)
3	20	30	4.48	89.6	42.34	846.8	6	4	18	12	1.64	2	70	(Fasakin, Serwata, and Davies, 2005)

3	15	30	1.93	28.95	10.39	155.85	6	4	18	12	1.24	2	56	(Fasakin *et al*., 2005)
1	10	37	1.24	12.4	14.92	149.2	10	4	37	14.8	5.92	2	42	(Loum *et al*., 2013)
1	10	32	1.21	12.1	13.87	138.7	10	4	32	12.8	5.85	2	42	(Loum *et al*., 2013)
1	20	32.6	30	600	408.2	8164	3.8	2.8	12.38	9.128	2.84	2	103	(Wu *et al*., 1994)
1	25	34.6	0.5	12.5	14.45	361.25	14	6.5	48.44	22.49	1.25	nd	56	(Wu, Rosati, and Brown, 1997)
1	25	34.6	0.5	12.5	12.1	302.5	14	6.5	48.44	22.49	1.43	nd	56	(Wu *et al*., 1997)
1	20	32	30	600	96.3	1926	3.8	3.1	12.16	9.92	1.85	2	75	(Wu *et al*., 1995)
1	20	32.1	30	600	91.8	1836	3.8	3.1	12.19	9.95	1.86	2	75	(Wu *et al*., 1995)
Strategies 5 and 6: Using a specific g kg^{-1} ‡														
1	5	40.7	41.9	209.5	84.5	422.5	a		7.3		1	nd	56	(Mamun *et al*., 2007)
1	40	32.5	56.4	2256	157	6280	b		6.6		1	nd	49	(Schneider *et al*., 2004)
1	15	32	2.8	42	9.6	144	c		2.4		0.41	nd	54	(Kasper, White, and Brown, 2000)
1	15	32	3.3	49.5	14	210	c		3.0		0.5	2	54	(Kasper *et al*., 2000)
1	15	32	3.3	49.5	13.4	201	c		3.0		0.51	2	54	(Kasper *et al*., 2000)
1	2	34.5	5	10	35.8	71.6	d		4.9		1	2	70	(Dongmeza *et al*., 2006)
1	40	42.88	45	1800	153.5	6140	e		7.5		0.98	4	56	(Amirkolaie *et al*., 2005)
1	7	35	10.09	70.63	37.19	260.33	d		2.6		0.9	2	119	(Richter, Siddhuraju, and Becker, 2003)
1	30	35	6.7	201	61.7	1851	f		5.0		1.2	5	84	(Fontaínhas-Fernandes, *et al*., 1999)

Notes:† The strategy variable body weight percentage shows two DPIs (initial (I) and final (F)) and two body weight percentages ((I%) initial and (F%) final). These data was calculated using the FCRs. ‡Explanation of the strategy data: (a) 3.0 g kg$^{0.8}$/day, (b) 17 g feed kg $^{0.8}$/day, (c) 5 g feed (100 g body/ weight) d), (d) 15 g feed per metabolic body weight kg$^{0.8}$, (e) 80-89.2 g per day, and (f) 4 g DM mean body weight/day. Note • = Satiation, nd = no.

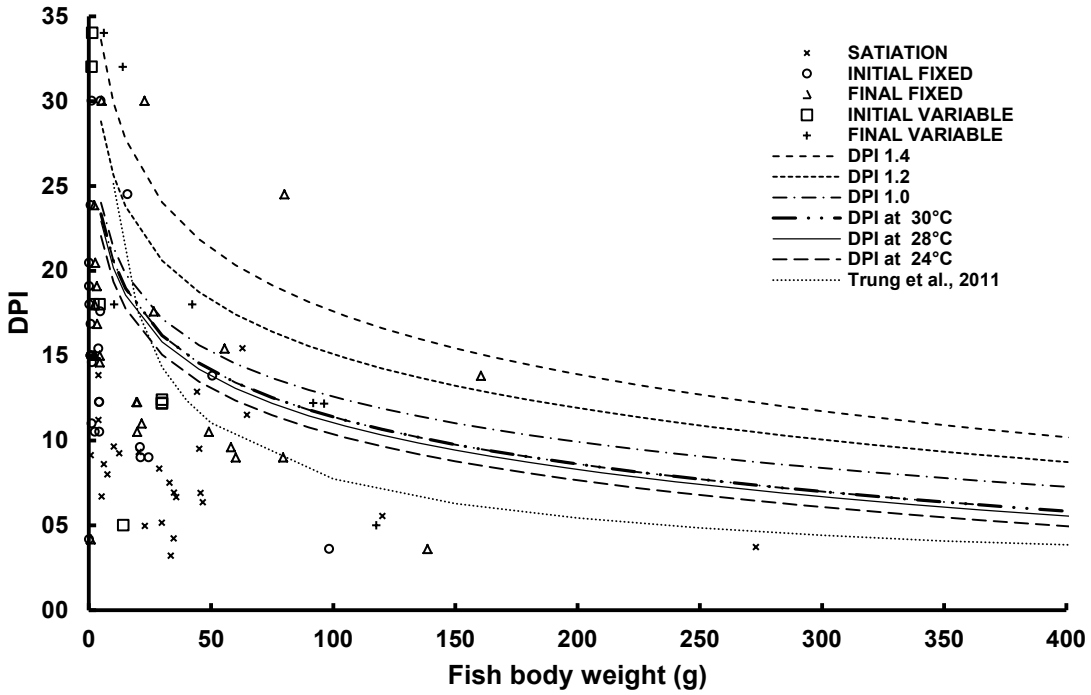

Figure 29. The symbols represent the DPI levels (g protein/kg fish biomass) related to fish body weight (BW) applied in different research papers using satiation feeding; circles indicate the initial time with the fixed body weight percentage (BW%); triangles indicate the final time with the fixed BW%; squares indicate the initial variable BW%; and crosses indicate the final variable BW% during the different experiments reported in 51 research papers (Table 6). Lines represent the DPI mathematical functions: DPI 1.0 =100% (DPI = -3.818 ln (BW) + 30.158), DPI 1.2 =120% (DPI = -4.582 ln (BW) + 36.19), and DPI 1.4=140% (DPI = -5.345 ln (BW) + 42.221). Different temperatures were assessed (DPI = -3.997 ln (BW) + 29.79 for 30°C, DPI = -3.965 ln (BW) + 29.305 for 28°C, and DPI = -3.902 ln (BW) + 28.334 for 24°C; recommended by the Fisheries Agency of Japan, 1995). DPI =-2.807*LN (BW)+21.259 for 28 °C (R^2 = 0.9725) using the original data from Van Trung, Diu, Hao, and Glencross (2011).

For tilapia farming, six feeding strategies were implemented: satiation and satiation with time, fixed biomass during all the experiment and changing biomass; specific gram for every kg was also implemented but only DPI included the amount of protein, digestibility, productivity in the system and weekly growth (Table XVI).

Table XVI. Comparison of the different feeding strategies implemented in tilapia culture.

	FEEDING STRATEGIES[1]						
Variables	**Satiation**	**Satiation with time**	**Fixed % Biomass**	**Change % biomass**	**g.Kg.Bw**	**g/100 g Bw**	**DPI**
Temperature	•						•
Average weight			•	•	•	•	•
Feeding rate			•	•			
Protein in feed							•
Digestibility							•
System productivity							•
Weekly growth							•
Growth tracking			•	•	•	•	•
Biomass			•	•	•	•	•
Satiation	•	•	•	•	•	•	•

Notes: [1]The black point indicated that the feed strategy met the indicated conditions.

EXPERIMENT II - BFT

7.2 Data obtained during the Biofloc Technology Experiment

The 40-week experimental period implemented the different trophic levels: autotrophic, heterotrophic, photoautotrophic in two growth phases (nursery and grow-out) with *Oreochromis niloticus*.

7.2.1 Water quality in Biofloc Technology experiment

Oxygen remained at an optimal range with no differences among treatments ($p > 0.05$; Table XVII). In the nursery phase, pH recorded significant differences among treatments (Table XVII). Salinity oscillated during the whole experiment with significant differences among treatments ($p < 0.05$; Table XVII); the highest values of these parameters were detected in treatments M and CV in the grow-out phase. For conductivity, Q and H had a lower level during the nursery phase, increasing to the highest level (> 6 dS/m) after week 11. In the grow-out phase, all treatment levels decreased with significant differences among treatments ($p < 0.05$), where the photoautotrophic treatments obtained the highest levels (Table XVII).

In the nursery phase, nitrogen compounds progressively accumulated NH_4-N, NO_3-N except for NO_2-N (Fig. 30a, c, b). Low NH_4-N values were detected during the first seven weeks with no significant differences among treatments. An increase of NH_4-N was observed after week eight in photoautotrophic treatments; after week 12 in treatment Q and in treatment H ($p < 0.05$; Fig. 30 a, b, c). In fish grow-out tanks, NH_4-N increased to values higher than 150 mg/L ($p < 0.05$). After 35 weeks, the level of NH_4-N was higher without significant difference ($p > 0.05$). In the nursery phase treatments, CS and CV showed high levels of NO_2-N only during the first six weeks, followed by a rapid decrease while a low level of this component was maintained (lower than 5 mg/L) in all treatments in both phases (Fig. 30b).

Table XVII. Description of rearing parameters (Temperature, dissolved oxygen (DO), pH, conductivity and salinity), total evaporation and evaporation per week during 40 weeks. The rearing period was divided into two nursery phases (weeks 1-20) and grow-out (weeks 21-40).

Physical parameters	Week	Q	H	M	CS	CV
Temperature (°C)	**10**	21.0±1.0^{c}	20.6±1.1^{bc}	19.9±1.3^{ab}	20.2±1.4^{ab}	19.7±1.4^{a}
	20	24.4±1.8	22.3±1.8	21.8±1.6	22.3±1.6	21.7±1.6
	30	26.5±1.3	26.6±1.3	25.8±1.8	25.7±1.8	25.6±1.6
	40	30.6±0.4	30.4±0.4	29.8±0.4	29.9±0.6	29.7±0.6
DO (O_2 mg/L)[1]	**10**	8.9±0.5	8.9±0.8	9.14±0.7	9.2±0.6	9.3±0.7
	20	7.6±1.0	7.8±1.0	7.6±1.0	7.5±1.1	7.8±1.0
	30	6.7±0.6	6.9±0.5	6.6±1.0	6.8±0.8	6.9±0.7
	40	6.3±0.6	6.4±0.9	5.9±0.7	5.9±0.8	6.0±0.8
pH[2]	**10**	7.9±0.8^{b}	8.0±0.5^{a}	7.2±0.9^{d}	7.3±0.9^{c}	7.2±0.9^{d}
	20	5.3±0.5^{b}	5.5±0.5^{a}	5.2±0.5^{b}	5.3±0.4^{b}	5.3±0.4^{b}
	30	5.6±0.6	5.4±0.7	5.5±0.5	5.5±0.5	5.6±0.6
	40	5.1±0.2	5.2±0.2	5.1±0.2	5.1±0.1	5.1±0.3
Conductivity[3] (dS/m)	**10**	1.1±0.1^{b}	1.2±0.4^{b}	3.5±0.3^{a}	3.4±0.3^{a}	3.4±0.4^{a}
	20	4.4±1.6^{a}	4.9±2.1^{a}	3.3±0.4^{b}	3.3±0.4^{b}	3.3±0.4^{b}
	30	2.2±0.3^{ab}	2.1±0.3^{b}	2.4±0.5^{a}	2.4±0.5^{a}	2.4±0.4^{a}
	40	3.7±0.4^{bc}	3.4±0.4^{c}	4.7±0.6^{a}	4.6±0.5^{a}	4.4±0.4^{ab}
Salinity (ppt)	**10**	0.6±0.05^{b}	0.6±0.04^{b}	2.0±0.1^{a}	2.0±0.1^{a}	2.0±0.1^{a}
	20	1.4±0.9^{b}	1.8±1.3^{a}	1.9±0.2^{a}	1.8±0.2^{a}	1.9±0.2^{a}
	30	1.0±0.2	1.1±0.1	1.2±0.2	1.2±0.2	1.2±0.2
	40	1.7±0.1^{bc}	1.6±0.1^{c}	2.2±0.2^{a}	2.2±0.1^{a}	2.0±0.2^{ab}
Total Evaporation (L)	**40**	1254.6±28.4	1381.0±127.9	1401.2±55.7	1349.1±72.0	1374.9±56.1
Evaporation per week (L)	**T**	31.3±0.7	34.5±3.2	35.0±1.4	34.4±1.4	33.7±1.8

Notes: The first phase was characterized by a high density (180 fish m^3) and low temperature (weeks 1-20). The second phase showed low density (55 fish m^3) and high temperature (weeks 21-40). [1]DO = Dissolved oxygen (mg/L); [2]pH ranges 0-14., [3]conductivity in dS/m. Each value represents the mean ± SD. Lower case letters indicate differences among treatments. Values in the same row with different superscripts are significantly different ($p < 0.05$). Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

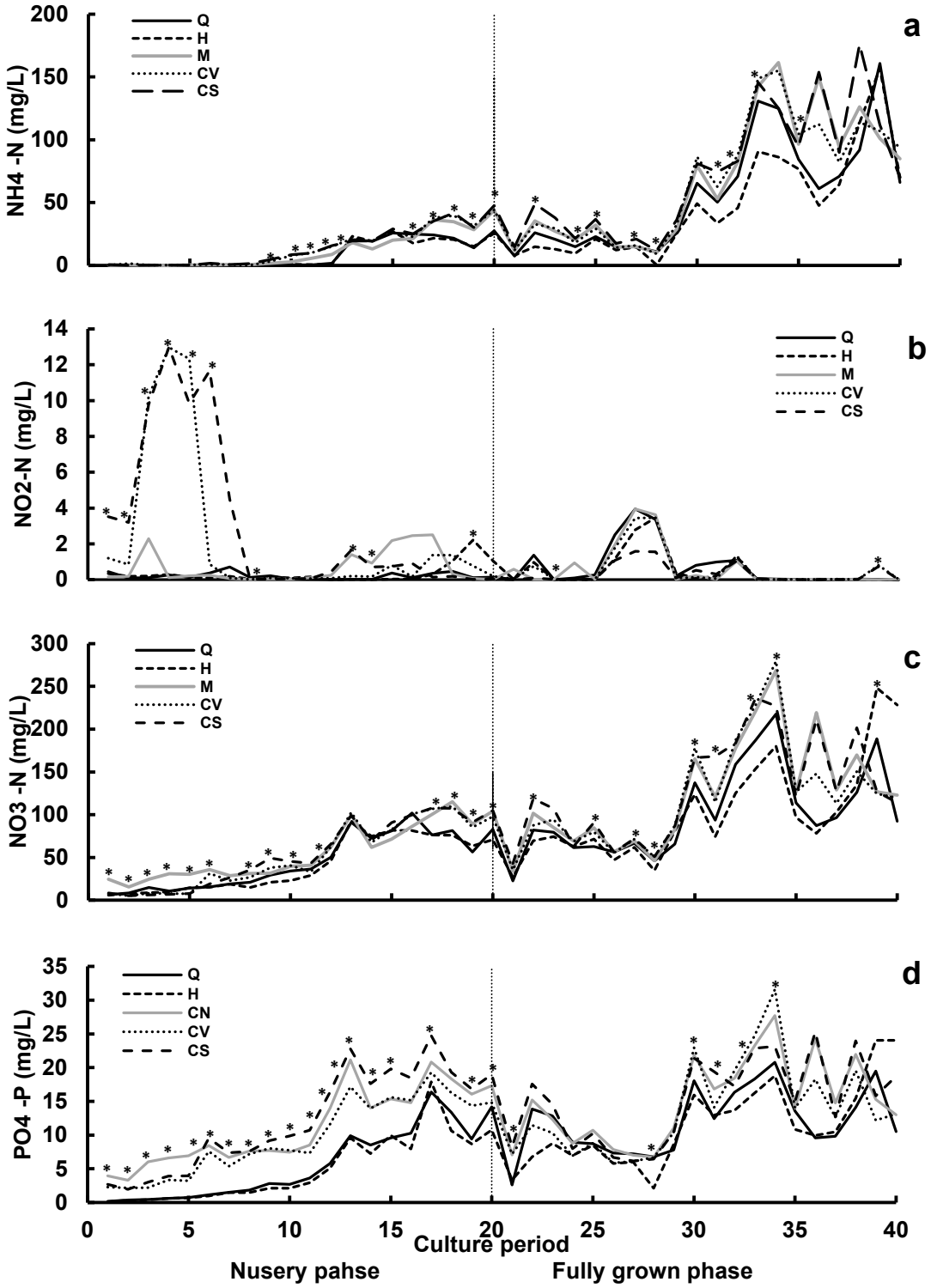

Figure 30. Description of water quality during the experimental period (40 weeks). (a) ammonia; (b) nitrites; (c) nitrates and (d) phosphates. The vertical grey line in the middle of each plot separates data into two phases: nursery (1 - 20 weeks) and tilapia grow out (21- 40 weeks) during tilapia rearing. Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

NO_3-N increased progressively with significant difference ($p < 0.05$) in the nursery phase. In the grow-out phase the photoautotrophic treatments showed the highest level of NO_3-N among weeks from 20 to 35, the highest value of this component in treatments M, CV and CS were observed at week 34 (Fig. 30 c). The highest phosphate levels were detected in the photoautotrophic treatments, which showed significant differences ($p < 0.05$) during most of the experiment in both phases (Fig. 30d).

7.2.2 Growth performance

Differences in both growth and weight gain were observed among the photoautotrophic treatments and treatments Q and H ($p < 0.05$, Table XVIII) during nursery and grow-out phases. The food conversion ratio showed significant differences among treatments in the initial time point of nursery and in the final time of grow-out ($p < 0.05$, Table XVIII). Photoautotrophic treatments showed the highest fish survival in the nursery phase ($p < 0.05$, Table XVIII). At the grow-out phase survival did not show significant differences (week 30) ($p > 0.05$, Table XVIII) and at week 40, the highest survival value was found in treatments Q, H and M with significant differences ($p < 0.05$, Table XVIII).

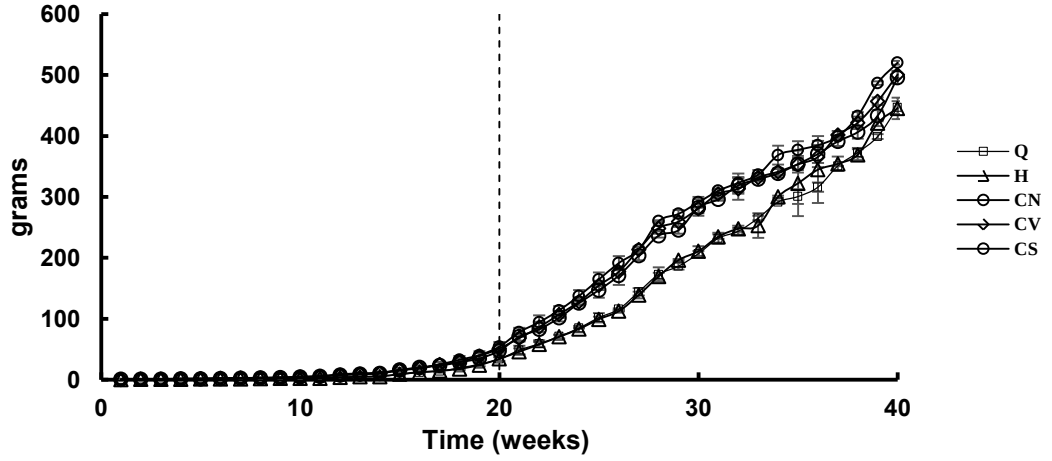

Figure 31. Growth during the 20-week rearing period for tilapia (*Oreochromis niloticus*) implementing five treatments in biofloc. The period was divided in winter and summer. First period with high density (180 fish/m^3) and low temperature (1-20 weeks); second period with low density (55 fish/m^3) and high temperature (weeks 21-30).

Table XVIII. Production parameters of tilapia (*Oreochromis niloticus*) (mean ± SD) (Body weight, weight gain, food conversion ratio, specific growth rate and survival) in five treatments Biofloc Technology (BFT) for 40 weeks.

Growth parameters	Week	Q	H	M	CS	CV
Body weight (g)	**10**	2.2 ± 0.2^{b}	2.4 ± 0.4^{b}	4.6 ± 0.6^{a}	4.6 ± 0.5^{a}	4.7 ± 0.3^{a}
	20	33.8 ± 3.2^{b}	34.2 ± 3.2^{b}	47.6 ± 3.2^{a}	51.3 ± 4.5^{a}	54.2 ± 7.7^{a}
	30	207 ± 4.4^{b}	210.9 ± 8.0^{b}	282.5 ± 13.7^{a}	281.1 ± 8.0^{a}	291.1 ± 8.9^{a}
	40	446.7 ± 10.5^{b}	445.4 ± 17.5^{b}	496.0 ± 10.5^{a}	500.6 ± 6.4^{a}	520.2 ± 3.1^{a}
Weight gain (g/kg)	**10**	1.9 ± 0.2^{b}	2.1 ± 0.4^{b}	4.2 ± 0.6^{a}	4.3 ± 0.5^{a}	4.3 ± 0.3^{a}
	20	31.5 ± 3.2^{b}	31.8 ± 2.8^{b}	43.0 ± 3.2^{ab}	46.7 ± 4.3^{a}	49.5 ± 7.6^{a}
	30	173.2 ± 1.2^{b}	176.7 ± 8.8^{b}	234.9 ± 16.1^{a}	229.8 ± 6.7^{a}	236.9 ± 12.8^{a}
	40	239.7±14.4	234.5±11.8	213.4±23.5	219.5±7.3	229.0±11.9
FCR[1]	**10**	2.8 ± 0.4^{Aa}	2.6 ± 0.3^{Aa}	2.0 ± 0.3^{Ab}	2.1 ± 0.3^{Ab}	2.0 ± 0.2^{Ab}
	20	1.4 ± 0.1^{B}	1.3 ± 0.1^{B}	1.4 ± 0.1^{B}	1.3 ± 0.1^{B}	1.3 ± 0.2^{B}
	30	1.7 ± 0.1^{B}	1.6 ± 0.1^{B}	1.7 ± 0.1^{AB}	1.7 ± 0.05^{AB}	1.8 ± 0.05^{A}
	40	1.5 ± 0.2^{Ba}	1.5 ± 0.2^{Ba}	1.7 ± 0.2^{Aab}	1.7 ± 0.2^{Aab}	1.7 ± 0.1^{Aab}
SGR (%)[2]	**10**	3.04 ± 0.1^{Db}	3.2 ± 0.3^{Db}	4.2 ± 0.2^{Aa}	4.2 ± 0.1^{Aa}	4.2 ± 0.1^{Aa}
	20	3.7 ± 0.1^{Aa}	3.8 ± 0.1^{Aa}	3.2 ± 0.1^{Db}	3.2 ± 0.1^{Db}	3.3 ± 0.2^{Db}
	30	2.1 ± 0.03^{Cab}	2.2 ± 0.1^{Ca}	2.0 ± 0.1^{Cbc}	2.1 ± 0.04^{Cb}	1.9 ± 0.05^{Cc}
	40	0.9 ± 0.03^{Ba}	0.9 ± 0.1^{Ba}	0.7 ± 0.03^{Bb}	0.7 ± 0.02^{Bb}	0.7 ± 0.01^{Bb}
Survival (%)	**10**	87.0 ± 0.02^{Cc}	78.3 ± 0.04^{Cd}	90.7 ± 0.01^{Cbc}	95.6 ± 0.01^{Ca}	93.7 ± 0.01^{Cab}
	20	98.0 ± 0.01^{Bbc}	96.6 ± 0.02^{Bc}	98.8 ± 0.003^{Bb}	99.0 ± 0.01^{Bb}	100.0 ± 0^{Aa}
	30	100.0^{A}	100.0^{A}	100.0^{A}	100.0^{A}	100.0^{A}
	40	100.0^{Aa}	100.0^{Aa}	99.3 ± 0.01^{Aa}	95.5 ± 0.02^{Cb}	95.0 ± 0.03^{Bb}
Production (kg)	**10**	0.35 ± 0.02^{b}	0.33 ± 0.04^{b}	0.74 ± 0.1^{a}	0.79 ± 0.1^{a}	0.78 ± 0.06^{a}
	20	5.3 ± 0.4^{b}	4.8 ± 0.6^{b}	7.8 ± 0.5^{a}	8.8 ± 0.8^{a}	9.1 ± 1.2^{a}
	30	11.4 ± 0.2^{b}	11.6 ± 0.4^{b}	15.5 ± 0.8^{a}	15.5 ± 0.4^{a}	16 ± 0.5^{a}
	40	21.4±0.5a^{b}	21.4 ± 0.8^{ab}	23.3 ± 0.5^{a}	21.2 ± 0.7^{ab}	19.4 ± 1.3^{b}

Notes: [1]FCR = Food conversion ratio, [2]SGR = Specific Growth Rate. The first phase was characterized by a high density (180 fish m^3) and low temperature (weeks 1-20); the second one showed low density (55 fish m^3) and high temperature (weeks 21-40). Lower case letters indicate differences among treatments and capital letters differences among weeks. Values in the same row with different superscripts are significantly different ($p < 0.05$). Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

7.2.3 Characterization of residuals in Bioflocculation technology

Settleable solids determined with Imhoff cones showed significant differences ($p < 0.05$) during the first weeks of the nursery phase. Treatments H and Q showed the highest values after week eight and until week 15 (Fig. 32 a, b). The highest level of TDS was found in H and Q in the nursery phase ($p < 0.05$) (Fig. 33)

The number of solids was estimated with the particle counter shown in Figure 34 a-d. At the start of the nursery phase (week 10) of our experiment, super colloidal and settleable solids (20-150 µm) were the most abundant in the

photoautotrophic treatments. In treatments H and Q > 60% of the particles were settleable solids (>150-500 µm); significant differences were found among size ranges (p < 0.05). Super colloidal and settleable solids (20-150 µm) (67.1–73.5%) were found from week 20 in all treatments. Particles > 500 µm were detected only for treatments Q and H, and those with significant differences (p < 0.05) were < 20 µm. During the grow-out phase, particle sizes were similar (20-150 µm) among treatments. No significant differences were found among treatments (p > 0.05) (Fig. 34 c, d).

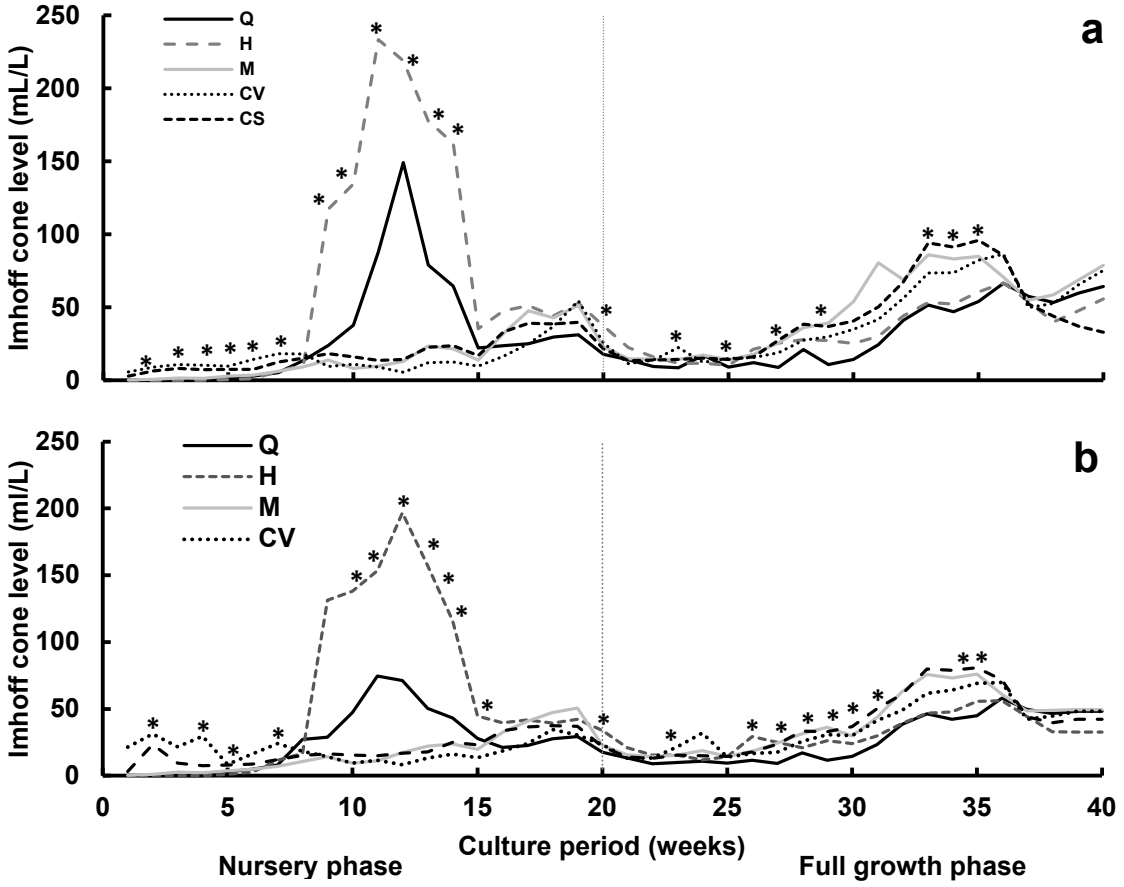

Figure 32. Settleable solids during the experimental phase were determined with an Imhoff cone. (a) Quantity of settleable solids at minute 15; (b) quantity of settleable solids at minute 30. Asterisks indicate significant differences (p < 0.05). The vertical gray line in the middle of each plot separates data into two phases: nursery (1 -20 weeks) and tilapia grow out (21- 40 weeks) during tilapia rearing. Q = chemotrophic treatment, H = heterotrophic treatment, M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

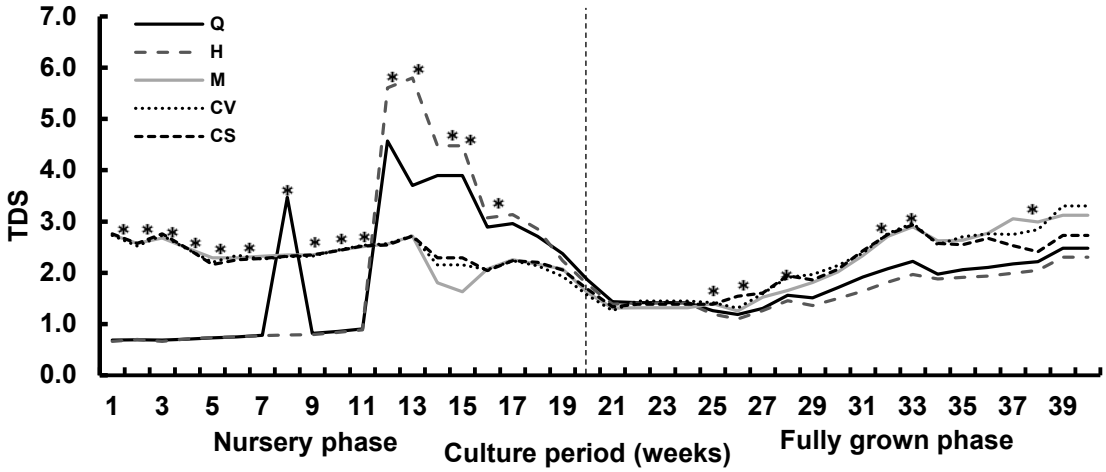

Figure 33. Total Dissolved Solids (TDS) during nursery (1 - 20 weeks) and fully-grown tilapia (21 - 40 weeks) in *Oreochromis niloticus* rearing period. The asterisk indicates significant differences ($p < 0.05$); Q = chemotrophic treatment, H = heterotrophic treatment, M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

7.2.4 *Oreochromis niloticus* and floc nutrient composition

For *O. niloticus* carcass, the protein level did not show significant differences among treatments ($p > 0.05$); only did the photoautotrophic treatments show variation among weeks; the lower level was obtained in week 40. For crude lipid and crude fiber the treatments did not show significant differences ($p > 0.05$); the amount of crude lipid only showed variation in treatments CV and for fiber only in M (Table XIX).

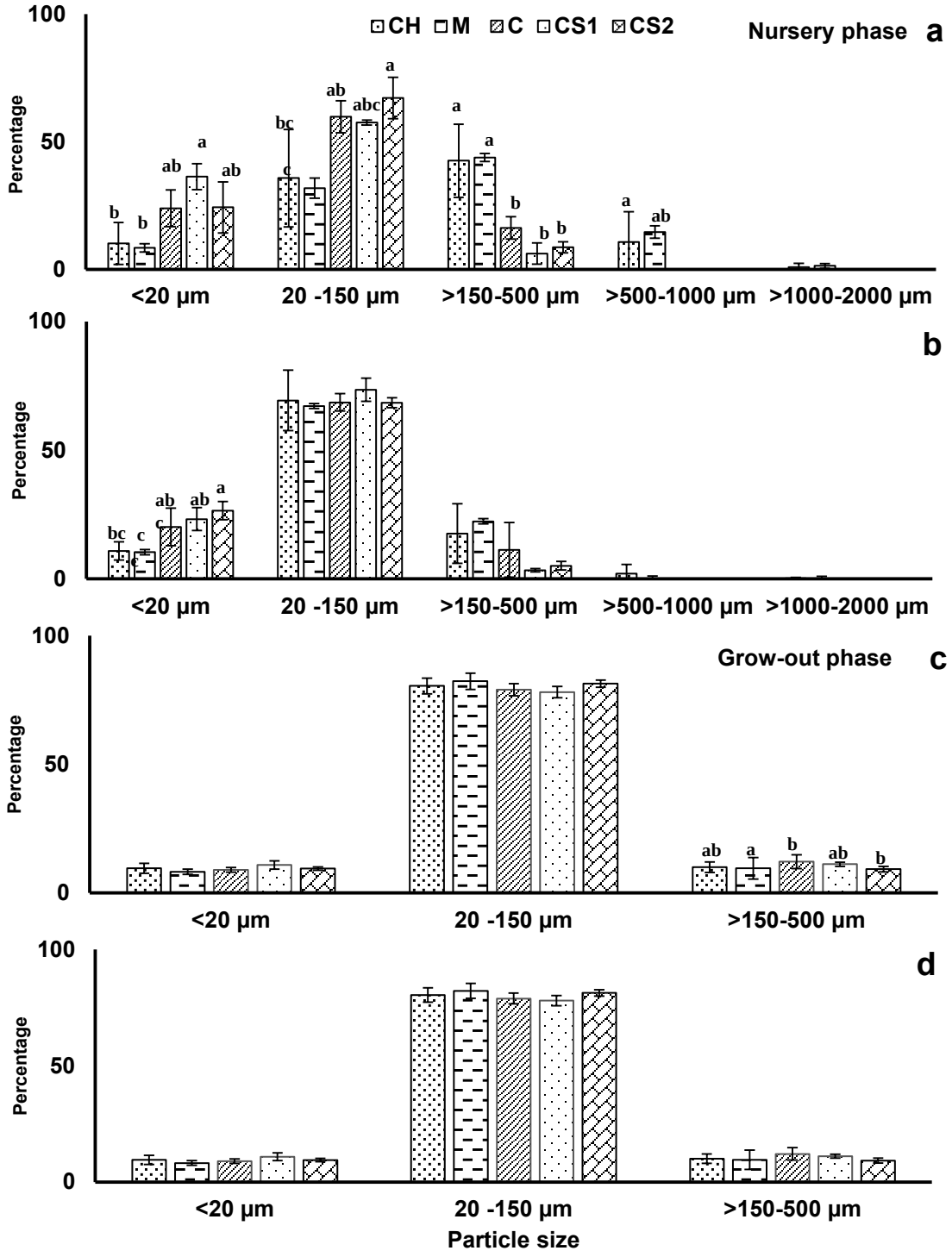

Figure 34. Size of particles from biofloc technology in all treatments during the experimental period (40 weeks). (a) Data obtained in week 10; (b) 20; (c) 30; (d) 40. Note: Each value represented in mean ± SD. Values in the same row with different superscripts are significantly different ($p < 0.05$). Q = chemotrophic treatment, H = heterotrophic treatment M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

Table XIX. Carcass analysis of *Oreochromis niloticus* during weeks 10 - 40 comparing the different treatments.

Proximal analyses	T[1]	Q	H	M	CV	CS
Moisture (%)	**10**	72.6 ± 2.9^{A}	70.6 ± 2^{AB}	62.3 ± 2.3^{B}	64.6 ± 5.6^{AB}	64.1 ± 5.4^{B}
	20	72.0 ± 0.4^{Aa}	71.1 ± 0.2^{ABa}	64.9 ± 0.9^{ABb}	63.1 ± 0.9^{Bb}	64.8 ± 0.9^{Bb}
	30	70.3 ± 1.8^{ABab}	73.3 ± 0.3^{Aa}	67.4 ± 0.9^{Ab}	69 ± 0.2^{Aab}	70.6 ± 3.5^{Aab}
	40	67 ± 1.4^{Bab}	68.2 ± 0.4^{Ba}	$66.7\pm1.8A^{Bab}$	64.8 ± 0.9^{ABb}	66.1 ± 0.7^{ABab}
Crude protein (%)	**10**	56 ± 1.6^{A}	53.68 ± 0.7^{A}	52.4 ± 1.3^{AB}	52.2 ± 0.06^{AB}	52.2 ± 0.6^{A}
	20	55.1 ± 0.4^{A}	54.3 ± 0.6^{A}	53.2 ± 1.0^{AB}	53 ± 1.0^{AB}	53.4 ± 0.4^{A}
	30	55.4 ± 1.7^{A}	54.2 ± 2.0^{A}	56.5 ± 2.6^{A}	56.9 ± 2.5^{A}	54.3 ± 3.9^{A}
	40	51.8 ± 3.2^{A}	52.9 ± 3.1^{A}	50.9 ± 0.3^{B}	51.5 ± 4.8^{B}	51.8 ± 3.2^{A}
Crude lipid (%)	**10**	26.3 ± 1.3^{A}	27.2 ± 2.6^{A}	29.8 ± 0.4^{A}	29.8 ± 1^{A}	28.9 ± 1.8^{A}
	20	26.4 ± 0.4^{A}	26.4 ± 0.9^{A}	27.2 ± 0.4^{A}	27.3 ± 1.3^{AB}	26.6 ± 0.45^{A}
	30	25 ± 1.1^{A}	23.1 ± 4.9^{A}	26.3 ± 1.4^{A}	23.5 ± 0.2^{B}	25.5 ± 4.7^{A}
	40	27.2 ± 1.8^{A}	26.9 ± 3.2^{A}	26.1 ± 2.3^{A}	29.7 ± 5.8^{A}	28.6 ± 5.5^{A}
Crude fiber (%)	**10**	0.3 ± 0.2^{A}	0.2 ± 0.2^{A}	0.3 ± 0.2^{A}	0.3 ± 0.1^{A}	0.3 ± 0.02^{A}
	20	0.4 ± 0.1^{A}	0.3 ± 0.3^{A}	0.2 ± 0.1^{AB}	0.3 ± 0.1^{A}	0.2 ± 0.03^{A}
	30	0.1 ± 0.03^{A}	0.1 ± 0.01^{A}	0.2 ± 0.02^{A}	0.2 ± 0.05^{A}	0.2 ± 0.07^{A}
	40	0.2 ± 0.02^{A}	0.2 ± 0.03^{A}	0.2 ± 0.8^{B}	0.3 ± 0.2^{A}	0.2 ± 0.06^{A}
Ash (%)	**10**	12.7 ± 0.5^{AB}	11.61 ± 0.9^{A}	10.6 ± 1.1^{A}	11.6 ± 1.4^{A}	11.19 ± 0.8^{A}
	20	12.1 ± 0.2^{A}	10.9 ± 0.6^{A}	10.9 ± 1.2^{A}	10.7 ± 0.3^{A}	10.4 ± 0.7^{A}
	30	11.4 ± 0.8^{B}	12.7 ± 1.0^{A}	8.8 ± 1.8^{A}	10 ± 3.0^{A}	9.4 ± 1.2^{A}
	40	12.2 ± 3.3^{AB}	13.2 ± 1.8^{A}	14.5 ± 2.1^{A}	11 ± 3.0^{A}	12.4 ± 1.8^{A}

Notes: [1] T=Time in weeks. Different letters in the same row indicate significant differences; lower case letters indicate differences among treatments; capital letters indicate differences among weeks. Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

The amino acid in carcass only showed significant differences in isoleucine and valine for essential amino acid and aspartate for nonessential amino acid. Tryptophan, asparagine and glutamine were not detected during all the experiment (Table XX).

Table XX. *Oreochromis niloticus* carcass amino acid analyses, reared in Bioflocculation Technology implementing different treatments.

Amino acid (g/100 g)	Time	Q	H	M	CV	CS
			Essential amino acids AA			
Arginine	**10**	2.2±0.3	2.3±0.3^{B}	2.6±0.2	2.1±0.2	2.7±0.3
	20	2.7±0.5	3.4±0.4^{A}	3.0±0.5	2.7±0.2	3.1±0.2
	30	2.8±0.2	2.6±0.9^{AB}	2.7±0.1	2.4±0.1	2.8±0.5
	40	2.2±0.3	2.3±0.3^{AB}	2.6±0.2	2.1±0.2	2.7±0.3
Histidine	**10**	0.6±0.1^{B}	0.7±0.02^{B}	0.8±0.1^{AB}	0.7±0.1	0.8±0.1
	20	0.9±0.2^{A}	1.0±0.1^{A}	0.9±0.1^{A}	0.8±0.1	0.9±0.03
	30	nd	nd	nd	nd	nd
	40	0.7±0.1^{B}	0.7±0.02^{B}	0.8±0.1^{B}	0.7±0.1	0.8±0.1
Isoleucine	**10**	1.1±0.2^{Bab}	1.0±0.1^{Bab}	1.1±0.1^{Bab}	0.9±0.1^{Bb}	1.3±0.1^{Ba}
	20	1.4±0.3^{AB}	1.6±0.1^{A}	1.7±0.3^{A}	1.5±0.2^{A}	0.6±0.2^{AB}
	30	1.5±0.2^{AB}	1.3±0.3^{AB}	1.5±0.1^{AB}	1.2±0.1^{AB}	1.4±0.3^{AB}
	40	1.1±0.2^{A}	1.0±0.1^{AB}	1.1±0.1^{A}	0.9±0.1^{A}	1.3±0.1^{A}
Leucine	**10**	2.6±0.3	2.6±0.2	3.0±0.2	2.5±0.5	3.3±0.1
	20	3.3±0.8	3.3±0.3	3.4±0.5	3.0±0.2	3.3±0.3
	30	3.5±0.1	3.2±1.0	3.3±0.3	3.3±0.1	3.3±0.8
	40	2.6±0.3	2.6±0.2	3.0±0.2	2.5±0.5	3.3±0.1
Lysine	**10**	2.5±0.3^{A}	2.6±0.3	2.2±0.1^{AB}	2.5±0.4^{A}	3.2±0.3^{A}
	20	3.8±0.7^{A}	3.3±0.4	3.7±0.5^{A}	3.3±0.2^{A}	3.6±0.3^{A}
	30	0.5±0.2^{B}	1.8±2.2	1.5±1.7^{B}	0.7±0.1^{B}	0.7±0.3^{B}
	40	2.5±0.3^{A}	2.6±0.3	2.2±0.1^{A}	2.5±0.4^{A}	3.2±0.3^{A}
Methionine	**10**	0.6±0.1	0.7±0.1^{AB}	0.6±0.2	0.6±0.1	0.6±0.1
	20	0.8±0.2	1.1±0.6^{A}	0.7±0.1	0.6±0.1	0.8±0.03
	30	0.5±0.1	0.6±0.4^{B}	0.5±0.2	0.5±0.1	0.5±0.2
	40	0.6±0.1	0.7±0.1^{AB}	0.6±0.2	0.6±0.1	0.6±0.1
Phenylalanine	**10**	1.6±0.2	1.6±0.1	1.8±0.2	1.6±0.3	1.9±0.1
	20	1.9±0.4	2.1±0.2	1.9±0.3	1.7±0.1	1.9±0.1
	30	1.7±0.1	1.8±0.6	1.7±0.1	1.6±0.1	1.7±0.3
	40	1.6±0.2	1.6±0.1	1.8±0.2	1.6±0.3	1.9±0.1
Threonine	**10**	1.7±0.2	1.7±0.2	1.7±0.6	1.6±0.2	2.1±0.1
	20	1.9±0.4	2.2±0.3	1.8±0.4	2±0.04	0.9±1.3
	30	2.1±0.1	2.1±0.7	2.1±0.2	2.0±0.1	2.1±0.4
	40	1.7±0.2	1.7±0.2	1.7±0.6	1.6±0.2	2.1±0.1
Tryptophan	**10**	nd	nd	nd	nd	nd
	20	nd	nd	nd	nd	nd
	30	nd	nd	nd	nd	nd
	40	0.2±0.05	0.1±0.04	nd	nd	0.3±0.04
Valine	**10**	1.2±0.2^{Bab}	1.2±0.2^{Bab}	1.3±0.05^{Bab}	1.0±0.1^{Bb}	1.5±0.2^{Ba}
	20	1.9±0.2^{A}	2.0±0.2^{A}	2.1±0.4^{A}	1.9±0.3^{A}	1.9±0.2^{AB}
	30	1.9±0.2^{Aab}	1.7±0.4^{ABab}	1.8±0.1^{ABab}	1.6±0.1^{ABab}	1.7±0.4^{ABa}
	40	1.2±0.2^{ABab}	1.2±0.2^{ABab}	1.3±0.05^{ABab}	1.0±0.1^{Ab}	1.5±0.2^{Aa}
			Non-essential amino acids			
Alanine	**10**	2.7±0.2	2.8±0.3	3.1±0.4	2.7±0.5	3.1±0.1
	20	3.2±0.4	3.2±0.4	3.3±0.5	3.0±0.2	3.4±0.2
	30	3.5±0.1	3.2±0.9	3.1±0.1	3.1±0.2	3.3±0.6
	40	2.7±0.2	2.8±0.3	3.1±0.4	2.7±0.5	3.1±0.1
Asparagine	**10**	nd	nd	nd	nd	nd
	20	nd	nd	nd	nd	nd
	30	nd	nd	nd	nd	nd
	40	nd	nd	nd	nd	nd
Aspartate	**10**	3.8±0.3^{ab}	4±0.2^{ab}	4.4±0.5^{a}	3.0±0.8^{Bb}	4.6±0.1^{a}

	20	4.7±0.9	4.7±0.1	4.7±0.7	4.1±0.2^{AB}	4.7±0.3
	30	4.9±0.2^{ab}	4.6±1.4^{ab}	4.6±0.5^{a}	4.8±0.3^{Ab}	4.8±1.0^{a}
	40	3.8±0.3^{ab}	4.0±0.2^{ab}	4.4±0.5^{a}	3.0±0.8^{Ab}	4.6±0.1^{a}
Glutamate	**10**	5.5±0.5	5.7±0.4	6.3±0.7	5.5±1.1	6.6±0.2
	20	6.9±1.3	6.9±0.1	6.8±1.0	6.1±0.2	6.9±0.5
	30	7.5±0.1	6.9±2.0	6.9±0.6	7.1±0.3	7.2±1.6
	40	5.5±0.5	5.7±0.4	6.3±0.7	5.5±1.1	6.6±0.2
Glycine	**10**	3.3±0.2	3.4±0.5	3.5±0.5	3.1±0.4	3.5±0.3
	20	3.4±0.03	4.0±0.3	4.1±0.8	3.7±0.3	4.2±0.2
	30	4.7±0.5	4.0±0.6	3.9±0.3	3.8±0.2	4.3±0.3
	40	3.3±0.2	3.4±0.5	3.5±0.5	3.1±0.4	3.5±0.3
Serine	**10**	1.9±0.2	2.0±0.1	2.2±0.2	1.9±0.3	2.2±0.1
	20	2.1±0.3	2.3±0.1	2.1±0.3	1.9±0.05	2.1±0.1
	30	2.1±0.04	2.0±0.6	1.9±0.2	2.0±0.1	2.0±0.3
	40	1.9±0.2	2±0.1	2.2±0.2	1.9±0.3	2.2±0.1
Conditionally essential AA						
Tyrosine	**10**	1.2±0.1	1.3±0.1^{B}	1.3±0.2	1.1±0.2	1.4±0.1
	20	1.5±0.3	1.9±0.5^{A}	1.5±0.2	1.3±0.1	1.5±0.1
	30	1.4±0.1	1.3±0.4^{A}	1.4±0.04	1.3±0.1	1.3±0.3
	40	1.2±0.1	1.3±0.1^{A}	1.3±0.2	1.1±0.2	1.4±0.1
Cysteine	**10**	nd	nd	0.1±0.01	0.1±0.01b	nd
	20	0.2±0.04	0.3±0.2	0.2±0.03^{Ba}	0.2±0.02	0.2±0.02
	30	nd	nd	nd	nd	nd
	40	nd	0.1±0.04^{B}	nd	nd	0.1±0.03^{B}
Glutamine	**10**	nd	nd	nd	nd	nd
	20	nd	nd	nd	nd	nd
	30	nd	nd	nd	nd	nd
	40	nd	nd	nd	nd	nd
Hydroxyproline	**10**	0.5±0.02^{C}	0.5±0.2	0.5±0.1^{B}	0.4±0.1	0.6±0.2^{B}
	20	0.7±0.1^{BC}	0.8±0.2	0.9±0.2^{A}	0.8±0.2	0.9±0.03^{AB}
	30	1.2±0.2^{A}	0.9±0.03	0.9±0.2^{AB}	0.9±0.1	1.2±0.4^{A}
	40	0.5±0.02^{AB}	0.5±0.2	0.5±0.1^{AB}	0.4±0.1	0.6±0.2^{A}
Proline	**10**	2.2±0.3^{B}	2.1±0.4	2.1±0.2	2.0±0.2	2.4±0.4
	20	2.4±0.1^{AB}	2.5±0.05	2.6±0.6	2.3±0.2	2.7±0.1
	30	3.0±0.03^{A}	2.7±0.4	2.8±0.5	2.6±0.4	2.6±0.2
	40	2.2±0.3^{AB}	2.1±0.4	2.1±0.2	2.0±0.2	2.4±0.4
Taurine	**10**	0.7±0.02 ^{A}	0.7±0.1^{A}	0.8±0.1^{A}	0.6±0.1	0.7±0.1
	20	0.7±0.2^{AB}	0.7±0.1^{C}	0.7±0.1^{AB}	0.6±0.1	0.7±0.1
	30	0.6±0.1^{AB}	0.4±0.1^{B}	0.5±0.04^{B}	0.5±0.03	0.5±0.1
	40	0.7±0.02^{B}	0.7±0.1^{BC}	0.8±0.1^{B}	0.6±0.1	0.7±0.1

Notes*:* Different letters in the same row indicate significant differences; lower case letters indicate differences among treatments; capital letters indicate differences among weeks. Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

The elemental content in carcass did not show significant differences among treatments, only in Mn where Q obtained the highest level for week 10, H for week 20, Q and H for week 30 and H for week 40 (Table XXI).

Table XXI. Macroelements (N, Ca, K, Mg, Na, Si, P, S) and microelements (B, Fe, Mn, Mo, Ni, Se, Zn) in carcass of *Oreochromis niloticus* culture Bioflocculation Technology, implementing different treatments: Q = chemotrophic, H = heterotrophic, M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805 during the initial time and at week 10.

Macroelements (g/L)	Week	N	P	K	Ca	Mg	S	Si	Na
Q	**10**	70.8±4.5	22.8±1.9	7.3±1.0	32.1±3.3	1.4±0.1	2.2±0.1	0.03±0.004	4.9±0.7
	20	69.9±3.7	18.2±1.4	4.1±0.7	27.8±1.2	1.1±0.04	1.9±0.1	0.01±0.001	2.5±0.6
	30	72.2±12.0	20.2±3.2	4.7±0.6	34.3±6.3	1.2±0.1	1.9±0.1	0.01±0.005	3.5±0.3
	40	65.8±8.1	17.5±3.9	4.6±0.3	24.2±7.2	1.1±0.2	1.7±0.1	0.03±0.005	2.8±0.4
H	**10**	69.8±8.2	20.1±0.9	8.0±2.7	29.2±1.8	1.3±0.1	1.9±0.2	0.05±0.05	6.0±2.4
	20	72.0±2.0	19.5±1.5	4.3±0.4	31.3±2.6	1.2±0.04	2.0±0.1	0.01±0.004	3.0±0.3
	30	80.6±7.2	22.9±5.7	4.5±0.4	40.9±12	1.2±0.3	1.8±0.1	0.01±0.004	3.3±0.3
	40	76.7±8.4	16.3±1.1	5.1±0.4	22.0±3.0	1.0±0.1	1.6±0.2	0.03±0.002	3.2±0.2
M	**10**	73.2±0.7	20.9±1.3	6.1±1.7	30.7±1.1	1.3±0.1	2.2±0.2	0.02±0.004	4.1±1.2
	20	72.7±0.7	17.3±0.2	4.0±0.7	27.4±1.0	1.0±0.05	1.9±0.1	0.01±0.002	2.7±0.4
	30	76.3±3.6	23.3±5.6	4.3±0.6	41.1±12.9	1.3±0.3	1.8±0.1	0.01±0.002	3.2±0.3
	40	65.5±11.4	24.1±6.3	4.6±0.5	36.5±10.9	1.4±0.3	1.5±0.2	0.04±0.004	3.2±0.2
CV	**10**	70.4±5.5	21.8±1.4	8.2±1.9	30.0±3.7	1.3±0.1[b]	2.0±0.2	0.03±0.01	5.6±1.5
	20	75.1±3.3	18.4±1.4	3.3±0.1	31.6±3.8	1.1±0.1	1.8±0.1	0.01±0.003	2.1±0.2
	30	65.5±2.4	19.4±1.7	4.4±0.5	33.3±4.0	1.1±0.1	1.8±0.1	0.01±0.003	3.4±0.8
	40	66.5±3.9	17.1±0.9	4.5±0.4	24.6±1.5	1.1±0.1	1.5±0.1	0.03±0.002	2.7±0.2
CS	**10**	73.5±6.5	20.5±1.1	6.5±1.1	29.7±1.5	1.3±0.004	2.1±0.03	0.02±0.001	4.3±1.1
	20	71.9±3.3	19.1±1.1	4.4±1.0	31.1±3.6	1.1±0.1	1.9±0.1	0.01±0.003	2.9±0.7
	30	74.8±8.9	20.1±1.8	4.8±0.6	33.5±4.1	1.2±0.2	1.8±0.1	0.01±0.003	3.4±0.2
	40	71.2±8.8	17.1±1.7	4.4±0.1	24.7±1.8	1.1±0.1	1.5±0.1	0.03±0.004	2.8±0.2

Microelements	B	Fe	Mn	Mo	Ni	Se	Zn

(mg/L)								
Q	**10**	23.8±0.8	171.6±16.3	10.1±4.0[a]	nd	1.5±1.0	12.0±11.0	80.2±3.9
	20	19.0±0.5	117.2±34.2	3.6±0.5[b]	0.4±0.6	0.8±1.4	30.9±3.5	56.0±3.5
	30	21.2±0.4	110.1±22.0	3.9±0.6[a]	1.1±1.9	6.1±7.3	23.2±5.2	56.6±7.6
	40	105.7±6.8	86.3±12.1	2.6±0.6[b]	1.3±1.0	2.3±0.5	10.2±5.8	43.6±7.7
H	**10**	36.9±31.8	260.4±150.8	7.7±0.5[ab]	7.2±7.7	6.2±7.7	4.9±2.7	72.3±5.0
	20	21.4±1.6	124.3±17.1	5.2±0.2[a]	nd	2.2±1.0	25.0±5.5	58.5±4.4
	30	20.8±0.8	115.6±23.6	4.2±0.1[a]	nd	2.7±1.6	23.5±13.1	61.5±0.4
	40	105.9±0.7	85.4±13.6	2.5±1.0[a]	nd	1.1±0.6	6.3±1.6	41.7±13.3
M	**10**	21.1±3.6	136.8±11.3	5.7±0.1[ab]	3.3±2.2	2.2±0.3	6.1±1.0	76.4±12.8
	20	19.7±0.9	147.9±50.5	3.3±0.4[b]	nd	1.3±2.2	22.3±14.5	52.7±6.5
	30	22.9±1.3	134.0±53.4	2.4±0.4[b]	nd	3.3±2.4	28.1±6.7	47.8±9.0
	40	109.5±3.6	87.1±12.4	3.0±0.2[b]	nd	1.7±0.2	8.7±6.0	37.1±3.1
CV	**10**	21.7±0.9	180.5±24.9	4.8±1.1[b]	2.1±0.3	2.0±0.7	12.5±8.8	68.2±3.0
	20	18.9±1.4	142.4±39.7	3.4±0.3[b]	2.7±4.8	1.4±1.1	18.1±2.2	59.3±2.1
	30	20.9±1.7	98.7±12.8	2.9±0.2[ab]	1.1±1.5	4.7±1.4	35.7±2.8	42.7±1.4
	40	109.9±3.7	87.9±12.1	2.5±0.3[b]	nd	2.4±1.4	6.4±5.7	40.3±5.8
CS	**20**	21.3±0.5	119.7±21.0	3.2±0.3[ab]	nd	1.9±0.7	24.5±9.4	58.4±6.0
	10	22.1±6.3	192.6±39.0	6.5±0.7[b]	nd	1.7±0.3	10.1±7.1	80.5±6.5
	30	20.5±1.6	119.6±34.4	3.3±1.0[ab]	2.2±3.8	6.6±4.0	19.9±1.9	47.2±11.3
	40	104.4±4.0	92.2±24.3	2.1±0.4[b]	1.7±1.7	2.5±0.2	8.9±4.7	35.7±6.0

Notes: Different letters in the same row indicate significant differences; lower case letters indicate differences among treatments; capital letters indicate differences among weeks. Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

For biofloc analyses, the highest moisture level was in M in week 10 with significant differences ($p < 0.05$, Table XXII), but no differences were observed in week 30 ($p > 0.05$). Q, M treatments obtained the greatest levels of crude protein and Q, M, CS crude lipid in week 10 ($p < 0.05$, Table XXII). In week 10, CS and M obtained the highest level of crude fiber and H the lowest level of protein and fiber and the greatest level of ash ($p < 0.05$, Table XXII). All the values for the proximal analyses decreased with time and did not show significant differences among treatments in week 30 ($p > 0.05$, Table XXII).

Table XXII. Proximal analyses of the Biofloc technology system during weeks 10 and 30 comparing the different treatments.

Proximal analyses (%)	Time	Q	H	M	CV	CS
Moisture	**10**	4.0 ± 0.01^{Be}	5.4 ± 0.02^{Bb}	6.1 ± 0.02^{Ba}	4.9 ± 0.02^{Bc}	4.6 ± 0.03^{Bd}
	30	8.4 ± 0.5^{A}	9.1 ± 0.7^{A}	8.6 ± 0.6^{A}	8.7 ± 1.0^{A}	9.0 ± 1.2^{A}
Crude	**10**	42.6 ± 0.2^{Aa}	34.7 ± 0.2^{Ad}	39.3 ± 0.4^{Ab}	38.2 ± 0.01^{Ac}	37.7 ± 0.2^{Ac}
protein	**30**	26.7 ± 0.7^{B}	26.4 ± 1.3^{B}	24.0 ± 3.4^{B}	27.6 ± 5.8^{B}	25.3 ± 5.1^{B}
Crude lipid	**10**	1.1 ± 0.01^{Aa}	0.5 ± 0.03^{Ac}	0.7 ± 0.01^{Ab}	0.7 ± 0.01^{Ab}	0.4 ± 0.01^{Ad}
	30	0.1 ± 0.04^{B}	0.1 ± 0.06^{B}	0.1 ± 0.02^{B}	0.11 ± 0.06^{B}	0.1 ± 0.01^{B}
Crude fiber	**10**	2.3 ± 0.1^{Bc}	2.0 ± 0.03^{Bd}	3.3 ± 0.03^{Bb}	4.9 ± 0.0^{Ba}	2.4 ± 0.0^{Bc}
	30	11.0 ± 2.0^{A}	11.9 ± 0.8^{A}	13.1 ± 1.5^{A}	10.4 ± 2.4^{A}	12.5 ± 1.0^{A}
Ash	**10**	17.8 ± 0.2^{Bd}	26.7 ± 0.3^{Aa}	24.9 ± 0.02^{Abc}	23.9 ± 0.3^{Bc}	25.2 ± 0.7^{Ab}
	30	19.6 ± 0.8^{A}	22.1 ± 2.4^{B}	20.2 ± 2.5^{B}	21.1 ± 2.5^{A}	21.3 ± 2.6^{B}

Notes*:* Different letters in the same row indicate significant differences; lower case letters indicate differences among treatments; capital letters indicate differences among weeks. Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

The highest content of amino acid in biofloc was obtained in the nursery phase. The highest content of isoleucine, lysine and valine were found in M, Q, CS and tyrosine obtained the highest level in M, Q, CV and CS. In the grow-out phase, the highest level of histidine was found in M, Q, H and CV. The other amino acids did not show significant differences ($p > 0.05$, Table XXVI).

In week 10, the highest level of isoleucine, valine and aspartate was found in Q, H, M, CS. In week 20, the amino acid did not show significant differences ($p > 0.05$). In week 30 and 40, valine and aspartate were found in Q, H, M, CS (Table XXIII).

Table XXIII Floc amino acid analyses (essential, non-essential, conditionally essential) during week 10 and 30 comparing the different treatments.

Amino acid (g/100 g)	Time	Q	H	M	CV	CS
			Essential AA			
Arginine	**10**	1.2±0.3^{A}	e	1.2±0.3^{A}	1.0±0.07^{A}	1.2±0.07
	30	0.7±0.09^{B}	0.9±0.3	0.8±0.09^{B}	0.6±0.03^{B}	0.9±0.05^{B}
Histidine	**10**	0.5±0.1^{A}	e	0.6±0.2^{A}	0.5±0.02^{A}	0.5±0.06^{A}
	30	0.3±0.03^{Bab}	0.3±0.08^{a}	0.4±0.03^{Bab}	0.2±0.007^{Bab}	0.3±0.009^{Bb}
Isoleucine	**10**	0.8±0.1^{Aab}	e	1.0±0.08^{Aa}	0.7±0.08^{Ab}	0.8±0.03^{Aab}
	30	0.5±0.1^{B}	0.6±0.4	0.5±0.03^{B}	0.4±0.06^{B}	0.5±0.06^{B}
Leucine	**10**	1.8±0.3^{A}	e	2.3±0.3^{A}	1.7±0.06^{A}	2.0±0.1^{A}
	30	1.1±0.1^{B}	1.4±0.4	1.2±0.1^{B}	1.0±0.07^{B}	1.4±0.08^{B}
Lysine	**10**	1.2±0.2^{Aab}	e	1.6±0.2^{Aa}	1.1±0.1^{Ab}	1.3±0.1^{Aab}
	30	0.7±0.07^{B}	0.9±0.3	0.8±0.09^{B}	0.6±0.03^{B}	0.9±0.04^{B}
Methionine	**10**	0.4±0.08^{A}	e	0.4±0.1^{A}	0.3±0.02^{A}	0.4±0.02^{A}
	30	0.2±0.06^{B}	0.3±0.1	0.3±0.03^{B}	0.2±0.03^{B}	0.3±0.007^{B}
Phenylalanine	**10**	1.1±0.2^{A}	e	1.4±0.2^{A}	1.1±0.1^{A}	1.3±0.07^{A}
	30	0.7±0.07^{B}	0.7±0.2	0.8±0.09^{B}	0.6±0.04^{B}	0.8±0.05^{B}
Threonine	**10**	1.3±0.2^{A}	e	1.4±0.3^{A}	1.1±0.08^{A}	1.3±0.05^{A}
	30	0.8±0.09^{B}	1.0±0.4	0.9±0.09^{B}	0.8±0.04^{B}	1.0±0.05^{B}
Tryptophan	**10**	nd	nd	nd	nd	nd
	30	nd	nd	nd	nd	nd
Valine	**10**	1.1±0.1^{Aab}	e	1.4±0.1^{Aa}	1.1±0.1^{Ab}	1.1±0.1^{Aab}
	30	0.7±0.08^{B}	0.9±0.4	0.7±0.06^{B}	0.6±0.04^{B}	0.8±0.06^{B}
			Nonessential AA			
Alanine	**10**	2.0±0.3^{A}	e	2.4±0.3^{A}	1.9±0.1^{A}	2.0±0.1^{A}
	30	1.1±0.2^{B}	1.5±0.7	1.3±0.2^{B}	1.0±0.05^{B}	1.5±0.04^{B}
Asparagine	**10**	nd	nd	nd	nd	nd
	30	nd	nd	nd	nd	nd
Aspartate	**10**	2.2±0.7^{A}	e	2.6±0.1^{A}	2.7±0.2^{A}	2.9±0.1^{A}
	30	1.5±0.2^{B}	1.9±0.7	1.7±0.2^{B}	1.4±0.08^{B}	2.0±0.09^{B}
Glutamate	**10**	3.0±0.5^{A}	e	3.5±0.6^{A}	2.8±0.2^{A}	3.0±0.2^{A}
	30	1.7±0.3^{B}	2.2±0.9	1.8±0.2^{B}	1.5±0.08^{B}	2.1±0.08^{B}
Glycine	**10**	2.0±0.4^{A}	e	2.1±0.3^{A}	1.7±0.1^{A}	1.7±0.05^{A}
	30	1.0±0.1^{B}	1.2±0.4	1.1±0.1^{B}	1.0±0.07^{B}	1.3±0.03^{B}
Serine	**10**	1.3±0.2^{A}	e	1.5±0.4^{A}	1.2±0.2^{A}	1.4±0.1^{A}
	30	0.8±0.09^{B}	1.0±0.3	0.9±0.1^{B}	0.8±0.04^{B}	1.0±0.05^{B}
Tyrosine	**10**	1.0±0.04^{Aab}	e	1.1±0.05^{Aa}	0.8±0.2^{Ab}	0.9±0.1^{ab}
	30	0.6±0.09^{B}	0.8±0.3	0.6±0.08^{B}	0.6±0.02^{B}	0.8±0.06
			Conditionally essential AA			
Cysteine	**10**	0.3±0.06^{A}	e	0.3±0.06^{A}	0.3±0.003^{A}	0.3±0.04^{A}
	30	0.2±0.01^{B}	0.2±0.03	0.2±0.02^{B}	0.2±0.01^{B}	0.2±0.02^{B}
Glutamine	**10**	nd	nd	nd	nd	nd
	30	nd	nd	nd	nd	nd
Hydroxyprolin e	**10**	0.5±0.07	e	0.4±0.1	0.2±0.08	nd
	30	nd	nd	nd	nd	0.2±0.005
Proline	**10**	1.5±0.3^{A}	e	1.7±0.3^{A}	1.3±0.1^{A}	1.4±0.1
	30	0.9±0.1^{B}	1.0±0.3^{B}	1.0±0.08^{B}	0.9±0.06^{B}	1.1±0.003
Taurine	**10**	0.02±0.003	e	0.04±0.004	0.04±0.03	0.03±0.005
	30	nd	nd	nd	nd	nd

Notes: Different letters in the same row indicate significant differences; lower case letters indicate differences among treatments; capital letters indicate differences among weeks. Treatments: Q = chemotrophic, H = heterotrophic, photoautotrophic: M = *Chlorella* sp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805.

The relationship lipids and protein in the different species of *Oreochromis niloticus* indicated that *O. niloticus x O. mossambicus* got the best relationship, following for *O. niloticus x O. aureus*. The results found in this experiment are based on the definite values *for O. niloticus* in other research studies (Fig. 35).

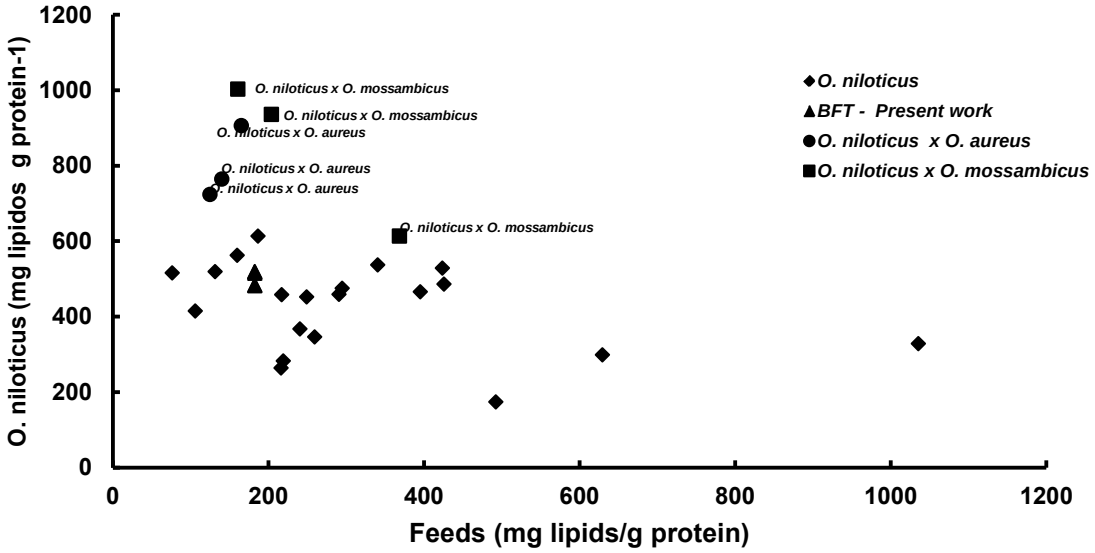

Figure 35 Relationship between lipids and proteins in the *Oreochromis* spp. carcass and feed. Circles are *Oreochromis niloticus x O. aureus* (mg lipids/g protein) (Lin *et al*., 1997), squares are *O. niloticus x O. mossambicus* (mg lipids/g protein) (De Silva, Gunasekera, and Shim, 1991), rhombuses are *O. niloticus* (Abdel-Tawwab, 2012; Cavalheiro *et al*., 2007; Diop *et al*., 2013; Dongmeza *et al*., 2006; Ergün *et al*., 2009; Gaber, 2006; Lara-Flores *et al*., 1995; Twibell and Brown, 1998; Yangthong *et al*., 2014), and triangles represents the data obtained in this research reared under: chemotrophic, heterotrophic, and photoautotrophic Bioflocculation Technology conditions.

The correlation of the amino acid content in *O. niloticus* and feed showed a deficiency of methionine, threonine and lysine. The correlation with *O. niloticus* and floc showed a deficiency of taurine, lysine and arginine (Fig. 36a).

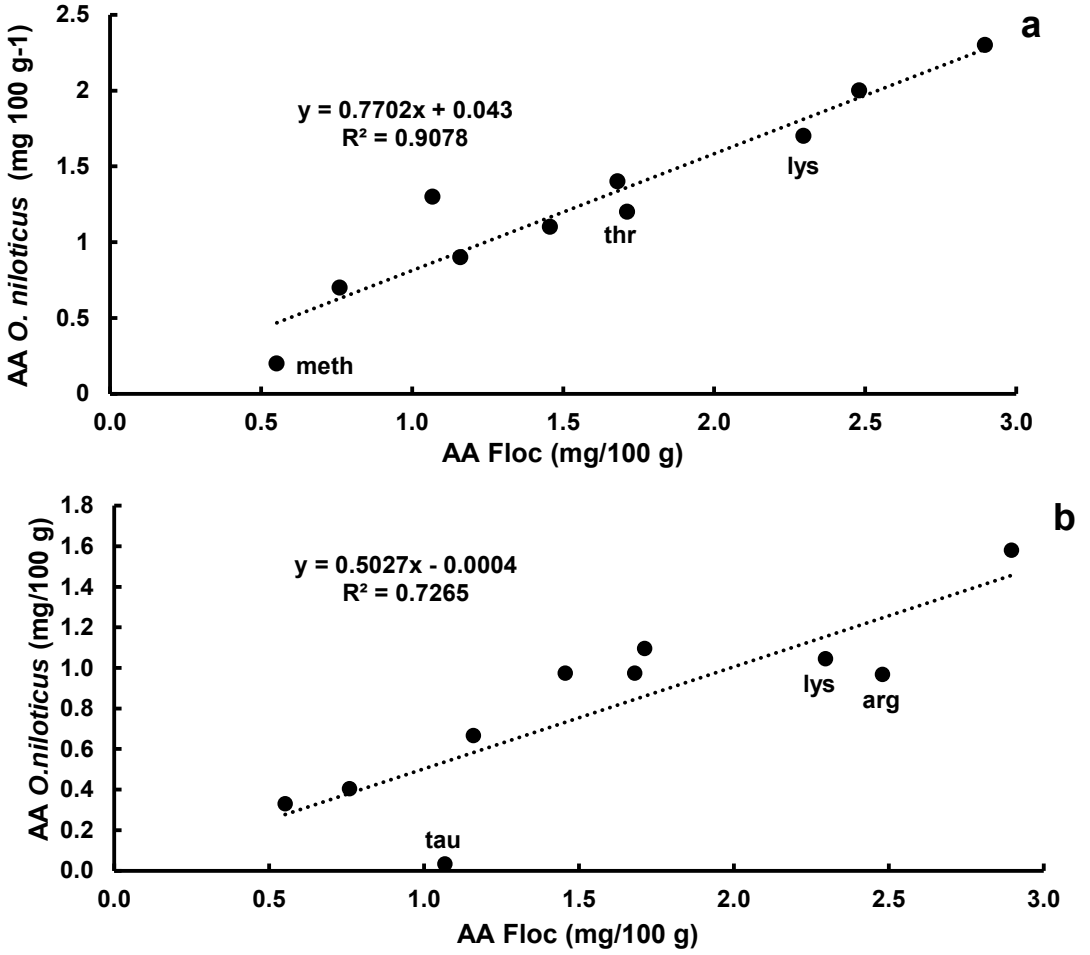

Figure 36. Relationship between *Oreochromis niloticus* and amino acid in feed and floc. (a) Correlation of the amino acid in *O. niloticus* carcass and amino acid in feed (mg/100 g), b) Correlation of the amino acid in *O. niloticus* carcass and amino acid in feed (mg/100), meth = methionine, thr = treonine, lys = lisine, tau = taurine, arg = arginine.

EXPERIMENT III- Mineralization

7.3 Elemental analysis of particulate fraction in RAS and BFT.

Nitrogen was maintained without significant differences ($p > 0.05$) throughout the grow-out period. P, K, B, Fe, Mn, Mo, Zn, Cu, Ni increased throughout the experiment. Ca declined as the experiment progressed in all treatments (Table XXIV).

7.4 Aerobic mineralization in RAS

In liquid fraction mineralization (MFL), N in the three treatments increased in the 100-day period and decreased in the 120-day period; the other elements (macro and micro) accumulated over time (Table XXVI).

The highest amount of P was found in the particulate fraction mineralization (MFP). Ca, Mg, K, Na and B were higher in MFL. Microelements were found in greater numbers in MFP (Table XXVII), DPI 1.4 regained a greater amount of N, P and Mn at 100 days, showing significant differences between treatments ($p > 0.05$). The other elements had no difference between treatments in any period (65, 100 and 120 days).

The description of the three macro elements N, P, K indicates that N, P and K increased from day 65 to 100. N and P decreased from day 100 to 120; K kept a constant increase (Fig. 37).

Table XXIV Macroelements (N, Ca, K, Mg, Na, Si, P, S) and microelements (B, Fe, Mn, Mo, Ni, Se, Zn) in the liquid fraction of treatments: Q = chemotrophic, H = heterotrophic, M = *Chlorella* spp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805 during the initial time and at week 10.

Macroelements (mg/L)	Week	Ca	K	Mg	N	Na	P	S	Si
Hoagland[1]	-	179-224	230-232	49	220-242	-	24-31	113	-
Q	0	59.5±1.1^{b}	0.5±0.2^{c}	24.2±0.3^{c}	6.0 ± 0.7	61.7±3.3^{c}	nd	2.5±0.3^{c}	30.3±2.3^{a}
	10	54.5±19.7	2.2±1.4^{b}	16.0±8.6^{ab}	8.5±1.6^{b}	44.7±23.7^{b}	3.0±0.3^{b}	3.8±1.3^{b}	31.9±0.4^{a}
	20	131.0±8.9	16.7±4.1	72.4±16.3	61.4±2.9	244.6±69.4	17.8±2.3	14.6±3.6	37.6±2.5
H	0	58.8 ±1.1^{b}	0.7^{c}	25.6±1.6^{c}	5.60 ±0.3	63.3±4.1^{c}	nd	2.5±0.2^{c}	30.4±1.0^{a}
	10	51.5±13.7	0.6±0.1^{b}	13.5±3.2^{b}	12.2 ±1.4^{ab}	33.8±6.4^{b}	2.2±0.7^{b}	2.6±0.8^{b}	28.1±5.5^{a}
	20	127.0±3.9	15.1±1.1	71.3±5.5	40.3 ±5.8	241.5±20.8	12.0±0.9	13.7±1.2	37.0±2.1
M	0	71.8±12.1^{b}	11.0±3.5^{b}	57.7±9.0^{b}	6.8±0.80	271.3±39.6^{b}	1.3±0.6	12.5±2.0^{b}	21.46±3.3^{b}
	10	83.6±4.0	11.0±4.4^{ab}	42.4±14.3^{ab}	15.1±3.4^{ab}	186.1±60.0^{a}	11.9±2.1^{a}	11.8±0.9^{a}	8.13±7.0^{b}
	20	148.0±10.2	21.4±2.1	58.2±1.9	81.8±7.2	153.8±3.9	20.5±4.6	12.6±0.8	34.4±5.2
CV	0	100.6±3.3^{a}	16.9±1.8^{a}	100.9±0.8^{a}	7.0±1.06	434.4±55.5^{a}	nd	19.5±0.5^{a}	3.4±1.0^{c}
	10	90.2±22.4	11.1±6.2^{ab}	47.3±22.7^{ab}	19.8 ±8.4^{ab}	185.3±72.8^{a}	13.5±0.8^{a}	13.3±2.8^{a}	3.0±0.7^{b}
	20	140.0±4.0	19.8±2.5	56.7±3.3	72.8±9.0	143.2±12.0	15.51±2.2	12.8±1.5	29.7±1.3
CS	0	104.0±2.9^{a}	19.4±1.0^{a}	104.3±2.2^{a}	7.6±1.05	453.8±25.1^{a}	1.7±0.5	21.0±0.5^{a}	7.0±0.9^{c}
	10	94.3±15.6	13.9±5.2^{a}	53.5±17.4^{a}	24.1±6.4^{a}	211.2±63.9^{a}	12.2±0.6^{a}	13.8±2.0^{a}	3.0±0.2^{b}
	20	147.3±1.5	21.2±1.8	58.8±1.6	92.3 ±2.9	146.2±10.8	22.0±3.4	13.2±0.4	30.5±5.5
Microelements (mg/L)	**Week**	**B**	**Fe**	**Mn**	**Mo**	**Ni**	**Cu**	**Zn**	
Hoagland	-	0.45	7	0.05-0.5	0.0106	-	-	0.48	
Q	0	0.1±0.02^{c}	nd	nd	0.02±0.01^{b}	0.01±0.01	nd	nd	
	10	0.1±0.04^{bc}	nd	nd	nd	nd	nd	nd	
	20	0.3±0.05	0.004	0.06±0.02	0.004	0.0025±0.001	nd	0.077±0.048	
H	0	0.1±0.001^{c}	nd	nd	0.04^{b}	0.01±0.001	nd	nd	
	10	0.1±0.02^{c}	nd	nd	0.03±0.02^{ab}	nd	nd	nd	
	20	0.3±0.02	0.05±0.03	0.06±0.001	0.055±0.003	0.006	nd	0.087±0.098	
M	0	0.4±0.07^{b}	nd	nd	0.1±0.04^{a}	0.02±0.01	0.01±0.008 ^{a}	0.09±0.02	
	10	0.3±0.06^{abc}	0.006	nd	0.02±0.0^{b}	nd	0.02±0.01	0.1±0.02^{a}	
	20	0.4±0.01	0.03±0.03	0.1±0.03	0.1±0.05	0.03±0.01	0.04±0.01^{ab}	0.2±0.1	
CV	0	0.5±0.007^{ab}	nd	nd	0.08±0.05^{ab}	0.01±0.01	0.02±0.01^{b}	0.02±0.03	

	10	0.3±0.107^{ab}	nd	0.01	0.08±0.03^{a}	nd	0.003±0.002	0.1±0.05^{a}
	20	0.4±0.09	0.02±0.01	0.1±0.06	0.03	0.02±0.02	0.001±0.001^{b}	0.1±0.07
CS	0	0.5±0.01^{a}	nd	nd	0.1±0.03^{ab}	0.02±0.01	0.01±0.006 ^{ab}	0.002
	10	0.3±0.08^{a}	nd	0.004	0.1±0.01^{ab}	nd	0.003±0.001	0.1±0.03^{ab}
	20	0.4±0.01	0.03±0.01	0.2±0.03	0.1±0.01	0.01±0.001	0.006±0.003^{a}	0.2±0.09

Notes: nd= non-detected, - = not present in the feed formula. [1]Hoagland is a commercial hydroponic formula (Hoagland and Arnon, 1950). Reference data (Jones, 2004). Each value is represented by mean ± SD. Lower case letters indicate differences among treatments. Values in the same row with different superscripts are significantly different ($p < 0.05$).

Table XXV. Elemental analysis of the particulate fraction recovered in Recirculating Aquaculture System for 120 days, implementing DPI 1.3, 1.2, 1.0.

		15 days			45 days			65 days			105 days			120 days		
	E	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**
Macronutrients g/L	**N**	3.4±0.1	3.3±0.6	3.2±1.0	7.0±0.4	6.3±0.4	6.0±0.4	5.3±1.7	5.8±1.4	5.7±1.5	4.3±0.4	4.5±0.4	4.1±0.3	4.2±.3	4.2±0.4	4.4±0.5
	P	4.6±1.0	6.7±5.8	3.2±1.7	13.5±1.5	12.7±2.2	11.6±3.2	17.7±4.1	16.2±3.3	18.8±4.0	21.7±4.5	22.3±1.5	19.1±6.2	20.4±1.2	21.4±2.3	20.0±1.6
	K	0.8±0.1	1.2±0.3	0.9±0.5	0.8±.2.0	0.8±0.2	0.8±0.2	2.4±1.8	1.1±0.4[b]	1.2±0.4[b]	2.4±0.3[a]	2.5±1.0	2.0±0.5	4.5±0.5	4.7±0.8	4.3±0.4
	Ca	103.0±29.0	121.7±40.0	104.6±68.9	34.9±1.0	35.3±4.1	34.2±2.8	40.4±2.4	37.9±1.0	41.6±6.1	45.3±6.1	47.5±2.3	44.5±4.3	45.7±1.6	47.7±6.2	49.1±6.2
	Mg	3.6±0.7	4.4±1.9	3.5±1.7	2.7±0.1	2.6±0.2	2.9±0.7	4.4±2.3	3.1±0.3	3.4±0.6	4.4±0.2	4.6±1.0	4.4±0.8	5.4±0.5	6.0±1.0	5.8±0.7
	S	1.8±0.4	2.3±1.8	1.5±0.8	3.2±0.1	2.9±0.5	3.1±0.2	2.6±0.6	2.4±0.6	2.5±0.4	2.0±0.2	2.1±0.2	2.3±0.5	2.2±0.1	2.3±0.2	2.2±0.1
	Na	2.2±0.4	3.3±1.2	2.3±0.8	2.9±0.3	3.1±0.8	4.5±3.5	5.2±1.4	4.3±1.6	5.5±1.8	8.5±2.8	9.4±5.1	9.6±4.3	14.7±2.0	16.7±3.7	16.8±3.4
Micronutrients mg/l	**B**	27.8±8.5	32.4±11.1	26.8±14.0	41.7±2.9	42.2±6.3	42.7±6.0	50.7±18.6	41.0±4.0	50.1±5.3	48.4±1.9	50.3±7.4	52.7±4.1	53.6±2.1	56.6±4.6	56.7±4.4
	Fe	1587.8±271.8	1482±542.6	1427.9±681.3	1370.5±659.0	1386.7±733.1	1601.7±409.2	1020.6±385.6	1390.7±1051.7	1593.4±508.7	1152.5±572.7	1326.9±395.7	1397.3±658.1	609.2±108.1	999.4±322.1	720.8±280.1
	Mn	77.4±19.9	101.5±89.6	65.3±35.5	220.4±27.6	235.6±120.1	195.7±41.5	193.8±28.7	184.8±25.3	186.9±20.0	207.0±50.6	202.6±9.2	169±30.1	168.9±31.0	177.6±32.4	167.5±26.4
	Mo	2.1	4.8	3.3±3.2	6.4	4.6	3.2	6.5	1.0±0.8	6.4±2.7	0.7	5.3±1.2	2.5±1.9	2.6±2.3	nd	nd
	Zn	630.6±132.3	724.6±391.4	610.7±304.8	786.5±63.6	733.0±89.2	839.0±35.9	865.3±92.5	950.4±63.1	1016.0±219.1	1116.5±198.2	1180.4±44.3	1120.1±141.9	991.9±93.9	1113.9±61.8	1010.3±63.9
	Cu	40.0±9.4	47.0±32.6	33.8±17.8	53.2±3.5	48.9±7.8	56.2±2.4	69.6±12.9	73.7±12.1	81.0±26.9	107.2±15.9	108.9±5.1	107.6±12.8	108.1±14.9	112.5±14.9	109.3±12.6
**	**Co**	7.9±3.5	9.3±4.3	9.6±7.1	5.9±2.6	6.7±2.7	9.8±4.1	3.9±1.3	5.5±2.9	4.7±2.1	4.1±2.9	4.2±1.8	7.2±5.0	2.5±0.9	3.1±1.5	3.2±2.5
	Ni	16.3±3.7	13.6±3.7	9.0±2.1	13.1±4.3	13.5±1.4	18.0±8.2	14.8±7.9	15.9±3.1	16.5±1.8	15.3±5.0	15.0±2.5	22.3±13.9	12.3±1.2	12.6±1.0	13.9±1.1

Notes*:* Each value represents the average value of SD. Values on the same line with different letter indicate a significant difference ($p < 0.05$). Treatments: Q = chemotrophic, H = heterotrophic, M = photoautotrophics: *Chlorella* spp., CV = *C. sorokiniana*-2714, and CS = *C. sorokiniana*-2805 during the maternity and fattening stages.

Table XXVI. Macroelements (N, Ca, K, Mg, Na, Si, P, S) and microelements (B, Fe, Mn, Mo, Ni, Se, Zn) contained in the particulate fraction obtained in Bioflocculation Technology.

	70 days					140 days				
E	**Q**	**H**	**M**	**CV**	**CS**	**Q**	**H**	**M**	**CV**	**CS**
N	60.6±5.2^{a}	45.3±2.4^{ab}	48.3±8.3^{ab}	52.4±9.0^{ab}	42.1±4.1^{b}	25.0±3.7	28.1±8.8	30.3±2.1	24.2±1.8	23.1±1.3
P	9.0±1.7	8.0±1.6	6.3±4.5	19.9±11.3	8.3±1.5	7.6±1.4^{b}	7.4±1.2^{b}	8.1±0.8^{b}	7.7±0.7^{b}	11.6±1.1^{a}
K	2.5±0.2^{ab}	3.2±0.7^{a}	2.5±0.3^{ab}	1.6±0.4^{b}	2.8±0.9^{ab}	1.1±0.1^{ab}	1.8±1.8^{b}	1.6±0.5^{a}	1.1±0.03^{ab}	1.7±0.3^{a}
Ca	18.1±4.9	17.8±2.1	20.8±3.2	40.1±24.9	18.4±2.2	17.2±3.6^{b}	15.7±0.3^{b}	19.1±1.5^{b}	17.8±1.9^{b}	25.7±3.3^{a}
Mg	6.3±1.6^{ab}	7.9±1.6^{a}	5.4±1.2^{ab}	4.0±0.7^{b}	5.4±0.9^{ab}	2.3±0.1	3.2±1.9	3.4±1.0	2.6±0.1	3.6±0.6
S	3.6±0.1^{a}	3.1±0.5^{ab}	3.8±0.8^{a}	2.2±0.5^{b}	3.1±0.4^{ab}	1.6±0.04	2.0±0.7	1.9±0.3	1.7±0.03	1.9±0.1
Na	14.5±3.5^{ab}	19.5±1.9^{a}	8.6±2.2^{bc}	5.0±1.3^{c}	10.2±1.8^{bc}	2.0±0.3	2.8±2.3	3.3±1.0	2.3±0.1	3.5±0.5
B	43.9±6.8^{ab}	49±1.4^{ab}	54.1±9.8^{a}	35.6±3.4^{b}	51.7±8.8^{ab}	29.6±3.9	34.9±6.5	41.8±9.1	34.4±1.1	42.7±5.3
Fe	2294.8±265.7	2181.5±237.3	1543.8±1344.5	2774.5±715.5	1651.3±1418.5	2479.3±232.7	2393.3±459.5	2572.5±213.8	2347.7±255.9	2694.0±155.9
Mn	41.2±7.7	42.3±3.8	39.5±34.7	103.4±56.0	36.4±31.2	66.1±14.0^{b}	52.2±7.4^{b}	68.9±2.7^{b}	72.0±6.6^{b}	108.8±22.1^{a}
Mo	3.9±4.5	5.6±5.4	9.4±13.2	15.3±2.7	10.4±9.1	6.9±7.9^{b}	5.9±1.1^{b}	26.4±3.8^{a}	25.2±4.7^{a}	12.5±2.2^{b}
Zn	267.6±33.0^{ab}	188.2±17.0^{b}	294.6±274.6^{ab}	590.6±262.7^{a}	223.8±192.8^{ab}	448.2±184.7	375.8±112.3	412.2±21.3	506.6±19.9	626.0±105.0
Cu	70.1±5.5^{bc}	54.5±4.5^{c}	119.2±2.8^{a}	90.5±13.8^{ab}	109.3±20.1^{a}	67.8±18.1^{b}	64.3±9.0^{b}	90.3±11.1^{ab}	94.6±1.3^{ab}	102.6±11.5^{a}
Co	1.0±0.6	1.2±0.6	1.0±0.8	2.2±0.8	1.1±0.9	1.0±0.4	0.7±0.6	1.4±0.8	0.9±0.6	1.4±0.3
Ni	8.0±1.9	8.0±1.6	5.3±4.5	11.1±4.1	5.4±4.8	10.6±2.6	10.5±0.8	11.9±2.8	11.7±4.9	13.1±0.6

Notes: Each value represents the average value of SD. Values on the same line with different letter indicate a significant difference ($p < 0.05$). Treatments: Q = chemotrophic, H = heterotrophic, M = photoautotrophics: *Chlorella* spp., CV = *C. sorokiniana*-2714, and CS = *C. sorokiniana*-2805 during the maternity and fattening stages.

Table XXVII. Elemental analysis of the liquid fraction obtained from aerobic mineralizers in Recirculating Aquaculture System.

E	Element	65 days			100 days			120 days		
		DPI 1.4	DPI 1.2	DPI 1.0	DPI 1.4	DPI 1.2	DPI 1.0	DPI 1.4	DPI 1.2	DPI 1.0
Macronutrients (g/L)	**N**	20.5±4.3	19.7±9.7	16.5±5.2	306.5±67.9[a]	281.0±63.9[ab]	177.1±37.5[b]	213.6±93.3	161.3±126.1	179.0±46.7
	P	0.3±0.2	0.07±0.05	nd	67.8±10.4[a]	59.4±20.9[ab]	39.5±6.5[b]	94.5±59.5	90.8±55.9	102.8±8.6
	K	6.0±0.9	5.9±0.6	6.0±1.4	66.2±12.0	68.2±10.0	49.4±9.6	107.0±15.5	102.4±28.1	85.0±11.9
	Ca	83.7±10.1	77.5±3.4	81.8±12.2	575.3±97.4	562.4±95.3	464.0±21.0	656.7±142.7	689.1±262.6	711.3±67.6
	Mg	45.6±4.8	44.5±2.0	44.8±7.1	152.7±43.1	152.1±21.2	130.4±24.4	188.6±26.6	193.9±62.9	182.9±16.3
	S	1.1±0.8	0.5±0.4	1.5±0.2	36.2±5.3	33.5±2.7	29.9±3.1	40.7±2.9	38.8±10.3	33.1±3.8
	Na	100.5±23.3	100.2±14.0	118.6±26.1	410.4±133.8	419.2±54.5	371.9±131.4	517.5±40.6	545.1±151.9	506.7±104.5
Micronutrients (mg/L)	**B**	0.2±0.04	0.2±0.01	0.3±0.1	0.9±0.3	0.8±0.1	0.7±0.2	1.2±0.2	1.2±0.3	1.1±0.2
	Fe	nd	nd	nd	0.3±0.1	0.3±0.03	0.2±0.03	0.2±0.1	0.4±0.2	0.2±0.04
	Mn	nd	nd	nd	0.9±0.3[a]	0.8±0.2[ab]	0.4±0.1[b]	1.1±0.8	0.9±0.7	1.3±0.3
	Mo	nd	0.1±0.03	0.04±0.02	0.1±0.1	0.05±0.02	0.1±0.04	0.1±0.001	0.1±0.1	0.1±1.1
	Zn	0.04±0.03	0.1±0.02	0.1±0.1	0.3±0.1	0.4±0.4	0.2±0.03	1.5±1.1	1.4±1.0	2.0±1.1
	Cu	nd	nd	nd	0.01±0.003	0.02±0.001	0.02±0.01	0.03±0.003	0.04±0.02	0.04±0.004
	Co	0.005±0.002	0.02±0.001	0.01±0.001	0.02±0.004	0.02±0.001	0.03±0.02	0.02±0.01	0.02±0.01	0.02±0.02
	Ni	0.002±0.01	nd	nd	0.02±0.001	0.03±0.02	0.02±0.01	0.05±0.01	0.03±0.02	0.1±0.007

Notes: Each value represents the average value of SD. Values on the same line with different letter indicate a significant difference ($p < 0.05$). Days 45, 75 and 105 indicate the exposure time that the particulate fraction was in the mineralization tanks

Table XXVIII. Elemental analysis obtained from the particulate fraction from the aerobic mineralizers in Recirculating Aquaculture System.

		65 days			100 days			120 days		
	Element	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**	**DPI 1.4**	**DPI 1.2**	**DPI 1.0**
Macronutrients (g/L)	**N**	6.0±1.0	6.8±1.9	4.6±0.7	4.9±0.5	5.1±0.5	5.1±0.4	4.1±1.1	5.1±0.7	4.2±0.8
	P	23.9±66.6	21.4±7.1	21.7±2.8	14.7±1.3	14.3±1.5	18.6±7.3	18.3±3.3	17.5±2.8	17.7±5.2
	K	0.9±1.9	0.6±0.3	0.8±0.1	1.7±0.2	1.9±0.2	2.1±0.4	2.0±0.2	2.0±0.2	1.9±0.3
	Ca	53.9±133.4	48.0±24.8	78.9±17.4	26.3±2.8^{b}	29.8±5.2^{ab}	36.2±6.1^{a}	31.6±4.8	29.5±7.4	30.9±10.6
	Mg	2.7±3.2	2.2±0.9	3.1±0.5	3.4±0.4	3.8±0.4	4.5±1.0	3.5±0.4	3.5±0.4	3.2±0.3
	S	3.0±3.7	2.4±1.2	2.5±0.3	2.9±0.1	2.8±0.2	2.6±0.7	3.3±0.4	3.0±0.2	3.0±0.2
	Na	4.3±12.7	3.3±1.4	4.0±1.5	8.4±1.2	10.4±1.0	12.2±5.1	9.1±1.2	8.9±1.4	7.9±1.2
Micronutrients (mg/L)	**B**	0.04±0.03	0.03±0.01	0.03±0.002	0.05±0.01	0.05±0.001	0.05±0.01	0.04±0.07	0.05±0.04	0.01±0.003
	Fe	2.5±1.2	1.9±0.7	2.6±0.2	2.1±0.2	2.0±0.3	1.6±1.0	2.0±0.1	2.1±0.3	2.5±0.7
	Mn	0.2±0.04^{a}	0.1±0.06^{b}	0.2±0.01^{ab}	0.1±0.01	0.1±0.03	0.2±0.04	0.5±0.01	0.1±0.01	0.2±0.04
	Mo	nd	nd	0.005±0.003	0.002±0.001	nd	0.002±0.001	0.003±0.0001^{b}	0.004±0.004^{a}	0.01±0.004^{b}
	Zn	3.0±3.8	2.4±1.2	2.5±0.3	2.8±0.1	2.8±0.2	2.6±0.7	3.2±0.3	3.0±0.2	3.0±0.2
	Cu	59.6±8.5	41.9±17.9	53.5±11.1	106.7±4.7	105.2±4.1	107.6±12.7	160.4±25.2	151.5±13.7	165.3±34
*	**Co**	7.4±0.3	5.9±2.5	10.9±8.3	8.0±2.7	7.3±0.7	8.5±1.1	9.6±2.3	8.5±1.2	11.2±7.2
*	**Ni**	12.2±2.0	9.9±2.9	12.9±3.1	17±3.8	16.2±1.8	18.4±4.4	22.0±5.8	17.3±4.4	17.9±3.6

Notes: N, Mn, Mo, Zn, Cu, Co, Ni are in mg/L, P, K, Ca, Mg, S, Na, B, Fe are in g/L. Days 45, 75 and 105 = indicate the exposure time that the particulate fraction was in the mineralization tanks.

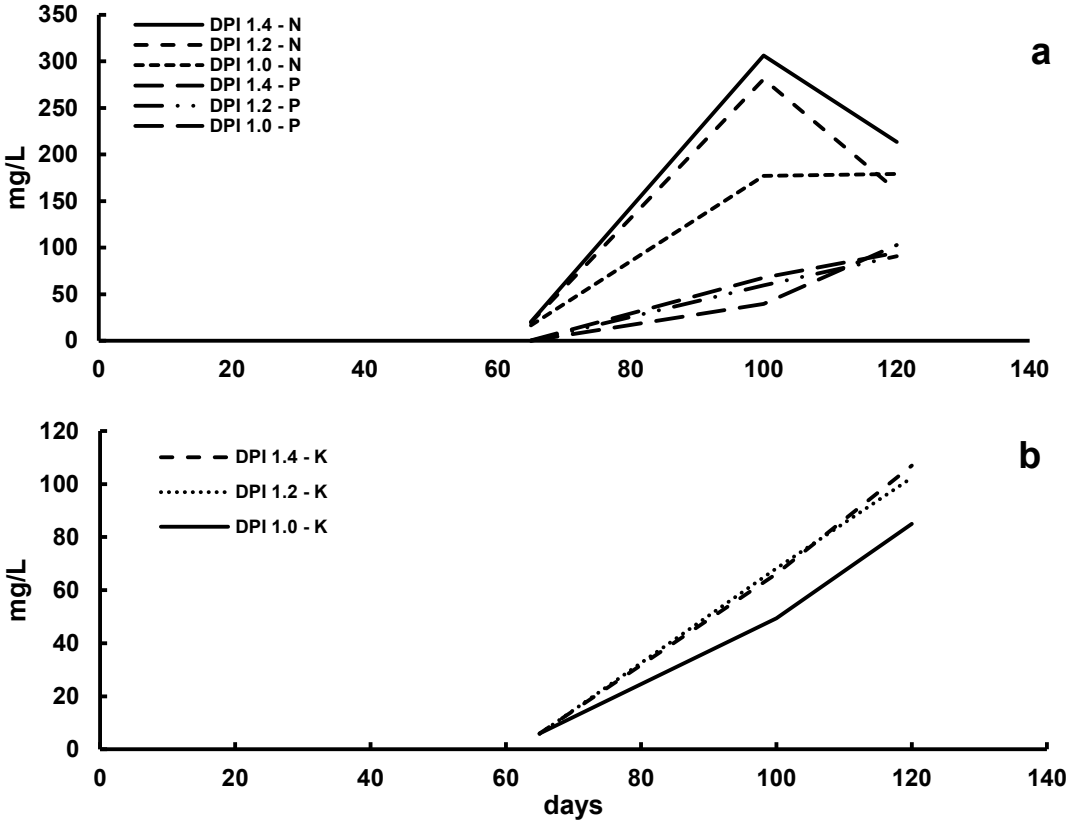

Figure 37. Nutrient flow during the Recirculating Aquaculture System period (Experiment I). (a) N and P flow during Experiment I, (b) K flow during Experiment I.

Table XXIX. Comparison of the liquid fraction and the particulate fraction against the mineralization of the liquid and particulate fractions.

	Hogland[1] (mg/L)	FL[2] (days)	MFL[3] (days)	FP[4] (days)	MFP[5] (days)
N	220-242	100	100	na	
P	24-31	na	100	*	*
K	230-232	na	na	*	*
Ca	179-224	65	<90	*+	*
Mg	49	30	65	*	na
S	113	na	na	*	*
Na	-	-	-	-	*
B	0.45	*	100	*	na
Fe	7	na	na	*	*
Mn	0.05 - 0.5	na	>120	*	<65
Mo	0.01	*	65 -100	*	>120
Zn	0.5	na	100	*	<65
Cu	0.02	na	100	*	*
Co	-	-	-	-	-
Ni	-	-	-	-	-

Notes: [1]Values from Hoagland solution, [2]FL- liquid fraction SAR, [3]MFL= aerobic mineralization of the liquid fraction, [4]FP = particulate fraction, [5]MFO = mineralization of the particulate fraction. Values in days represent the time each fraction takes to match the values of the Hoagland hydroponic solution. *The values were reached without waiting for time.

The MFL retained lower values compared to the liquid fraction that recirculated in the tank. The P in the FP and MFP reached the value of Hoagland. MFP was devoid of K, Mg, B, but it was a good source of microelements. MFL took about 90 days to obtain Hoagland values (Table XXVIII). FL and MFL showed very low levels of K, S, Na, Fe, Mn, Mo, and Cu.

7.5 Chemical mineralization

HNO_3 and H_2SO_4 allowed to recover more P, Ca, S, Fe and maintained ideal levels of N and Mn. Incineration regained a lower level of P, K, but near-optimal level for Mg, Fe, B, and Cu (Table XXIX).

Table XXX. Elemental analysis obtained from chemical mineralization: acid mineralization with H_2SO_4, HNO_3 and incineration for the particulate fraction obtained in SAR.

#	Elements	Hoagland[1]	Steiner[2]	HNO_3[3]	H_2SO_4[4]	IN[5]
	N	220-242	170	218	221	159
	P	24-31	50	312.8	436.6	46.6
	K	230-232	320	33.7	46.8	16.5
	Ca	179-224	183	192.5	227.8	122.2
	Mg	49	50	7.1	17.7	19.4
	S	113	148	78.9	90.4	6.8
	Na	-	-	97.9	1058.4	nd
	B	0.45	1-2	0.08	0.4	3.0
	Fe	7	3-4	0.03	25.2	46.4
	Mn	0.05-0.50	1-2	0.03	3.6	0.1
	Mo	0.0106	0.1	0.02	0.05	nd
	Zn	0.48	0.2	0.02	28.4	0.5
	Cu	0.02	0.5	0.01	1.5	1.4
	Co	-	-	0.03	0.1	nd
	Ni	-	-	nd	nd	nd

Notes: undetected nd-, [1,2]Hoagland and Steiner = Commercial Hydroponic Solutions. [3]HNO_3=acid mineralization with nitric acid, [4]H_2SO_4 = acid mineralization with sulfuric acid, [5]IN = incineration at 650°C in a mufla oven. For comparative purposes, the average results of each technique are displayed. Mineralization was carried out at laboratory level, 3 µg of dried sample per triplicate.

7.6 Specific hydroponic formulations and chemical mineralization

The profile closest to the chemical formulation for lettuce was thrown by incineration, only with surplus of Fe. Co was only detected in acid mineralization. No Na values were detected for incineration (Table XXX).

Table XXXI. Elemental analysis results from the particulate fraction obtained from Recirculating Aquaculture System aerobic mineralizers.

#	E	Lettuce[1]	HNO_3[2]	H_2SO_4[3]	IN[4]
1	**N**	142	218	221	159
2	**P**	31	312.8	436.6	46.6
3	**K**	215	33.7	46.8	16.5
4	**Ca**	90	192.5	227.8	122.2
5	**Mg**	12	7.1	17.7	19.4
6	**S**	18	78.9	90.4	6.8
7	**Na**	-	97.9	1058.4	nd
8	**B**	0.16	0.08	0.4	3.0
9	**Fe**	1.1	0.03	25.2	46.4
10	**Mn**	0.140	0.03	3.6	0.1
11	**Mo**	0.02	0.02	0.05	nd
12	**Zn**	0.13	0.02	28.4	0.5
13	**Cu**	0.024	0.01	1.5	1.4
14	**Co**	-	0.03	0.1	nd
15	Ni	-	nd	nd	nd

Notes: [1]Hydroponic solution formulated for the cultivation of lettuce. [2]HNO_3 = acid mineralization with nitric acid, [3]H_2SO_4 = acid mineralization with sulfuric acid, [4]IN = incineration at 650°C in a mufla oven. For comparative purposes, the average results of each technique are displayed. Mineralization was carried out at laboratory level,3 µg of dried sample per triplicate.

Table XXXII. Comparison of the chemical mineralization of the particulate fraction of Recirculating Aquaculture System against specific hydroponic solutions for the cultivation of tomato, strawberry, cucumber, chili and melon obtained from aerobic mineralizers in RAS.

#	E	Tomato[1]	Strawberry[1]	Cucumber[1]	Chili[1]	Melon[1]	HNO_2[2]	H_2SO_4[3]	IN[4]
1	**N**	192	140	210	152	223.2	218	221	159
2	**P**	46	39	24	39	32	312.8	436.6	46.6
3	**K**	275	205	217.5	245	217.5	33.7	46.8	16.5
4	**Ca**	144	110	157.5	110	157.5	192.5	227.8	122.2
5	**Mg**	32	27	48	29	36	7.1	17.7	19.4
6	**S**	42	36	64	32	48	78.9	90.4	6.8
7	**Na**	-	-	-	-		97.9	1058.4	nd
8	**B**	0.5	0.3	0.2	0.3	0.2	0.08	0.4	3.0
9	**Fe**	0.5	1.0	2.0	3.7	2.0	0.03	25.2	46.4
10	**Mn**	0.5	0.6	0.2	0.4	0.2	0.03	3.6	0.1
11	**Mo**	0.05	0.05	0.005	0.05	0.005	0.02	0.05	nd
12	**Zn**	0.1	0.05	0.02	0.3	0.02	0.02	28.4	0.5
13	**Cu**	0.05	0.05	0.01	0.05	0.01	0.01	1.5	1.4
14	**Co**	-	-	-	-		0.03	0.1	nd
15	**Ni**	-	-	-	-		nd	nd	nd

Notes: [1]The values shown by the different vegetables correspond to hydroponic solutions specific to each. [2]HNO_3 = acid mineralization with nitric acid, [3]H_2SO_4 = acid mineralization with sulfuric acid, [4]IN = incineration at 650°C in a mufla oven. For comparative purposes, the average results of each technique are displayed. Mineralization was carried out at laboratory level; 3 µg of dried sample per triplicate.

The incineration generated a profile very similar to that required by tomato and chili, all mineralizations had deficiencies in Mg, K. The recovery of P was greater in acid mineralizations, greater than the requirements of tomato, strawberry, cucumber, chilli and melon. The greatest recovery of micronutrients was obtained by mineralization with H_2SO_4. The accumulation of Na is present in acid mineralizations (Table XXXI).

EXPERIMENT IV

7.7 Implementation of the TBF effluents in hydroponics

7.7.1 Elemental analyses in the liquid fraction from BFT rearing

In the liquid fraction, the dominant macroelements were Na and Ca for all treatments. Potassium, N, P and Si accumulated during nursery and grow-out phases in all treatments ($p < 0.05$; Table 2). Calcium, Mg, Na and Si were found from the beginning of the experiment to week 30. Regarding micronutrients, Fe and Mn were detected until the end of the grow-out phase ($p < 0.05$). Molybdenum, Se and Zn fluctuated during the whole experiment ($p < 0.05$). Treatment CS showed the highest level of B and Ca. During the experimental period, mixotrophic treatments showed the highest levels of micro- and macronutrients among all treatments.

7.7.2 Water quality and nutrients in BFT

The parameters obtained within the cultivation of *O. niloticus* are within the optimal values for vegetables grown in hydroponics (Table XXXIII). The salinity accumulated in the system conditions growth to halophyte plants or salinity resistant plants. Nitrogenous residuals accumulated over time presenting significant differences between treatments ($p<0.05$) (Table XXXIII).

The relationship NH_4-N: NO_3-N was lower in the nursery phase with significant differences between treatments after week nine ($p < 0.05$) when the level started to increase in CV and CS; after week 13 an increase was observed in all treatments when the grow-out phase obtained the highest levels (Fig. 38a).

The relationship between NT and PO_4-P was lower in the photoautotrophic treatments (M, CS and CV) in the nursery phase and higher in Q and H. In the grow-out phase the relationship exceeded in all the treatments with respect to hydroponics solutions (Hoagland, Steiner, Cooper, Hewitt solutions) (Fig. 38b). Evaporation increased throughout the experimental period; treatment H had the highest evaporation level at the nursery phase ($p < 0.05$) (Fig. 39a), but the total evaporation did not show significant differences between treatments ($p > 0.05$, Table XXXIII). Conductivity Q and H showed the lowest level during the nursery phase, and after week 11 they increased to the highest level (> 6 dS/m maximum level) until week 21. In the grow-out phase the level of all treatments decreased without significant differences until week 26 when the photoautotrophic treatments obtained the highest level (Fig. 40).

Table XXXIII. Description of culture parameters (DO, pH, conductivity and salinity), total evaporation and NH_4-N, NO_2-N, NO_3-N, PO_4-P for 40 weeks. *Oerochromis niloticus* BFT rearing was divided into two periods, representing winter and summer.

Physical parameters	Time	Q	H	M	CV	CS
Temperature (°C)	**T**	25.6±4.9	25.6±5.5	24.3±4.1	24.2±4.1	24.5±4.0
DO (O_2 mg/L)[1]	**T**	7.4±1.2	7.5±1.3	7.3±1.5	7.3±1.4	7.5±1.3
pH[2]	**T**	5.9±1.2	6.0±1.2	5.7±0.9	5.8±0.9	5.8±1.0
Salinity (ppt)	**T**	1.4±0.8	1.5±1.1	1.8±0.4	1.8±0.4	1.8±0.4
Total Evaporation (L)	**T**	1254.6±28.4	1381.0±12 7.9	1401.2±55.7	1374.9±56.1	1349.1±72.0
NH_4-N (mg/L)	**10**	$0.3±0.2^{b}$	$0.4±0.3^{b}$	$0.7±0.6^{b}$	$1.3±1.8^{a}$	$1.6±2.0^{a}$
	20	$18.0±9.8^{c}$	$17.2±9.4^{c}$	$23.1±12.5^{b}$	$26.8±11.2^{a}$	$27.4±11.9^{a}$
	30	$22.4±16.3^{bc}$	$16.8±13.2^{c}$	$27.5±20.3^{ab}$	$28.0±22.3^{ab}$	$32.8±20.9^{a}$
	40	91.2±36.0	78.5±36.3	109.1±34.5	106.8±28.5	112.4±36.4
NO_2-N (mg/L)	**10**	$0.3±0.2^{c}$	$0.2±0.1^{c}$	$0.3±0.7^{c}$	$3.8±5.6^{b}$	$5.6±5.0^{a}$
	20	0.2±0.2	0.5±0.7	1.0±1.0	0.5±0.5	0.5±0.5
	30	1.3±1.5	0.9±1.3	1.1±1.5	1.0±1.4	0.5±0.6
	40	0.2±0.4	0.2±0.4	0.1±0.3	0.2±0.5	0.2±0.4
NO_3-N(mg/L)	**10**	$17.4±8.9^{bc}$	$13.9±5.8^{c}$	$29.3±6.7^{a}$	$19.6±13.9^{bc}$	$21.0±17.1^{b}$
	20	$73.0±19.8^{bc}$	$69.2±18.7^{c}$	$83.1±23.3^{ab}$	$84.9±24.3^{a}$	$87.9±21.5^{a}$
	30	$68.0±29.6^{bc}$	$65.4±26.9^{c}$	$79.5±36.3^{ab}$	$80.9±38.9^{a}$	$85.5±37.6^{a}$
	40	136.2±48.1	142.2±60.4	168.3±52.6	159.2±55.9	171.8±46.9
PO_4-P (mg/L)	**10**	$1.2±1.0^{c}$	$1.0±0.7^{c}$	$6.5±1.7^{a}$	$4.9±2.5^{b}$	$5.9±3.1^{a}$
	20	$10.1±3.8^{b}$	$9.0±4.0^{b}$	$16.0±4.7^{a}$	$14.6±3.2^{a}$	$18.6±3.8^{a}$
	30	9.4±4.4	7.3±3.8	10.8±3.7	9.7±5.0	11.0±5.2
	40	14.5±4.0	15.6±5.2	19.0±5.0	18.0±5.0	19.4±4.3

Notes: [1]DO = Dissolved oxygen (mg/L); [2]pH ranges 0-14. Each value represents the mean ± SD. Lower case letters indicate differences among treatments, capital letters indicate differences among weeks. Values in the same row with different superscripts are significantly different ($p < 0.05$). Experimental period, nursery (1 – 20 weeks) and grow-out phase (21 - 40 weeks). Treatments: Q = chemotrophic, H = heterotrophic, M = photoautotrophics: *Chlorella* spp., CV = *C. sorokiniana*-2714, and CS = *C. sorokiniana*-2805 during the maternity and fattening stages.

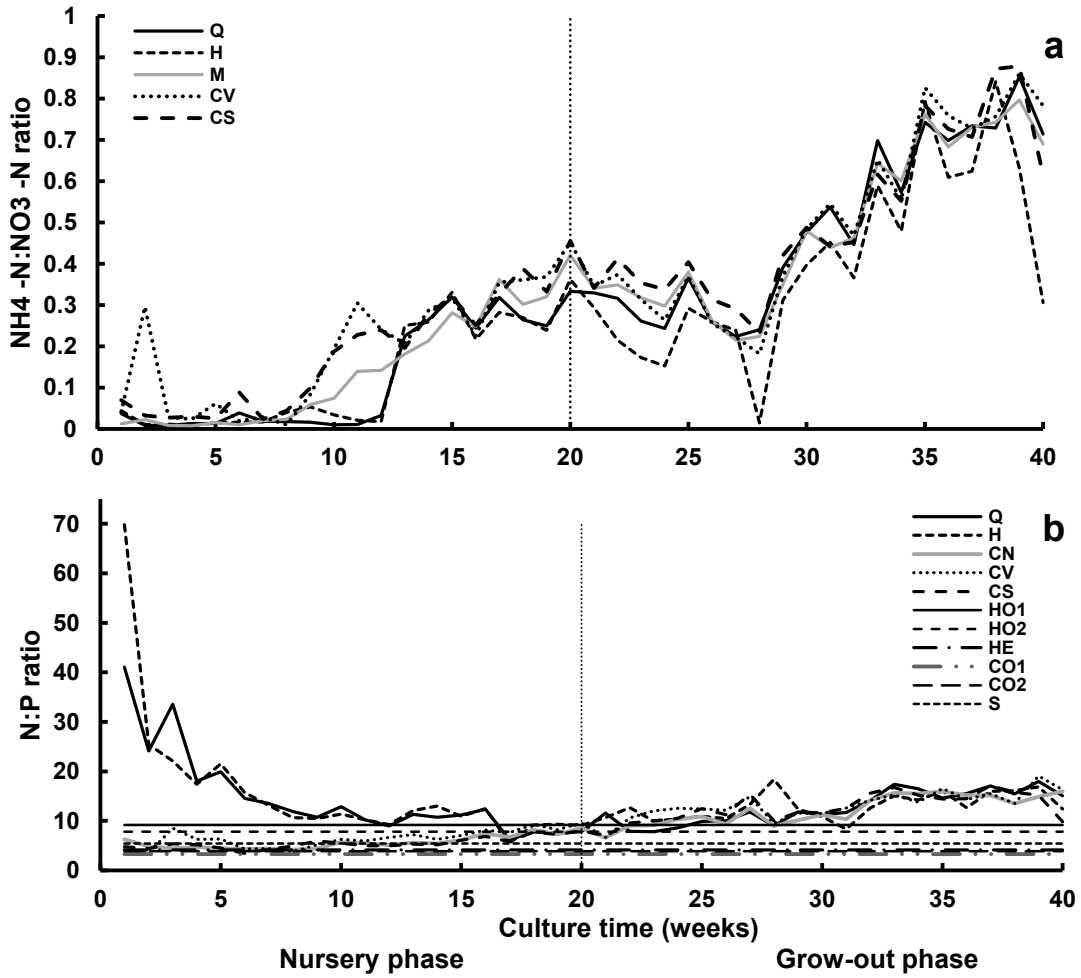

Figure 38. Description of water quality during the experimental period (40 weeks). (a) relationship NH_4-N: NO_3-N, (b) relationship N:P = NT:PO_4-P. Concentration ranges of N and P according to various authors: HO1 = Hoagland solution 1, HO2 = Hoagland solution 2 maximum and minimum of Hoagland data used in hydroponics solutions (1960), Hewitt = HE (1966), Cooper solution 1 = CO, CO2 = Cooper solution 2 (1979), Steiner = S (1984) (Data obtained in Trejo-Téllez and Gómez-Merino, (2012) .The vertical grey line in the middle of each plot separates data into two periods: nursery (1 -20 weeks) and grow-out (21- 40 weeks), during tilapia rearing. Treatments: Q = chemotrophic, H = heterotrophic, M = photoautotrophics: *Chlorella* spp., CV = *C. sorokiniana*-2714, and CS = *C. sorokiniana*-2805 during the maternity and fattening stages.

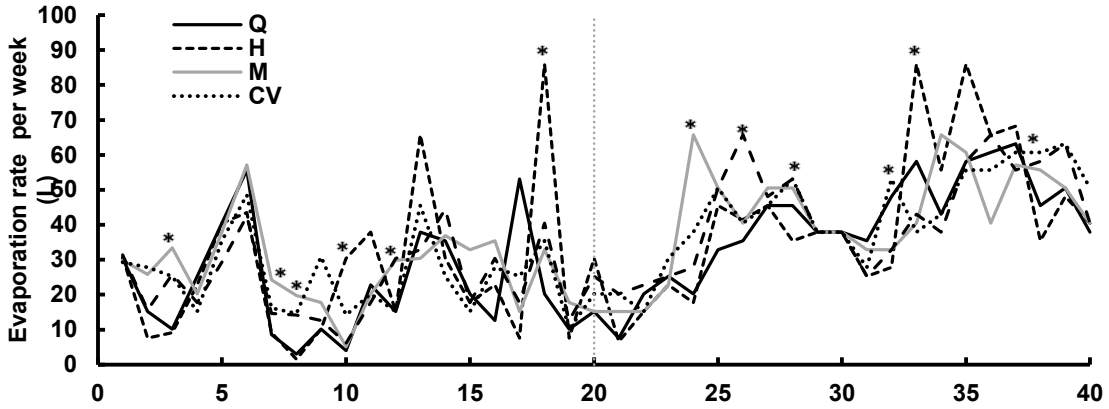

Figure 39. Evaporation (L) during the experimental period; the vertical black line in the middle of each plot separates data into two periods: tilapia nursery (1 -20 weeks) and grow-out (21- 40 weeks) during tilapia rearing. (a) Weekly evaporation. Asterisk indicates significant differences ($p < 0.05$). Treatments: Q = chemotrophic, H = heterotrophic, M = photoautotrophics: *Chlorella* spp., CV = *C. sorokiniana*-2714, and CS = *C. sorokiniana*-2805 during the maternity and fattening stages.

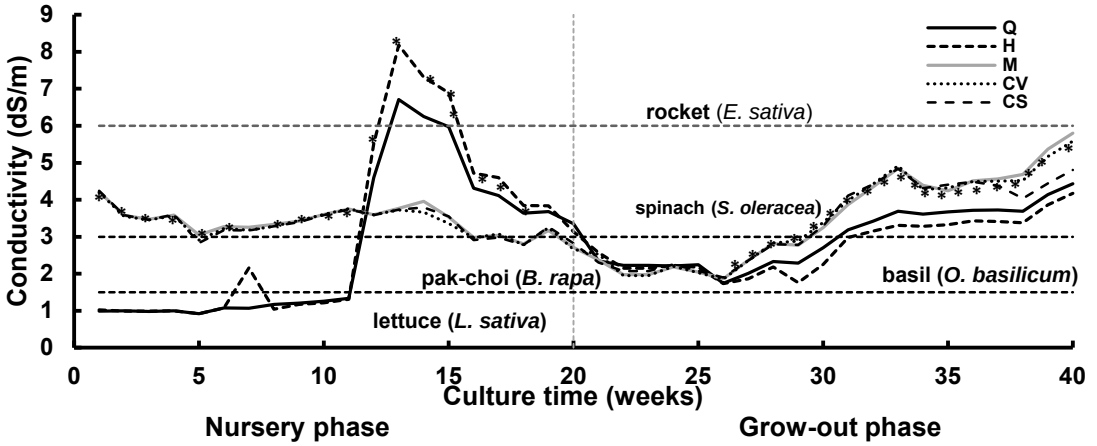

Figure 40. Conductivity during tilapia rearing. Q = chemotrophic treatment, H = heterotrophic treatment, M = *Chlorella* spp., CV = *C. sorokiniana*-2714, and CS = *C. sorokiniana*-2805. The vertical grey line in the middle of each plot separates data into two periods: nursery (1 -20 weeks) and grow-out phase (21-40 weeks) during *Oreochromis niloticus* BFT rearing. Q = chemotrophic treatment, H = heterotrophic treatment, M = *Chlorella* spp., CV = *C. sorokiniana*-2714 and CS = *C. sorokiniana*-2805. The first horizontal line represents the sensitivy rate (0 -1.5 dS/m); second line represents moderately sensitive (1.5 - 3.0 dS/m); thirth line represents moderately tolerant (3.0 to 6 dS/m). Data for stablishing this line was obtained in Trejo-Tellez and Gómez-Merino, 2012 and Shannon and Grieve, 1998. The name of the plants indicated the affinity to conductivity: lettuce (*L. sativa*) is a sensitive plant; pak-choi (*B. rapa*) moderately sensitive; spinach (*S. oleracea*) moderately tolerant; and rocker is tolerant (Shannon and Grieve, 1998).

7.7.3 Hydroponic horticulture experiment with green leaf plants

The greatest plant growth was reached in the middle of the experimental period, except for pak-choi (*B. rapa*), which did not show significant differences ($p > 0.05$) (Table XXXIV). By the end of the experiment, basil (*O. basilicum*) had not shown significant differences among treatments ($p > 0.05$); rocket (*E. sativa*), and spinach (*S. oleracea*) showed the highest wet growth with treatment Q ($p < 0.05$), and lettuce (*L. sativa*) reached the highest growth with Hoagland solution; no statistical differences were found among the rest of the treatments ($p > 0.05$) (Table XXXIV). The best growth of pak-choi (*B. rapa*) occurred with treatment CS. According to growth percentage, lettuce (*L. sativa*) and pak-choi (*B. rapa*) did not show significant differences ($p > 0.05$) while for basil (*O. basilicum*) and spinach (*S. oleracea*), the best results were obtained with treatment CS ($p < 0.05$), and rocket (*E. sativa*) with treatment Q ($p < 0.05$) (Table XXXIV).

Table XXXIV. Nutrient Film Technique in hydroponic horticulture experiment with green leaf plant species for 40 weeks. Plant growth using liquid fractions from biofloc cultures. Growth data is shown for the middle and final periods of the experimental period.

Plant parameters Middle weight	Plant[2]	QH+	Q+	H+	M+	CV+	CS+
Wet weight leaves (g/plant)	AL	22.9±8.8^{a}	4.6±1.8^{b}	5.9±3.4^{b}	5.8±2.9^{b}	6.8±2.5^{b}	3.0±0.9^{b}
	AR	14.2±5.7^{a}	12.0±4.8^{ab}	4.5±1.8^{c}	8.9±5.3^{abc}	8.0±3.2^{abc}	6.7±1.8^{bc}
	ES	5.9±1.9^{a}	4.6±2.7^{ab}	2.3±1.6^{b}	2.3±0.4^{b}	2.3±0.8^{b}	2.5±0.9^{b}
	LO	75.6±38.5^{a}	6.3±1.6^{b}	4.7±1.8^{b}	4.0±0.9^{b}	7.2±3.7^{b}	3.5±0.6^{b}
	PC	87.6±68.2	51.8±37.0	31.2±12.5	43.4±25.7	28.9±20.3	32.5±10.7
Dry weight leaves (g/plant)	AL	1.9±0.7^{a}	0.4±0.2^{b}	0.6±0.3^{b}	0.6±0.3^{b}	0.7±0.2^{b}	0.3±0.1^{b}
	AR	1.2±0.5^{a}	1.2±0.5^{a}	0.4±0.1^{b}	0.9±0.5^{ab}	0.8±0.3^{ab}	0.7±0.2^{ab}
	ES	0.5±0.1^{a}	0.5±0.3^{ab}	0.2±0.1^{b}	0.2±0.03^{b}	0.3±0.1^{ab}	0.2±0.1^{ab}
	LO	2.7±1.0^{a}	0.6±0.2^{b}	0.5±0.1^{b}	0.6±0.1^{b}	0.6±0.3^{b}	0.7±0.1^{b}
	PC	2.9±1.3	3.0±1.6	2.0±0.8	3.1±1.7	2.4±1.1	2.4±0.8
Wet weight roots (g/plant)	AL	14.8±5.5^{a}	4.8±1.9^{b}	3.8±2.3^{b}	3.9±2.2^{b}	4.2±1.5^{b}	1.8±0.8^{b}
	AR	7.7±2.6^{a}	4.2±2.8^{ab}	2.2±0.6^{b}	4.4±2.0^{ab}	3.6±2.5^{b}	2.9±1.0^{b}
	ES	2.7±0.5^{a}	2.0±1.5^{ab}	1.0±0.4^{b}	1.0±0.3^{b}	1.0±0.2^{b}	1.0±0.3^{b}
	LO	13.5±4.3^{a}	2.1±0.8^{b}	1.7±0.3^{b}	1.6±0.3^{b}	2.3±0.7^{b}	12.8±0.2^{b}
	PC	14.7±9.4	12.6±7.9	8.4±4.0	12.3±6.3	10.8±5.7	7.1±1.9
Dry weight roots (g/plant)	AL	0.7±0.2^{a}	0.2±0.1^{b}	0.2±0.1^{b}	0.2±0.1^{b}	0.2±0.1^{b}	0.1±0.1^{b}
	AR	0.4±0.2^{a}	0.4±0.2^{a}	0.2±0.1^{a}	0.4±0.1^{a}	0.3±0.2^{a}	0.3±0.1^{a}
	ES	0.2±0.02^{a}	0.2±0.1^{ab}	0.1±0.03^{b}	0.1±0.03^{b}	0.1±0.02^{b}	0.1±0.03^{b}
	LO	0.7±0.03^{a}	0.1±0.04^{b}	0.1±0.03^{b}	0.1±0.02^{b}	0.3±0.03^{b}	0.1±0.02^{b}
	PC	0.7±0.4	0.7±0.4	0.5±0.2	0.8±0.3	0.5±0.2	0.4±0.2
Final weight							
Wet weight leaves (g/plant)	AL	87.8±33.5	78.7±36.8	53.0±32.5	62.5±21.5	48.6±37.8	57.3±13.8
	AR	76.4±80.3^{ab}	92.9±47.0^{a}	14.1±13.6^{b}	20.0±10.7^{b}	28.6±27.6^{b}	8.2±4.9^{b}
	ES	7.0±4.8^{b}	33.8±20.9^{a}	7.2±5.9^{b}	11.8±5.9^{b}	11.4±5.6^{b}	24.4±16.4^{ab}
	LO	320.9±63.2^{a}	112.9±41.5^{b}	86.0±30.5^{b}	49.2±22.8^{b}	71.8±46.6^{b}	35.1±24.6^{b}
	PC	470.8±171.7^{ab}	308.0±100.1^{b}	427.1±172.9^{ab}	334.4±168.1^{ab}	323.7±100.7^{ab}	340.1±77.7^{a}

Dry weight leaves (g/plant^{-})	**PC**	122.8±39.7^{ab}	65.8±30.2^{b}	122.0±59.5^{ab}	119.0±44.5^{ab}	127.1±56.0^{ab}	160.8±25.1^{a}
	AL	7.3±2.6	8.5±3.4	4.5±2.9	5.3±1.9	4.4±3.4	6.2±3.3
	AR	6.4±4.9^{ab}	8.5±5.5^{a}	1.4±1.1^{b}	2.1±1.0^{b}	2.8±1.2^{ab}	0.9±0.5^{b}
	ES	1.0±0.3^{b}	2.9±1.7^{a}	1.0±0.7^{b}	1.0±0.5^{b}	1.4±0.5^{b}	2.0±1.2^{ab}
	LO	6.2±2.0^{a}	5.0±0.7^{b}	5.4±3.2^{b}	3.3±1.3^{b}	3.4±2.0^{b}	2.5±1.5^{b}
Wet weight roots (g/plant)	**PC**	6.7±2.0	4.0±2.9	6.9±3.3	7.7±3.2	6.9±3.3	7.7±2.7
	AL	74.4±23.7	49.8±21.3	48.5±29.3	56.1±22.1	42.4±27.7	31.1±13.0
	AR	39.9±15.8	38.7±17.7	12.7±10.6	13.9±7.8	27.1±16.7	6.0±2.9
	ES	5.2±2.3^{b}	24.7±12.4^{a}	7.0±6.4^{b}	9.6±4.9^{b}	10.5±6.0^{b}	19.2±11.1^{ab}
	LO	43.8±7.4^{a}	29.0±9.8^{b}	23.2±10.0^{b}	10.6±1.7^{b}	13.1±9.2^{b}	10.2±7.2^{b}
Dry weight roots (g/plant)	**PC**	21.3±0.3^{a}	1.1±0.3^{b}	0.7±0.3^{bc}	0.5±0.2^{c}	0.7±0.5^{bc}	0.4±0.3^{c}
	AL	2.5±1.0	2.4±1.2	1.5±1.0	1.3±0.5	1.6±1.1	1.3±0.5
	AR	1.8±0.8a^{b}	2.3±1.3^{a}	0.8±0.4^{ab}	0.9±0.5^{ab}	1.6±0.8^{ab}	0.4±0.1^{b}
	ES	0.2±0.02^{a}	0.2±0.1^{ab}	0.1±0.03^{b}	0.1±0.03^{b}	0.1±0.02^{b}	0.07±0.03^{b}
	LO	21.3±0.2^{a}	1.1±0.3^{b}	0.8±0.3^{b}	0.5±0.2^{b}	0.7±0.4^{b}	0.4±0.2^{b}
% Growth	**PC**	21.3±0.25^{a}	1.1±0.3^{b}	0.7±0.3^{bc}	0.5±0.2^{c}	0.7±0.5^{bc}	0.4±0.2^{c}
	AL	450.7±239.4^{b}	1812.0±780.0^{a}	961.0±480.0^{ab}	1373±882^{ab}	935.0±987.0^{ab}	2076.0±752.0^{a}
	AR	517.0±453.0^{ab}	882.0±562.0^{a}	313.7±222.7^{ab}	262.1±122.5^{b}	384.0±369.0^{ab}	169.0±60.4^{b}
	ES	188.9±115.6^{b}	765.0±333.0^{ab}	343.5±229.3^{ab}	505.2±235.7^{ab}	591.0±294.0^{ab}	1228±1186^{a}
	LO	493.1±211.9	2031.0±1322.0	2291.0±1629.0	1306±747	1398.0±1427.0	1174.0±1063.0
SGR (%/day)[1]	**PC**	2042.0±3516.0	506.0±377.0	1634.0±1198.0	1928±1224	1111.0±898.0	1260.0±496.0
	AL	7.9±3.9^{c}	16.7±2.4^{ab}	12.8±2.6^{abc}	14.3±4.2^{abc}	10.1±6.9^{bc}	17.5±2.3^{a}
	AR	7.7±5.4^{ab}	11.2±5.6^{a}	5.5±4.2^{ab}	5.1±2.9^{ab}	5.8±5.3^{ab}	2.8±1.5^{b}
	ES	3±2.9^{b}	11.4±2.9^{a}	6.0±4.4^{ab}	8.7±4^{ab}	9.1±4.7^{ab}	12.0±7.1^{a}
	LO	8.9±2.6	16.8±3.4	17.4±3.8	14.3±3.5	10.9±9.0	11.6±7.4
	PC	12±8.5	8.2±4.3	15.4±3.6	12±8.4	12.6±4.6	14.0±2.9

Notes: Each value is represented by mean ± Standard Deviation. Lower case letters indicate differences among treatments. Values in the same row with different superscript letters are significantly different ($p < 0.05$). Row without letter indicates no significant differences among groups ($p > 0.05$). [1]SGR specific growth rate (SGR= [(ln final weight − ln initial weight) × time−1] × 100) (% day−1). [2]AL= *Ocium basilicum* (basil), AR = *Eruca sativa* (rucula), ES = *Spinacia oleracea* (spinach), *LO* = *Lactuca sativa* (letucce), PC= *Brassica rapa* subsp. *chinensis* (pak-choi). Treatments: Q = chemotrophic, H = heterotrophic, M = photoautotrophics: *Chlorella* spp., CV = *C. sorokiniana*-2714, and CS = *C. sorokiniana*-2805 during the maternity and fattening stages.

8. DISCUSSION

8.1 The integrated systems in the immediate future

Integrated systems have gained ground as sustainable models, which allow recycling nutrients, taking advantage of the raw materials, incorporating residual nutrients to the crops, and above all managing a dynamic equilibrium in the production of nutrients improving efficiency in each phase of the system (Blidariu and Grozea, 2011; Klinger and Naylor, 2012; Rakocy, Masser, and Losordo, 2006). Waste produced in a part of the system is recycled by the other one; this ability to reuse waste has granted success to the integrated systems, which reuse effluents that allow lower investment costs, generate less residual matter, and solve two or more crops (plants crop) with the same raw material (Figs. 2, 5, and 6). The characteristics of two integrated models RAS and BFT are discussed in this thesis research.

EXPERIMENT I - RAS

8.2 Designed a RAS for arid regions and tilapia rearing

The development of the RAS worked around three lines (1) improving recirculation techniques; (2) recycling nutrients into crops; (3) obtaining high yields (Martins *et al*., 2010). The main feature of the RAS system in this project was unifying an integrated system of three phases (a) rearing tilapia in RAS; (b) performing hydroponic cultivation on floating bed; (c) cultivating in soil by ferti-irrigation where nutrient flow will be one-way, which allows having three production systems supported with the residual nutrients of tilapia farming. For this pilot stage, the decision was to handle high densities in rearing tilapia (100 fish/m^3), using *O. niloticus* fed with high protein throughout the entire experimentation (40%) with high feed rates (DPI 1.4, DPI 1.2, DPI 1.0), starting farming in summer with greenhouse inner temperatures of up to 50 °C (Table VI). All the features implemented in the first experiment had a fixed objective, be able to design a system that allowed handling high densities of total waste production recycling, applying both the particulate and liquid fractions attached to the system crops, carrying out and developing methodologies that allow optimizing the use of the residuals in hydroponic crops.

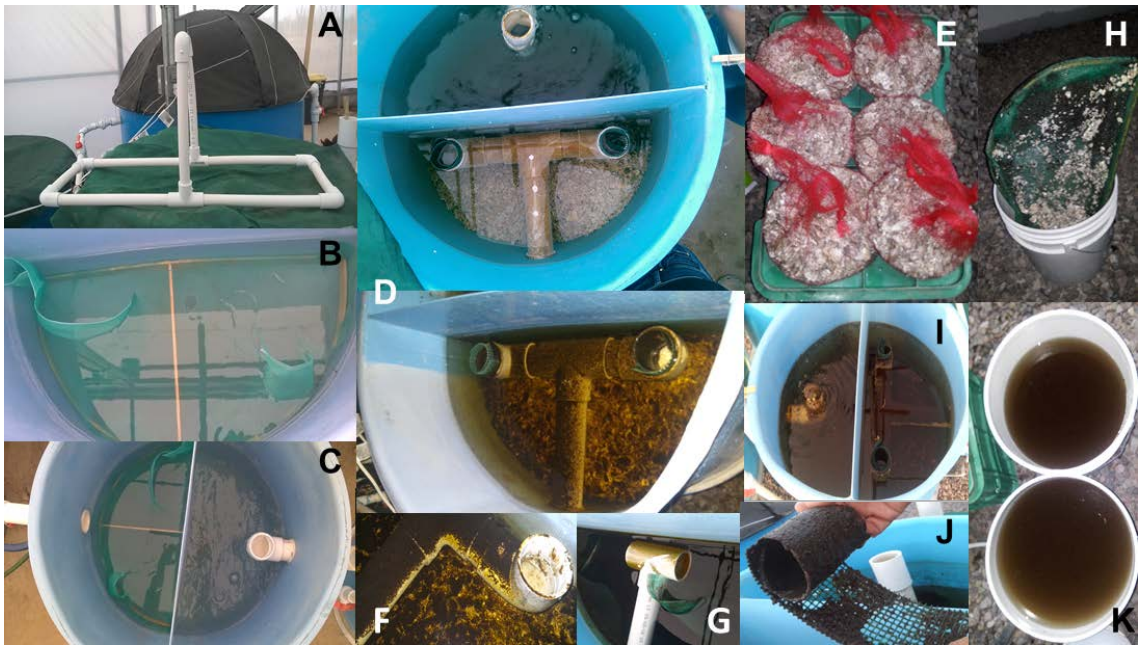

Figure 41. Modifications made to the settling tank, A. frame support, B. Mesh frame, C. location of support and frame, D. PVC "t" to cause slow flow, E. shells, F and G, settling tank in operation, H, I, J, K. Cleaning of settling tank (handwork).

Among the improvements developed in RAS, there were modifications to the sedimentator: adding three phases: contact adhesion, sedimentation and pH damping (Fig. 41). These modifications were necessary because the implementation of a good sedimentator offers many advantages since it allows to maintain higher quality water, prevents the overcrowding of organic matter in the biofilter or in the growing tanks, avoids anoxic areas or ammonia accumulation in the system (Fig. 22). The implementation of the modified sedimentation system was ideal as it allowed to have a production of 50 kg/m^3. It is important that the systems that handle the residuals split allow the retention and evacuation of organic matter (Fimbres, 2015), For this reason is developed a process of control of organic matter of four phases: first, stopping particles easily sedimentable (sedimentator), the second obtaining of the suspended particles, decreasing the current flow, having contact surfaces, third contributing to the pH damping (clarifier-buffer), and finally the fourth, a section for the

flocculation of dissolved particles and their filtration, to reduce the load of organic matter and keep the pH balanced (Fig. 41).

The biofilter plays a very specific role in carrying out nitrification; this component that works with the liquid fraction can carry out the conversion by oxidation of ammonium NH_4 (toxic fish waste component) and conversion by the nitrification process of nitrites NO_2 (toxic for fish) nitrates NO_3 (nitrogenous compound non-toxic to aquatic organism and high requirement for plants). The biofilter performance was adequate, as seen in the graph of the chemical parameters (Fig. 22, 37). Nitrite values in the system were maintained at acceptable levels (1.5 mg/L for tilapia); ammonium was raised to concentrations at levels > 50 mg/L, dangerous for tilapia values; the loss of the biofilter function may have been due to fish density growth or problems of poor alkalinity because of pH (5.6- 5.8) decline in the last rearing seasons (Table VI), which might have caused a lower efficiency in the nitrification process; the optimum pH for nitrification is 7.5 to 8.6. The decrease of pH in biomass levels higher than 18 kg/m^3 indicated that the pH buffer was undersized (6 kg shell by system) and inadequate neutralization by shells. Thus we proceeded to increase to 9 kg of shells by system, and implemented a pH neutralization with potassium hydroxide (KOH) solution to maintain the pH at levels higher than 6.2

An additional attachment of the RAS system was the aerobic digestion, which consisted of a conical cylinder with ventilation through a PVC-hose diffuser arm for uniform air intake. Half of the harvested organic matter was settled weekly in this container for 34 weeks of rearing. Aerobic mineralization is a slow method (30-60 days), but efficient to process residual organic matter. This method consists of generating microbiological communities that mineralize fecal matter, which allows odorless compost rich in nutrients with important features to apply in crops. Aerobic mineralization is currently a widely used process for its low cost and its easy application (Uggetti *et al*., 2010; Rakocy *et al*., 2005).

Importantly, the success of RAS lies in understanding the functions that each component performs. RAS is not a simple system, it is a biotechnological system that requires constant monitoring, is considered a high-tech production system (Badiola *et al*., 2012) so training has to be careful and needs a direct interaction between the operator and fish.

8.3 RAS Experiment

8.3.1 Water quality in RAS

RAS offers several advantages and produces wastewater nutrients with high yields that provide the conditions for greater economic efficiency (Badiola *et al*., 2012; Graber and Junge, 2009); however, one of the main problems for intensive culture is the accumulation of residual nitrogen (Ebeling *et al*., 2006). No differences ($p > 0.05$) were found between treatments with respect to N-NO_3, and DPI 1.0 had the highest level (220 mg/L) only in weeks 29 and 33 ($p < 0.05$) (Fig. 24).

The N-NO_2 concentration increased after week five in all treatments, and the highest level was obtained in this week (1.26, 1.4, and 0.91 m/L for DPI 1.4, DPI 1.2 and DPI 1.0, respectively) (Fig. 24), which could have been caused by fish density and size (Abdul *et al*., 1999). Fleckenstein *et al*. (2018) and Li *et al*. (2018) obtained lower values for these components. Luo *et al*. (2014) compared a biofloc system and a RAS with tilapia rearing; lower values for N-NO_2 and TAN were found in the RAS than with the biofloc technology, and the values were lower than those reported in this research, which showed a stable system with the nitrification process (Fig. 14). The accumulation of P-PO_4 increased after week 17 and continued until week 25 (1.1-1.6 mg/L). Then, the concentration continued to increase (19.6- 32.0 mg/L) without a significant difference ($p > 0.05$) (Fig. 24).

N-NH_4 concentration recorded high fluctuations starting in week four that continued throughout the experiment with values higher than 50 mg/L (Fig. 24). These values could have been caused by the protein in the diet, temperature

variation, change in pH and biofilter performance (Avnimelech, 2006; Badiola *et al*., 2012b; Piedrahita, 2003); however, despite the variations in the parameters, rearing was stable because survival was high and no external damage was observed in fish. The plastic film contributed to the high temperature in the greenhouse and in the fish tank water. All nutrients increased over time in the experiment at similar rates (Fig. 24).

The experiment was conducted throughout three seasons (i.e., summer, autumn and winter) although the temperature variation increased during the 34 weeks (Table VI). Despite such high variations in the greenhouse temperature, the culture tanks remained stable and showed no elevated temperatures and low evaporation (< 7%), and water loss was replaced with clean water (data not shown) (Black arrow, Fig. 24). This result could have been caused by the shade cloth at the top of the greenhouse (Fig. 13). The physical parameters of culture water were all within appropriate values for tilapia rearing (DeLong, Losordo, and Rakocy, 2009), and none of these data recorded significant differences between treatments ($p > 0.05$) (Table VI). Salinity and conductivity increased, and the DO and pH decreased at the end of the experiment (Table VI), but these changes did not affect the development of the experiment.

8.3.2 Growth performance

Fish growth in this research (0.2 to 60 g) (Table V) was higher than that reported by Siddiqui, Howlader and Adam, (1988) where fish growth was 0.83 g to 22.7 g during 98 days with a FCR of 1.86 higher than that obtained in this study (FCR of 0.4 to 0.6, Table V). El-Sayed, (2002) obtained different density values of FCR 2.65 to 3.45 with a SGR from 7.78 to 10.0 lower than this study (SGR 23.9 to 25.7), also obtaining FCR 1.27 to 2.98 testing different percentages of feeding levels (10 to 35). De Oliveira *et al*., (2017) reported FCR from 1.0 to 1.2 in fish with body weight from 42.8 to 50.3 g with SGR from 4.5 to 4.7% and survival from 75 to 96.3%. Abo-State, Tahoun and Hamouda (2009) analyzed the growth of tilapia offspring with five different diets (soybeans and dry grains distilled combined with phytase). The experiment began with 1.96-

2.05 g weight, and reported 1.64-1.83 FCR, survival > 99.5, and SGR (specific growth rate) with 3.3 - 3.5 between treatments. The SGR was higher compared to that obtained in this experiment, which was 2.63 and 2.94 g/day for T1 and T2 (Table V); survival was high with values similar to Abo-State and collaborators; however, the values of the FCR in this experiment were very low, that is, values not found in other publications (FCR 0.4-0.5), which could have been due to the nursery phase of this experiment carried out in with biofloc technology where nutrients, feces and not consumed feed are recycled, allowing to generate an extra supply of food for the organisms, and the feed contained 44.0% protein (Fig. 23). Ogunji and Wirth, (2000) tested eight different levels of protein diets (7.2 - 44.2%) where they obtained a FCR from 1.19 - 1.7 for *O. niloticus* offspring from 4.09-4.82 g initial weight, 2.32-3.39 values, which were similar to those obtained in this experiment for SGR. The authors concluded that variations in the biological parameters were related to the amount of protein contained in the feed, as more protein was provided, better results were obtained in growth and biological factors. These results agree with those reported by (Abdel-Tawwab, Ahmad, Khattab, and Shalaby, (2010), who also tested different percentages of protein feeds and found that the best feed for rearing tilapia was 35% protein. The nursery was in continuous light; this factor has been marked as a growth stimulator in fish (Biswas and Takeuchi, 2003). Various authors (Crovatto-Veras *et al*., 2013; El-Sayed and Kawanna, 2004) have linked the increase in growth with light incidence and frequency. The reason could be that fish are kept alert and food was available every three hours, which could favor the search for food. Providing high-protein feed in the early stages of development in offspring of *O. niloticus* promotes increase in height and weight, survival, and biological parameters, which could be observed in the retrieved results in nursery.

FCR values from 1.5 (Martínez-Córdova, Emerenciano, Miranda-Baeza, and Martínez-Porchas, 2015) to 2.0 are considered the optimum range for most species (DeLong *et al*., 2009). In tilapia, some studies have reported SGR values from 1.0-2.2 to 0.8-3.5 in dirt ponds and >1.5 in ponds (Leenhouwers,

Ortega, Verreth, and Schrama, 2007; Little *et al*., 2008; Martins, Ochola, Ende, Eding, and Verreth, 2009). Luo *et al*. (2014) recorded an FCR of 1.5 and an SGR of 1.9 in a RAS with fish that weighed 138.3 ± 34.6 g fed 43% protein. In the experiment in this study, these sizes were obtained at weeks 4 and 5 with FCR values of 0.6, 0.6 and 0.7 in DPI 1.4, DPI 1.2, and DPI 1.0, respectively (Fig. 28). At 500 g, the FCR was from 1.3 to 1.5 in all treatments (Table VIII). For obtaining 800 g of fish, the best FCR was recorded in DPI 1.2, and this trend continued throughout the experiment; at the end, the FCR values were 2.4 in DPI 1.4 and 2.4 in DPI 1.2, and the SGR had a lower level, 0.43 in DPI 1.4 and DPI 1.2 and 0.39 in DPI 1.0 ($p < 0.05$) (Table VIII). The FCR values recorded in Mexico in tilapia rearing in tank systems were higher (exogenous sources of food) (Watanabe, *et al*., 2002). As a result, the reported values were important and could be a reference for future studies based on the density of farmed fish; therefore, FCR and SGR estimates allowed us to determine the effectiveness of feeding in RAS for tilapia (Table VIII). The duration of the rearing experiment was ideal for obtaining 350 g fish, which took 11.8 ± 0.5, 12.0 ± 0.4 and 14.0 ± 1.7 weeks in DPI 1.4, DPI 1.2 and DPI 1.0, respectively (Table VIII). The initial goal was to reach an average weight of 500 g; the fish in DPI 1.4 reached 502.2 g at 15.8 weeks, and those in DPI 1.2 reached 503.2 g at 16.8 weeks; furthermore, a production level of 50 kg/m^3 was achieved at 20.5 weeks in all treatments (Table VIII).

The difference in average growth between the DPI 1.2 and DPI 1.0 treatments was approximately 185 g, and the growth in DPI 1.0 did not reach 800 g (Table VIII). The second phase was to challenge the production system to reach 80 kg/m^3, which occurred at 26.9 weeks for DPI 1.4 and at 28.2 weeks for DPI 1.2 (Table VIII). This production is innovative in Mexico where the common production has been 10 – 12 fish/m^3 with a total production of 6 kg/m^3 (data obtained from aquaculture producers from Sinaloa, Mexico). No significant differences were observed in growth between DPI 1.4 and DPI 1.2 ($p > 0.05$) (Fig. 25) (Table VII), which means it was possible to reduce food intake without affecting fish growth. Meeting the protein requirement of fish during growth has

been essential for the success of the crop; protein is the main component of fish body and the most expensive macronutrient in feeds (El-Sayed, 2004; Hargreaves, 1998). Abdel-Tawwab, Ahmad, Khattab, and Shalaby (2010a) and Larumbe-Móran, Hernandez-Vergara, Olvera-Novoa, and Pérez -Rostro (2010) indicated that the protein level in feed had a strong relationship with growth and was significantly affected at low levels.

8.3.3 Nutritional characterization of feed

The analysis of a commercial food with 41.2% protein had Fe 544 mg / kg, Mn of 161 mg/kg, Zn 384 mg/kg and Cu 18 mg/kg. In our case, the amount of Fe (172.9 - 327.8 mg/kg) was lower, Mn (1725.6 to 2476.1 mg/kg) and Zn (122.6 to 255.4 mg/kg) had a higher level, and for Cu the data were similar (9.3 to 23.1 mg/kg); the variation of these compositions, especially with the elements that are not required by the plants since they will accumulate in the residuals and cannot be used by the second phase, as with the Na. An advantage of the formulation of 35% and 40% diets of this research was not finding Na (Table IX).

Köprücü and Özdemir, (2005) designed different diets and analyzed the digestibility of different raw materials (Anchovy meal, corn gluten meal, soybean meal, gammarid meal and crayfish exoskeleton meal) and the best results with values of protein 49.5%, crude lipid 5.2%, crude fiber 2.2% and ash 8.25 and amino acid content of arginine 3.0, histidine 1.2, isoleucine 2.2, leucine 3.5, lysine 3.4, methionine 1.2, phenylalanine 2.0, threonine 2.7, valine 2.5 were obtained with anchovy meal (Table X). Furuya *et al*. (2004) found the best growth with a diet of 31.0% protein, 3.8% crude lipid and 5.6% crude fiber; the diet used in this research showed the highest level of lipids of (Köprücü and Özdemir, 2005; Michelato *et al*., 2016) but the levels of amino acids were lower (Table IX and XII), which indicates a low level of essential amino acids in the diet, affecting their accumulation in the farmed organisms.

8.3.4 Proximal analyses

With respect to the proximal analysis, Azim and Little (2008) found levels of 55% protein, 26% crude lipid, and 17% ash in a juvenile *O. niloticus* (wet weight, clean water, 35% of protein in feed). Hassaan *et al*., (2017) described tilapia (whole body) with sizes of 34.1-38.8 g and supplemented the basal diet with *Bacillus subtillis*; the malic acid values of protein were 57.6-62.2%, crude lipid values 17.0-21.8%, and ash content 13.4-15.0%. Mansour *et al*. (2018) determined values (wet weight) from 15.8-17.0% protein, 6.9-7.4% crude lipids and 3.3-3.9% ash for juvenile tilapia (38.7-49.9 g). In this study, the results from the proximal analyses of whole body (wet weight) indicated percentages of protein (48.4-53.9% in week 17 and 51.0-60.5% in week 34) (Table X) that were similar to those mentioned by Azim and Little; however, the percentage of crude lipid was higher than that found in other studies, with values from 20.7-26.2% in week 17 and 8.7-28.2% in week 34. Ash values were high in DPI 1.0 in week 34, at 24.7% (Table XI).

Crude lipids in the carcasses were significantly different ($p < 0.05$) at the final time point among DPI 1.0 (recorded a low level 8.7%) and the remaining treatments. High levels of ash (24.7%) and protein (60.6%) were obtained even though the values of crude lipids (DPI 1.4, DRI 1.2) were high for weeks 17 and 34 compared with those determined by Michelato, Furuya, and Gatlin III (2018), who found 6.7-3.8% (chloroform: methanol extraction) in tilapia reared with supplementation of methionine and taurine; levels from 5.7-10.7% were found in 80.1-90.3 g fish that were supplemented with methionine (He *et al*., 2016). The high values in the carcasses could have been affected by protein in feed (40% protein during all rearing times). Taşbozan *et al*. (2013) reported values of crude lipids (2.6-3.5%), protein (18.8-20.5%) and ash (1.1-1.2%) in muscle for five different tilapia species from a river (Turkey). Liu, Wen, and Luo (2018) found values of crude lipids (0.5-1.3%) and protein (17.8-19.4%) in muscle (wet weight) of juvenile tilapia (34.6–81.1 g), which were lower than the values reported in this research; DPI 1.0 obtained the highest value of crude lipids (3.9%) followed by DPI 1.4 (3.6%) and DPI 1.2 (3.3%) ($p > 0.05$). DPI 1.2 had

the highest level of protein (87.0%) and the best growth with no significant differences ($p > 0.05$) between the highest DPI treatments.

The mg lipid/g protein relationship in the middle of the experiment (week 17) was related to the amount of food that every treatment received: 540.7 mg/g, 424.3 mg/g and 384.9 mg/g in DPI 1.4, DPI 1.2, DPI 1.0, respectively (Table XI). The final time did not differ between DPI 1.4 and DPI 1.2 (546.6 mg/g and 541.4 mg/g), and only DPI 1.0 (144.4 mg/g) had a lower level. However, for the fillets, the highest level was obtained in DPI 1.0 (47.5 mg/g), followed by DPI 1.4 (44.2 mg/g) and DPI 1.0 (37.5 mg/g) ($p < 0.05$) (Table XI). Ayisi, Zhao, and Rupia (2017) indicated values from 206.7–290.2 mg/g for whole body (44.4-49.5 g/fish) (values calculated with the information from the research paper) and 41.9–53.7 mg/g for muscle in treatments with a high level of palm oil (0% to 8%) in the feed diets. Ali and Al-Asgah (2001) reported values from 299.6–341.6 mg/g (data calculated with the information reported in the research paper) in *O. niloticus* using diets with different levels of carbohydrates and lipids. The mg lipid/g protein relationship was higher in the results for the middle and final time periods for DPI 1.4 and DPI 1.2; for muscle in this research study, only DPI 1.2 was lower (Table XI).

For DP:DE, the level was higher in DPI 1.0 in the middle and final time periods in the carcasses (23.6 and 36.2 g/MJ); however, for the fillets, the highest level was found in DPI 1.2 (38.4 g/MJ) (Table XI). These data were compared with Van Trung, Diu, Hao, and Glencross (2011); *O. niloticus* with body weights from 10 –1000 g had values from 32.7 - 21.4 g/MJ where they decreased as the fish grew. In this study, the values increased as the size of the fish increased. The amount of feed that every treatment received conditioned fish growth and lipid proportion including protein in *O. niloticus* bodies but not in the fillets, and this proportion was higher in bodies than in fillets.

Seawright, Stickney, and Walker (1998) found that *O. niloticus* retained 85 - 102% of Ca, 4-6% of Fe, 23 -26% of K, 19-21% Mg, 3% Mn, 42-47% N, 43-57%

Na, 51-59% P and 35-39% Zn. In this research, the values were higher 2- 257% Ca, 55- 178% Fe, 44-141% K, 36-1575 Mg, 0.3 – 1519%, 6.7 -13.9% N, 32-222% Na, 18.5 -224% P, 24.1-70 % Zn (Table XII), which could have occurred due to the type of diet, or sizes of the organisms, even the kind of system.

The amount of Mn retained by the organisms showed a higher variation during the different stages; the highest values were obtained at middle time, lower at the final time and in fillet (Table XII). When comparing the percentages of the fillet with and without depuration, the value of K decreased. Jaeger *et al*. (2019) showing that tilapia retained 19.4 – 24.3% of N from feed, common carp 18.7 – 22.6 and Atlantic salmon 36.5 – 47.1. In this study the N retention in *O. niloticus* carcass and fillet was lower (Table XIII).

8.3.5 Effluents Characterization in RAS

Endo (2012) analyzed nitrogen and phosphorous flow and found that 38.2% nitrogen and 50.7% phosphorus from diet was retained by fish; 3.4 N% and 38.5% P was in the particulate fraction, 49.3% N (as nitrate) and 1.4% of P in the liquid fraction; these data agreed with the results in this research where the highest amount of phosphorous was obtained in the particulate fraction. As to the liquid fraction, Ca, K and Mg were higher (Table XIV), compared with Clarkson and South, (1991) who obtained 54.4 mg/L Ca, 7.1 mg/L K, and 4.1 mg/L of Mg in rearing water.

Goddek *et al*. (2015) analyzed solutions from hydroponics and aquaponics and found values for Ca 12 -200 mg/L, Mg 6-50 mg/L, Na 14-50 mg/L, K 27-430 mg/L, Fe 0.2 – 5 mg/L, Mn 0.2 -0.8 mg/L, Cu 0.03 - 0.15 mg/L, Zn 0.3 – 0.44 mg/L; the important point with this data is the high difference among the levels of the different macro nutrients. The quality of the residuals is dependent on several characteristics, such as water quality and chemical composition, protein in feed, formulation and quality of mineral in feed, frequency of feeding, stages and density of culture (Goddek *et al*., 2015). For the success of integrated systems, knowing the characteristics of the residuals and considering the three

components (fish – residuals – plants) is important. The macro nutrients increase with time (Table XIV), so it is a priority to know what kind of culture should be implemented to allow managing rearing time and amount of residual accumulation.

8.3.6 DPI and feeding strategies

Understanding the implementation of protein in culture is the first step for obtaining more successful and sustainable crops. In general, a requirement is that conforms to the level of protein in feed (%) is established, and it is not based on DPI (g protein/kg biomass). Most feed companies recommend feeding based on a body weight percentage: 12% in the first stages (nursery and juveniles) and lower percentages (2%) in the last stage (grow out). Additionally, protein and lipid levels in the feed increased from 50% protein and 5-15% lipids in the first stage and lowered 25- 30% protein (25-30%) and lipid (5-10%) levels in the final stages (Al Hafedh, 1999; Chou and Shiau, 1996; El-Saidy and Gaber, 2005; Ng and Romano, 2013; Watanabe, 1982).

At least six different feeding strategies were assessed in the combined analyses; the contents (Table XV), include information from 49 research papers in which the main topic was tilapia nutrition, and all these data provided interesting information. For example, the main feeding strategy detected was satiation, which was used in two different ways: satiation during a specific time (e.g., 10, 30, and 40 min), once or twice per day, or satiation where the fish marked the ration. Only one research paper reached the level achieved in DPI 1.0 while others underestimated the DPI compared with the results and the DPI function from TUMSAT in this study (Fig. 29). In the feed analyses that used a fixed body weight percentage and variable body weight percentage almost all the studies reported underfeeding while others were overfeeding, and only a few were feeding adequately (Fig. 29). In these analyses, different sizes and densities of tilapia were considered (Table XV). Notable differences were observed in the mathematical DPI levels for *O. niloticus* calculated in this study (Fig. 29) compared to those previously published (Table XV). Almost all the

information reported underestimated the daily protein required for tilapia and had the same results in terms of final growth.

The other two strategies fixed 1.0 g of feed per kg of metabolic body weight per day or 5 g of feed per every 100 g of body weight per day; Table XV indicates the variations in the results and underfeeding. Nevertheless, no specific pattern was observed for managing protein in feed and rations in any of the strategies. The last strategy was to implement DPI through mathematical functions. Comparing rearing conditions, temperature, average weight, feeding rate, protein in feed, productivity, weekly growth and biomass only DPI was found to be working with all these conditions; fixing and changing biomass only met 5 conditions (feeding rate, average weight, growth tracking, biomass and satiation), and satiation only two (temperature and satiation). DPI is a good tool for feeding in RAS (Table XVI).

For RASs, no clear information was found about how to implement the protein feeding level. All the nutrition received by RASs must come from the feed; therefore, it is important to implement an appropriate feeding strategy. The advantage of using the DPI metrics is that the value can be adjusted to conform to the characteristics of the culture, temperature, density, average weight, feeding rate, level of protein in the feed, digestibility, productivity in the system, weekly growth, biomass, and adequate feed quantity (satiation). The value can be updated every week, which allows for major control of the ration that the fish receive and generates fewer residuals. This strategy can be good for RAS crops even with temperatures ≥ 30°C and with high densities (> 50 kg/m^3).

The results obtained in this research indicated that implementing a feeding strategy related to the DPI metric is an efficient and necessary alternative for increasing the performance of *O. niloticus* aquaculture. This method allows supplying the indicated amount of protein food at different stages. Furthermore, it is not based only on fish weight, but it also considers the protein content in feed, which avoids overfeeding or underfeeding (Table XVI). Therefore, a high

protein feed of 40% was used in all the RAS replicates and modulated according to tilapia weight. This feeding strategy generates excellent results and allows fish to be fed with a high level of protein, which is specifically recommended for production in RAS (Craig and Helfrich, 2017). The results indicated that the best treatment was DPI 1.2. This level allowed an optimal FCR (1.3) at 500 g and FCR (1.7) at 800 g. A difference was observed between treatments DPI 1.2 and DPI 1.0 (almost 180 g). DPI 1.2 allowed for more efficient feeding and reduced waste and production costs. With all these results, a base was established for using the DPI metrics to feed in high density RAS in warm climates and in cultures with temperatures above 30°C.

EXPERIMENT II - BFT

8.4 Biofloc Technology Experiment

8.4.1 Water analyses

Emerenciano *et al.* (2017) determined the ideal parameters of biofloc culture in fish and shrimp. Comparing their values to those in this research, DO remained in an optimal range during all the experiment; however, pH values in the grow-out phase showed a tendency towards acidification (Table XVII), which could be due to water retention time, size of fish and temperature. The optimal temperature range for rearing tilapia is 24 °C–32 °C (El-Sayed and Kawanna, 2008); this value was obtained during the grow-out phase, showing a strong relationship with growth (Table XVIII). This parameter is also related to evaporation (amount of 31.3 – 45 L per week); however, total evaporation did not show differences among treatments (p >0.05) (Table XVII).

For the number of nitrogen compounds, treatment H had low levels of nitrites < 3.5 mg/L and Q the lowest level of nitrate (> 217.7 mg/L) while the photoautotrophic treatments showed the highest values of these residuals in both phases CS for NH_4 (< 175.9 mg/L); NO_2 for CV and CS (< 56.1-67.8 mg/L); NO_3 for M and CV (< 269-279.4 mg/L) and CV for PO_4 (31.6 mg/L) (Fig. 30). These results can be explained by the interaction of several components, such

as “die-offs”, algal density, photosynthetic processes related to “stored ammonia/nitrogen” and feed protein level (Brune *et al.,* 2003).

The concentration of NH_4-N obtained in this experiment was higher than in the study of Luo *et al*. (2014), where the highest concentration of TAN was 60 ± 0.45 mg/L and also higher than Nootong & Pavasant (2011) (< 20 mg/L) and Serra *et al.* (2015) (<5 mg/L). In this experiment, NH_4 was > 100 mg/L after week 28 (Fig. 30), which was due to the combination of several factors, such as: high stock density, low temperature (nitrifying community were sensitive to changes in DO, pH and temperature) at the beginning of the experiment, immaturity of the system during the first weeks, level of protein in feed (Azim and Little, 2008b; Ebeling *et al*., 2006; Figueroa and Silverstein, 1992), as well as water harvest for the hydroponic experiment (data of weeks 19, 21 and 28 are not shown).

Moreover, the high accumulation of nitrites exhibited the low nitrification process in the system and high accumulation of nitrates (> 250 mg/L) and phosphates (> 30 mg/L), compared to the values found by the authors mentioned earlier (nitrates < 25 mg/L; < 100 mg/L; < 30 mg/L; phosphates< 40 mg/L) (Fig. 30). Something important to considerer in photoautotrophic biofloc is the microalgae life cycle. Jung *et al.* (2017) described that microalgae grew for three days and showed a constant concentration for six days before they entered in a death phase. Therefore the methodology implemented in this study for photoautotrophic treatments where a weekly inoculation was performed, it was ideal to keep microalgae concentrations close to 10^7; in the fattening phase, it is important to make weekly crops to remove microalgae and prevent them from entering the stationary or death phase.

For photoautotrophic treatments, each tank was inoculated with each species of microalgae every seven days. Taking into account the results of these experiments, all treatments showed normal processes of oxidation and nitrification of ammonium during the cultivation period, especially before week

25, where it was suspected that the chemoautotrophic community was formed in heterotrophic and photoautotrophic treatments, but after this week a significant accumulation of ammonium was detected. Even heterotrophic treatment showed lower levels of ammonium and nitrates, indicating that the C/N ratio (13:1) was not sufficient to counteract the accumulation of ammonium.

In addition, in all photoautotrophic treatments, the microalgae community could not counteract the generation of ammonium, which suggests a shadow effect of the biofloc biomass, thus allowing oxidation and nitrification processes of natural ammonia. After week 25, the generation of ammonium was not neutralized in any of the treatments, and all showed high levels of ammonium, pH levels below 5.6 and elevated nitrate levels. Since ammonium oxidation and nitrification events are acidification processes that transform NH4 into NO3 and lower pH levels to less than 7.0, which influences the processing of ammonium, the results suggested that the chemoautotrophic or photoautotrophic phases at this level of biomass required periodic pH neutralization to control NH4. The heterotrophic phase needs a C / N> 13: 1 ratio and an effort to neutralize the pH, as mentioned above. When selecting the appropriate alkaline mixture (Ca/Mg) to neutralize pH, the magnesium and calcium concentrations necessary to improve growth of microalgae and the needs of hydroponic experiments should be considered when necessary (Fig. 30).

8.5 Tilapia growth

Some authors such as Jung *et al*. (2017) found no differences in growth, survival, FCA and TCE (p> 0.05) when using *C. vulgaries* and *Scenedesmus obliquus* in biofloc tilapia rearing for 8 weeks, but protein and lipid contents were higher in the photoautotrophic treatment (p < 0.05). De Araújo *et al*. (2019) analyzed the growth of *O. niloticus* with different inoculation densities of *C. sorokiniana*-2714 (Control, 2.5 x10^4, 5.0 x10^4, 10 x10^4 mg/L), and in 63 days, they found values of TCE 3.8 - 4.0, FCA 1.4-1.5, survival 80-85% and weight gain of 18.7-21.6 g. Badwy *et al*. (2008) found values of TCE 1.7 and FCA 2.0 (30.7 g of fish) in a diet with 50% replacement for *Chlorella* spp. in fry.

Comparing this information with the data obtained in this research, the maternity phase in the photoautotrophic treatments at 47.6-54.2 g (week 20), FCA values (1.3-1.4) were lower, and those of TCE (3.2-3.3) higher (Table XVIII). In our case, the photoautotrophic treatments (M, CV, CS) showed the highest growth (47.6- 54.2 g), TCE (3.2-3.3), FCA 1.3-1.4 and survival (> 98.8%) (weeks 1 to 20), with significant difference ($p < 0.05$), even with low temperature and high density (180 fish/m^3) (Table XVIII, Fig. 31). The implementation of photoautotrophic treatments in the maternity phase for *O. niloticus* is a good performance strategy, even in non-optimal conditions (low temperatures, high density, Fig. 31).

The use of microalgae in biofloc allowed obtaining greater survival and better growth during the maternity phase where mortality was frequent (> 20%). The advantages of growth continued until week 30 for the grow-out phase, even with good survival, but at the end of this phase, the biological parameters did not show significant differences ($p> 0.05$) between treatments.

8.6 TDS and particle size

In water from aquaculture, solids generated due to uneaten feed, fish feces, fish metabolites, microorganisms and biofilm. Settleable solids are easy to remove (Cripps and Bergheim, 2000), but biofloc has particles in a wide variety of sizes, which are classified into the following ranges: dissolved (< 0.001 µm), colloidal (0.001~1 µm), super colloidal (1-100 µm) and settleable (>100 µm) solids (Lekang 2013; Timmons and Ebeling 2013 on Bao, Zhu, Jin, and Ye, (2018)). In contrast to the results in this research where super colloidal particles and settleable solids dominated in the nursery and grow-out phases, respectively (Fig. 34). Ekasari *et al*., (2014) detected a dominance of small particles (< 48 µm) (44.8%), followed by particles >100 µm (29.2%) and 48–100 µm (26%) in a BFT experiment of shrimp culture; these results were similar to Castine, Paul, Magnusson, Bird, and de Nys, (2013), who found that particles ranging 11-20 µm were the most abundant. The divergence of those studies

with the results in this study could be due to water harvest, mechanical aeration and BFT harvest, intrinsic to our study (Lekang *et al*., 2000) (Fig. 34).

Knowing the particle size can help control the interaction of nitrifying bacteria in the system. Lara *et al*., 2017 indicated that the reduction in particle size interfered negatively in the nitrification process of BFT. In this study, smaller particles reduced the nitrification process under photoautotrophic conditions and resulted in a lower biofloc size in the maternity phase (Fig. 34). On the other hand, excess biofloc can be harmful to shrimp and fish (Emerenciano *et al*., 2017; Schveitzer *et al*., 2013). Araújo *et al*. (2019) analyzed sedimentable solids (Imhoff cones, 30 min) for 63 days in biofloc with *C. sorokiniana*-2714 and found levels of 15-32 mL/L. These levels were lower than those obtained in this research (<200 ml/L for treatment with H and <100 ml/L in photoautotrophic and Q treatments. Significant differences were found in weeks 9-14 in biofloc volumes between treatments H (100-233 mL/L) and Q (38-149 mL/L) compared to all photoautotrophic treatments (<24 mL/L) (Fig. 32); this result can be attributed to the formation of a Colloidal ("Gravity Immune" gel type Graham, 1861 in Gustafsson and Gschwend, 1997) structure during the development of the biofloc in dark conditions. As a result, the estimated volumes through Imhoff cones were not similar to those obtained with sunny conditions. Photoautotrophic or biofloc formed after 15 weeks in dark conditions under heterotrophic and chemoautotrophic conditions. Colloidal particles of biofloc were more noticeable in heterotrophic conditions than in chemoautotrophic conditions.

The levels of TDS recorded in treatments Q and H during the maternity phase were lower (> 7 mg/L) (Fig. 33) than those obtained by Gallardo-Collí *et al*., (2019) using clear water 157 mg/L and reused water (biofloc) 1543.8 mg/L in filling tanks; These values decreased at the end of the rearing period. The low values obtained in this study could be due to microbiological communities at different trophic levels (Natrah *et al*., 2014).

8.7 Nutritional analyses in Tilapia and floc

8.7.1 Proximal analyses

The BFT has a good effect on the values of the proximal compositions in cultured organisms (Ekasari, Crab, and Verstraete, 2010). Different authors have suggested that implementing BFT in the nursery and grow-out phases provides advantages for organisms' growth and development (de Oliveira *et al*., 2017; García-Ríos *et al.*, 2019; Green *et al.*, 2019; Nahar *et al*., 2015). The whole-body proximal analyses in *O. niloticus* did not show significant differences ($p < 0.05$) among treatments and growth phases (nursery and grow-out) in BFT (Table XIX), which could be due to the effect of biofloc and the interactions of the different microbiological communities in the different trophic levels; when these results were compared with those obtained in RAS for 400 weeks (using the same feed and species *O. niloticus*) (Table XI), we found that crude protein and lipid values changed over time and between treatments (half time (48.5 -53.9% protein and 20.7 -26.2% lipids), final time (51%-60.6% protein, 8.8 -28.2% lipids).

De Sousa *et al*. (2019) implemented a by-product from a pizzeria in tilapia rearing with heterotrophic biofloc and obtained 60% inclusion values of 45.69 % for crude protein and 37.54 % for crude lipids in *O. niloticus* whole body (10.68 g). Azim and Little (2008a) reported values of 53.41% and 27.83% for crude protein and lipids, respectively, with 35% protein in biofloc when compared with clean water and heterotrophic BFT system; Long *et al*. (2015) reported 51.9 % of protein and 18.3% of crude lipids in whole body of reared O. *niloticus* in heterotrophic biofloc. Comparing the crude lipid and crude protein contents in the previous studies (heterotrophic biofloc), our results had a greater level of protein component (50.9% – 56.9%) and lower level of crude lipids (23.1% - 29.8%) (Table XIX) than those obtained from De Sousa *et al*. (2019) but similar values than Azim and Little (2008) and Long *et al*. (2015) when working with high protein level in their cultures (35% P and 46% respectively).

In this study, the proximal analyzes of the whole body and the ratio of lipids / g protein in *O. niloticus* showed no significant differences (p <0.05) between trophic treatments (Fig. 35), even between photoautotrophic treatments with *Chlorella* spp., *C. sorokiniana*-2714 and *C. sorokiniana*-2805 or between growth phases (maternity and grow-ou). Using 186.6 mg of lipids/g protein in the diet (Table X, Fig. 35), an average of 503.5 mg of lipids / g protein was obtained in *O. niloticus*. compared with *O. niloticus* rearing in RAS for 40 weeks using the same food (40% protein) with 186.6 mg of lipids / g protein, showing an average of 512.5 mg of lipids / g protein with a daily intake of similar protein (DPI) and 264.5 mg of lipid g protein when the DPI was reduced by 20% (Table XI).

Emerenciano *et al*. (2017) discussed information with respect to proximal analysis in biofloc rearing shrimp, obtaining values from 18.2 – 43.0% of protein, < 0.1- 8.0% lipids, 0.8-16.2% crude fiber and 13.4-44.8% ash. In general, for tilapia biofloc the values were within the percentage of shrimp biofloc (Table XXII). Martínez-Córdova *et al.* (2015) described protein content in biofloc from 14 to 50% and 1.2 to 9.0% of lipid and discussed information with respect to proximate analysis in biofloc with shrimp culture and obtained values from 18.2 to 43.0% of protein, 18.1-36.4% carbohydrates, < 0.1 -8.0% lipids, 0.8-16.2% crude fiber and 13.4-44.8% ash. Azim & Little (2008a); Becerril-Cortés *et al*. (2018); López-Elías *et al*. (2015) found 2.5% - 3.5% of crude lipid and 24.1% - 42.0 % of crude protein for biofloc in *O. niloticus* rearing. The protein values reported by these authors are similar than those obtain in this research, protein 24-42.6%, but the level of lipids in biofloc were lower when compared with all these studies.

The data with autotrophic biofloc (*C. sorokiniana*-2714) in this study was compared with Jung *et al*. (2017) with values of protein (18.4%) and crude lipid (5.9%) and with Badwy *et al*. (2008), who implemented a diet with 50% replacement of *Chlorella* spp. and obtained crude protein 65.5%, crude lipid 13.8% and ash 18.1% values. We concluded that all the these results of lipids

are lower than ours (Table XIX). These results could have been due to biofloc, periodical harvest of the solid fraction, inoculation of microalgae in the photoautotrophic treatment and also the implementation of 40% protein in feed.

In this research, the values of protein and lipid showed the highest values in the nursery phase with (0.4 -1.1%) of crude lipid and (34.7-42.6%) crude protein (Table XXII). Mabroke *et al*. (2019) tested the substitution of fish meal by soybean meal at 25% and 50% in tilapia biofloc system and found 2.4% and 2.9% of crude lipid and 36.3 and 34 of crude protein, respectively. Gallardo-Collí *et al*. (2019) obtained a greater level of crude protein in biofloc during the experiment with levels from 31.9%-47.1% and 29.7%-46.6%, where protein increased with time; in our study, the greatest level of protein was obtained in Q treatment with 42.6% in week 10; in all cases the values were lower and decreased with time in all treatments (Table XXII). For week 30, all the protein levels stabilized not showing significant differences (24.0% – 27.6%). For crude lipids, the level was higher in their study, but for ash, this research showed a higher level (Table XXIII). In both cases, the level of lipids reduced in time, 8.0% to 1.8% and 10.5% to 1.1% from weeks 14 to 25 and in this study 1.2% - 0.06% from weeks 10 – 40.

8.7.2 Amino acid analyses

Fish body protein is composed of approximately 20 amino acids, the essential amino acids must be supplied through food (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine (Akiyama *et al*., 1997). Santiago and Lovell, (1988), described the amino acid requirement for tilapia, 5.1% lysine, arginine 4.2%, histidine 1.7%, threonine 3.8%, valine 2.8%, leucine 3.4%, isoleucine 3.1%, methionine 2.7%, phenylalanine 3.8, and tryptophan 1% protein. Köprücü and Özdemir (2005) obtained the best growth result with feed composed of anchovy flour, finding values in the diet of arginine 3.0%, histidine 1.2%, isoleucine 2.2%, leucine 3.5 %, lysine 3.4%, methionine 1.2%, phenylalanine 2.0%, threonine 2.7% and valine 2.5% The diet used in the present research showed similar values to the previous authors; arginine 3.4%, histidine 0.8%, isoleucine 1.4%, leucine 3.3%, lysine 3.5%, metionine 0.9%,

phenylalanine 1.8%, threonine 2.3%, valine 1.9% (Table X), but differed with Santiago and Lovell, (1988) for tryptophan that was not detected and lower levels of methionine, phenylalanine and threonine in the diet.

Taşbozan *et al*. (2013) reported the amino acid composition for *O. niloticus* (60 ± 10 g, from farming), our results showed lower levels in almost all the amino acids (except for leucine). Jung *et al*. (2017) found a higher level in essential and non-essential amino acids implementing biofloc with *C. vulgaris* and *Scenedesmus* than water with 50% of recharge. Comparing data for essential amino acids, this study obtained a higher percentage level for arginine > 2.1%, threonine > 0.9%, valine > 1.2%, isoleucine >1.0%, leucine > 2.5%, methionine > 0.4%, lysine > 0.6%, phenylalanine >1.5%, histidine > 0.6% (Table XX) than 1.1%, 0.7%, 0.8%, 0.7%,1.2%, 0.5%, 1.4%, 0.7% and 0.5%, respectively.

Logan *et al*. (2010) reported values for amino acid produced in a large scale commercial bioreactor: arginine 3.6%, threonine 3.1%, valine 3.5%, isoleucine 3.4%, leucine 5.0%, methionine 1.4%, lysine 4.3%, phenylalanine 3.3%, histidine 1.5%. Kuhn *et al*., (2010) analyzed a biofloc from confectionary food effluent water and obtained values of arginine 3.6%, threonine 1.7%, valine 2.4%, isoleucine 1.5%, leucine 2.3%, methionine 0.5%, lysine 1.5%, phenylalanine 2.0%, histidine 0.9%; all these data were higher than those obtained in the different levels of biofloc (Table XXI).

The analysis of the relationships among the contents of amino acids in food, biofloc and throughout the body (carcass) of *O. niloticus* is displayed in figures 36 a-b, where amino acid content in food was deficient in lysine, methionine and threonine; substantial deficiencies in lysine, arginine and taurine were also detected in amino acid content of biofloc, which could be related to an increase in the use of plant ingredients in food (Akiyama *et al*., 1997). These data are relevant because Furuya *et al*. (2001, 2004) indicated that lysine and methionine (He *et al*., 2013), among others, are the most limiting amino acids in fish nutrition, related to fish growth, yield of the steaks and cost of the diets. Therefore, it is important to take these results into account in future research

related to the content of amino acids in foods used in aquaculture of BFT to improve the use of protein in the biofloc.

EXPERIMENT III - Mineralization

8.9 Particulate fraction mineralization

8.9.1 Particulate fraction in RAS, BFT

Aquaculture residuals are nitrogenous compounds, phosphorus, among others (Bao *et al*., 2018). Years ago the elemental characteristics of solid and liquid aquaculture residuals have been described, finding favorable characteristics for reuse in agricultural crops (Cripps and Bergheim, 2000; Guangzhi and Au-unsson, 2001; Dediu, 2012); however, they also have characteristics that are not as favorable or prevent their application from being widespread (accumulation of Na, antibiotics, pathogens) (Cole *et al*., 2009; Sapkota *et al*., 2008). The reuse of aquaculture waste lies in the liquid fraction, which has been incorporated into aquaponics, hydroponics or soil cultivation systems (Rakocy *et al*., 2003; Turcios and Papenbrock, 2014).

Usually, 15 mineral nutrients are grouped into two categories: macronutrients, including nitrogen (N), sulfur (S), potassium (K), calcium (Ca), phosphorus (P) and magnesium (Mg), which are required in large amount, and trace micronutrients or minerals, such as iron (Fe), zinc (Zn), manganese (Mn), fluorine (F), copper (Cu), boron (B), molybdenum (Mo), chromium (Cr) , iodine (I) and selenium (Se), which are needed in a relatively small amount (µg / d); others, such as sodium (Na) and cobalt (Co) are only required in specific cases (plants halophytes); all these nutrients can be found in the recovered particulate fraction from aquaculture activity (RAS and BFT) (Table XXIV and XXI).

Total N and P contained in food, 69-86% of P (Lazzari and Baldisserotto, 2008) and 54- 72 % of N (Mallekh *et al*., 2015) are estimated end up as residuals. The relevance of these values is that P is a finite resource, which is necessary for plant growth (Ragnarsdottir *et al*., 2011; Sverdrup and Ragnarsdottir, 2011) besides agricultural soils lack P, so it can be implemented through fertilizers;

thus its cost has increased 800% since the last decade (McGill, 2012 in Goddek *et al*., 2019). Its recovery and implementation is necessary, taking into account that aquaculture is increasing in production and intensity of cultivation, which would indicate that these residuals will continue to generate, so attaching this fraction within attached systems is necessary.

Therefore, in recent years a breakthrough of new technologies for the recovery and implementation of nutrients has increased (Cerozi, 2016; Lovley and Phillips, 1986; Parameswaran and Anderson, 2007; Summerfelt *et al*., 1999); for example, the recovery of the particulate fraction and implementation in plant nutrition, is a very recent activity (Cerozi, 2016; Pinho *et al*., 2017). This activity allows us to recover important nutrients, such as phosphorus, potassium, calcium, magnesium (Fimbres, 2015), nutrients with a very important role in the nutrition of plants and high price in the market.

When the particulate fraction from RAS and BFT was analyzed at 65 and 70 days (Table XXV and XXVI), we found a high recovery of macronutrients P and Ca in the particulate fraction of RAS and greater in Na in BFT; similar values were found in K, Mg, S. In micronutrients, RAS recovered higher values of Mn, Zn, and Ni, while Fe, Mo, Cu were recovered in BFT (Table XXV and XXVI). The liquid fraction was compared with the particulate fraction, finding that P and micronutrients were in the particulate fraction; for the coupling of aquaponics, it is necessary to supplement Fe, Mg elements that are found in the particulate fraction (Delaide *et al*., 2017). Thus it is necessary to implement processes that allow incorporating and retrieving this fraction.

Among the factors that various authors have described for nutrient accumulation are feeds and the amount of protein they contain, fish density and the farmed species (Mallekh *et al*., 2015; Schneider *et al*., 2005, 2004; Sun *et al*., 2016). For this experiment, the same feed and the same species were used, the difference could have been generated by the microorganisms contained in

the biofloc that hijack elements, such as Mg, Zn, or physicochemical factors of the crop as pH.

8.8.2 Residual characterization in *Oreochromis niloticus* BFT rearing

The three major essential nutrients for plant growth are N, K and P, of which P is a non- renewable resource and N is in depletion for aquaculture and agriculture (Goddek *et al*., 2019). The implementation of integrated systems and their importance consist of reusing these resources. The effluents from aquaculture are rich in N and P (Lazzari and Baldisserotto, 2008); at the same time, agriculture lacks these nutrients, so here is where hydroponics has an important role coupling this technique with aquaculture to be successful. The most common coupling is with RAS, but BFT has been more frequently adopted with *O. niloticus*, which is why coupling with biofloc is necessary.

Therefore, analyzing the nutritional quality of the residuals is necessary for hydroponics; in general, fifteen macro and micronutrients are essential for plants (Das and Mandal, 2015; Fageria, 2015). Rakocy *et al.* (1997) reported that aquaculture effluents supplied at least ten nutrients required by plants, and commonly Ca, K and Fe should be added to the aquaculture nutrient solutions. Seawright, Stickney, and Walker, (1998) suggestedd that changes in concentration of dissolved Ca, Cu, Fe, K, Mg, Mn, Na, P and Zn are in function of increases in biomass of *O. niloticus,* which was observed in this research where macronutrients accumulated according to the organism's growth (Table XXIV).

The elemental analysis in the solid fraction (biofloc) from *O. niloticus* BFT showed the highest level of microelements in contrast with the liquid fraction, which retained lower levels and frequently lacked elements, such as Fe, Mn, Mo and Ni. Instead, the solid fraction retained the highest level of micronutrients Fe, Mn, Co and macronutrients as P, Mg, K (Table XXVI). Seawright *et al*. (1998) indicated that the solid fraction compared with *O. niloticus* and plant elemental content retained the highest percentages of Cu, Ca, Fe, and Zn; they also

observed a reduction of the elemental content percentage in the solid fraction as biomass in culture increased. These results differed from those in this research where almost all nutrients increased as biomass in the culture increased. Jiménez- Montealegre *et al.* (2002) found values from 20 mg/g to 40 mg/g of Fe in pond soils from *O. niloticus* rearing; in contrast, our results showed lower Fe (1.5-2.7 mg/g) values, which could be explained because the culture tanks were isolated from soil in *O. niloticus* BFT.

To integrate horticulture hydroponics with *O. niloticus* BFT the need to develop methodologies that allow recovering nutrients containing the biofloc particulate fraction is imminent, especially P, Fe and micronutrients. Several authors have described methodologies that allow recovering these micronutrients by diverse methodologies, such as aerobic mineralization, anaerobic, aerobic-anaerobic mix, and acid digestion (Crohn, 2004; Cerozi, 2016; Delaide *et al*., 2018; Parameswaran and Anderson, 2007). The development of mineralization methodologies (Goddek *et al*., 2019) to process the particulate fraction could be a sustainable way to recover important macro and micronutrients and stop releasing waste in the environment. For example, P is an important residual found in the particulate fraction in high quantities; therefore, solubilization from the solid fraction could be an efficient process for reusing a non-renewable resource in an integrated *O. niloticus* BFT and hydroponics horticulture.

8.8.3 Aerobic mineralization in RAS

The most common processes for treating particulate fraction are storing it in geomembrane tanks (anaerobic processes) and drying them in the sun; storing it in polyethylene bags and reusing leachate for agriculture; or including aerobic processes. However, its application is sparse. Another process that is beginning to be described and used is mineralization, which allows converting complex compounds of organic matter to simple compounds (individual elements) performed by heterotrophic bacteria under aerobic and anaerobic conditions (Goddek *et al*., 2019).

Aerobic mineralization is performed by oxidation of organic matter, which is done by breathing of the heterotrophic community (Goddek *et al*., 2019). By comparing the results of the particulate fractions of RAS and BFT against the results of aerobic mineralization of RAS, we found an increase in the release of P, Fe, Ca, but a significant decrease in K, Zn and Ni (Table XXVII). Concerning the mineralization of the liquid fraction, we found that it is efficient for macronutrients such as Ca, Mg, Na but not for micronutrients (Table XXVI).

Monsees *et al*. (2017) indicated that during the aerobic mineralization process the pH changed, which caused greater release of P; the results obtained were consistent with that described above since this process had the greatest amount of P detected with 21.7-23.9 g/L at 65 days and 98-102.8 g/L for day 120. They also described that P and Ca were the elements that would recover in greater numbers; results that are consistent with this research.

With respect to P, N, variations were observed between increments and decreases during the 120 days of cultivation, but K remained in constant accumulation (Fig. 38); however, the K values found in aerobic mineralization were higher than those observed in RAS and BFT, which may be due to the hijacking of this element by the microorganisms or the low proportion of K in feed. Therefore, in reusing these residuals, it is important to supplement them with KOH, which serves to cushion pH drops in the crops, taking care and supplementing the low K values for hydroponic crops or implementing a chemical mineralization to release these nutrients. Another important factor is the accumulation of Na; this macroelement can limit the reuse of these residuals.

Macronutrients in aerobic mineralization were increasing over time (P, K, Ca, Mg, S, Na, B, Mn, Zn), so aerobic mineralization was not only favorable for the release of P but also for most of the essential elements for nutrition (Fageria, 2015; Graber and Junge, 2009); Goddek *et al*. (2019), indicated that the

maturation of aerobic mineralization could take five to 30 days depending on the system; in our case comparing the requirements of Hoagland and Steiner (Hoagland and Arnon, 1950; Steiner, 1961) aerobic mineralization reached its values from the earliest days. These results indicated that the implementation of aerobic mineralization can be a pathway for management and reuse of the particulate fraction. It is a simple process that allows storing the particulate fraction and reusing it when necessary, which would allow for an additional source of nutrients that can be incorporated as required by the growing system. This process agrees with Monsees *et al.* (2017) who promote the coupling of an aerobic or anaerobic process as a source of nutrients that can be reincorporated into hydroponic systems. Within the disadvantages that have been mentioned is the additional cost for keeping aeration constant; however, harnessing the nutrients released in the long run could promote the independence of high-cost chemical fertilizers. Although aerobic mineralization is a known process (Delaide *et al*., 2018; Goddek *et al*., 2019; Parameswaran and Anderson, 2007), its implementation is still rare.

Analyzing Table XXVIII we could observe that aerobic mineralization allowed us to have a high-value nutrient storage; even mineralization of the liquid fraction could accumulate a good level of nutrients, but this process required more time, which was worse due to the feasibility of handling, implementation and cost; aerobic mineralization could be a good implement for farms that combine aquaculture and horticultural processes.

8.8.4 Chemical mineralization and hydroponics solutions

Information on use, application and nutrition of plants through chemical fertilizers is extensive (Wang *et al*., 2008), but one important thing is the need to develop more sustainable systems to reduce environmental and economic costs that these fertilizers generate (Bugbee, 2004), and in this line hydroponic crops are not exempt.

The search for processes or methodologies that allow recovering finite elements, such as P, has led to the implementation of chemical mineralization and incineration, among others. Endo and Takeuchi (2009) described the solubilization of macro elements by implementing H_2SO_4 and hydrogen peroxide to the particulate fraction recovered from rearing *O. niloticus*, finding that mineralization with H_2SO_4 (using various concentrations) obtained better results for P, Ca , Fe, ZN, Mg and Cu. The implementation of methodologies that allow processing the particulate fraction and recovering nutrients is a path that is under development.

Steiner and Hoagland's solutions are the most common commercial fertilizers in hydroponics designed for cosmopolitan needs (Hoagland and Arnon, 1950; Steiner, 1961). Comparing these solutions with the results obtained in chemical mineralization, we found that the implementation of a mineralization process allows recovering important nutrients such as phosphorus that in many regions is scarce (Jones *et al*., 2015; Sattari *et al*., 2012; Wiel *et al*., 2016). Table XXIX shows the values obtained by applying chemical and physical mineralization, observing that acid mineralization allows greater recovery of P, but acid mineralization with H_2SO_4 allows greater recovery of micronutrients (Table XXIX).

For tomato and fruit plants, phosphorus is a very important element that promotes root growth, improves efficiency in the use of nutrients and water, increases yield (Sattari *et al*., 2012). The best method to recover phosphorus is acid mineralization with HNO_2, which allows the recovery of 312.8 mg/L and with H_2SO_4, 436.6 mg/L (Table XXIX). Additionally, the mineralization process may provide some important nutrients, such as potassium, calcium, making it a good source of microelements (Table XXX).

Tomato is a plant with very specific nutrient needs, this plant needs nitrogen, calcium and potassium at very high levels (Herrera, 2000; Lopez *et al*., 2011). When analyzing the nutrients recovered in the implementation of chemical and

physical mineralization and nutritional requirements, we found that any process obtains the necessary K and N values that the plant needs, but Ca complementation is needed (Table XXXI). K complementation is required in all mineralization processes, but P, Ca and S are ideal (Table XXXI). Sainju *et al*. (2014) indicated that tomato plants can develop in soils with Na at rates of 80-100 mg/L, only HNO_2 obtained a lower rate (Table XXXI). The values obtained for P, K, Mg and Ca were higher than those indicated as suitable for tomato by Barrdoso (2018). One important thing to consider is that tomato requires higher levels of P, N and K (Sainju *et al*., 2014) in spring. The formulation in this research study is concerning spring; a special formulation should be considered for different seasons.

In an aquaponics cultivation with strawberry and tilapia using NFT (Villarroel *et al*., 2011), values of K 7.98 x 4.35 mg/L, Ca 13.0 s 0.96 mg/L, Mg 1.39 s 0.77 mg/L in the control solution were implemented, lower than those recovered in this study (Table XXIX). Strawberry has a lower requirement than tomato concerning nitrogen, magnesium and boron; however, a higher magnesium requirement, in this case by mineralization with H_2SO_4, is suitable for microelement recovery (Table XXXI). Moreover, nutrients containing the particulate fraction are sufficient for strawberry cultivation in hydroponic solutions, but the level of Na should be highlighted. Villarroel *et al*. (2011) indicated that the level of Na in strawberry is 64.6 x 20.03 to 96.9 x 41.9 mg/L, so HNO_2 and IN are a good source to be used in strawberry cultivation (Table XXXI).

Cucumber has the lowest phosphorus requirement than other plants, so all mineralization processes are good, but it needs magnesium and sulfur supplementation. Melon needs high levels of nitrogen and calcium, but the levels this plant needs are included in almost all treatments (Table XXXI). Lettuce has the lowest nutrient requirement, so all treatments yielded optimal values for this plant (Table XXX).

Na has not yet shown to be essential for most upper plants (certain types of C4 plants are an exception). Cabbage, beans and celery are a good prototype for being used in hydroponic waters with a high Na level of more than 5 ppm; in this case, the H_2SO_4 solution could be good for this type of plants. The level of sodium, cobalt and selenium that the entire mineralization process showed was important, only incineration did not show the level of these elements.

Sahrawat *et al*. (2006) indicated that sulfuric acid allowed recovering more elements; they also mentioned that it was an easy process to be used in the laboratory. This method needs a wet, ventilated and safe area for handling (Endo and Takeuchi, 2009). Analyzing the results in Table XXIX, we concluded it was the best method for nutrient recovery. Incineration was a good method for recovering iron and sulfur. Mineralization with H_2SO_4 and incineration were good tools for microelement recovery (Table XXIX).

Chemical mineralization with HNO_3 and H_2SO_4 allows recovering nutrients of great value. However, this technique has a high cost if used on a large scale, so it is necessary to perform it under specialized supervision due to the vapors released from the reaction; thus implementing any of these techniques by producers or on farms without the proper facilities is not feasible. Further research on the processes of mineralization or processing of the particulate fraction is necessary.

8.8.5 Handling the particulate fraction

The solid fraction is withdrawn regularly from RAS and BFT; other crops do not implement it. Within the flow circulation of the RAS flow, the particulate fraction is constantly removed through filters, sedimentators or foam fractionators; however, in BFT no joint process exists to remove this fraction, so to unify it, the integration of aquaculture systems with hydroponic systems without recirculation is necessary.

EXPERIMENT IV - Hydroponic

8.9 Hydroponic experiment in NFT

8.9.1 Biofloc monitoring

Fifteen macro and micronutrients are essential for plants (Das and Mandal, 2015; Fageria, 2015). Delaide *et al*. (2017); Rafiee and Saad (2005) and Siddiqui and Al-Harbi (1999) noted a deficiency of elements, such as P, K, Fe, Mn and S in RAS effluents. In this research, only five (P, Mg, Mo, B and Mn) essential macro and micronutrients were detected in optimal quantities; hence, Ca, K, N, S, Fe and Zn (Hoagland's solutions) should be added to BFT effluents (Table XXXII). Even though some nutrients were lacking or resulted in low concentrations in the treatments in this study, lower concentrations of these elements have been obtained from RAS effluents (*e.g.*, Rafiee and Saad, 2005). Furthermore, macronutrient retention was higher in the mixotrophic treatments compared to treatments H and Q, which could have been related to the formulation used for microalgal growth (in the culture period) and to the nature of microalgal composition (Mandalam and Palsson, 1998).

8.9.2 Water quality and nutrients in BFT

Temperature is one of the most important factors that determine fish and plant growth and development (Yan and Hunt, 1999); it also influences water and nutrient uptake in crop (Trejo-Téllez and Gómez-Merino, 2012) and has a direct relationship with the amount of oxygen consumed by the plant and a reverse relationship with dissolved oxygen. The optimum temperature for almost all plants is 15-30°C, but the ideal temperature for every species could be different: spinach (*S. oleracea*) (24-28°C), lettuce (*L. sativa*) (20-25°C) and pak-choi (*B. rapa*) (21 ideal, 18-25°C), finding that all plants were within the optimal value (Table XXXIII) (Mahmud *et al*., 1999; Trejo-Téllez and Gómez-Merino, 2012).

The pH determines nutrient availability for plants. The ideal pH for plants is 5.6-6.5 (Parks and Murray, 2011), so nutrient deficiencies could occur below 5 and above 7.5 because pH affects the availability of some nutrients (Parks and Murray, 2011). Considering this aspect, the pH was in average in all the

treatments from 5.7 - 6.0, which were suitable values for NFT hydroponics horticulture.

Sodium ions (Na^+) are important to monitor in the recirculating nutrient solution; sodium level in the nutrient solution should not exceed 100 mg/L for lettuce (*L. sativa*) and 150 mg/L for leafy Asian vegetables as pak-choi (*B. rapa*) (Parks and Murray, 2011); taking these data into account, all these levels exceeded during *O. niloticus* rearing in BFT heterotrophic, chemoautotrophic and photoautotrophic treatments. In these cases, Na^+ excess could have originated, among other factors in (a) sodium in the nutrient solution used in the culture of *Chlorella* inoculums, which should be avoided in future phototrophic BFT using sodium-free nutrient solution as possible; (b) sodium content in feeds must be reduced to the minimum level possible by better ingredient selection; (c) Na^+ content in the original water used in *O. niloticus* BFT aquaculture. As in this mode, integrating BFT with NFT horticulture, residual water from BFT is used by nutrient batches where critical nutrients (N and P) reach a certain level instead of a continuous recirculation as in traditional aquaponics models; in the time elapsed between the aquaculture process and the first nutrient batch, transferred Na^+ was accumulated. To avoid these problems, sodium levels can be a reference to transfer nutrient batches; if it is not possible, salt-tolerant plant species should be selected.

With respect to the physicochemical parameters obtained in *O. niloticus* rearing in arid regions in winter and summer, they were ideal for hydroponics cultivation (Table XXXIII). Given the affinity of plants for nitrates and phosphates, the values obtained in this study indicated that the system functioned; the nitrogen level that could be retained in the biofloc system was optimal for plant cultivation when compared with the hydroponics solution, for example, Hogland solution (220-240 mg/L of N and 20-32 mg/L of P). Nootong *et al.* (2011) found levels of nitrite > 40 mg/L and nitrate > 80 mg/L, which were similar to those recorded in this research for nitrate 136.2 -171.8 mg/L and higher for nitrite 0.1-0.2 mg/L in their final values (week 40) (Table XXXIII).

Nitrate is one of the major N sources, and N is a key nutrient for plant growth and development (Wang *et al*., 2012). The accumulation level of these nutrient residuals in biofloc is greater than in clear water (Fleckenstein *et al.,* 2018; Nootong *et al.,* 2011), but it depends on the exchange rates and the extent of nitrification and nitrate removal in clean water (van Rijn *et al*., 2006), size of floc in biofloc system (Ekasari *et al*., 2014b); additionally, these values could increase if the biofloc is reused (Gallardo-Collí *et al*., 2019b), which is why it is important to control accumulation in the system; when it is integrated with hydroponics, nitrate is a major source of N available in higher plants, but a higher level could be counterproductive because nitrates can be accumulated in plants (Medina *et al*., 2016).

Moreover, the increase of residual nitrogen NH_4-N after week 18 (Fig. 30, Table XXXIII) could occur additionally to the low pH, related to the nitrification process; Gallardo-Collí *et al*. (2019a) found that partial removal of biofloc from the culture tank might be involved in the decrease of the microbial community attached to the biofloc associated to the chemoautotrophic process.

Phosphates require a considerable amount of energy; they are expensive and progressively scarce. Therefore, it is necessary to search for alternatives for this resource in the near future (Khan *et al*., 2009). Moreover, phosphate is ubiquitous in soil and could play an important role in supplying P to plants in a more environmentally friendly, efficient and sustainable manner (Gyaneshwar *et al*., 2002), recycling the effluents from aquaculture and implementing the diverse system as a good alternative for arid zones. In this study the amount of phosphates was higher in the autotrophic treatments in nursery phase (week 1-20); this residual did not show significant differences among treatments (Table XXXIII).

The use of P into the system depended on the microorganisms and the interaction among different communities (Khan *et al*., 2009). Water transfer from BFT tanks to hydroponics started at week 17 and continued for six weeks,

which could have caused the standardization of the amount of phosphates in the treatments (Table XXXIII).

González *et al.* (2009) reported that the optimal combination of NO_3 with NH_4 produced higher growth in certain plants, but the proportion NH_4:NO_3 changed with plant species and development level. The best relationship NH_4:NO_3 for blueberry was 50:50 NH_4:NO_3 and 67:33 NH_4:NO_3, which was described by Crisóstomo *et al.* (2014) showing the highest growth in fruit. Nevertheless, the treatment without NH_4 got the best results for accumulation of K, Ca, Mg in leaves. For onion the best growth occurred in the relationship 0:100 NH_4:NO_3; for basil (*O. basilicum*), it was 20:80 NH_4:NO_3; dill growth did not show significant differences in all treatments 10:80, 100:0, 0:100, 4:60 NH_4:NO_3 (González *et al*., 2009); the best relationship in tomato cultivation in NFT was 0:100 NH_4:NO_3 and 12.5:87.5 NH_4:NO_3; these proportions obtained the best growth, the highest leaf area and leaf growth (Ismail and Othman, 1995). In this study the ratio was higher in the nursery period with values over 150, which showed a disequilibrium with the concentration of this N-residuals (high level of NH_4-N); after week 13 the values decreased close to one, equivalent to 50:50 (Fig. 38a), which was related to the time where water was collected for the hydroponic experiment.

Koerselman and Meuleman (2007) described the relationship N:P in tissue plants around 14:1, and if this ratio was high >16, it indicated an excess of nitrogen and lack of phosphorous; if the ratio was low < 14, it indicated an excess of P and insufficiency of N. Taking this premise into consideration, diverse hydroponics solutions were compared with this ratio and found values of 3.3 - 9.2 (Fig. 41b). Considering these examples, Q and H in the nursery phase had a higher relationship that was recommended in all hydroponics solutions, which caused the excessive amount of ammonium in the liquid fraction and the reduced presence of phosphorous. The optimal relationship was obtained during weeks 16 to 22 in the photoautotrophic treatments rocket (*E. sativa*) and

lettuce (*L. sativa*) grew well in levels from 150-200 mg/L of N at EC 2.0 dS/m and pH 5.6 (Fig. 38b) (Petropoulos *et al*., 2016)

The maximum evaporation (90 L per tank) occurred in week 18 in treatment H (Fig. 39); in general, the average was 31.4 - 35 L per week. This parameter was relevant in arid zones (Han *et al*., 2019) where it could help to design better strategies for cultivation. The general strategy for controlling evaporation is to use reflective (Avnimelech, 2011), suspending and floating covers (Han *et al*., 2019), but they are difficult to find and costly (Verdegem and Bosma, 2009). Other strategies are increasing production per surface unit, reducing the cultivation area (small model), implementing an alternative cultivation (hydroponics), reducing the feed implemented, using natural resources (biofloc) allowing increasing the production with the same water resource; a plus would be unifying biofloc with hydroponics where this synergism is necessary to exploit with different plants species.

Another important consideration for the integration of BFT and NFT is the characteristics of the effluents: TDS, physical parameters, especially conductivity, and the macro and micronutrient quantities that could be recovered in liquid and solid fractions and used in hydroponics. Hydroponic plants absorb and assimilate soluble compounds from wastewater although these plants are not skilled removers of suspended solids (Pan *et al*., 2007). Thus, the first step to reuse wastewater from aquaculture is to settle the solids, which must be followed by filtration (Bao *et al*., 2018). Pan *et al*. (2007) found that filtration effectiveness was directly related to the number of layers in the filter; in other words, the greatest the number of layers in the filter, the lowest the level of dissolved, total and volatile solids.

In this study, wastewater was filtered with a 5-µm filter prior to be transferred to the hydroponic tanks; despite particles larger than 1 µm dominated the solid fraction, particles smaller than 5 µm adhered to the roots of rocket (*E. sativa*) and spinach (*S. oleracea*), which might have affected their growth. Thus,

implementing good filtration techniques is necessary in further studies since controlling particle size can be beneficial to hydroponics.

The ideal conductivity for hydroponics was 1.5 to 2.5 dS/m; also conductivity tolerance can be classified from sensitive 0 to 1.5 dS/m; moderately sensitive 1.5 to 3.0 dS/m, moderately tolerant 3.0 to 6.0 dS/m and tolerant > 10 dS/m (Trejo-Téllez and Gómez-Merino, 2012). Figure 43 shows the diverse conductivity clasiffication; in this case Q and H had the best conductivy profile for sensitive plants, so photoautotrophic treatments were ideal for moderate tolerance in the nursery phase (Fig. 43). In the grow-out phase from weeks 18-26, conductivity did not show significant differences, and the values were ideal for moderate sensitivity (Fig. 43). This situation occurred because water culture started for the hydroponics experiment in week 18 and continued for six weeks more; the parameters indicated the importance to harvest water regularly from biofloc culture.

The nutrient concentration must be continuously monitored and water replacement used to correct the nutrient deficiencies and conductivity. Shannon and Grieve (1998) indicated that lettuce *Lactuca sativa* L. was moderately sensitive to salt (1.3 dS/m), and its tolerance increased with age; the romaine types were more tolerant; pak-choi (*B. rapa*) tolerated values 3-23 dS/m by reducing its growth 4% every 1 dS/m; rocket (*E. sativa*) was relatively salt-tolerant depending on the lineage (up to 30 dS/m), and spinach (*S. oleracea*) tolerated 2.0 to 4.4 dS/m. In this case the effluents from Q and H water were ideal for sensitive and moderately sensitive plants, and the photoautotrophic treatments were good for moderately tolerant and tolerant plants (Fig. 40; Table XXXII).

Conductivity is one of the factors that most affects the succes of the hydroponic system (Abou-Hadid *et al.,* 1996). One advantage to implement biofloc residuals in hydroponics culture is modulating the quality of these residuals. Time of of biofloc culture maturity and water collection are good tools for

residual management. In integrated systems, it is important to considerer fish and plant necessities. Implementing individual management for biofloc and hydroponics could be a good technique for arid zones because this methodology allows collecting water when the biofloc system needs it and applying hydroponics when the plants require it.

Modulating conductivity is an indispensable tool to integrate BFT with hydroponics and contribute to diversify the potential for horticulture; for example, Shannon and Grieve (1998) developed an extensive study following this clasification; the characteristics of conductivity could be conditioned to the kind of plants: sensitive (0 to 1.5 dS/m) lettuce, cassava, radish, fennel, parsnip, celery, carrot, strawberry, onion; moderately sensitive (1.5 to 3.0 dS/m) brocoli, garlic, turnip, pak-choi, chinese cabagge, cabbage, tomato, cucmber, radish, pepper; moderately tolerant (3.0 to 6.0 dS/m) sweet potato, table beet, asparagus, spinach, soybean, kale, ryegrass, potato, purslane; and tolerant (> 10 dS/m): orach, bermuda-grass, sugarbeet, cotton (Shannon and Grieve, 1998; Trejo-Téllez and Gómez-Merino, 2012).

Due to conductivity increase originated from evaporation rates, increase in nutrient residuals along the BFT rearing process and possible restrictions in plant species selection deriving from conductivity tolerance and stablishing a conductivity limit according to the plants selected for horticulture is very important when transferring water batchtes from BFT to NFT hydroponics. If this is not possible, new water with low conductivity can be used to dilute the conductivity level.

8.9.3 Hydroponic horticulture experiment with green leaf plants

According to Diem *et al*. (2017); Licamele (2016); Rakocy (2012); Rakocy *et al*. (1997); Turcios and Papenbrock (2014), the *O. niloticus* aquaculture effluents in aquaponics systems were favorable for lettuce (*L. sativa*) and basil (*O. basilicum*); however, implementation of effluents from BFT in hydroponics has only been scarcely described (Pinho *et al*., 2017); therefore, to our knowledge,

this is one of the few research studies where residual water from *O. niloticus* BFT is described and used in water batches and non-continuous recirculation with hydroponics horticulture. According to our results, basil (*O. basilicum*) could reach good growth with *O. niloticus* BFT effluents at any trophic level tested in this study; rocket (*E. sativa*) grew better with treatment Q and lettuce (*L. sativa*) with Hoagland, and the best growth was observed in spinach (*S. oleracea*) with treatment CS (1228%; five weeks) (Table XXXIV). Salam *et al.* (2014) obtained 926.18% of plant growth using an effluent from *O. niloticus* RAS rearing, and Liang and Chien (2013) detected 175% of plant growth in four weeks using a six-time feeding frequency for red tilapia rearing and raft aquaponics.

Pinho *et al*. (2017) implemented heterotrophic biofloc with different lettuces in hydroponics environment and obtained favorable results for butter lettuce (17.7 SGR); in this study, the SGR value obtained for lettuce (*L. sativa*) was 8.9-17.4 SGR ($p > 0.05$). In addition, Castillo-Castellanos *et al*. (2016) detected a wet weight of 18.8 g/plant for lettuce (*L. sativa*) with a yield of 47.9 g/m^2, using effluents from aquaponics with *O. niloticus*; in this study, lettuce (*L. sativa*) wet weigh was 320 g/plant with Hoagland, and values from 35.1-112.9 g/plant were obtained with the rest of the treatments ($p > 0.05$); thus, lettuce (*L. sativa*) growth was irregular and highly variable. Diver and Rinehart, (2010) stated that the most common combination in aquaponics is tilapia with lettuce while our results evidenced that for *O. niloticus* BFT, the best combination was with spinach (*S. oleracea*) and/or pak-choi (*B. rapa*) and/or lettuce (*L. sativa*).

da Rocha *et al*. (2017) compared growth of lettuce (*L. sativa*) in hydroponics, aquaponics and aquaponics with biofloc with catfish and found the best fresh weight 38.9 g, root fresh weight 32.1 g and height 14.5 cm in aquaponics with biofloc, treatments similar to this research. The results from basil (*O. basilicum*), pak-choi (*B. rapa*) and rocket (*E. sativa*) growth in shared hydroponic beds is novel besides the description of the different trophic levels in *O. niloticus* BFT

and water-batch transfer to hydroponics horticulture instead of recirculation as in the aquaponics model.

Oreochromis niloticus BFT could contain heterotrophic, autotrophic and photoautotrophic microorganisms (Burford, Thompson, McIntosh, Bauman, and Pearson, 2003), but most BFT systems in commercial farms use “green water” biofloc systems (Hargreaves, 2013); in this sense, photoautotrophic treatments may be a guideline for future research because different trophic levels in *O. niloticus* BFT are indispensable to help in the design of diverse coupling methods with hydroponic horticulture. The success in the future will be in diversifying systems and biological production models, for example, basil (*O. basilicum*), Pak-choi (*B. rapa*) and rocket (*E. sativa*) that are important plants in horticulture.

Biofloc technology is a beneficial system for rearing several aquatic species, including *Oreochromis* and *Penaeus* species. Our results are framed within the tendency to diversify aquaculture and production and constitute the baseline to integrate biofloc+hydroponic cultivation. Love *et al*. (2015) used information from 22 countries and detected a growth in cultivation and consumption of plants combined with aquaponics, such as basil (81%), salad greens (76%), herbs (not basil) 73%, lettuce and tomatoes (68%), kale (56%), chard (55%), pak-choi (51%), peppers (48%) and cucumbers (45%). This information, together with our results, confirms that implementing effluents from aquaculture systems (in our case from BFT) for plant growth is a favorable alternative to diversify production models. The effluents from *O. niloticus* BFT showed deficiencies in micronutrients content in the liquid fraction, so implementing processes for nutrient recovery from the particulate fraction is a feasible way to develop more sustainable systems. Moreover, water scarcity in arid zones is an important and relevant factor to integrate aquaculture with agriculture where the development for more efficient water use systems is a priority.

Therefore, *O. niloticus* BFT rearing in the photoautotrophic mode and its integration with NFT hydroponics horticulture in a non-recirculating system is beneficial in coastal arid zones where water is scarce and there is a need to improve aquaculture performance, reuse water, BFT nutrients and increase profit.

9. CONCLUSIONS

The design and implementation of the Daily Protein Intake (DPI) allowed improving growth rate, FCR, and survival during the whole crop cycle of *Oreochromis niloticus* in Recirculating Aquaculture System with a biomass of 50 kg/m^3 up to 80 kg/m^3.

The DPI allowed improving the yield in protein and lipid content in the body of tilapia but not in the fillet.

RAS was a suitable model for the cultivation of *O. niloticus* at high temperatures and densities.

The implementation of the photoautotrophic level allowed greater growth, better yield and survival in crops with 1 kg/m^3.

The photoautotrophic phase generated a nitrogen cycle suitable for integrated systems with hydroponic horticulture.

Commercial foods for tilapia showed a deficiency in methionine, lysine and threonine that is reflected in the content of *O. niloticus* and floc in Bioflocculation Technology systems.

The implementation of mineralization allowed accumulating and recovering high-value nutrients generated in the particulate fraction of RAS and BFT and used in hydroponic horticulture.

The effluents generated in a BFT crop at different trophic states were suitable for hydroponic cultivation of lettuce (*Lactuca sativa*), pak-choi (*Brassica rapa* subsp. *Chinensis*), arugula (*Eruca sativa*), spinach (*Spinacia oleracea*), and basil (*Ocium basilicum*).

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