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Proximate composition, nutritional properties and attractiveness of Aerobic, Anaerobic and Anoxic bioflocs as a fish feed

Esther Wairimu Magondu, Verdegem MCJ, J Nyamora, Kobingi Nyakeya

Abstract
This study involved testing use of bioflocs made under aerobic, anaerobic and anoxic conditions as a fish feed. Previously made bioflocs were administered to Nile tilapia larvae while video recording the feeding behavior. The main goal was to compare the attractiveness of the different types of biofloc to fish. Proximate and nutritional compositions of the bioflocs were analyzed and fish growth on diets containing bioflocs was recorded. The experiment was carried out in 12 glass aquaria each with 30 one month old tilapia fish. Feeding behaviors observed included approaching, eating and nibbling parameters. Approaching time was higher in anaerobic and anoxic treatments as compared to aerobic and control ($P<0.05$). Time invested in eating was higher in aerobic and control treatments as compared to anaerobic and anoxic while nibbling time was higher in control treatment as compared to other treatments ($P<0.05$). Anaerobic flocs had the highest crude protein, crude fat and volatile fatty acids values. Fish growth from the floc feed was evident with a specific growth rate of 5 % but not as in control which had a specific growth rate of 10 %.

Keywords: Bioflocs, Aerobic, Anaerobic, Anoxic

1. Introduction

Fish feed accounts for over 50% of the total cost of fish production [1]. Sustainability of the aquaculture industry therefore depends on the source of feeds and management. Previous studies have shown that microbial flocs produced in biological reactors can be used as an alternative cheap protein source in fish feed pellets [2].

In most instances farmed fish are fed pelleted feed that provide a balanced diet with the aim to achieve optimum growth rates. Fish diets often contain fishmeal and fish oil, resources presently over exploited but preferred for their optimal nutritional quality [3]. Bio-floc based diets eaten by fish provide a supplementary protein rich low cost feed. This happens when the microbial community develops forming a mixture of microorganisms consisting of bacteria, colloids, particles polymers and dead cells [4, 5]. The formed bioflocs can be consumed as a food source by the cultured fish thereby creating a nutrient recycling process within the culture system [6].

It was showed that the nutritional composition of bioflocs makes it potentially cheap feed ingredient contributing to the production of healthy and high quality products [7]. In aquaculture the average diets used comprise of ash (<8.5%), lipid (10–25%), phosphorus (<1.5%), carbohydrate (15–20%), water (<10%), protein (18– 50%) and trace amounts of minerals and vitamins [1]. Thus the nutritional composition of produced flocs should be compared with these values if they have to benefit the cultured species. Protein, lipid content and polyunsaturated fatty acid (PUFA) are considered most important parameters in determining the viability of the bio-flocs as feed in aquaculture [5]. In addition to the nutritional value of the bio-flocs other internal compounds may also be beneficial to the cultured species.

Current research should therefore mainly focus on the nutritional quality and microbial composition of bioflocs, maximizing their energy content and digestibility for the aquaculture species [5]. It has been reported that in ponds fish and shrimps have been known to avoid areas of reduced sediments and look for food in sediments with rich oxygen supply [7]. It has not been known yet whether these aquatic animals don't like flocs produced in low or reduced oxygen conditions or is it that they cannot reach the food because of low oxygen levels.

In answering these questions, the current study investigated the proximate composition, nutritional properties and attractiveness of bioflocs produced from aerobic, anaerobic and anoxic systems as a fish diet. We hypothesized that the oxic state under which bioflocs are produced affect the composition of the flocs, since microbial community composition changes depending on oxygen availability. It is expected that this approach will provide valuable information on the possibility to use bioflocs to minimize the environmental impacts from aquaculture while benefiting the cultured organisms.

2. Materials and methods
2.1 Study area
The experiments were conducted at experimental facility ("De Haar Visen") of the Wageningen University. All procedures involving fish were carried out in accordance with the Dutch law of experimental animals, approved by the Ethical committee for animal experiments (DEC) of Wageningen University, The Netherlands.

2.2 Experimental fish
A red phenotype strain of Nile tilapia (*Oreochromis niloticus*), purchased from a commercial tilapia farm, Til-Aqua, Velden, The Netherlands was used for (sludge production) [8]. Three hundred 72 g tilapia were stocked in 7 glass tanks equipped with sludge collectors for sludge collection. 360 juvenile 1 g Nile tilapia (*Oreochromis niloticus*) of a normal colored strain were used for feeding experiment.

2.3 Experimental design
The reactors were operated under aerobic, anaerobic and anoxic conditions with three replicates per condition. A 45% crude protein feed was fed to 20 kg adult tilapia with the aim of producing sludge. The latter was collected daily and transferred to 9 incubators: 3 were operated aerobically, 3 anaerobically and 3 anoxically [8]. The incubators were run continuously during the study period to provide bioflocs for nutritional studies with the juvenile fish. One month old tilapia juveniles grown previously on a commercial starter diet were fed pure floc pellets in a completely randomized design. Thirty larvae fish were randomly assigned to four treatment groups; aerobic, anaerobic, anoxic and control (Table 1). Feeding events were recorded on video for a period of 7 days and feeding behavior compared between treatments.

Table 1: Order of feeding experimental units

	Tank											
Number	1	2	3	4	5	6	7	8	9	10	11	12
Treatment*	Ana	Ano	Con	Ana	Aer	Ano	Con	Ana	Aer	Ano	Aer	Con

*Aer=Aerobic; Ana=Anaerobic; Ano=Anoxic; Con=Control

2.4 Experimental conditions
Mixed sex tilapia (*Oreochromis niloticus)* larvae of approximately 1 g body weight each were weighed and stocked in 20 l glass aquariums (Figure 1) and left to acclimatize for two days before introduction of the treatment frozen floc diets. The daily measured water quality parameters stayed within the required range: water temperature (25-26.5 °C), pH (8.0- 8.5), dissolved oxygen (6.5–7.0), NO_3-N (10-20) mg/l, NO_2-N <0.2 mg/l and TAN < 0.4mg/l. Thirty fish occupied the whole aquarium and were observed to swim actively and randomly in all directions.

Fig 1: Diagram showing larvae stocked in glass aquarium and camera set up for recording feeding behavior.

3. Feed and feeding
Ice pellets (Figure 2) of flocs prepared under aerobic, anaerobic and anoxic conditions and a commercial feed were used as test diets. Five-mm frozen pellets were prepared after harvesting the bioflocs from the 9 reactors at the end of the first experiment [8]. For ease of feeding and recording a trial was first conducted to find out the most appropriate way of introducing the flocs into the aquariums. Either oven drying overnight at 60°C or use of ice cubes were tested. Floc ice cubes proved better since it was possible for fish to eat them with ease before melting in water and the feeding behavior was visible with video recording. Feeding was done 3 times a day. The feeding behavior was recorded on video 2 times to judge the attractiveness of the flocs to the fish. This was by introducing 1 ice pellet per aquarium. One restricted feeding of 1.6% body weight per day with a commercial diet was administered to all the treatment tanks at 1700hrs. After each floc feeding all uneaten feeds were removed by siphoning. Fish in control tanks were fed at 5% body weight per day divided in 3 feeding periods per day.

Fig 2: Ice floc pellets used for the feeding experiment. (From top, aerobic, anaerobic and anoxic).

3.1 Experimental System
Twelve 40 X 29 X 24 cm glass aquariums were maintained at a water volume of 19.2 l of water and a total of 30 larvae fish. Aeration supply facilities with diffuser stones were placed on one edge of the glass aquarium in order to prevent possible effects on the images captured from the rearing tank and any influence on fish behaviour. Water was replenished through a

RAS system at a flow rate of 1.35 l/min in every aquarium. Images were acquired by a computer vision system using the observer XT 9.0 software package (Noldus, Wageningen the Netherlands) consisting of a black and white video camera. A wooden flame was made to hold the cameras in position with the camera facing one side of the aquarium (Figure 1). Image size was 720 x 576 pixels for the frame width and height respectively.

3.2 Behavioral test on attractiveness.

Testing was performed between 0800-0900hrs and 1300-1400hrs. Fishes were observed 1 minute before feeding started followed by introduction of ice floc pellet and recording for 40 minutes. After the seven days, 168 recorded videos were digitally transferred to the computer using windows media player and files stored on external hard drives for later replay and analysis. Behavior of a single fish is highly random so aggregated behavior activity of the 30 fish in each tank was studied. The reaction of the fish to the floc pellet was analyzed by hand at tank level following the ethogram (catalogue of different behavior patterns; Table 2). This was by doing a 10 minutes scan sampling of every video. For testing attractiveness the time spent on the activity patterns was expressed as absolute values for further statistical analyses.

Table 2: Ethogram used to categorize feeding behaviour responses.

Behavior element	Event	description
Approaching time in seconds	State	After introducing the ice floc pellet, time elapsed between the immersion of the pellet in water and start of fish eating. (Fish either first or cautiously approached the pellet).
Eating time in seconds	State	If floc pellet consumption occurred, time spent during the feed intake process. (Fish is seen eating the floc).
Nibbling time in seconds	State	When the ice floc pellet melted, time spent by the fish taking small amounts of the left over or melted ice floc. (Fish is seen making attempts to eat from the disintegrated floc).

3.3 Biofloc proximate composition

Proximate composition analysis of crude protein, crude fat, dry matter and ash content of the experimental diets and biofloc samples were performed by the standard methods of [9] Crude protein was determined by measuring nitrogen using Kjeldal method and multiplying by the international protein factor of 6.25. Crude fat was determined by ether extraction using soxhlet and bentrop procedures. Dry matter was determined by oven drying at 102 °C for 4 hours until stable weight and crude ash by combustion at 550 °C for 3 hours until stable weight.

3.4 Biofloc nutritional properties

Volatile Fatty acid analysis was done by a modified direct methylation method [10] using an N-Evap 112 Liquid Gas Chromatograph and according to protocols of Animal Nutrition Aquaculture and Fisheries laboratory. Minerals were analyzed by (ICP-AES) Inductively coupled plasma atomic emission spectrophotometry.

3.5 Fish growth comparison

Fish growth was compared among the treatments fed with aerobic, anaerobic and anoxic bioflocs and control. To measure the fish growth, all the fish from the different treatments were weighed after 7 days of feeding. At the end of the experiment all fish were harvested and weighed up to the nearest 0.1 g. Specific growth rate (% body weight day^{-1}), geometric mean body weight and metabolic growth rate were determined.

Specific growth rate

SGR= (lnWTF−lnWTI)* 100/T where WTF=average final fish weight (g), WTI=average initial fish weight (g), T=duration of the experiment (days).

Geometric mean body weight (Wg)

Geometric mean body weight was calculated to determine the estimate for the body weight of the fish at the middle of the experiment period.

Wg=e ((lnWTF + lnWTI)/2)

Metabolic growth (RGRm)

Metabolic growth was calculated to determine the growth achieved by the fish after utilizing the available food to generate energy for metabolism during the experiment period. This formula was used **RGRm= (WTF-WTI) /Wg$^{0.8}$/T.**

3.6 Data Analysis and Statistics

Results on attractiveness studies were analyzed by ANOVA with repeated measures with treatments as the main factors and day and day period as sub factors. For both analyses day period was nested in day. Comparison of fish growth in the different treatments was done by one way ANOVA. Results of proximate and nutritional composition (minerals and volatile fatty acids) were presented in table format. These results were end points of the floc material that was used to prepare the flocs fed to the fish. Prior to ANOVA data was checked for normality using Shapiro-Wilk test and homogeneity of variance using Mauchly's sphericity test for repeated measures and Levene’s test for one way ANOVA. The significance level was set at (P < 0.05). Means were compared by Tukey test. Statistical analysis was performed using SPSS version 18.

4. Results

4.1 Biofloc attractiveness test

Mean values of three measurements that determined attractiveness of the floc to the fish from the four different treatments and outcomes of ANOVA are shown in Table 3. The activities that defined attractiveness were approaching, eating and nibbling. There was a significant interaction effect among the treatments and sampling time as shown in the graphs for all the feeding related activities.

Table 3: Comparison of feeding related activity among four treatments and time based on two way repeated measures ANOVA. Units are in seconds. * Day factor not included in the table. The mean values followed by the different superscript letter within factor indicate significant difference at (P<0.05) a>b>c. If the effects were significant, ANOVA was followed by Tukey test. *P<0.05; ** P<0.01; ***P<0.001

Activity	Means Tukey test						
	Treatment				Day Period		Interaction
	Aerobic	Anaerobic	Anoxic	Control	Morning	Afternoon	Trt X day *
Approaching	5.26^{b}	12.45^{a}	13.76^{a}	2.97^{b}	8.87	8.36	**
Eating	109.52^{a}	88.05^{b}	91.97^{b}	108.74^{a}	98.51	100.63	**
Nibbling	97.19^{bc}	97.09^{bc}	81.12^{d}	113.88^{a}	96.35	98.29	**

4.1.1 Approaching
In this study the mean approaching time of the fish to the floc in aerobic and control was significantly different from anaerobic and anoxic treatments ($P<0.05$). Fish in anoxic and anaerobic treatments took the longest time to approach the floc as shown in Table 3 while fish in aerobic and control took the shortest time. The approaching time for all the treatments decreased gradually ($P < 0.05$) with the number of days as the fish got used to the flocs (Figure 3). Results of ANOVA did not show significant differences between morning and afternoon feedings (when sampling was done).

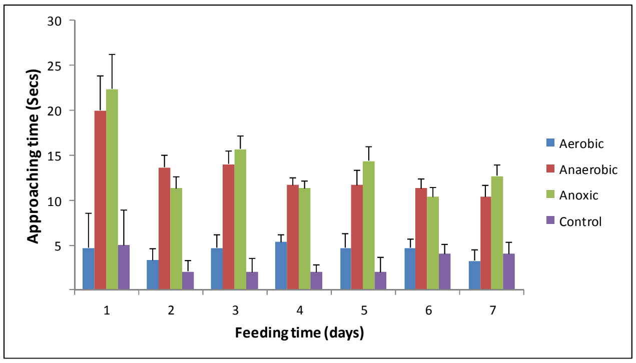

Fig 3: Mean approaching time of the fish to the floc in the different treatments over the 7 days feeding period. Error bars represent the standard error.

4.1.2 Eating
The mean floc eating time in aerobic and control was significantly different from anaerobic and anoxic treatments ($P<0.05$). Fish in the aerobic and control treatments took more time eating the floc while those in anaerobic and anoxic treatments took less time as shown in (Table 3). The time taken eating was higher during the first days of feeding and gradually decreased as time progressed (Figure 4). Results of ANOVA did not show significant differences in the time of the day when the sampling was done.

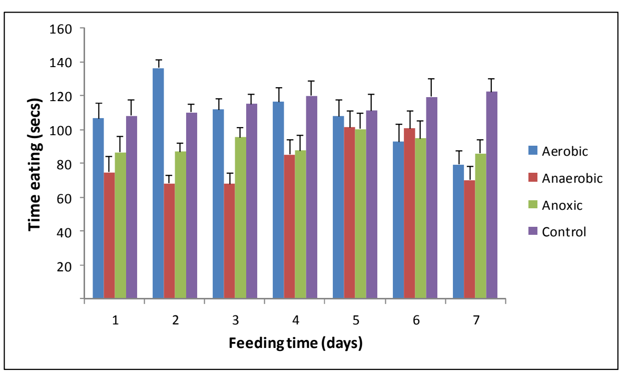

Fig 4: Mean floc eating time in the different treatments over the 7 days feeding period. Error bars represent the standard error.

4.1.3 Nibbling
There was no significant difference in the mean nibbling time between the aerobic and anaerobic treatments ($P>0.05$). Fish in the control treatment took on average 114 seconds nibbling the feed (Table 3) which was significantly higher than the 81 seconds nibbling observed in the anoxic treatments ($P<0.05$). The time spent nibbling was not constant over time as shown (Figure 5). Results of ANOVA did not show significant differences between the times of the day when feeding were recorded.

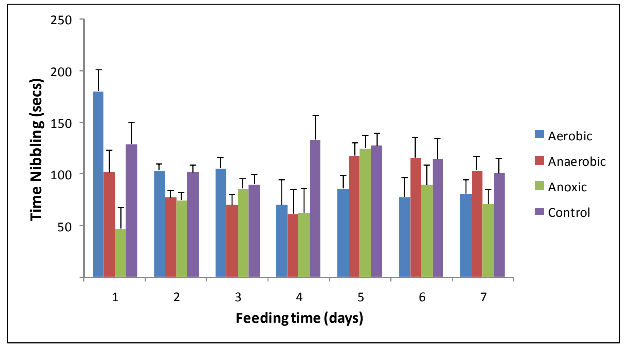

Fig 5: Mean floc nibbling time in the different treatments over the 7 days feeding period. Error bars represent the standard error.

4.2 Proximate composition of the flocs
Mean values on dry matter basis of the proximate analysis done on the different treatments are presented in (Table 4). Fresh sludge results were from a cumulative sample collected during the three weeks of sludge incubation. The floc treatment results were from pooled samples collected during floc harvesting. Fresh sludge had the highest dry matter content of 32.04 g/kg and anaerobic the lowest of 15.52 g/kg. Anoxic bioflocs had the highest ash content of 473.21 g/kg and fresh sludge the lowest of 224.74 g/kg. Anaerobic bioflocs had the highest crude protein value of 216.56 g/kg and fresh sludge the lowest of 129.08 g/kg. Anaerobic bioflocs had the highest fat content of 42.86 g/kg while fresh sludge and aerobic had the lowest concentrations of 19.47 and 19.46 g/kg, respectively. Carbohydrate values were calculated by subtracting values of ash, crude protein and fat from 1000. Fresh sludge had the highest carbohydrate content of 626.71 g while anaerobic and anoxic sludge contained 313.7 g and 313.31 g, respectively. Table 5 shows the calculated yield of bioflocs at harvest from the different treatments. The values were calculated by multiplying the proximate composition value with the volume of the sample used for proximate analysis (0.53 liters). During the sludge incubation experiment one pure feed was used to feed the adult fish. One control commercial larvae feed was used in the feeding experiment. The mean values of the proximate composition for both feeds are also shown in Table 4.

Table 4: Proximate composition on dry matter basis of fresh sludge, bioflocs from the different treatments and fish feeds used in the experiment. 3 g dry matter of sludge was added to the reactors everyday hence for a number of 22 days, a total of 66 g dry matter was added. Efficiency of the reactors was therefore calculated as (output/input x 100) aerobic 22.3 %, anaerobic 12.3 % and anoxic 21.1 %.

Treatment	DM (g/kg)	Ash(g/kg dm)	Cp g/kg dm	Fat g/kg dm	Carbohydrate g/kg dm
Fresh sludge	32.02	224.74	129.08	19.47	626.71
Aerobic	28.27	463.72	193.24	19.46	323.57
Anaerobic	15.52	426.87	216.56	42.86	313.70
Anoxic	26.85	473.21	191.36	22.12	313.31
Control feed	877.90	102.37	641.06	161.66	94.91
Adult fish feed	965.66	77.95	422.71	77.86	421.49

Table 5: Yield of bioflocs (g) from the different treatments at harvest.

Treatment	DM (g)	Ash (g)	Cp (g)	Fat (g)
Aerobic	14.70	241.13	100.48	10.11
Anaerobic	8.07	221.97	112.61	22.26
Anoxic	13.96	246.06	99.50	11.50

4.3 Biofloc nutritional properties

In this study biofloc nutritional properties were assessed from analyses of mineral composition and volatile fatty acids.

4.3.1 Minerals

The results of mineral composition are shown in Table 6. Total organic carbon (TOC) and N-NH4 content was 682 mg/l in anoxic bioflocs and lowest with 452 mg/l in anaerobic bioflocs with aerobic bioflocs and fresh sludge being in the middle range. N-($NO_3 + NO_2$), total nitrogen (Nts) and orthophosphate (P-PO_4) content was high in the fresh sludge and lowest in anaerobic bioflocs with aerobic bioflocs and anoxic bioflocs being in the middle range. Ammonia is the available form that is usually utilized for bacteria synthesis [11]. It was observed that a bacteria cell yield of 0.12 mg biomass /mg NH_4^+-N in flow reactors [12]. Based on this information the calculated bacteria yield in the different treatments in the present study would be; Aerobic 2.3 mg biomass/mg NH_4^+-N, anaerobic 1.6 mg biomass/ NH_4^+-N, anoxic 2.4 mg biomass/ NH_4^+-N.

Table 6: Composition of minerals in fresh sludge, aerobic, anaerobic and anoxic bioflocs.

Treatment	TOC(mg/l)	N-NH_4 (mg/l)	N-(NO_3 +NO_2) (mg/l)	Nts (mg/l)	P-PO_4 (mg/l)	Organic N (mg/l)
Fresh sludge	669	16.1	1042	1089	113	30.9
Aerobic	623	19.4	20.3	125	13.6	85.3
Anaerobic	452	13.3	0.22	57.9	13.3	44.4
Anoxic	682	20.1	15.9	128	29	92

4.3.2 Volatile fatty acids

The results of volatile fatty acids composition are shown in Table 7. Analyses gave the values for acetic acid, propionic acid and valeric acid in the treatments. All other values indicated by <0.1 were below detection limit. Fresh sludge had the highest values of acetic acid 3.51 milli-moles /liter while aerobic bioflocs had the lowest value of 0.48 milli-moles /liter, with anaerobic and anoxic bioflocs in between.

The calculated yield of acetic acid at harvest was 109.2mg/l in fresh, 15.6 mg/l in aerobic, 33.8 mg/l in anaerobic and 20.8 mg/l in anoxic sludge.

Table 7: Composition of volatile fatty acids in fresh sludge, aerobic, anaerobic and anoxic bioflocs.

Treatment	Acetic acid mM/L	Propionic acid mM/L	Iso butyric acid mM/L	Butyric mM/L	Iso Valeric acid mM/L	Valeric acid mM/L
Fresh sludge	3.5067	0.14	<0.1	<0.1	<0.1	0.1
Aerobic	0.4840	<0.1	<0.1	<0.1	<0.1	<0.1
Anaerobic	1.0886	<0.1	<0.1	<0.1	<0.1	<0.1
Anoxic	0.6796	<0.1	<0.1	<0.1	<0.1	<0.1

4.4 Fish growth

Seven days growth was measured from a total of 30 fish from all the 12 tanks. Mean values of the four treatment feeds fed to the fish and outcomes of ANOVA are shown in Table 8. The results showed an increase in weight gain in all the treatments; however weight gain in the control treatment was significantly different from the three biofloc treatments ($P< 0.05$). Within the floc treatments aerobic treatment had the highest mean of 9.76 g which was not significantly different from the anaerobic 8.3 g and anoxic 8.4 g. The specific growth rate, geometric mean body weight and metabolic growth were higher in the control experiment and significantly different from the three biofloc treatments ($P<0.05$).

Table 8: Effects of different treatments (with control) on growth of tilapia juveniles based on one way ANOVA. The mean values followed by the different superscript letter within factor indicate significant difference at ($P<0.05$) a>b. If the effects were significant, ANOVA was followed by Tukey test. *$P<0.05$;**$P<0.01$; ***$P<0.001$.

Growth parameters	Means Turkey test			
	Reactors			
30 fish per treatment	Aerobic	Anaerobic	Anoxic	Control
Stocking weight (g)	20.63	20.6	21.53	19.26
Harvest weight (g)	30.4^b	28.9^b	29.96^b	39.26^a
Weight gain (g)	9.76^b	8.3^b	8.4^b	20.1^a
Specific growth rate (%bwd-1)	5.55^b	4.84^b	4.72^b	10.17^a
Geometric mean body weight (g)	0.83^b	0.81^b	0.84^{ab}	0.91^a
Metabolic growth (g bw/d/kg^0.8)	13.6^b	12^b	11.3^b	25.6^a

5. Discussion

5.1 Proximate analysis and assessment of bioflocs

The nutritional benefits of bioflocs as a natural food source for tilapia are still under investigation. Previous studies with shrimps used bioflocs at different inclusion levels in the feed [2]. In ponds, active suspension systems have been studied showing the ability of tilapia to harvest microbial flocs from the culture water hence doubling utilization of feed and protein by the fish [13]. It has been pointed out that harvesting of bioflocs and subsequent use as an alternative protein source can potentially reduce the amount of marine meal required in aquaculture diets [14]. Very few details have been shown on bioflocs formed under aerobic, anaerobic and anoxic conditions more so their components, their ability to be manipulated and engineered and application as a food source in fish culture systems. Bioflocs can contain high levels of crude proteins and crude fats [15]. Proximate analysis of biofloc from the current study indicates the presence of 21.6 % crude protein, 4.2% crude fat and 42.6 % crude ash in the anaerobic bioflocs which was higher than for the other treatments (Table 4). This proximate composition profile (though from a reduced state) is comparable with the work of [15] who studying biofloc composition from tilapia found crude protein levels of 38 %, crude lipids 3% and crude ash 12%. The high protein and fat concentration in anaerobic bioflocs may be related to the chemical composition of anaerobic bacteria and other micro-organisms associated to bioflocs and biofilms. Dry matter and carbohydrate contents were high in the fresh sludge possibly because fresh sludge did not go through the mechanical breakdown by stirring as compared to the three floc treatments. Anaerobic reactors had the lowest dry matter content with might have been attributed to anaerobic digestion that led to production of volatile fatty acids. The composition of sludge carbohydrate was influenced by the state characteristics with anaerobic and anoxic reactors having the lowest CH concentration. This could probably be due to uptake by bacteria during the biochemical reactions that were taking place in the reactors. Ash content was generally high in the three treatments because of the organic carbon accumulation in the reactors. Ash was higher in the anoxic bioflocs which might have been one of the reasons for high mineral contents and presence of acid insoluble oxides [16].

5.2 Nutritional value of bioflocs

Bioflocs have been shown to contain nutrients such as fatty acids and amino acids, vitamins and minerals [15, 17]. This has been shown to contribute to fish growth by an average of 4 to 67% in lab scale trials with shrimp. Thus manipulation of microbial flocs not only provides an option for disposing of bioflocs as part of normal suspended growth in biological operations, but also provides a sustainable alternative as a fish feed. In this study anoxic bioflocs had the highest mineral content among the biofloc treatment which agrees with the high ash content in the anoxic proximate composition. Anaerobic bioflocs had lowest mineral composition which might have been as a result of nitrogen containing compounds released by dead bacteria and higher life forms. In anaerobic biological treatments two macro nutrients; ammonium-nitrogen (NH_4^+-N) and phosphorous-orthophosphate (P-HPO_4^-) are available to bacteria in soluble form [18]. Anoxic reactors had high levels of P-PO_4 as compared to oxic and anaerobic reactors. This might have been because of high nitrate concentration a well-known inhibitor of phosphorus release in anoxic systems due to its uptake by denitrifying phosphorous reducing bacteria [19]. The high levels of total nitrogen (Nts) and phosphates in the analyzed fresh sludge samples could also be associated with the large amounts of waste in form of TAN that originated from the uneaten feeds and feces in the collected sludge. Occurrence of anaerobic digestion with denitrification might have contributed to the low total organic carbon in anaerobic treatments. The low inorganic nitrogen values recorded in anaerobic treatments might have been a result of denitrification, the most probable reduction pathway of nitrogenous compounds in such systems [20]. In aerobic and anoxic reactors higher organic carbon and total nitrogen concentrations were observed probably due to ammonia oxidation to nitrite. Waste waters have been shown to contain high concentrations of volatile fatty acids which concur with the high acetic acid and propionic acid findings in the present study. Comparing the three floc treatments in this study anaerobic bioflocs had the highest volatile fatty acids content of 1.09 milli-moles per liter. The presence of fatty acids is associated with degradation of animal fat *(*triglyceride) and death of bacteria [18]. In addition phospholipids released after death of bacteria serve as surface active agents that favor foam production which dissipates as the concentration of volatile fatty acids goes down. The biological treatments under anaerobic conditions, phosphate accumulating organisms (PAOs) use the energy released from the hydrolysis of intracellular polyphosphate to transport VFA (mainly acetic acid) across their cell membranes producing polyhydroxyalkonates (PHA) and polyhydroxybutyrate (PHB) (21). It has been shown that apart from supplying nutrients to fish, microorganisms in the flocs also exert positive effects on digestive enzymes activity and gut microflora [22].

5.3. Floc feed attractiveness

5.3.1 Approaching

The observed low mean time taken by the fish to approach the floc in the aerobic and control treatments in this study might have been because the fish liked the smell of the feed more as compared to the bioflocs from the anaerobic and anoxic treatments. Fish might have also approached the flocs out of curiosity which was determined with the percentage of fish that went ahead eating as observed from the video. The decreasing trend of approaching the treatment feed by the fish with the number of feeding days might have been caused by lack of interest by the fish as it got used to the flocs fed.

5.3.2 Eating

Fish in the aerobic and control treatments took longer time eating the feed as compared to the anoxic and anaerobic treatments. This observation shows that the fish liked the aerobic and control feeds as compared to the anaerobic and anoxic feeds. From the fifth day of feeding a decrease in eating activity was observed which could also be related to the fish reduced interest in the bioflocs apart from the fish in the control experiment. It is deliberated on the reason behind fish and shrimps that are known to avoid areas with reduced sediments and search for food in oxygen rich sediments [7]. The floc attractiveness test showed that at least fish spent some time either approaching, eating or nibbling on the flocs from the three states with aerobic treatment performing better then anaerobic and finally anoxic. In addition to the floc feed fish were also given a normal diet at the end of the day for maintance purposes and supply of nutrients that were lacking in the floc feed. However the growth data aimed at assessing the attractiveness of the flocs showed a significant increase in weight gain from the three floc treatments. In a pond situation it can thus be inferred that fish and shrimps actually like the flocs under reduced conditions only that they cannot reach them because of insufficient oxygen levels in the sediment. Reports show that available oxygen in the sediment water interface does not penetrate deeper than 1 to 2 mm in intensive and semi-intensive fish pond sediments [23]. Furthermore ponds are shallow so processes that occur across the sediment water interface will have a greater impact on water quality due to accumulation of ammonia and inorganic phosphorus. Decomposition of organic matter at the sediment–water interface and respiration in the water column reduces dissolved oxygen concentration near the pond bottom [24].

5.3.3 Nibbling

The observed high mean time taken by the fish in the control experiment nibbling the feed might be because the feed was palatable and hence more interest to feed on all the feed particles in the water. There was comparatively less nibbling time taken by fish in the other treatments which might be because of smell or taste of the feed, with fish in the anoxic treatments taking the least mean nibbling time of 81 seconds.

5.4 Fish growth with floc diet

The average fish growth among the growth parameters during the feeding experiment were higher in the control as compared to the biofloc treatments which was as a consequence of rich nutrient supply in control feed. Since the preceding nutrients before the experiment were constant and not limiting in the control and experimental diets the differences in growth when the biofloc feed was introduced is probably not due to any of these nutrients. Fish in all the treatments responded to feed application by jumping towards the floc and control feed and feeding on it as seen from the attractiveness test. This led `to a significant increase in weight at harvest in all the treatments.

In culture systems, together with microbial flocs acting as a feed also do play some important ecological roles. The deterioration of water quality due to unconsumed feed, fecal matter of cultured organisms or the presence of other organic matter in culture facilities is nullified because the floc microbes act as conditioner for water. These always control excess nitrogen. The subsequent uptake of nitrogen from the water facilitated synthesis of microbial protein. Hence biofloc based aquaculture system also offers potential to use as zero exchange recirculation aquaculture system [4]. In biofloc, 10-

90% has been found to be living things, which serve as feed for cultured organism simulating natural environment and hence there is an increment in production. So biofloc based aquaculture system can be compared with estuarine ecosystem which are considered to be the world's most productive system because these flocs are essentially the same as the suspended detritus and planktonic organisms of nutrient rich estuaries [25]. Probiotic bacteria in the microbial floc continuously surround the stocked fish or shrimp and provide natural disease prevention and control [14]. Effect of metals in biofloc technology was not studied in the present study. However it has been shown that presence of calcium in biofloc protects the cultured species against heavy metal toxicity [5].
Bioflocs grown under aerobic conditions have been used as a natural food source in shellfish and finfish aquaculture systems and has shown promise for reducing feed costs and achievement of sustainable aquaculture [25, 28, 29]. It has previously been demonstrated that microbial biomass is used as a food source by hybrid tilapia, *Oreochromis niloticus Oreochromis aureus*, and by shrimp, *Penaeus monodon*, in aquaculture systems. The microbial protein supplied by the stocked fish biomass was enough to supplement the protein provided by the fish feed [25]. The role of the floc in food utilization by growing tilapia can be answered by analyzing which species or groups of microbes in floc contribute most to the nutritive value of the flocs. Therefore an important step toward understanding and utilizing microbial floc would be microbial community composition and changes occurring in aerobic, anaerobic and anoxic environments. The latter was beyond the scope of this study. Besides bioflocs are generated from a process that cleans waste from culture facilities. Therefore, this is an added benefit for dealing with aquaculture effluent.

6. Conclusion
Results of proximate and nutritional composition differed between the treatments with anaerobic bioflocs having the highest crude protein, crude fat and volatile fatty acids composition. Anoxic treatments had the highest mineral composition. Aerobic and fresh sludge treatments were within the middle range of the analyses done. Floc attractiveness test showed that fish in aerobic and control treatments took less time to approach the floc and also spent more time eating and nibbling. This indicates that the aerobic and control treatments were more attractive to the fish as compared to anaerobic and anoxic treatments. Our study suggests that also anaerobic and anoxic flocs could be a food source. The disadvantage is however that the yield is lower, and that the risk for poisoning is higher than with aerobic flocs. Research on the presence of PHB (poly-b-hydroxybutyrate) in the different types of sludge and its effect on the cultured organisms is recommended. Inoculation of bioflocs from the aerobic, anaerobic and anoxic conditions with probiotics merits further. A more interesting field of research would be looking at the carbon sources that would increase attractiveness of the bioflocs towards fish and shrimp. To advance the use of microbial floc as a feed supplement, it will be necessary to manipulate its microbial components which require comprehensive characterization of the microbial communities.

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