Qualitative and Biochemical Analysis of Biofloc from an Indoor Biofloc Technology System Culturing *Oreo-Chromis Karongae*

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DOI 10.53974/unza.jabs.7.2.1121

ABSTRACT

The structure of microbial communities in a Biofloc Technology (BFT) system has a great influence on the optimal functioning of the system. This makes understanding the microbial composition in BFT of paramount importance. A study was conducted to characterise the microorganisms in an indoor biofloc technology system. The microorganisms were qualitatively and biochemically analysed. Qualitative analysis of microorganisms in BFT was carried out using microscopy and plate culture of bacteria and fungi. Isolated bacteria were identified by colony morphology, gram staining, and microscopic observation. Catalase test, as a biochemical test, was also used to identify bacteria. Biochemical analysis of the biofloc was achieved through proximate analysis of nutrients following AOAC (2002) methods. The biofloc sample used in the present study was obtained from culture water of three 1000l circular fiberglass tanks with a working volume of 580l of biofloc water per tank. Oreochromis karongae fingerlings were stocked in the three tanks at a stocking density of 6kg/m^3 per tank and cultured for 12 weeks. The fish were fed 30% CP on-farm formulated feed (2mm pelleted) with a feeding rate of 1.5% of the total estimated fish biomass. Maize flour was added in tanks to maintain an optimum C/N ratio (above 10) for heterotrophic bacteria production. Microscopy of the biofloc water revealed various types of microorganisms, which included Protozoa (e.g. ciliates), Zooplanktons (e.g. Rotifers and copepods) and nematodes, heterotrophic bacteria and fungi. It was noted that the bacteria were all heterotrophic, confirming their utilisation of organic carbon in the BFT system to produce microbial protein. The biochemical composition of biofloc showed that the nutritional quality of biofloc was appropriate for tilapia with the exception of lipid content, which was found to be 3.25%. This value is considered low for aquaculture feed as a range of 5- 10% lipid content in feed is recommended.

BFT is a promising field, which will enable aquaculture to grow towards an environmentally friendly approach because of the ability of microorganisms to recycle nutrients in the system.

Keywords: Biofloc technology, qualitative analysis, biochemical composition, *oreochromis karongae*

INTRODUCTION

The demand for fish is ever-increasing due to the world's population, which was projected to grow from about 7 billion people in 2012 to 9.6 billion in 2050 (FAO, 2014). Global demand for fish and other aquatic foods will double by 2050 (Naylor et al., 2021). There is a need to find sustainable solutions in producing fish. The demand for fish calls for the expansion and intensification of aquaculture production (Crab et al., 2012) and Ragasa et al., (2022). To grow sustainably, aquaculture must produce more fish per unit of land and water and reduce its reliance on wild-caught fish for feed. Sustainable aquaculture production must include producing without significantly increasing the usage of basic natural resources, being environmentally friendly, and being cost-effective to support economic and social sustainability (Naylor et al., 2000; Piedrahita, 2003).

A relatively new approach to sustainable intensive aquaculture production is Biofloc Technology (BFT) aquaculture (Avnimelech, 2006), Sharma et al., (2023) and Melaku et al., (2023). At the core of this technology are microorganisms, which are co-cultured with fish under controlled conditions within the culture pond or tank. The roles of these microorganisms are mainly to maintain water quality by the uptake of nitrogenous compounds, thereby generating microbial proteins and providing an additional source of high-quality fish feed (De Schryver et al., 2008; Bossier and Ekasari, 2017).

BFT facilitates intensive culture while reducing investment and maintenance costs and incorporating the potential to recycle feed. The technology works based on recycling nutrients by maintaining a high Carbon/Nitrogen (C/N) ratio in the system. This stimulates heterotrophic bacteria that converts ammonia into microbial biomass (biofloc), which is eaten as a natural food within the culture system, making it possible for a protein to 'be eaten twice' (in feed and microorganism) by the cultured fish (Azim and Little, 2008; Hargreaves, 2013; Ogello et al., 2014; Melaku et al. 2023). Culture feasibility is increased by reducing the feed conversion ratio through higher protein utilisation and lower inputs of commercial feed, hence decreasing feed cost. The present study aimed at characterising the diversity of microorganisms in an indoor biofloc technology system.

The specific objectives were to: (1) qualitatively analyse the microorganisms in the biofloc by direct microscopy, (2) isolate and identify bacteria and fungi in the biofloc using the plate culture method and (3) determine the biochemical composition of biofloc through proximate analysis.

2. MATERIALS AND METHODS

The study was conducted at Bunda Fish Farm, Lilongwe University of Agriculture and Natural Resources (LUANAR) in Malawi. The farm is located at 14° South latitude 33° East longitude and is elevated at 1126 meters above sea level.

2.1 Culture facilities

Biofloc was obtained from an already established biofloc farm (Chambo Fisheries Farm in Blantyre, Malawi). The tanks containing biofloc were housed in the farm's wet laboratory to avoid sunlight. Three 1000*l* circular fiberglass tanks with a working volume of 580*l* of biofloc water per tank were used. The stocking density of *Oreochromis karongae* was 6kgm^3 per tank. The fish were fed 30% CP on-farm formulated feed (2mm pelleted) with a feeding rate of 1.5% of the total estimated biomass. Maize flour was added to the tanks at 60% of the feed given to maintain an optimum C/N ratio (above 10) for bacteria, as suggested by Avnimelech (2012) and Azim and Little (2008). Aeration was provided by blowing air (from a root blower) through submerged air stones that dispersed fine streams of bubbles through the water. Simple airlift pumps were also included in the tanks to achieve constant intensive turbulent mixing of the biofloc water to keep flocs in suspension. Heating of the water was achieved through the use of water heaters (KY5-1000) to maintain temperatures around 25 to 27°C. Sodium bicarbonate was added to the biofloc to maintain the pH in the range of 7.2 to 7.8, which is ideal for the bacteria to flourish. This was only done whenever a decline in pH was noticed. Floc volume was checked everyday to keep it at an average of 20ml/L. The determination was carried out as described by Avnimelech (2012). An Imhoff cone (LIC1 PENTAIR) was fully dipped in the sub-surface water of the biofloc tanks. It was then raised from the tank, and the water level in the cone was adjusted to the 1000ml mark. The cone was placed on a rack and left to stand for about 20 minutes for the flocs to aggregate and settle. The volume of the floc was read from the graduations in ml/L. Hand sanitiser was used after every contact with biofloc water.

Direct Microscopic Analysis of Microorganisms in Biofloc Water

Well-mixed water samples from the three biofloc tanks were collected in plastic bottles. One (1) ml sample was placed on the microscope slide and left for several minutes to allow organisms to settle. The organisms were then observed using a compound microscope (x10) and photographed through the microscope lens using a mobile phone.

Isolation and Identification of Bacteria and Fungi in Biofloc using Plate Culture Method

Media Preparation

Six (6) types of media were used. These constituted five (5) used for bacteria and one for the isolation of fungi. The names of the media and the methods of their preparation are summarised in Table 1.

Table 1: Media used in the experiment and their preparation (following manufacturer's instruction).

No.	MEDIA	METHOD OF PREPARATION
1	Trypticase Soy Agar (TSA) (General purpose medium)	4.0 grams of the medium was suspended in 100ml of distilled water in a beaker. The mixture was heated with frequent agitation and boiled for 1 minute. This was followed by sterilization of the medium in an autoclave at 121oC for 15 minutes. Thereafter, the medium was cooled, poured into Petri dishes and left to solidify. The plates were labeled and stored in the refrigerator.
2		2.3 gram dehydrated nutrient agar was dissolved in 100 ml distilled water in a beaker whilst heating with frequent agitation. The solution was left to boil for 1 minute to completely dissolve the powder. Sterilization of the medium by autoclaving (121°C for 15 min) followed. The medium was dispensed on the sterilized petri dishes and left to solidify. The plates were labeled and stored in the refrigerator.
3.	Blood agar (5% sheep blood) (for determination of hemolytic reactions)	This was prepared by dissolving 4.0 grams of TSA into 100ml of distilled water in a beaker and brought to boil. After complete dissolution, the medium was cooled and sterilised by autoclaving at 121°C for 15 minutes. After the agar base was cooled to 50°C, 5ml of sheep's blood was added to the medium and mixed well, taking care to avoid air bubbles. 15ml was dispensed per sterile Petri plate. The plates were labelled and stored at 2-8°C.
4.	MacConkey (Selective media for isolation of gram-negative bacteria displaying lactose fermentation)	4.953 grams of dehydrated MacConkey agar was dissolved in 100 ml of distilled water. The solution was heated to boiling until the medium dissolved completely. The medium was then sterilized by autoclaving at 121°C for 15 min. The medium was then cooled to 45-50oC and thereafter dispensed on sterilized petri dishes and left to solidify. The plates were then stored in the refrigerator.
5.	Thiosulfate Citrate Bilesalts Sucrose (TCBS) (Selective for isolation of Vibrio spp.)	8.81grams of commercial powder was added to 100 ml distilled water. The mixture was slowly brought to boil whilst stirring with constant agitation to dissolve the medium completely. This medium was not autoclaved but was poured directly into the sterilised petri dishes after cooling to about 50oC. After solidification, the plates were labeled and stored in the refrigerator.

6.	Potato dextrose agar (PDA) (selective media for isolation of fungi)	To prepare the media, 3.9 grams of commercial PDA powder was added to 100 ml of distilled water. The mixture was heated to boiling whilst stirring to dissolve the medium completely. This was followed by sterilization (autoclaving at 121°C for 15 minutes). After cooling, about 15-20ml was poured into sterilised Petri dishes, labeled and stored in the refrigerator.

Culture of the Biofloc Sample on the Media

The biofloc sample was obtained by mixing small volumes of biofloc water from each of the three tanks into one bottle. The sample was sedimented at relative centrifugal forces (RCFs) of about 10,000 times gravity for 10 minutes. The supernatant water was removed, and the sediment was re-suspended in a small volume of water for subsequent analysis and characterisation. The sample was first cultured on TSA plates. This was done using an inoculating loop, which was flame sterilised. The loop was dipped in the sample and then streaked on the plates in a zigzag pattern. The plates were then incubated at 37^{o}C for 24 hours except for fungi, which were then incubated at 25^{o}C for three days. After the incubation period had elapsed, the plates were removed from the incubator and observed. Representative colonies were identified along the streak and picked for gram staining.

Additionally, a catalase test (a biochemical test) was carried out on the different colonies. This was achieved by transferring a small amount of bacterial colony to the surface of a clean, dry glass slide using a sterilised inoculating loop. A drop of 3% hydrogen peroxide (H_2O_2) was mixed on the slide. Production of gas bubbles indicated a positive result.

2.4 Determination of the Biochemical Composition of Biofloc through Proximate Analysis

Biofloc samples were collected continuously throughout the trial period (12 weeks). This was done by sieving concentrated biofloc water using a 45micron sieve. After drying, the samples were ground using a laboratory motor and pestle and stored in the refrigerator at -4^{o}C to await analysis. At the end of the experiment, pooled samples were processed for proximate analysis following AOAC (2002). Crude protein was determined by the micro-Kjeldahl method after acid digestion. The amount of protein in the sample was calculated by multiplying the amount of total nitrogen in the sample by 6.25. Lipids were determined by petroleum ether extraction for 16 hours in a soxhlet apparatus. The fat flasks were dried in an oven for 8 hours at 85^{o}C. The ether was evaporated, and the crude fat was weighed and recorded. The crude fiber was determined by subjecting the residue from ether extraction to boiling

in diluted sulfuric acid (1.25%) for 30 minutes, then boiling in a dilute sodium hydroxide (1.25%) for another 30 minutes and then passing the solution through the filter. The material was then dried and weighed. The material was burnt in a furnace at 550°C for 5 hours to oxidise off the crude fiber, and ash weight was obtained. The ash left was weighed, and the amount of crude fiber was calculated by subtracting the weight of the ash from the weight of the material left after boiling and drying. Ash content was determined by complete incineration of the sample in a muffle furnace at 550oC for 5 hours.

RESULTS AND DISCUSSION

Direct microscopy of the biofloc water revealed various types of microorganisms, which were observed feeding on the biofloc particles. These included protozoa (e.g. ciliates), Zooplanktons (e.g. Rotifers and copepods) and nematodes (Fig. 1).

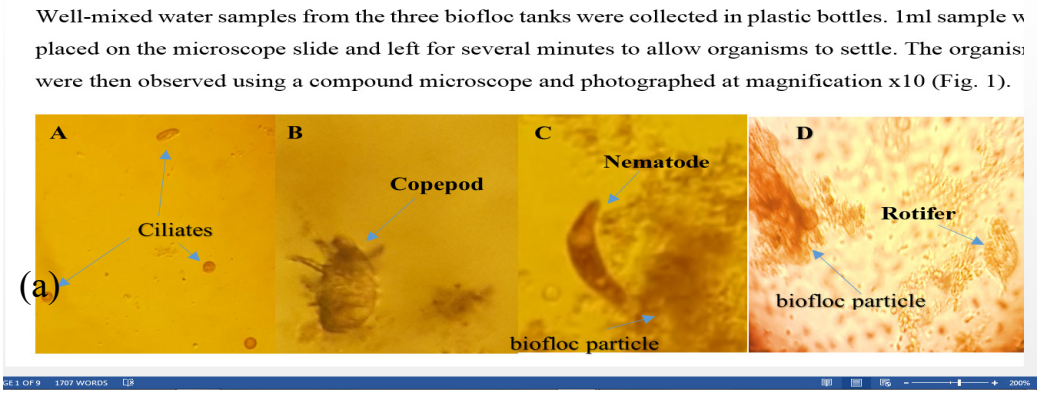


Figure 1: Larger microorganisms observed in the biofloc water: Ciliates (A), Copepod (B), Nematode (C) and Rotifer (D) (10x magnification).

Microscopy is the most common way of identifying the presence and type of microorganisms in a given sample. The present study used it to identify the larger microscopic organisms in the biofloc water and, later on, bacteria and fungi. The analysis was carried out to help understand the biofloc technology system.

Floc particles were observed, as well as floc-associated microorganisms. These were rotifers, nematodes, ciliates and copepods. Rotifers, copepods and nematodes were observed to be grazing on the floc particles. The observed microorganisms are similar to what Azim and Little (2008), Emerenciano et al. (2013), Hussain et al. (2014) and Zablon et al. (2022) found in their studies confirming their presence in biofloc systems. These results give a glimpse of what takes place in the biofloc water community to appreciate the interactions between the microorganisms.

The identified microorganisms implemented the symbiotic processes in the system. Apart from contributing to nutrient recycling, zooplanktons consume bacteria and are known to significantly contribute to the protein and energy requirements of fish (Ray *et al*., 2010; Ibrahim et al., 2015;

Dewil et al, 2020). The floc particles were essentially made up of bacteria (Hussain et al., 2014), which acted as natural water stabilisers in regulating the nutrients in the culture tanks whilst generating microbial protein. The floc grazers also served as decomposers for dead organic matter in the system. The microbial protein was, in turn, fed on directly by the fish, which at the same time also fed on the floc grazers.

Isolation and Identification of Bacteria and Fungi in Biofloc

Different colonies were observed growing on the general media TSA (Fig. 2). The characteristics of the different colonies included dry, flat, irregular, round, shiny and smooth with convex elevation.

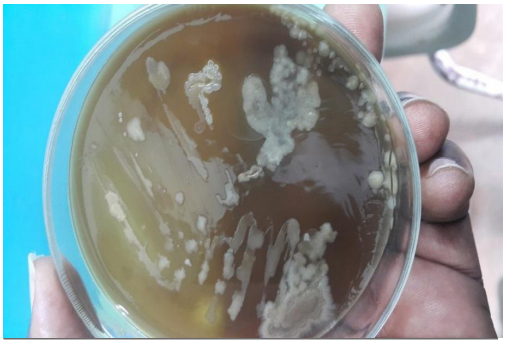
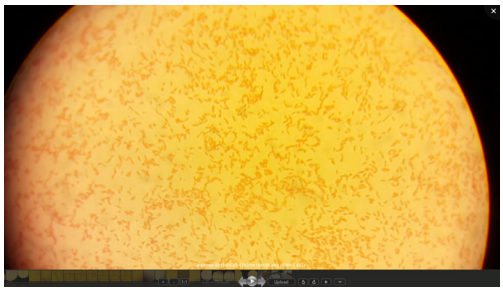
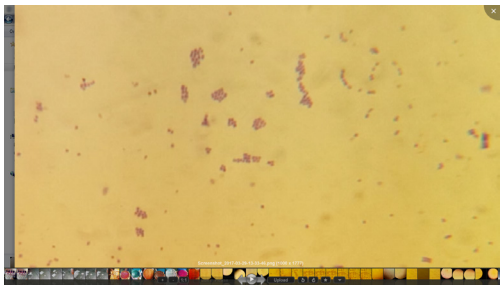


Figure 2: TSA medium plate displaying bacterial growth from biofloc water with different types of colonies.

Representative colonies were picked along the streak line for gram staining. Both gram-negative (rod-shaped) (Fig. 3a) and gram-positive (spherical-shaped) (Fig.3b) were observed using a compound microscope (x100).



(a)

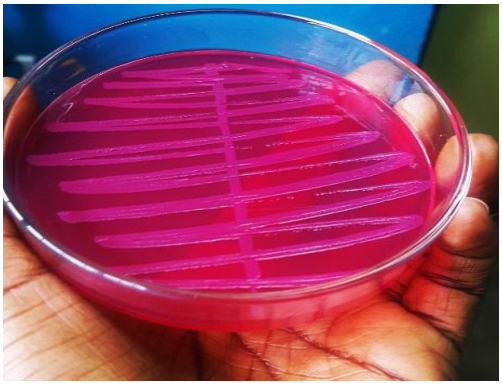


(b)

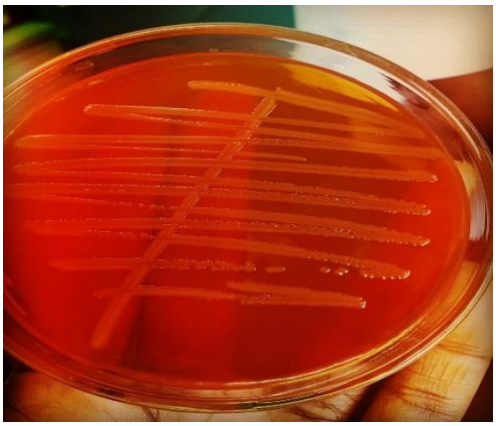
Figure 3: Gram-negative rod-shaped bacteria (a) and gram-positive spherical-shaped bacteria (b)

The gram-negative colonies were sub-cultured on MacConkey medium to check for lactose fermenters. After incubation, a mixed culture of both lactose fermenting (appearing as circular pink colonies) and non-lactose fermenting (appearing to be circular clear colonies) bacteria were observed (Fig 4a). After that, some representative pink and clear colonies were each subcultured on MacConkey to obtain pure cultures of each type (Fig. 4b and 4c, respectively). The pure colonies were also gram-stained and observed under a compound microscope (Magnification X100).

(a)



(b)

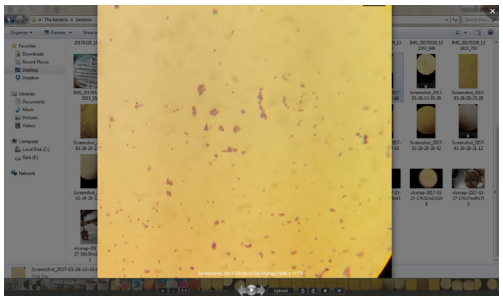


(c)

Figure 4: Mixed culture of both lactose fermenting (pink colonies) and non-lactose fermenting (clear colonies) bacteria (a), pure culture of the lactose fermenters (b) and pure culture of non-lactose fermenters (c).

Gram-positive bacteria were sub-cultured on nutrient agar, and smooth pure white bacteria colonies were observed (Fig. 5a). Samples were picked for gram staining and observed under a compound microscope (x100). Bacteria that were gram-positive and spherical were observed (Fig. 5b).

(a)



(b)

Figure 5: Growth of bacteria on nutrient agar (a) and microscopic view of the stained bacteria from the nutrient agar colonies (b)

Detection of Vibrio spp. of bacteria in the biofloc was done using Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar plates. No growth of bacteria was observed after incubation on the plates (Fig. 6). This indicated the absence of Vibrio spp. of bacteria in the biofloc.

Figure 6: TCBS agar shows no bacteria growth after 24hr incubation at 37oC of biofloc water

Fungi were detected using potato dextrose agar. Growth of fungi was observed (Fig. 7).

Figure 7: PDA plate containing fungal growth after incubation of biofloc water

Representative colonies along the streak on the PDA plates were picked and gram-stained. The fungi observed were gram-positive. Some colonies had yeast cells and hyphae (Fig. 8a), whilst only yeast cells could be observed in others (Fig. 8b).

Figure 8: A combination of yeast cells and hyphae (a) and Yeast cells only (b)

Various kinds of bacteria were detected from biofloc. The characteristics of the colonies were observed, and each type of bacterial colony had a distinctive feature distinguishing them from the rest, although some had common features such as being circular and smooth. Gram staining also distinguished the gram-positive colonies from the gram-negative ones. Catalase reaction and hemolysis of the bacteria on blood agar were also observed in some bacteria. The observed bacteria and the characteristics used in identifying them are summarised in **Table 2**.

Table 2: Observed bacteria from biofloc water and their characteristics

BACTERIA	CHARACTERISTICS USED IN IDENTIFYING
Aeromonas spp.	• Was detectable on TSA as smooth, convex, rounded colonies • Gram-negative, straight rods • Non-lactose fermenting on MacConkey medium (appeared as clear colonies) • β-hemolytic on blood agar • Catalase positive
Enterobacteriaceae spp.	• Colonies appeared pink on MacConkey medium(lactose fermenting) • Gram-negative straight rods • Catalase positive
Pseudomonas spp.	• Detectable on TSA as colonies with convex elevation • Gram-negative rods with some appearing straight and some slightly curved
Lactococcus spp.	• Could not be detected on TSA but grew on nutrient agar • Gram-positive spherical-shaped • Bacteria appearing singly • Catalase negative
Micrococcus spp.	• Detectable on TSA. Colonies appeared circular and smooth • Gram-positive spherical shapes appearing in irregular clusters • Catalase positive

Trypticase Soy Agar (TSA) is a general-purpose media that allows several types of bacteria to be cultured. As described in the results section, the observed TSA culture colonies were diverse and exhibited different characteristics. This showed that there was a variety of bacteria present in the biofloc. From the plate of mixed culture on MacConkey, it could be noted that there were more colonies of lactose fermenting bacteria than the non-lactose fermenting bacteria in the biofloc sample. According to

Kang et al. (1982), most expected lactose fermenters that grow on MacConkey medium are primarily members of *Enterobacteriaceae* and *Pseudomonas* sp. The slight colour difference in the MacConkey medium was due to the difference in the ability of the gram-negative enteric bacteria that grow on the medium to ferment lactose. Waltman (2000) reported that if the bacteria ferment the lactose, the production of acid drops the pH of the media. The drop in pH is indicated by the change of neutral red indicator to pink (neutral red appears pink at pH below 6.8). The non-lactose fermenters appeared colourless on the medium, and since there was no acid production, the agar surrounding the bacteria remained relatively transparent and neutral red. Lactose fermenting bacteria utilise lactose as an organic carbon source to derive energy. This is a typical characteristic of heterotrophic bacteria, and, therefore, the implication of having more lactose fermenters in the biofloc samples proved that most of the bacteria in BFT were heterotrophic in nature, which use organic carbon as a source of energy.

Based on the colony morphology exhibited on TSA (smooth, convex and rounded), the non-lactose fermenters were suspected to be *Aeromonas* spp. To confirm this, a sample from the pure culture plate was subcultured on blood agar to test for hemolysis. After incubation, the growth of bacteria was observed with some evidence of β-hemolysis. This was due to the lysis of red blood cells in the media around and under the area where the bacteria were growing: the area appeared lightened (yellow) and transparent. Additionally, the Catalase (biochemical) test was carried out on the suspected *Aeromonas* spp, giving a positive result. The spherical gram-positive bacteria were found to be of the *Micrococcus* spp and *Lactococcu*s spp. These are well known to have probiotic effects in aquaculture. *Vibrio* spp. of bacteria are well known to be very pathogenic and a major problem in aquaculture, especially in shrimp farming. This pathogen was not detected in the biofloc. The detected *Aeromonas* spp. and some *Enterobacteriaceae* spp. are known to be opportunistic pathogens. However, Monroy-Dosta et al. (2015), reported that microbial communities such as BFT systems have a competitive exclusion effect from the probiotic bacteria against other microbial groups. This is because probiotic bacteria secrete many exoenzymes and polymers, creating a hostile environment for pathogenic bacteria. Emerenciano et al. (2013), also reported that the 'natural probiotic effect' in BFT systems helped reduce pathogenic bacteria and ectoparasites; hence, these are usually not a threat to causing disease in BFT systems. It could be noted that the bacteria discussed above were all heterotrophic bacteria, which confirms their utilisation of organic carbon in the BFT system to produce microbial protein (Avnimelech, 1999; Ekasari et al., 2010).

Yeasts were detected on PDA culture, confirming the presence of fungi in biofloc. Yeasts are unicellular true microbes that behave much like bacteria. It has been shown that yeast

cells retain Gram's Stain more firmly than the common Gram-positive bacteria (Henrici, 1914). Like the heterotrophic bacteria, fungi use organic compounds as a source of energy. The carbon is mostly obtained from hexose sugars such as glucose and fructose (Emerenciano et al., 2017). Monroy-Dosta et al. (2013), reported the presence of fungi (*Rhodotorula* sp.) in their biofloc experiment.

In general, the zooplanktons, protozoans, nematodes, bacteria and fungi all have key roles to play in biofloc technology. The most important roles are recycling organic waste and producing high-value food for fish. The strength of the zero water exchange/ minimal exchange BFT system relies on the dynamic interaction of these microorganisms. These interactions are similar to those that occur in natural water systems (Emerenciano et al., 2017).

Crude protein was found to be 33.07 ± 1.31%. Carbohydrates expressed as Nitrogen-Free Extracts were 28.09 ± 0.83% and the lowest nutritional component of biofloc was found to be crude lipid (3.25 ± 0.13%). The proximate composition of biofloc is presented in Table 3.

Table 3: Biochemical composition of biofloc assessed from the study. (Data are mean ± SEM).

Parameter	BFT	BFT Theoretical values*
Crude protein (%)	33.07 ± 1.31	30.9, 37.93 – 38.41, 41.13
Lipids (%)	3.25 ± 0.13	0.3 – 0.7, 1.03, 1.27, 3.16
Crude fiber %	6.90 ± 0.83	5.71 – 6.27, 12.6, 16.2
Ash (%)	23.53 ± 0.21	11.83 – 13.38, 13.4, 34.5
NFE (%)	28.09 ± 0.83	18 –16, 18.62 – 19, 20.2- 35.7
Moisture (%)	5.08 ± 0.50	-

*BFT Theoretical values derived from Azim and Little (2008), Emerenciano et al. (2++013), Luo *et al*. (2014) and Long et al. (2015). Higher Crude protein values have been reported by Long et al. (2015), Azim and Little (2008) and Widanarni et al. (2012), who reported 41.13%, 37.93–38.41% and 39-48%, respectively. Peiro-Alcantar et al. (2019), reported a similar range of 33.4% to 37.6%**.** Luo et al. (2014), reported a lower value of 30.90 ± 9.04% (on a dry-matter basis). Protein levels in aquaculture feed generally

average 32-38% for tilapia (Abowei and Ekubo, 2011) and, therefore, the value of crude protein found in the present study (33.07 ± 1.31%) indicates that biofloc is a potential suitable source of crude protein for omnivorous and herbivorous fish such as tilapia. Ash content was found to be 23.53 ± 0.21%. Tacon and Maciocci (1988) and Craig and Helfinch (2009), suggested less than 13% of ash as suitable in feed for tilapia. Shirma et al. (2018), reported an ash content of 15.8%. The biofloc sample, in this case, contained more than enough ash content to meet the requirements for tilapia feed. Azim et al. (2007), and Hussain et al. (2014), who also found similar ash content levels, elaborated that the high proportion of ash content in biofloc is related to the presence of acid-insoluble oxides and mixed silicates.

The lipid content of biofloc was 3.25 ± 0.13%. Azim and Little (2008) and El-Sayed (2021), reported similar results of between 3.16 to 3.23%. Luo et al. (2014), and Long et al. (2015), reported even lower lipid content in biofloc, 1.03% and 1.27%, respectively. Azim et al. (2007) found crude lipid levels at 1.6% and 2.6%. The dietary lipid requirement for tilapia is 5-12% (Lim et al., 2009) and Zablon et al. (2022). In this case, some lipid supplementation from commercial feed could be necessary in biofloc systems. Emeranciano et al. (2013), in a review of different studies on biofloc, reported that crude protein, carbohydrates, crude fiber and ash content of biofloc vary considerably. The ranges reported were 12-49% for crude protein, 18-36% for carbohydrates, 0.8-16.2% for crude fiber and 13-46% for ash. The results obtained from the present study nominally fit into the given ranges.

The present study established qualitatively some of the types of microorganisms found in an indoor biofloc technology system, which brought about a deeper understanding and appreciation of how the BFT system functions. Understanding how biofloc microorganisms function is crucial in successfully operating a BFT system. The present study also showed that the nutritional composition of biofloc, which influences the growth performance of the fish, was appropriate for the production of tilapia.

CONCLUSION

Analysis of the microbial composition of biofloc revealed a diversity of the microorganisms present in the BFT system, and all the microorganisms identified were heterotrophs (zooplanktons, nematodes, fungi and bacteria). The biochemical composition of biofloc proved to be fairly nutritious and appropriate for tilapia, but supplementation in lipids from commercial diets may be necessary as lipid levels in biofloc were lower than the recommended levels in fish feed. Further studies are being recommended to determine the amino acid profile of biofloc to appreciate what biofloc might offer, especially in terms of essential amino acid composition.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the following people for their help in successfully implementing the study:

Staff in the Aquaculture and Fisheries Science Department of Lilongwe University of Agriculture and Natural Resources (LUANAR) and the LUANAR Fish Farmworkers. Special thanks go to Ramon Kourie (Former Engineering Manager at Chambo Fisheries) and Joseph Chapman (Former Operations Manager at Chambo Fisheries) for their technical advice, support and encouragement during the study. Our gratitude is also expressed to the NEPAD/SANBio office for providing the water quality checker used in the study. Funding for the study was provided by the EU through the regional Intra-ACP Mobility project (Share Capacity) coordinated by Makerere University.

REFERENCES

AOAC (Association of Official Analytical Chemists), 2002. Methods of Analysis of the association of official analytical chemists. Association of Official Analytical Chemists, Inc., Arlington, USA.

Abowei, J.F.N., and Ekubo, A.T., 2011. Some principles and requirements in fish nutrition. British Journal of Pharmacology and Toxicology, 2(4): 163-179. DOI: 10.1111/j.1749-7345.2007.00145.x

Avnimelech, Y., 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. Aquaculture, 176(3): 227-235. DOI: 10.1016/S0044-8486(99)00085-X

Avnimelech, Y., 2006. Bio-filters: the need for a new comprehensive approach. Aquacultural Engineering, 34(3): 172-178. DOI: 10.1016/j.aquaeng.2005.04.001.

Avnimelech, Y., 2012. Biofloc technology: A Practical guide book, 2nd ed. The World Aquaculture Society, Baton Rouge, LA, USA.

Azim, M.E., and Little, D.C., 2008. The biofloc technology (BFT) in indoor tanks: Water quality, biofloc composition, and growth and welfare of Nile tilapia (Oreochromis niloticus). Aquaculture, 283(1): 29-35. DOI: 10.1016/j.aquaculture.2008.06.036.

Azim, M.E., Little, D.C., Bron, J.E., 2007. Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. Bioresource Technology, 99: 3590-3599. DOI: 10.1016/j.biortech.2007.07.063.

Bossier P, Ekasari J. Biofloc technology application in aquaculture to support sustainable development goals. Microb Biotechnol. 2017 Sep;10(5):1012-1016. DOI: 10.1111 /1751-7915.12836. Epub 2017 Aug 14. PMID: 28941177; PMCID: PMC5609229.

Crab, R., Defoirdt, T., Bossier, P., and Verstraete, W., 2012. Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture, 356: 351 -356. https://doi.org/10.1016/j.aquaculture.2012.04.046

Craig, S., Helfrich, L.A., 2009 Understanding fish nutrition, feeds, and feeding. Virginia State Publication, Cooperative Extension No. 420-256:4, Virginia Polytechnic Institute, USA. : https://pubs.ext.vt.edu/420/420-256.html

De Schryver, P., Crab, R., Defoirdt, T., Boon, N., and Verstraete, W., 2008. The basics of bio-flocs technology: the added value for aquaculture. Aquaculture, 277(3): 125-137. https://doi.org / 10.1016 / j.aquaculture.2012.04.046

Dewi1 E.R.S.; Nugroho A.S and Ulfah M. 2020.Water Quality in the Maintenance of Oreochromis niloticus through Environmentally Friendly Biofloc Technology. IOP Conference

Series: Materials Science and Engineering 835 (2020) 012008 IOP Publishing doi:10.1088/1757-899X/835/1/012008.

Ekasari, J., Crab, R. and Verstraete, W., 2010. Primary nutritional content of bio-flocs cultured with different organic carbon sources and salinity. HAYATI Journal of Biosciences, 17(3): 125. DOI: 10.4308/hjb.17.3.125.

El-Sayed, A.F.M. Use of biofloc technology in shrimp aquaculture: A comprehensive review, with emphasis on the last decade. Rev. Aquac. 2021, 13, 676–705.

Emerenciano, M.G.C., and Cuzon, G., 2013. Biofloc Technology (BFT): A review for aquaculture application and animal food industry. In: Matovic MD (ed) Biomass now - cultivation and utilisation. InTech, Rijeka, Croatia: 301–328. : https://www.intechopen.com/books/biomass-now-cultivation-and-utilisation/biofloc-technology-bft-a-review-for-aquaculture-application-and-animal-food-industry.

Emerenciano, M.G.C., Martínez-Córdova, L.R., Martínez-Porchas, M. and Miranda-Baeza, A., 2017. Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture. In Water Quality. InTech. Rijeka, Croatia: 91-109. https://www.intechopen.com/books/water-quality/biofloc-technology-bft-a-tool-for-water-quality-management-in-aquaculture.

FAO., 2014. The state of world fisheries and aquaculture. ISBN 978-92-5-108275-1. FAO Fisheries Department, Rome.

Hargreaves, J.A., 2013. Biofloc production systems for aquaculture. Southern Regional Aquaculture Center, publication 4503. United States Department of Agriculture, National Institute of Food and Agriculture, Washington, DC.

Henrici, A.T., 1914. The staining of yeasts by Gram's Method. The Journal of Medical Research, 30(3): 409-415. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2094430.

Hussain, A.S., Mohammad, D.A., Ali, E.M. and Sallam, W.S., 2014. Nutrient Optimization for the Production of Microbial Flocs in Suspended Growth Bioreactors. Journal of the Arabian Aquaculture Society, 9 (1): 113-130. https://www.researchgate.net/publication/274311012_ Nutrient_ Optimization_for_the_Production_ links/551acafb0cf2fdce8437c2cd/Nutrient-Optimization-for-the-Production-of-Microbial-Flocs-in-Suspended-Growth-Bioreactors.pdf.

Ibrahim, A.N., Noll, M.S. and Valenti, W.C., 2015. Zooplankton capturing by Nile Tilapia, Oreochromis niloticus (Teleostei: Cichlidae) throughout post-larval development. Zoologia (Curitiba), 32(6): 469-475. DOI:10.1590/s1984-46702015000600006.

Kang, K.S., Veeder, G.T., Mirrasoul, P.J., Kaneko, T., and Cottrell, I.W., 1982. Agar-like polysaccharide produced by a Pseudomonas species: Production and basic properties. Applied and environmental microbiology, 43(5): 1086-1091. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC244190.

Lim, C., Yildirim-Aksoy, M., Li, M.H., Welker, T.L., Klesius, P.H., 2009. Influence of dietary levels of lipid and vitamin E on growth and resistance of Nile tilapia to Streptococcus iniae challenge. Aquaculture, 298: 76-82. DOI: 10.1016/j.aquaculture.2009.09.025.

Long, L., Yang, J., Li, Y., Guan, C. and Wu, F., 2015. Effect of biofloc technology on growth, digestive enzyme activity, hematology, and immune response of genetically improved farmed tilapia (Oreochromis niloticus). Aquaculture,

448: 135-141. DOI: 10.1016/j.aquaculture.2015.05.017.

Luo, G., Gao, Q., Wang, C., Liu, W., Sun, D., Li, L. and Tan, H., 2014. Growth, digestive activity, welfare, and partial cost-effectiveness of genetically improved farmed tilapia (Oreochromis niloticus) cultured in a recirculating aquaculture system and an indoor biofloc system. Aquaculture, 422: 1-7. DOI: 10.1016/j.aquaculture.2013.11.023.

Melaku S., Getahun A, Mengestou S,, Geremew A and Belay A. 2023 Bioflocs Technology in Freshwater Aquaculture: Variations in Carbon Sources and Carbon-to-Nitrogen Ratios In book: Aquaculture Industry - Recent Advances and Applications. Publisher: IntechOpen DOI: 10.5772/intechopen.112529.

Monroy-Dosta, M., Lara-Andrade, D., Castro-Mejía, J., Castro-Mejía, G., Emerenciano M.G.C., 2013. Composition and abundance of microbial communities associated with biofloc in a tilapia culture. Journal of Marine Biology and Oceanography, 48(3): 511–520. DOI: 10.4067/s0718-19572013000300009.

Monroy-Dosta M., Rodríguez-Montes de Oca G., Castro-Mejia J., and Becerril-Cortés D., 2015. Importance and function of microbial communities in aquaculture systems with no water exchange. Scientific Journal of Science, 4:103-110. DOI: 10.14196/sjas.v4i9.1941

Naylor, R.L., Goldburg, R.J., Primavera, J.H., Kautsky, N., Beveridge, M.C., Clay, J., Folke, C., Lubchenco, J., Mooney, H., and Troell, M., 2000. Effect of aquaculture on world fish supplies. Nature, 405(6790): 1017-1024. DOI: 10.1038/35016500.

Naylor R.L., Kishore A., Sumaila U.R., Issifu I., Hunter B.P., Belton B., Crona B. (2021). Blue food demand across geographic and temporal scales. Nat. Commun., 12 (1) (2021), pp. 1-14, 10.1038/s41467-021-25516-4.

Ogello, E.O., Musa, S.F., Aura, C.M., Abwao, J.O., and Munguti, J.M., 2014. An appraisal of the feasibility of tilapia production in ponds using biofloc technology: A review. International Journal of Aquatic Science, 5(1): 21-39. http://www.researchgate.net/publication/259617878.

Peiro-Alcan tar C., Rivas - Vega M.E., Martínez-Porchas M., Lizárraga-Armenta J.A., Miranda-Baeza A., and Martínez-Córdova L.R. 2019. Effect of adding vegetable substrates on Penaeus vannamei pre-grown in biofloc system on shrimp performance, water quality and biofloc composition. Latin American Journal of Aquatic Research [online]. 2019, vol.47, n.5, pp.784-790. ISSN 0718-560X. http://dx.doi.org/10.3856/vol47-issue5-fulltext-7.

Piedrahita, R.H., 2003. Reducing the potential environmental impact of tank aquaculture effluents through intensification and recirculation. Aquaculture, 226(1): 35-44. DOI: 10.1016/S0044-8486(03)00465-4.

Ragasa, C., Charo-Karisa, H., Rurangwa, E. et al. Sustainable aquaculture development in sub-Saharan Africa. Nat Food 3, 92–94 (2022). https://doi.org/10.1038/s43016-022-00467-1.

Ray A.J., Lewis B.L., Browdy C.L., and Leffler J.W., 2010, Suspended solids removal to improve shrimp (Litopenaeus vannamei) production and an evaluation of a plant-based feed in minimal-exchange superintensive culture systems, Aquaculture, 299(1-4): 89-98. DOI: 10.1016/j.aquaculture.2009.11.021.

Sharma A., Sharma B., Singh R., and Sangotra R. (2018). biotic and biochemical composition of biofloc cultured in laboratory conditions. IJCRT Volume 6, Issue 1 ISSN: 2320-2882.

Sharma N., Bania B., Kakati A., and Das P. 2023 Biofloc technology: Innovative approach towards sustainable aquaculture. Biotica. Research Today 5(1):39-42.

Tacon, A.G.J., and Maciocci, G., 1988. The nutrition and feeding of farmed fish and shrimp-A Training Manual.3. Feeding Methods. FAO Field Document. Projet GCP/RLA/075/ ITA, Field Document, 7. Rome.

Waltman, W.D., 2000. Methods for cultural isolation. Salmonella in domestic animals. CAB International, London, UK.

Widanarni, W., Ekasari, J., and Maryam, S., (2012). Evaluation of biofloc technology application on water quality and production performance of Red tilapia Oreochromis sp. cultured at different stocking densities. HAYATI Journal of Biosciences, 19:73–80. https://doi.org/10.4308/hjb.19.2.73.

Zablon, W. O., Ogello, E. O., Getabu, A., and Omondi, R. (2022). Biofloc system improves protein utilisation efficiency and growth performance of Nile tilapia, (Oreochromis niloticus) fry: Experimental evidence. Aquaculture, Fish and Fisheries, 2(2), 94–103.