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Comparison of water quality and composition of bioflocs reared in indoor and outdoor conditions

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Abstract

The Optimum fish production is entirely dependent on the physicochemical and biological qualities of water. Therefore, understanding of water quality is required for successful pond management. In the present study, a 60-day experiment was carried out to culture bioflocs in indoor as well as outdoor conditions, with an objective to generate data of their water quality and its impact on the formation of bioflocs. For this, tubs were prepared by filling them with tap water and adding a small amount of cow dung along with pond soil and pond water in order to inoculate them with nitrifying and heterotrophic bacteria as well as phyto-and zooplanktons. Fingerlings of Cyprinus carpio were then introduced in the tubs for the accumulation of nitrogenous waste. Furthermore, molasses was added to the culture water as a cheap carbon source, to kick off the formation of bioflocs. Water analysis was done from time to time for the physico-chemical parameters viz. air temperature, water temperature, pH, DO, FCO_2, ammonia, nitrites and nitrates. Analysis of physico-chemical parameters thus revealed optimum range for fish survival and therefore these can be successfully cultured in biofloc production systems. The data further suggested that the addition of molasses could effectively reduce inorganic nitrogen concentrations (i.e., ammonia and nitrite).

Keywords: Bioflocs, molasses, *Cyprinus carpio*, imhoff cones, ammonia.

Introduction

With almost seven billion people on earth, the demand for aquatic food carries on to increase and hence, expansion and intensification of aquaculture production are highly required to provide aquatic food for future generations without putting much stress on our diminishing land and water resources. Therefore, aquaculture globally has undergone tremendous growth during the last 50 years from a production of less than a million tons in the early 1950s to over 50 million tons during the present times. The growth of the fishes generally depends upon the kind of food, ration, feeding frequency, food intake and its ability to absorb nutrients. Among these, feeding frequency is an important aspect for growth and survival of fish[1]. The fishes should have access to feed upto satiation for their optimum growth. But to the best of our knowledge, only 20-25% of the nitrogen produced from the diet of fish in ponds is actually retained, with the remainder being lost as ammonia and organic nitrogen in faeces and feed residue[2]. Therefore, overfeeding leads to reduction in feed conversion efficiency along with increase in input cost, accumulation of wastes that adversely affects the water quality[3], disease outbreaks and heavy financial losses[4-6]. Owing to this, the aquaculture industry in the recent years has added to environmental degradation as intensive aquaculture coincides with pollution of the culture water by excessive organic material and nutrients that cause acute toxic effects and long term environmental risks[7]. It is due to the environmental concern that more new technologies are required and most efficient among them is Biofloc Technology (BFT) which focuses on a more efficient use of nutrient input with limited or zero water exchange. It is regarded as a resourceful alternative system as the nutrients are continuously recycled within the system and are consumed by culture species. The sustainable approach of this system is based on growth of microorganism in the culture medium, benefited by the minimum or zero water exchange. These microorganisms perform two major roles: i. Maintenance of water quality, by the uptake of nitrogen compounds generating "in situ" microbial protein; ii. reduction in feed conversion ratio and thus feed cost thereby increasing culture feasibility.

Effluent discharge from ponds and tanks contain high concentration of nitrogen and phosphorus that induce algae growth, which may cause severe eutrophication in natural water bodies. As a closed system, BFT has primordial advantage of minimizing the release of water into rivers, lakes and estuaries containing escaped animals, nutrients, organic matter and pathogens. The surrounding areas are also benefitted by the "vertically growth" in terms of productivity, preventing coastal or inland area destruction, induced eutrophication and natural resource losses. The minimum water discharge and reuse of water in BFT convert such system in a real "environmentally friendly system" with a "green" approach by preventing environment degradation[8]. Also, minimum water exchange maintains the heat and fluctuation of temperature is prevented[9], allowing growth of tropical species in cold areas.

Keeping in view the significance of Bioflocs, an attempt has been made to access the water quality of the biofloc production system along with biotic as well as biochemical composition of the bioflocs cultured in indoor and outdoor conditions.

Materials and methods

Procurement and acclimatization of experimental fish: The fingerlings used in the present experiment were of *Cyprinus carpio* which were procured from the fish farm at Doomi, Akhnoor Road, Jammu. For acclimatization, they were kept in tubs for 3-4 days. The dead fingerlings were removed from the tubs from time to time. They were then weighed accurately with the help of weighing balance and segregated according to their weight.

Setting up of experimental tubs and culture of bioflocs: Experimental units were prepared by filling two tubs each having a capacity of 65L, with clean water. One of the two tubs was kept inside under laboratory conditions and the other outside under natural conditions. In order to inoculate them with heterotrophic and nitrifying bacteria, a small amount of cow dung along with some pond soil from the botanical garden was added to the tubs. Pond water was added to expedite the growth of phytoplankton and zooplankton. For the accumulation of nitrogenous waste, each tub was stocked with 10 acclimatized and segregated fingerlings and covered with net to prevent the escape of fingerlings. They were fed twice a day at the rate of 2% body weight with commercial diet consisting of 32% protein and 4% lipid for few days. Same water containing uneaten feed and faecal matter of the fishes was used for the culture of bioflocs. Ammonia level of this waste water was measured regularly using standard methodology and when its level in the tubs increased, locally available cheap carbon source, i.e. molasses was added into the system to maintain C/N ratio for the growth of bioflocs. The amount of organic carbon source to be added was calculated as oer the methodology proposed by Avnimelech[10]. As the molasses contained 50% carbon, hence 20g molasses were used for each 1g of Total Ammonia Nitrogen (TAN) in order to maintain the C/N ratio above 10. Pre-weighed molasses were mixed in a glass beaker with the water taken from the corresponding culture tub and poured directly to the water column. After the addition of molasses and continuous aeration, froth appeared within 2-3 days. During the consequent days, froth disappeared and small suspended particles known as 'Bioflocs', started appearing in the water column. Artificial feed given to the fingerlings was stopped once the bioflocs were formed.

Water quality assessment: Water samples were collected and monitored for various physicochemical parameters, viz. air and water temperature, pH, DO, free CO_2, ammonia, nitrites and nitrates. Air and water temperature was measured in the morning and evening using mercury bulb thermometer. pH was monitored once daily with the help of a portable field pH meter (Hanna). DO and free CO_2 were analysed thrice a week by Winkler's method and Titrimetric method[11] respectively. The concentration of ammonia, nitrites and nitrates were determined twice a week as per the standard methods[11]. Floc volume was measured once a week starting from Week IV using Imhoff cones[12].

Biochemical analysis of Bioflocs: The filtrate containing bioflocs was oven dried at 106˚C and processed for biochemical composition. The protein, lipid, moisture and ash content was determined by standard methods using Lowry *et al.*[13], Folch *et al.*[14], hot air oven and muffle furnance[15] respectively.

Results and discussion

In aquaculture, the water quality plays a very important role for the growth and survival of cultured species as well as bioflocs. Any change in the physicochemical parameters may affect the growth, development and maturity of fish[16]. So regular monitoring of water quality parameters was done during the course of the experiment.

Water quality: Colour: The colour of the culture water was different in both the experimental tubs. The biofloc tub kept outside under natural light (Figure-1) was having green coloured water owing to more algal content due to direct exposure to sunlight. Such systems are also known as 'green water' biofloc system where a complex mixture of algal and bacterial processes controls water quality. The other tub installed indoor (Figure-2), operated as 'brown water' biofloc system since the water had brown colouration and here bacterial processes controlled the water quality. This concept of 'green and brown water' biofloc system is very well explained by Hargreaves[17] and substantiated by Perez–Rostro *et al.*[18] and Choo and Caipang.

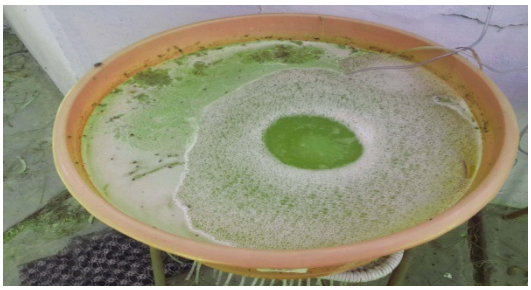

Figure-1: Outdoor tub.

Figure-2: Indoor tub.

Air and water temperature: In both indoor and outdoor tubs, water temperature closely followed air temperature (Figure-3 and 4) and remained high in outdoor conditions (20.5-28.1˚C) and low in the indoor conditions (20.7-30.2˚C). It was observed that the temperature was suitable for carp culture (24-30˚C) as validated by Santhosh and Singh[20].

pH: During the culture period, pH remained constant (alkaline) in both treatment sets for the first 3 week, followed by a slight decline due to addition of molasses for rest of the period (Figure-3 and 4). The weekly pH values were found to be well within the normal range (6.9–8.2) suitable for fish culture as well as biofloc culture. The findings of Santhosh and Singh[20] also indicated that a pH range between 6.7 and 9.5 is suitable for fish culture. However, Chen *et al.*[21] and Furtado *et al.*[22] recommended pH range of 7-9 and 7- 8, respectively for the best performance of nitrifying bacteria. Latter further reported that pH values ranging from 6 to 7 apparently do not cause any significant effects on heterotrophic bacteria metabolism.

DO: DO level fluctuated between 4-8mg/l in both the Treatment sets and this level of DO seems to be favourable for the growth of bioflocs as advocated by Martinez-Cordova *et al.*[23] who stressed that DO levels above 4-5mg/l can be highly suitable for adequate development of bioflocs. DO concentration remained high for the first 3 weeks, thereafter; it showed a decline (Figure-3 and 4) that may be attributable to the addition of molasses for maintaining the C:N ratio. Overall DO concentration was found to be slightly higher in tub kept under indoor conditions and may be ascribed to its lower water temperature. The above observation was analogous to the findings of Kalff[24] who put forward an inverse relationship between oxygen solubility and water temperature.

Free CO_2: During the whole experimental period, free CO_2 level remained high (Figure-3 and 4) due to formation of bioflocs as their growth causes O_2 depletion and consequent increase in free CO_2[25] but was within the desirable limits for fish culture as suggested by Boyd and Lichtkoppler[26], who were of the view that most fish species can survive in waters containing up to 60mg/l carbon dioxide, provided DO concentrations are high and many others have also suggested that fishes can survive at the maximum and minimum value of free CO_2 (9.0-34.0mg/l) recorded presently. Lowest concentration of free CO_2 was observed in Week I and highest in Week VII in both Treatment I and II. This may be due to high DO concentration during this period, justifying inverse relationship between the two. The overall mean free CO_2 concentration showed higher values in outdoor conditions than in indoor conditions.

Ammonia: The ammonia concentration recorded in both the treatments showed a steady increase for the first 3 weeks of the experiment due to the accumulation of excretory waste of fishes. Thereafter, a pattern of continuous decrease and increase in its concentration emerged in the Treatments (Figure-5 and 6). The decline in ammonia concentration may be because of the addition of molasses for the growth of heteretrophic bacteria which effectively remove ammonia-N[27,28,10]. However, decomposition of algal and bacterial cells[29], reduction in nitrification process due to lack of carbohydrate supplementation making bacteria to starve of carbon and die off[10,30] and increase in temperature causing bulking of sludge forming anaerobic zones[31] releasing ammonia along with other toxic compounds[17] may be the reasons of spike in its concentration. It was further observed that overall concentration of ammonia was higher in outdoor than indoor condition due to slightly higher water temperature in the former. During the present experiment, the ammonia concentration was maintained below the toxic level of 1.0mg/l and this level of ammonia supported the production of bioflocs as the microorganisms use this form of nitrogen for their biomass production along with the provided carbon source (molasses). It has been well documented that NH_3 concentration beyond this is highly toxic to aquatic organisms[32,20] while Neori *et al.*[33] postulated that ammonia is toxic to most commercial fishes at concentration above 1.5mg N/L.

Nitrites: In the present experiment, the presence of NO_2–N indicated the occurrence of nitrification process in the culture system. The low concentration of nitrite observed during the present culture period suggested the complete oxidation of ammonia to nitrate[34]. In contrary to this, Lin and Chen[35] observed the accumulation of nitrite in culture medium reaching concentrations higher than safe levels (15.2mg/l) reducing the growth and survival of fish and shrimps. It was also observed (Figure-5 and 6) that the concentration of nitrite followed the same trend as that of ammonia showing higher concentration in outdoor tubs, with steady increase in concentration for first 3 weeks followed by decreasing and increasing trend during rest of the culture period in both the conditions. The decrease in nitrite–N could be due to conversion of nitrite into nitrate during of nitrification as suggested by Liao *et al.*[36] and Kim *et al.*[37]. However, its increase may be due to oxidation of ammonia to nitrite.

Nitrates: Nitrates showed a steady increase in its concentration throughout the culture period for both the Treatments (Figure-5 and 6) but was below the maximum level of 2mg NO_3–N/l, which is considered appropriate for protecting the most sensitive fresh water species[38]. However, it became predominant and continued to accumulate in the culture water. This is the safest form of nitrogen in the aquatic system for the growth of biota therein. In the present sets, increase in the nitrates clearly reflected the activity of microorganisms/ nitrifying bacteria of the bioflocs. Several authors suggested the accumulation of nitrate in low exchange biofloc systems and also confirmed the important role of nitrification in the culture water[36,6,37,17].

Floc volume: Settling solids or 'biofloc volume' is another factor to consider for the management of biofloc based systems. They were managed at levels below 15ml/l[39]. During the present investigation, it was observed that temperature played major

role in the production and stability of the bioflocs as the floc volume showed negative correlation with air and water temperature (Table-1 and 2). Stable flocs i.e. 20-25ml/l were formed at intermediate water temperature (20-25˚C) and at high temperature i.e. 30-35˚C floc volume decreased due to loss of floatability and sinking of flocs to the bottom (Figure-7). It was also observed that floc volume showed negative correlation with DO and positive with FCO_2 under indoor condition and vice-versa, because in indoor tub algal content was less[17], hence DO production was low and whatever DO was available, it was subsequently consumed by the bacteria. However, in outdoor conditions DO production was much more than its consumption due to high algal growth in natural light. Furthermore, floc volume showed negative correlation with ammonia in both the conditions because with the increase in temperature, sinking of the flocs occur leading to release of ammonia along with other toxic compounds[17].

Biotic composition of bioflocs: The water sample from the culture system was collected in the imhoff cones and allowed to settle for 15 minutes. The filterate when seen under the compound microscope showed the aggregates of heterotrophic bacteria, algae, entangled zooplankton including protozoa, rotifers, diatoms, uneaten feed and other dead organic matter, ultimately resulting in the formation of suspended particles known as bioflocs. Outdoor bioflocs were found to be green in colour, dominated by algae (Figure-8a,b,c) whereas the indoor bioflocs were brown in colour and were dominated by zooplanktons (Figure-9a,b,c).

Biochemical composition of bioflocs: Biochemical composition of bioflocs (Table-3) in the indoor condition revealed 34% protein, 12.6% lipids, 31% moisture and 15.2% ash whereas in outdoor conditions the composition of proteins, lipids, moisture and ash was 32%, 11.8%, 37% and 16%, respectively. This falls in line with Ju *et al*.[40], who reported that the variation in proximate composition of bioflocs mainly depend upon the living component of biofloc and found that chlorophyll dominated bioflocs contain 26-34% protein and bacteria containing bioflocs contain 38% protein.

Conclusion

The results of the present study showed that physicochemical parameters of water were in the optimum range required for the proper growth of bioflocs as well as the fingerlings. In the present experimental studies, the bioflocs cultured in in-door conditions in the absence of sunlight were brown in colour and mainly dominated by animal protein while green bioflocs reared in sunlight (outdoor conditions) mainly composed of plant proteins as also suggested by Hargreaves[17]. The latter have less digestibility and are less beneficial as compared to animal protein[41]. During the course of experiment, the fingerlings were cultured simultaneously along with the bioflocs, that acted as feed for them. Hence, the source of nitrogen for the growth of bioflocs was the faecal matter of the fishes which was found to be effectively recycled by proper maintenance of the water quality of biofloc production system.

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Table-1: Correlation coefficient (r) between various physico-chemical parameters in Treatment I (Indoor condition).

Parameters	Air temp.	Water temp.	pH	DO	Free CO_2	Ammonia	Nitrite	Nitrate	FV
Air temp.	-								
Water temp.	0.979	-							
pH	-0.96	-0.942	-						
DO	-0.67	-0.628	0.826	-					
Free CO_2	0.529	0.591	-0.699	-0.839	-				
Ammonia	0.823	0.88	-0.796	-0.466	0.605	-			
Nitrite	0.888	0.889	-0.783	-0.415	0.354	0.846	-		
Nitrate	0.855	0.864	-0.787	-0.414	0.395	0.861	0.966	-	
FV	-0.886	-0.94	0.836	-0.587	0.224	-0.944	-0.953	-0.953	-

Table-2: Correlation coefficient (r) between various physico-chemical parameters in Treatment II (Outdoor condition).

Parameters	Air temp.	Water temp.	pH	DO	Free CO_2	Ammonia	Nitrite	Nitrate	FV
Air temp.	-								
Water temp.	0.952	-							
pH	-0.887	-0.933	-						
DO	-0.806	-0.837	0.918	-					
Free CO2	0.695	0.776	-0.685	-0.736	-				
Ammonia	0.667	0.782	-0.627	-0.673	0.931	-			
Nitrite	0.927	0.866	-0.739	-0.747	0.666	0.698	-		
Nitrate	0.943	0.833	-0.699	-0.649	0.649	0.622	0.953	-	
FV	-0.92	-0.998	0.899	0.519	-0.461	-0.619	-0.99	-0.929	-

Table-3: Biochemical composition of bioflocs.

Biochemical composition	Indoor condition	Outdoor condition
Protein	34%	32%
Lipid	12.6%	11.8%
Moisture	31%	37%
Ash	15.2%	16%

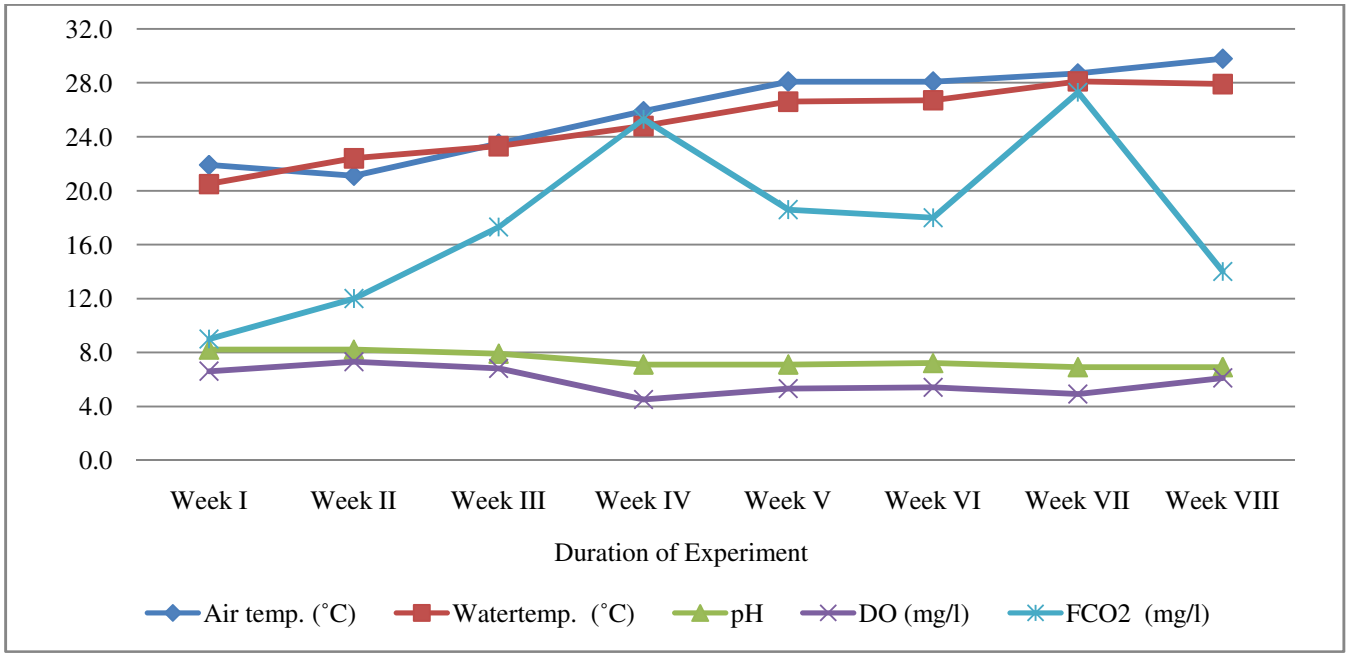

Figure-3: Weekly variations in some physico-chemical parameters in indoor conditions (Treatment I).

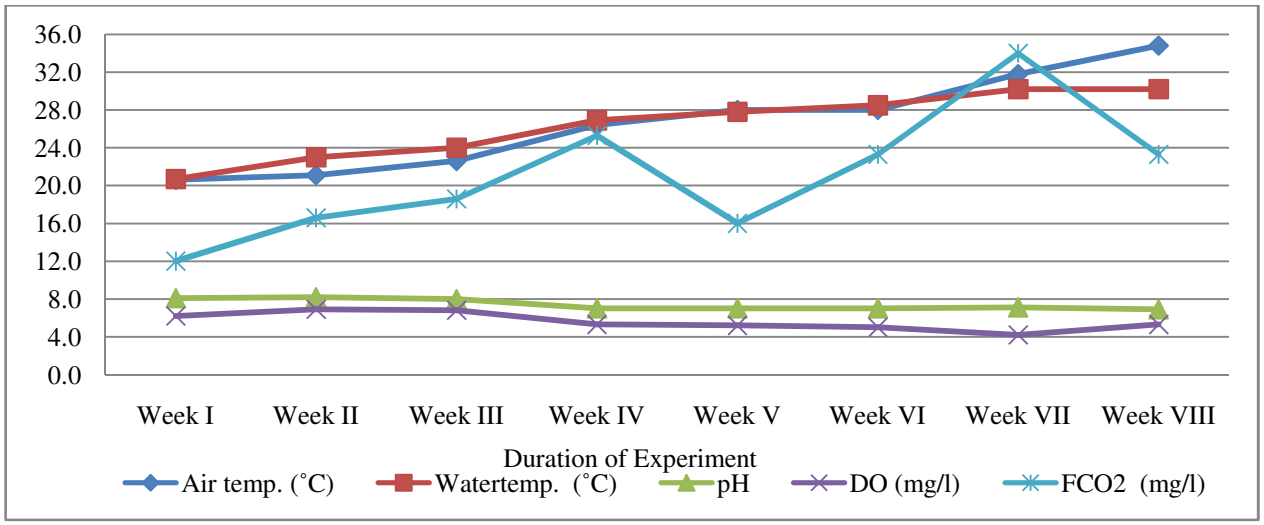

Figure-4: Weekly variations in some physico-chemical parameters in outdoor conditions (Treatment II).

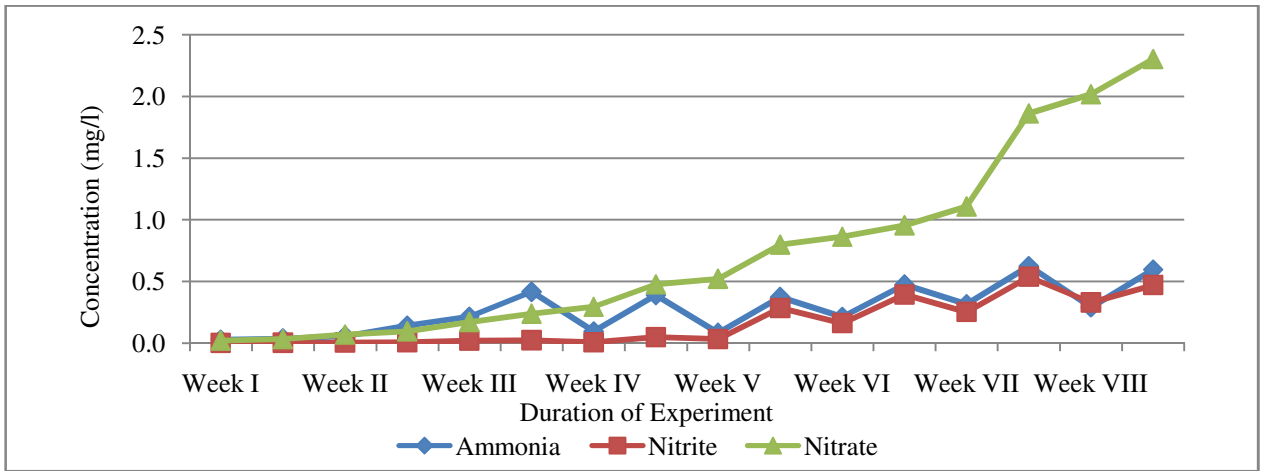

Figure-5: Weekly variations in concentration of ammonia, nitrite and nitrate in indoor conditions in Treatment I.

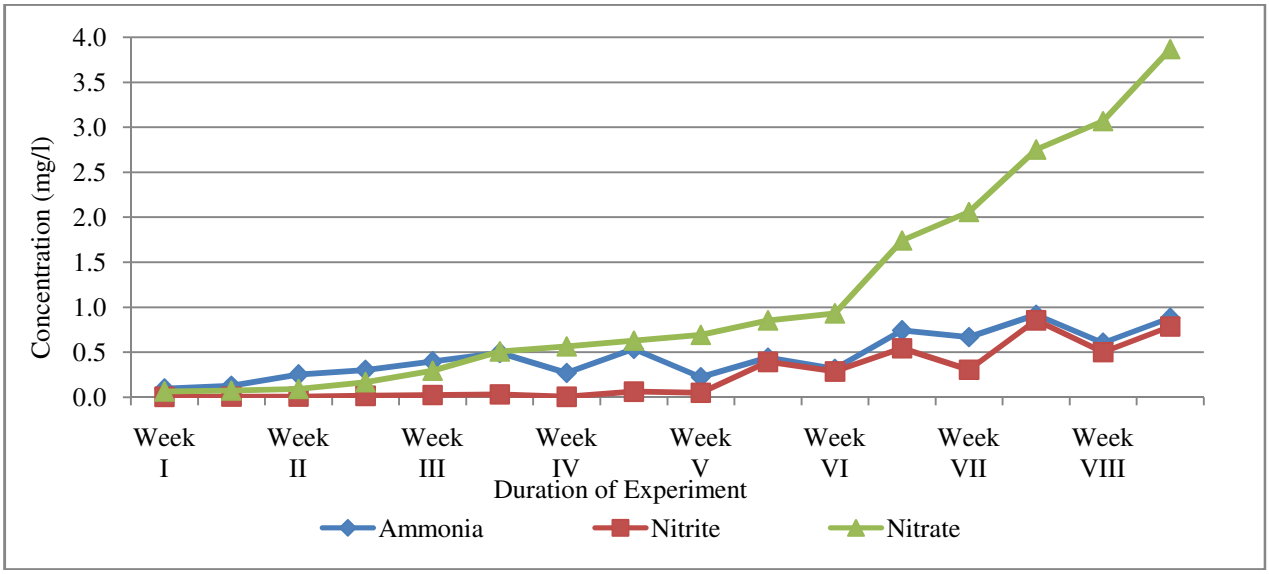

Figure-6: Weekly variations in concentration of ammonia, nitrite and nitrate in outdoor conditions in Treatment I.

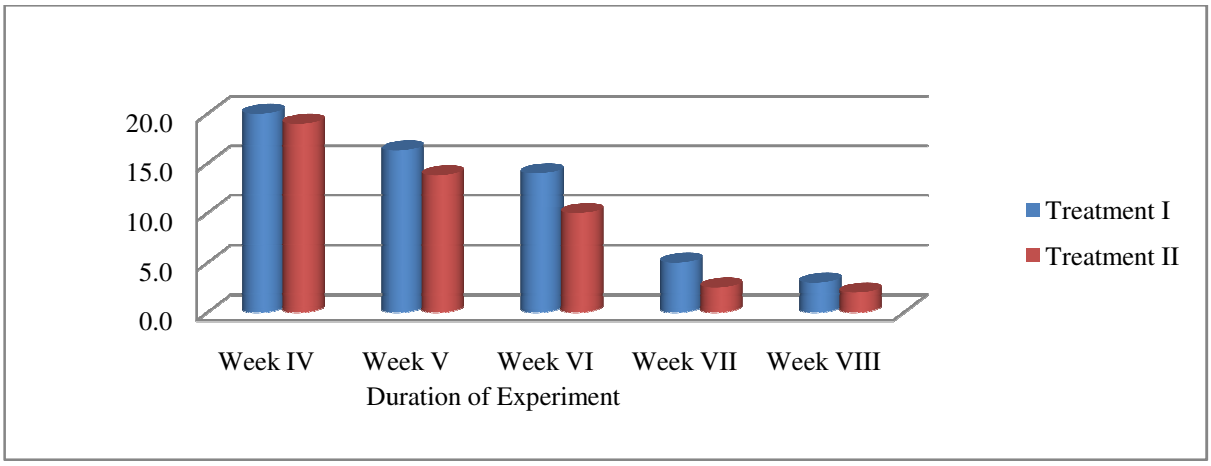

Figure-7: Weekly variations in Floc volume (ml/l) in different Treatment sets.

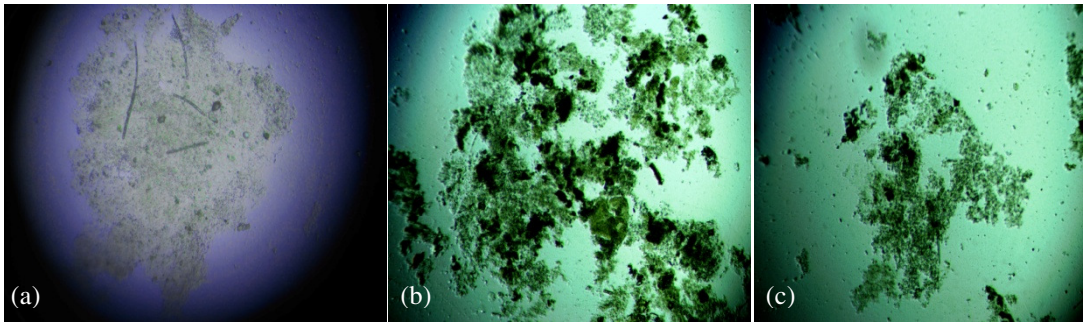

Figure-8(a,b,c): Microscopic view of bioflocs cultured in outdoor conditions.

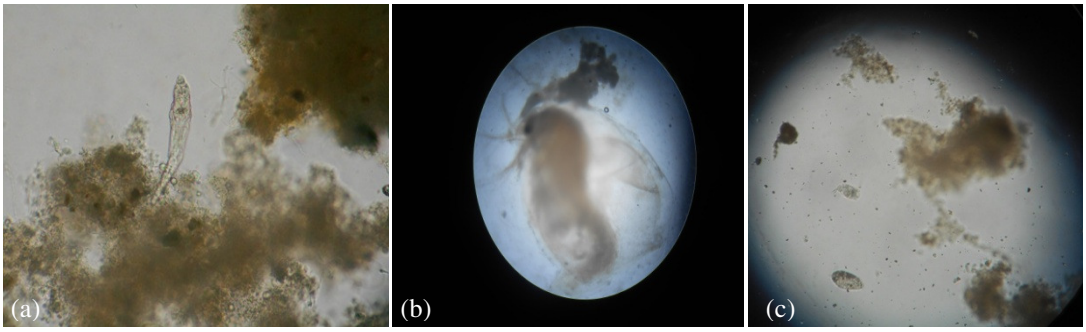

Figure-9(a,b,c): Microscopic view of bioflocs cultured in laboratory conditions.

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