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Effect of Biofloc on the Growth of *Oreochromis niloticus* and *Pampus argenteus*

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ABSTRACT

In the present world, with almost 7.98 billion population on earth (United Nations, 2022), there is always an increase in demand for aquatic food and hence, expansion and intensification of aquaculture production are highly required (Avinimelech, 2009). Presently, in India, the rate of commercial fish farming is rapidly increasing. Though fish culture is highly profitable, there are some inevitable problems faced by the aquafarmers during culture period. Among various factors which influence the culturing and production of fish, water availability, toxic nitrogenous wastes and diseases caused by various pathogens are the most important. To meet the need of increasing population, various strategies have been developed through different techniques, one of which is Biofloc Technology. In the present study, *Oreochromis niloticus* and *Pampus argenteus* were chosen to study the effect of biofloc on the growth of the fish. Fishes were cultured in three tanks which includes two individual cultures and one mixed culture. The culture was carried out for four months. During this culture period, the water quality parameters such as temperature, *p*H, ammonia, nitrate, nitrite and dissolved oxygen were analysed once in two days. The microbes present in the biofloc and the water samples from the three culture tanks were analysed. In the biofloc different bacterial species which include *Bacillus sp.*, *Klebsiella oxytoca*, *Shigella sp.*, and fungal colonies *Mucor sp.* and *Rhizophus sp.* were identified. In the water samples from the culture tanks bacterial species namely *Bacillus sp.*, *Klebsiella oxytoca*, *Shigella sp.*, *Salmonella paratyphii A, Pseudomonas sp.* and fungal colonies namely *Mucor sp.*, *Aspergillus niger, Penicillium sp.,* and *Saccharomyces cerevisiae*were isolated. The growth, protein content and the fat content were found to be significantly higher in the fishes cultured under biofloc technology when compared to the control fishes (pond fishes). In the experimental groups of fishes, the parameters studied were significantly higher in the mixed culture. The taste and quality of the fishes was also found to be good in the case of biofloc cultured fishes. After the culture, the fishes were harvested and sold in the market. For 4 months culture, there was an income of Rs. 99,250. The investment for this culture was Rs.93,500 for the first culture. For further culturing there will not be any investment for the culture tanks and other facilities required for the culture. Further standardisation of the culture technique would pave the way for sustainable eco-friendly aquaculture which would be highly beneficial for the aqua farmers and the consumers.

Key words *: Biofloc, Oreochromis niloticus, Pampus argenteus, Bacteria, Fungi*

Introduction

Aquaculture is one of the major food production industries that provide protein rich food for the human population which includes the farming of aquatic organisms like fish, molluscs, crustaceans and aquatic plants. The farming also implies some form of intervention in rearing process to enhance production such as regular stocking, feeding and protection from predators (Lucas *et al.*, 2019). The

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primary objective of aquaculture is to produce more aquaculture products and provide equitable cost/benefit ratio without increasing the usage of basic water and land resources (Avnimelech, 2009) and also to develop sustainable system not detrimental to the existing environment (Naylor *et al*., 2000). With almost seven billion people on earth, the demand for aquatic food carries to increase and hence, expansion and intensification of aquaculture production are highly required (Avinimelech, 2009). In India, the rate of commercial fish farming is rapidly increasing because the fishes and products from the fishes have huge demand nowadays. The common fishes that are used for fish culture in India are Carp (Silver, Grass and common), various types of Catfish, Tilapia, Koi, Pomfret, etc. High concentration of protein is required in the feeds of fishes as their energy production depends on the oxidation and the catabolism of the proteins. Supplementary feed forms the major input and plays a decisive role in the freshwater aquaculture. Fish meal acts as a primary source of protein in fish feed mainly for the carnivorous fishes (Olivera *et al*., 2002). Thus, a large quality of protein is supplied to the fishes but only 20-25% protein in the feed is assimilated and the remaining is decomposed by microbes that generates harmful nitrogenous compounds such as ammonia, nitrite and nitrate (Avnimelech and Ritvo, 2003). A fish pellet usually contain protein no less than 25%. The consequence of high feed input in intensive aquaculture system is a high accumulation of ammonia (Brune *et al*. 2003), which is highly toxic for aquatic organism (Stickney 2005). Though fish culture provides more profit there are some problems that are faced during culture. The major issues in aquaculture systems are the inorganic compounds such as ammonia and nitrites which constitute the major excretory materials.

Biofloc Technology has been reviewed to overcome the problems faced in the aquaculture industries. It is considered as "Blue Revolution" in aquaculture. This technique is based on *in situ* microorganism production which plays the major roles such as maintenance of water quality (by up taking the nitrogen compounds), increasing nutrition, and also providing competition with pathogens.

It is an innovative farming technology which potentially exploits the capacity to harness the natural microbial food web, remedy for water quality and also helps in disease management in aquaculture by boosting the innate immunity of the fish (Ahmad *et al*., 2017). The basis of biofloc technology are the bioflocs which are nothing but conglomerates of microbes, algae, protozoa and others together with detritus, dead organic particles. Bioflocs are porous, light and usually have a diameter of 0.1 to few mm (Avnimelech, 2009). Microbial flocs form these bioflocs are rich in nutrient level, thus they provide proteins, fatty acids and ammino acids for the rearing organisms (Burford *et al.*, 2003). Biofloc technology is an eco-friendly process. In recent years, the biofloc technology has gained attention as it is known for high stocking densities with little to no water exchange (Avnimelech, 1999).

The aim of the present was directed to understand the effect of bio floc's on the growth of the fishes and also to standardize the technology to minimize the cost and make it cost efficient. This also involves the investigation on microbes present in biofloc inoculum and the water samples from the culture tanks.

Materials and Methods

Experimental animals and maintenance

In the present studyTilapia (*Oreochromis niloticus*) and Pomfret (*Pampus argenteus*) were chosen to study the effect of biofloc on their growth. At first, 10g of fish fries were released into the culture tanks. The fishes were maintained for 4 months in the culture tanks. Three different tanks were used to maintain three experimental groups of fishes. In culture tank 1 both Tilapia and Freshwater Pomfret were maintained. Culture tank 2 and 3 were used to maintain the Tilapia. In culture tank 2 Tilapia were less densely populated, whereas culture tank 3 different sizes of Tilapia were maintained. The feed was provided to the fishes twice a day. Based on the growth of fishes the feed was increased by 2.5 kg/tank. CP 200 was the fish feed given. The fish feed contains 25-30% protein.

Biofloc inoculum

Biofloc was prepared by mixing the cow dung, cow urine, ghee, milk and curd. It was well fermented along with jaggery and water with help of aerator before adding it into the Culture tanks. Biofloc and probiotics were used in Tanks for the maintenance of culture. The inoculum was added 2 times per week.

Culture tanks

There were 3 Culture Tanks in the study area. The tanks used for the culturing of fishes was covered with PVC Tarpaulin sheet. The rectangular shaped drain tank was constructed deep in the ground. The outlet pipe from each tank was connected individually with drain tank which was used to collect the waste which gets settled in the bottom of the culture tanks. The Culture tanks were provided with proper aeration system. The Culture Tank 1 is mixed culture of *Oreochromis niloticus* and *Pampus argenteus*. Culture Tank 2 and 3 were used to maintain the culture of *Oreochromis niloticus*. In culture tank 1(mixed Culture) 500 fishes were maintained. Whereas inculture tank 2, 900 and culture tank 3, between 600-700 Tilapia were maintained. The population density of fish in culture tank 2 is greater when compared to other two culture tanks. The fishes were maintained for 4 months until the time of harvesting.

Analysis of water quality parameters

The water quality parameters such ammonia, nitrate, nitrite, dissolved oxygen, TDS and pH were measured for nearly 81 days with a time gap of 8 days in between during the culture in all three tanks and were recorded.

Temperature

The temperature is measured using thermometers which can measure from -50 ºC to 110 ºC. The temperature of all the three tanks were recorded. To check the temperature levels in tanks, the thermometers were vertically dipped into the water. The readings are recorded for nearly 81 days, twice a week with 3 days interval.

pH

The pH of the water sample was determined using pH Litmus paper. The level of pH was noted using pH papers in all the tanks, for 81 days, twice a week with 3 days interval. The pH paper was dipped into the water and based on the colour change the pH is determined.

Total dissolved solids (TDS)

The Total Dissolved Solids in all the Culture tanks were measured using TDS meter. The Total Dissolved Solids were measured using TDS meter in all tanks, twice a week with 3 days interval. The TDS meter was dipped into the water for measuring Total Dissolved Solids.

Dissolved oxygen

The dissolved oxygen was recorded for all the three tanks, for 81 days with a time gap of 8 days. The dissolved oxygen levels were estimated by Winkler's method (Carpenter, 1965).

Ammonia

The Ammonia for all the three tanks were noted for 81 days with a time gap of 8 days. The estimation of ammonia levels in culture tanks was carried out by using spectrophotometric method. In this method Nessler's reagent was used for ammonia estimation (Wu, 2013).

Nitrite

Nitrite was checked for 81 days in all three tanks for nearly 81 days with time gap of 8 days. In the spectrophotometric assay for the estimation of nitrite levels, Griess Reagent was used.

Nitrate

The nitrate levels in all three tanks were estimated by diazotization reaction (Buxton, 2011).

Identification of bacteria

Biochemical characterization was done to identify each bacterial species, based on the differences in their biochemical activities exhibited by them. Biochemical characterization of the isolated bacterial strains was carried out by using standard methods (Pelczar *et al.*, 1957). Different biochemical properties of the bacterial isolates such as enzyme activity Indole, Voges Proskauer test, citrate test, ability to produce hydrogen sulphide, were tested by standard methods (Technic *et al.*, 1957). Gram staining was carried out using standard protocol (Wood *et al.*, 1989). Cell motility was determined by observing under microscope. The isolated colonies were identified according to Bergey's Manual of Determinative Bacteriology for each tests such as Indole production test, Methyl Red test, Voges-Proskauer test, Citrate Utilization Test and Triple Sugar Iron Agar Test (Holt *et al.*, 1984, Vos *et al.*, 2009).

Gram's staining and microscopic examination of *Bacilli*

Using Gram's Staining technique (Smith, 2005), the Bacteria *Bacilli* was observed under microscope us-

ing reagents such as Crystal violet, Gram's Iodine.

Microscopic examination of fungi by Lactophenol cotton blue preparation

Identification of fungi from the fungal isolates was carried out by the examination of slides prepared by the Lactophenol cotton blue method (Leck, 1999).

Data analysis

The growth rate of the fish in three different tanks was analysed by using one way-ANOVA.

Results and Discussion

In the present study, three circular culture Tanks 1, 2 and 3 with a sloping bottom were constructed, because they provide uniform water quality and are good for culturing (Jer-Vui lee and Joo-Ling Loo, 2013). The culture tank is 4 meter in diameter and 1.8m in depth (Figure 1 & 2). The volume of the tank is 10,000 liters. The tank is constructed in outdoors

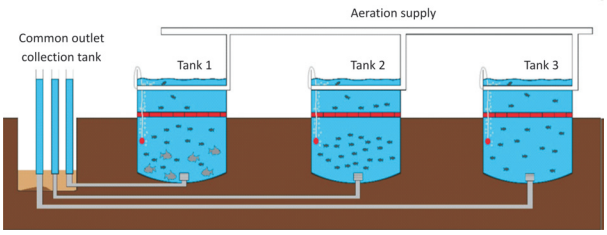

Fig. 1. Schematic representation of the biofloc culture system with aeration system and the outlets collecting the waste into a common tank

Fig. 2. Three culture tanks in the biofloc culture

and 3 feet of the tank is constructed below the ground to maintain the water temperature. The sloping bottom makes it easy to collect the waste sediments and send it to the drain tank through the outlet from each tank. The inorganic waste and sediments were removed and 30% of the water is changed once in eight days to maintain the water quality.

In culture tank 1 mixed culture of Tilapia (*Oreochromis niloticus*) and Pomfret (*Pampus argenteus*) were maintained and in culture tanks 2 and 3 Tilapia with different sizes and low population density were respectively maintained during the culture period for 81 days. These culture tanks were treated with biofloc and the parameters such ammonia, nitrate, nitrite and dissolved oxygen were estimated during the culture period. All the above said parameters tested were in the optimum range throughout the culture period (Figures 3).

Ammonia is the primary nitrogenous waste product excreted by the fishes. In the culture tanks the level of ammonia did not show any significant alterations during the culture period. In tank 1(mixed culture) the levels of ammonia recorded were high when compared to tanks 2 and 3. On the other hand, in tank 3 lowest levels of ammonia were recorded throughout the culture period except for one day. High levels of suspended solids may cause poor growth, fusion of gill lamellae (Mettam, 2005) and susceptibility to bacteria or other parasitic infections (Nobel and Summerfelt, 1996). The solid wastes were removed regularly everyday morning and evening. The raise in concentration of ammonia during the culture on different days may be due to the solid waste or fish excreta. The increase in levels of ammonia concentration may cause fish death.

High concentration of nitrogen can affect the oxygen transport and osmoregulation. The increase in concentration of ammonia can also raise the nitrate concentrations. During high concentrations of nitrate, the fishes were inactive and the decreased feed intake was observed. Nitrite is the innate component of nitrogen cycle and highly toxic. The high concentration degrades water quality, reduces growth and even cause mortality (Lin and Chen, 2003). Dissolved oxygen is an important aspect in the culturing of fish. The dissolved oxygen levels were recorded below 4 ppm in all the three tanks during experimental period. The dissolved oxygen levels are important for the maintenance of the favorable conditions for the heterotrophic bacteria and

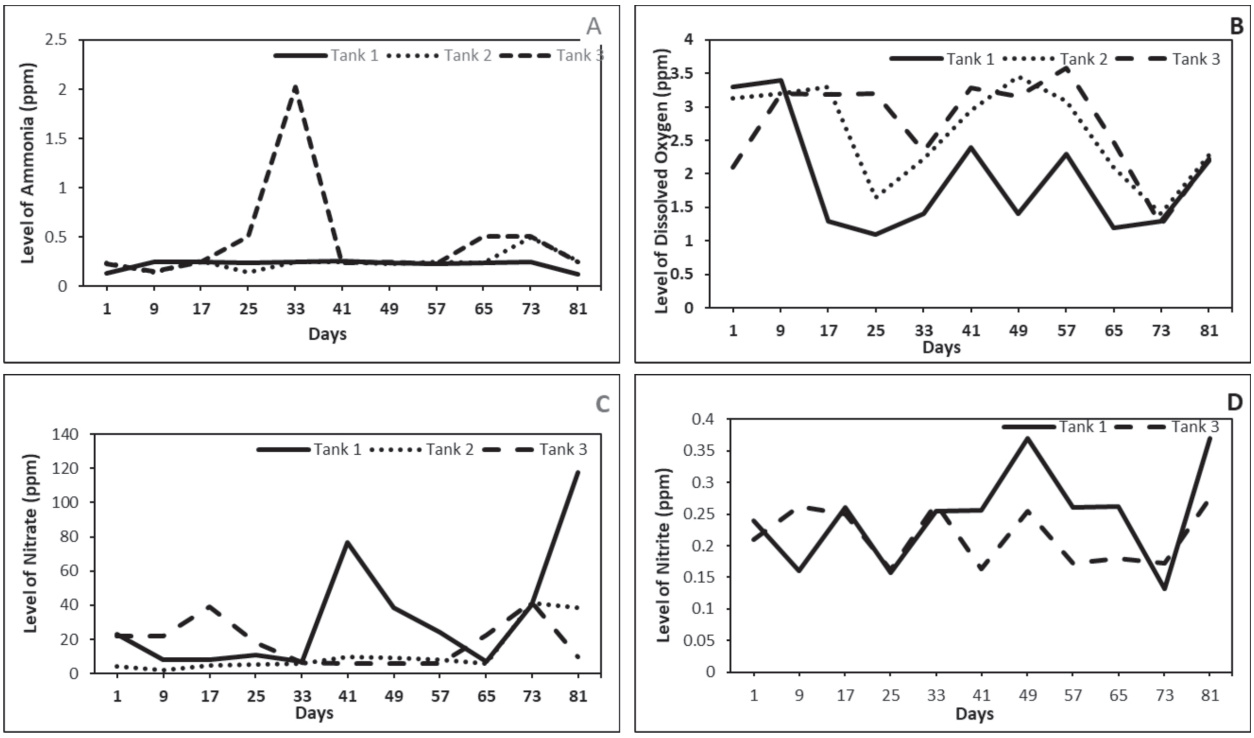

Fig. 3. Levels of Ammonia (A), Dissolved Oxygen (B), Nitrate (C) and of Nitrite (D) in the water samples from the culture tanks1, 2 and 3 during the culture period (81 days).

for the respiration of fishes. The reduced concentration of the dissolved oxygen levels recorded in the three tanks during the culture period at different time points of testing may be due to some undissolved biofloc suspension which consumes nearby dissolved oxygen. The uncleaned aerator ball can also affect dissolved oxygen levels. Zeolite was added whenever the oxygen levels were low. The tilapia is seen on the surface of water during low oxygen levels to get more air. Addition of Biofloc, might also be a reason for the maintenance of dissolved oxygen levels. More amount of feed wasn't required, this is because the fish has been continuously feeding on biofloc in water which may reduce the fish feeding response (Avnimelech, 2007) or the high concentration of suspended solids visually prevent the fish to consume their feed (Azim and Little, 2008).

In the present study, three cultured tanks were treated with biofloc inoculum once in 7 days. The tank 1 is mixed culture (*Oreochromis niloticus* and *Pampus argentus)* thus there will be symbiotic relationship between those two species. In the tank 2 *Orechromis niloticus* (Nile Tilapia) with more population density were cultured. On the other hand, in the tank 3, *Oreochromis niloticus* with less population density were maintained. Some of the bacteria present in these cultures and bio-floc inoculum were isolated using nutrient agar medium and were identified using biochemical tests. Biochemical analyses showed the presence of the bacteria such as *Bacillus sp.*, *Pseudomonas sp.*, *Salmonella paratyphii* A, *Shigella sp.*, *Klebsiella oxytoca.*

Bacillus sp. plays a significant role in nitrogen cycle (Hui *et al*., 2019) and therefore it indirectly maintains the ammonia levels, nitrate levels and nitrite levels. Throughout the culture period, in all the three tanks the ammonia levels, nitrate levels and nitrite levels were normal, this might be due to the presence of this bacterium *Bacillus sp.* which was introduced through biofloc inoculum. During some days of the testing ammonia, nitrate and nitrite levels were high, which might be due to the presence of excretory waste in the tanks. Increase in the concentration of these substances might cause the mortality of fishes. In order to avoid fish mortality, whenever the ammonia, nitrate and nitrite levels were high, biofloc inoculum was introduced into the tanks (Putra *et al*., 2020) which restored the normal levels. It might be due to the presence of *Bacillus sp.*,

and other useful microorganisms in the culture tanks. Nitrite in tank 2 was below the detection levels throughout the culture period.

Success in fish production depends on good oxygen management (Mallya, 2007). During the experimental period whenever there were low levels of dissolved oxygen, Zeolites were added to maintain the levels. Zeolites are a group of crystalline microporous, aluminosilicate minerals with chemically neutral basic formed in a honeycomb like structure. These are used to improve water quality (Abdel-Rahim, 2017).

In bioflocinoculum and culture tank 2, the presence of Enterobacteria such as, *Klebsiella oxytoca* and *Shigella sp.*, was observed. *Klebsiella* is a heterotrophic bacterium that can remove ammonia quickly (Aswiyanti *et al.*, 2021). Usually, the bacterium, *Klebsiella* has the ability to grow at a temperature of 30^0C. Throughout the culture, temperature in culture tank 1 ranged between 30^0C- 31^0C, this may be reason for the growth of this bacterium which plays an important role in nitrogen fixation. Even though *Klebsiella oxytoca* are useful, sometimes they can be pathogenic to the fishes in the culture. The mortality seen in culture tank 2 may be due to the presence of this bacterium *Klebsiella oxytoca*. In culture tank 2 *Shigella sp.* was identified. It is usually found in waters and they are harmful to humans than Tilapia. Further, studies must be conducted to know the role of *Shigella sp* in Tilapia Culture (Alejandro *et al.*, 2021). In the culture tank1, since it was mixed culture, both the fishes i.e., *Oreochromis niloticus* and *Pampus argenteus* has symbiotic relationship thus no diseases and mortality were noted. In culture tank 1, the *Pseudomonas sp.* was also identified. Since it is a denitrifying bacterium, it usually converts the ionic nitrogen into nitrogenous gas into atmosphere (Rajta *et al.*, 2019). Thus, because of the presence of both *Bacillus sp*., and *Pseudomonas sp*., in culture tank 1 the water quality parameters such as ammonia, nitrate and nitrite levels were maintained normal throughout the culture period (Duman *et al.*, 2021). *Salmonella paratyphii A* was identified in culture tanks 1 and 3. Usually, *Salmonella paratyphii A* does not affect any fishes. It might be due to the faecal contamination. This might affect the humans

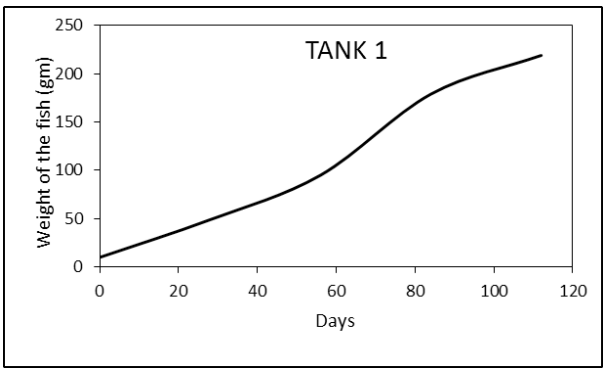

Fig. 4. Growth of the *Oreochromis niloticus* during the culture period under biofloc in the culture tank 1

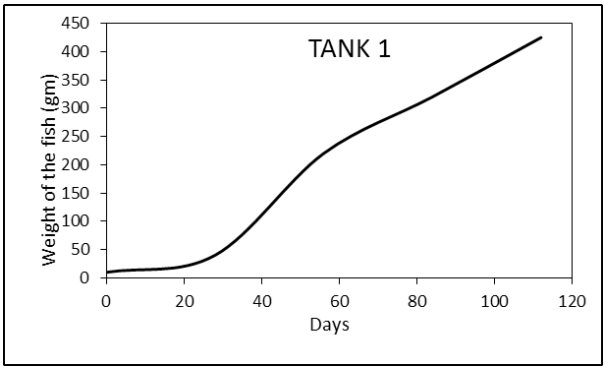

Fig. 5. Growth of the *Pampus argenteus* during the culture period under biofloc in the culture tank 1

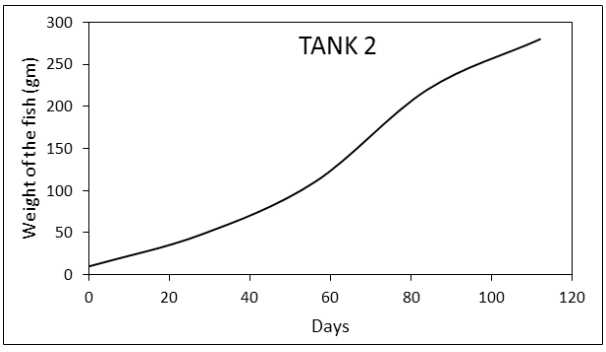

Fig. 6. Growth of the *Oreochromis niloticus* during the cultureperiod under biofloc in the culture tank 2

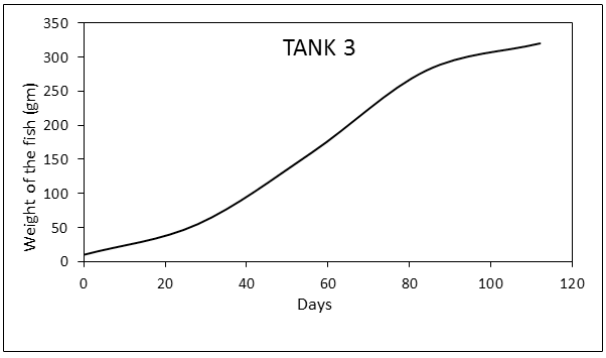

Fig. 7. Growth of the *Oreochromis niloticus* during the culture period under biofloc in the culture tank 3

during consumption. In culture tank 3, since there was only *Bacillus sp.* and *Salmonella paratyphii A*, the fishes were healthy and no mortality was seen during the culture period.

The fungi present in Biofloc Inoculum and Culture Tank 1, 2 & 3 were isolated in dextrose agar medium and identified under microscope using Lactophenol Cotton Blue stain. Totally five fungi species were isolated and identified from the samples. *Mucor sp.,* was seen in both biofloc inoculum and culture tank 1. The presence of *Mucor sp.*, in culture tank 1 is usually due to the fish feed and biofloc inoculum that has been introduced. *Rhizopus sp.* was identified only in Biofloc Inoculum, and not in any Culture tanks. This could be attributed to the lack of favourable conditions in culture tanks for the growth of *Rhizopus sp.* But this species is neither pathogenic nor useful to the fishes. In culture tanks 1 & 3, the fungi *Aspergillus niger* was identified. Some reports say, *Aspergillomycosis*, is principally a disease of tilapia (*Oreochromis niloticus*) caused by *Aspergillus* sp.. These fungal species are infectious through contamination of fish feed. But, in the present study the culture tanks which has this fungi *Aspergillus* doesn't show any infectious symptoms. The presence of *Penicillium sp.* was identified in culture tank 2. Many studies have shown that most feeds have species of the genera *Aspergillus sp.,* and *Penicilliumsp.* as predominant in pelleted feed (Khattak *et al*., 2014). Our results showed the presence of *Aspergillus niger*(culture tank 1 & 3) and *Penicillium sp*. (culture tank 3). In culture tank 2 the presence of *Saccharomyces cerevisiae* was identified which usually grows on food waste. Though it is grown on food waste it is a good protein for the fishes in culture. This fungus usually increases the crude protein level in fishes (Hassaana *et al.,* 2015).

Though the fungi identified in Culture Tank 2 is non-pathogenic, mortality was seen in this Culture Tank 2. The presence of bacteria such as *Klebsiella oxytoca* and *Shigella sp.*, might be the reason for mortality (Yimer, 2000). Since biofloc produces microbes that are useful for maintaining the water quality parameters it provides suitable environment for the growth of fishes and thus the fishes had good growth and were healthy in culture tanks 1 &2. Biofloc in culture tanks reduces the spread and effectiveness of pathogens and thus no pathogenic microbes were seen in all the three culture tanks. The redness around mouth and paleness of eyes were observed in fishes of culture tank 2 would be attributed to the other reasons such as fish lice or presence of some viruses (Khan, 1943).

Though only few microbes that have been identified in the culture tanks, the presence of microbial content and total dissolved solids has the effect on water turbidity and water transparency. In culture tank 1 nearly 6 different species have been identified and since it is a mixed culture there would be some symbiotic relationship between the two species and thus the microbial activities for the culture tanks would have happened, the microbes present in biofloc will absorb accumulated dissolved inorganic nutrients by making the water clear.

The growth rate of fishes was presented in the Figures 4, 5, 6 and 7. The tilapia which grew along with the pomfret were bigger in length, weight and had high growth rate. The significant difference in the growth of the fish among the three culture tanks was observed during the culture period. Fishes in the mixed culture tank showed more significant growth compared with the other two groups. Fishes in the culture tank 3 showed slower growth rate than fish in the culture tank 1, but was higher than the fishes in culture tank 2. Biofloc helps in the growth of the fish and maintaining the water quality.

Table 1. Expenses for Materials used for the construction of Culture Tanks.

Materials Used for Construction (for each tank)	Amount (Rs)
PVC taurpaulin sheets	9000
Pipes used for constructing tanks	2000
Mesh material + bricks	30,000
Air motor connection	7000
Inverter with battery	14,000
Green net	2000
Structure + labour	5000
Ground leveling	2000
Total	72,000

Table 2. Other Expenses During Culture Period (Each Tank)

Materials Used	Amount (Rs)
Elecricity	1000
5 Bags of Food (Each Bag 1500)	7500
Panchagavyam	1500
Probiotics (Biofloc)	1500
Labour Charges	10,000
Total	21,500

Table 3. Income from Biofloc Culture

Fishes Sold From Tank 1 (Both Tilapia And Pomfret- Total 195 Kgs)	= Rs 39,250
Fishes Sold From Tank 2 (Mixed Size Tilapia)	= Rs 30,000
Fishes Sold From Tank Three (Same Size Tilapia)	= Rs 30,000
Total Income From All Three Tanks (For 4 Months Culture)	= Rs 99,250
If, Culture Is Done For 3 Times A Year, Then Income Per Year	= Rs 99,250 X 3 = Rs 2,97,750

The cost for the construction of the culture tanks and the whole experimental setup reached up to Rs.93,500 per tank and Rs. 21,500 was spent for other expenditures (Tables 1 & 2).So the total expenditure was Rs. 2,37,500.The fishes were harvested sold in the market once the culture is over. There was an income of Rs.99,250 from all the culture tanks during the culture period (4 months) (Table 3). If the culture is done four times a year, the probable income per year from the culture would be Rs.2,97,750. Further standardization of biofloc technology to improve the production of cultured fish would pave the way for the ecofriendly sustainable aquaculture.

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Conflict of Interest

The authors declare no conflict of interest.

References

Abdel-Rahim, M.M. 2017. Sustainable Use of Natural Zeolites in Aquaculture: A short Review. *Juniper*. 2(4): 001-006.

Ahmad, I., Babitha Rani, A. M., Verma, A. K. and Maqsood, M. 2017. Biofloc technology: An emerging avenue in aquatic animal healthcare and nutrition. *Aquaculture International.* 25(3): 1215–1226.

Alejandro, De and Espinosa-Chaurand, Luis and García-Barrientos, Raquel and Alejandra, Lorena. 2021. Fish, Tilapia, and Shigellosis: A review. *African Journal of Agricultural Research.* 17: 498-512. 10.5897/AJAR2021.15436.

Aswiyanti, I., Istiqomah, I. and Isnansetyo, A. Isolation and identification of nitrifying bacteria from tilapia (*Oreochromis* sp.) pond in Sleman Yogyakarta Indonesia. 2021. *The 4th International Symposium on Marine and Fisheries Research IOP Conf. Series: Earth and Environmental Science.* 919.

Avnimelech, Y. and Ritvo, G. 2003. Shrimp and fish pond soils: processes and management. *Aquaculture.* 220: 549-567.

Avnimelech, Y. 2007. Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture.* (264) : 140-147.

Avnimelech, Y. 1999. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture.* 176: 227–235.

Avnimelech, Y.2009. Biofloc technology-A practical guide book. The World Aquaculture Society. *Aquaculture.* 176: 227-235.

Azim, M.E., Little, D.C. and Bron, J.E. 2008. Microbial protein production in activated suspension tanks manipulating C:N ratio in feed and the implications for fish culture. *Bioresour Techno.* 99: 3590-3599.

Brune, D.E., Schwartz, G., Eversole, A.G., Collier, J.A., Schwedler, T.E. 2003. Intensification of pond aquaculture and high-rate photosynthetic systems. *Aquac Eng.* 28 : 65-86.

Burford, M.A., Thompson, P.J., Bauman, R.H. and Pearson D.C. 2003. Nutrient and microbial dynamics in high intensive, zero-exchange shrimp ponds in Belize. *Aquaculture.* 219: 393-411.

Buxton, R. 2011. Nitrate and nitrite reduction test protocols.

Carpenter, J.H. 1965. The Chesapeake Bay Institute. Technique for the Winkler oxygen method. *Limnol. Oceanogr.* (10) : 141–143.

Duman, M., Altun, M.M.S., Saticioglu, I.B., Ozdemir, B., Ajmi, N., Lalucet, J. and Valdès, E.G. 2021. The diversity of *Pseudomonas* species isolated from fish farms in Turkey. *Aquaculture* (535).

Hassaana, M.S., Soltanb, M.A., Abdel-Moezc, A.M. 2015. Nutritive value of soybean meal after solid state fermentation with *Saccharomyces cerevisiae* for Nile tilapia, *Oreochromis niloticus*. *Animal Feed Science and Technology*. 201: 89-98.

Hlordzi, V., Kuebutornye, F.K.A., Afriyie, G., Abarike, E.D., Lu, Y., Chi, S. and Anokyewaa, M.A. 2020. The use of *Bacillus* species in maintenance of water qual-

ity in aquculture: A review. *Aquaculture Reports.* (18)

Holt, J. G., Krieg, N. R. and Sneath, P. 1984. *Bergey's Manual of Systematic Bacteriology.* Williams and Wilkins, Baltimore. 4 : 2784-2797.

Hui, C., Wei, R., Jiang, H., Y., Xu, L. 2019. Characterization of the ammonification, the relevant protease production and activity in a high-efficiency ammonifier *Bacillus amyloliquefaciens* DT. *Int. Biodeterior. Biodegredation.* (142): 11-17.

John, S.L., Paul, C.S. and Craig, S.T. 2019. *Aquaculture Farming Aquatics Animals and Plants Aquaculture* (3rd ed.). Wiley Blackwell Publ.1-16

Kasan, N.A., Ghazali, N.A., Ikhwanuddin, M. and Ibrahim Z. 2017. Isolation of Potential Bacteria as Inoculum for Biofloc Formation in Pacific Whiteleg Shrimp, Litopenaeusvannamei Culture Ponds. *Pak J Biol Sci.* 20(6) : 306-313.

Khan, H. 1943. Study in diseases of fish: Infestation of fish with leeches and fish lice. 171-174.

Leck A. 1999. Preparation of lactophenol cotton blue slide mounts**.** *Community Eye Health.* 12(30) : 24.

Lin, Y. and Chen, J. 2003. Acute toxicity of nitrite on Litopenaeusvannamei (Boone) juveniles at different salinity levels. *Aquaculture* (224): 193-201.

Mallya, Y.J. 2007. The effects of dissolved oxygen on fish growth in aquaculture. *Kingolwira National Fish Farming Centre, Fisheries Division Ministry of Natural Resources and Tourism* Tanzania.

Mettam, J., 2005. *An investigation into the use of gill pathologies in rainbow trout (Oncorhynchus mykiss) as a welfare score reflecting water quality*. Thesis, University of Stirling, UK.

Noble, A.C. and Summerfelt, S.T. 1996. Disease encountered in rainbow trout cultured in recirculating system. *Annual Review of Fish Diseases* (6): 65–92.

Olivera Novoa MA, Martinez Palacios CA and Olivera Castillo, L. 2002. Utilization of torula yeast as a protein source in diet for Tilapia (*Oreochromis mossambicus*) fry. *Aquaculture Nutrition.* 8(4): 257-264.

Pelczar, M. J., Technic, C. O. B., Bard, R. and Burnett, G. W. 1957. *Manual of Microbiological Methods.* McGraw-Hill Book Company.

Putra, I., Effendi, I., Lukistyowati, I., Tang, U.M., Fauzi, M., Suharman, I. and Muchlisin, Z.A. 2020. Effect of different biofloc starters on ammonia, nitrate, and nitrite concentrations in the cultured tilapia *Oreochromis niloticus* system. F1000Res.

Rajta, A., Bhatia, R., Setia, H. and Pathania, P. 2019. Role of heterotrophic aerobic denitrifying bacteria in nitrate removal from wastewater. *Journal of Applied Microbiology*. 1364-5072

Smith, A.C. and Hussey, M.A. 2005. Gram Stain Protocols. *American Society for Microbiology.* 1-8.

Stickney, R.R. 2005. *Aquaculture: An Introductory Text.* Cambridge (Mass.): CABI publ.

Technic, C. O. B., Pelczar, M. J., Bard, R. and Burnett, G. W. 1957. *Manual of microbiological methods.* McGraw-Hill Book Company.

Thompson, F.L., Abrea, P.C. and Wasielesky, W. 2002. Importance of biofilm for water quality and nourishment in intensive shrimp culture. *Aquaculture.* 203: 263-278.

Vos, P.D., Garrity, G.M., Jones, D., Kreig, N.R., Ludwig, W., Rainey, F.A, Scleifer, K.H and Whitman, W.B. 2009. *Bergey's Manual of Sysytematic Bacteriology.* 3: The Firmicutes. Springer, Springer-Verlag New York.

Wood, W. A. and Krieg, N. R. 1989. *Methods for General and Molecular Bacteriology.* ASM Press, Washington DC

Wu, H. and Cao, A. 2013. Preparation and Adding Methods of Nessler's Reagent Having Effects on Determination of Water Quality Ammonia Nitrogen. *Advanced Materials Research.* 726-731 : 1362-1366.

Yimer, E. 2000. Preliminary Survey of Parasites and Bacterial Pathogens of Fish at Lake Ziway. *Ethiop. Journal of Science***.** 23(1) : 25-33.