ISSN: 2347-5129
IJFAS 2015; 2(5): 347-352

www.fisheriesjournal.com
Received: 08-03-2015
Accepted: 11-04-2015

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Production of aerobic, anaerobic and anoxic bioflocs from tilapia sludge

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Abstract
Bioflocs produced in suspended growth bioreactors under aerobic, anaerobic and anoxic conditions could offer the fish feed industry a novel alternative feed. In this study, an experiment involving incubation of sludge in aerobic, anaerobic and anoxic systems for 22 days was conducted. The experiment was carried out in 9 glass aquaria tanks that were used as reactors for daily sludge incubation. These reactors were fed tilapia manure. Aerobic, anaerobic and anoxic conditions were confirmed by measurement of redox potential, pH, temperature, NO_3 and O_2 concentration. We succeeded in creating the right environmental conditions for biofloc development.

Keywords: Bioflocs, Aerobic, Anaerobic, Anoxic

1. Introduction
To meet the growing demand in aquaculture efforts are shifting from extensive rearing systems to more intensive rearing systems. The water purification method using biofloc technology (BFT) was developed to make fish farming more cost effective and to increase the nutrient utilization efficiency, reduce water use, provide additional feed, reduce effluent discharges and improve biosecurity [1, 2]. Research has also shown that microbial flocs produced in biological reactors can be used as an alternative cheap protein source in fish feed pellets [3].
New biomass of micro-algae and heterotrophic bacteria can be grown on nutrient wastes thus providing an alternative food source [4, 5, 6, 7, 8]. Recycling of the non-utilized fraction of the feed and waste from the culture system was shown to double the utilization of protein and feed by fish or shrimp [9, 10, 11]. The nutritional added value of bioflocs to aquatic animals is reliant on factors like food preference, ability to both ingest and digest and the size and density of the suspended flocs [12].
Different studies showed that fish like tilapia is capable of both filter feeding and detritivory making them a most ideal candidate for biofloc technology systems (BFT) [13]. Tilapia can also grow and flourish in intensive culture and is disease and stress resistant. Tilapia culture tank or pond effluents contains valuable nutrients such as organic and suspended matter [14, 15]. and converting these nutrients into microbial flocs can be accomplished in aerobic bioreactors [16]. The technology combines the removal of nutrients from the water with the production of microbial biomass, which in turn can be used by the culture species as additional food source.
Sludge from intensive fish farm effluents has been used as the culture medium for microalgae cultures and the algal meal produced used as ingredient in fish feeds [17, 18].evaluated results of growing algae on inorganic nutrients from sludge to feed *Oreochromis mossambicus, (*Fish*) Macrobranchium hainenese (*shrimp*) and Moina macrocopa (*Cradocera*).* The yields were unsatisfactory when fed the sludge grown algae directly. Thus their study recommended further analyses of sludge composition and nutritional properties. Also any other potential negative effects like microbiological risk and /or disease transmission and the issue of market perception regarding the use of fish or other animal waste on feed. Conclusions were that bio-flocs are not a complete replacement of traditional fish food but can decrease feeding cost, which still represent 40-50% of production costs, when used as a feed ingredient [19].
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 [20, 21]. Further reported that in ponds fish and shrimps have been known to avoid area

of reduced sediments and look for food in sediments with rich oxygen supply. 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. 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. Three hundred 72 g tilapia were stocked in 7 glass tanks equipped with sludge collectors.

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 the approximately 21 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. The incubators were run continuously during the study period to provide bioflocs meant for further nutritional studies.

2.4 Biofloc growing experiment

2.4.1 Sludge collection

The set up consisted of 7 fish tanks for sludge collection with a daily supply of feeds to the fish at 2 % body weight Fig 1. Each tank held 43 fish with an average weight of 72 g each. In each tank, sludge collectors were cleaned and connected at 4:30 pm after evening feeding for sludge collection overnight. To collect sludge, the outlet of each tank was connected to a swirl separator (height 45 cm, diameter 24.8 cm) equipped with a detachable bottle. Sludge was collected in a detachable bottle (volume 250 ml) at the bottom of swirl separator. Each detachable bottle was cooled using ice cubes to minimize sludge decomposition by bacteria. Sludge from collectors was then carefully transferred to the incubation reactors and divided equally among the reactors. A water flow of 7 L/min was maintained over each tank.

Fig 1: Sludge collection set up

2.4.2 Sludge incubation for biofloc development

A set of 9 reactors were divided equally between aerobic, anaerobic and anoxic states Fig 2. Each reactor contained 20 liters working volume and was continuously mixed using a magnetic stirrer during 22 days until floc harvesting. Approximately 150 ml sludge was added to every reactor every morning at 09.00. Nitrogen gas was bubbled through the anoxic and anaerobic reactors to remove atmospheric oxygen. Twice daily, pH, redox potential, temperature and nitrate and oxygen concentration were measured. Oxic conditions were created by continuous aeration in open aquaria. To create anaerobic conditions the aquaria tanks were put in sealed plastic bags through which nitrogen gas was bubbled. The same was done for creation of anoxic conditions except that sodium nitrate was added. Appropriate fittings were placed on each reactor; for the covered ones, the inlets were passing through the tightly wrapped opening of the bags. This allowed bubbling nitrogen gas through the anoxic and anaerobic reactors, while gases like carbon dioxide and methane could escape. Fittings having electrodes, thermistors and heaters were installed to monitor pH, redox, temperature and oxygen saturation in each reactor. Three piped syringes for the different reactors were used to extract 10 ml samples for nitrate-N concentration determination. Anoxic conditions were created by adding sodium nitrate ($NaNO_3$) to the reactors. Nitrate levels were maintained at 40 ±5 mg/l NO_3-N, by adding a calculated amount of sodium nitrate anytime nitrate concentration levels fell below 200 mg/l. Continuous stirring by a magnetic stirrer ensured complete mixing of the introduced sludge in the water column. Nitrogen gas was bubbled through the anoxic and anaerobic reactors during the entire sludge incubation period at a constant rate of 15 ml/min. Temperature, pH, and redox potential (ORP) measurements were determined using a pH/Oxi 340i electrode (WTW, Germany). Dissolved oxygen saturation was determined by Oxi 340i electrode (WTW, Germany). Nitrate concentration was determined by *test* strips (*Merck*, Darmstadt, Germany). Sampling of the flocs for quality was done on day 0, 7 and harvesting day. Samples were acidified with nitric acid and stored at -20°C for later chemical analysis.

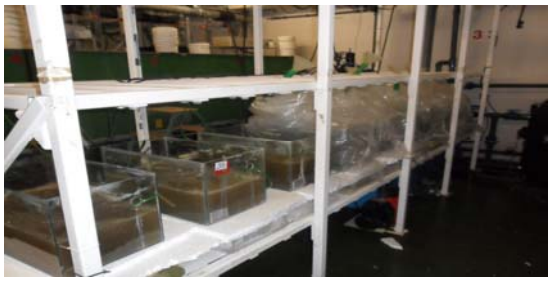

Fig 2: Diagram showing 9 reactors used for biofloc development. Open (aerobic reactors) covered (anaerobic and anoxic reactors).

2.4.3. Operations in the reactors

The main maintenance operations of the reactors included filling during which fresh sludge was added daily as explained above, mixing of water and sludge by use of a magnetic stirrer and continuous aeration. This ensured that the bioflocs remained in suspension and that the 20 l sludge in the reactor was constantly mixed. At harvesting the sludge was first allowed to settle by stopping the bubbling and mixing. After 22 days the sludge water was removed from the bioreactors leaving the settled bioflocs behind. The latter was siphoned out for further use and analyses. The collected sludge was filtered through Whatman filters (320mm) to get rid of excess water.

The harvested bioflocs colors differed between treatments as shown in Figure 3. From the residue, floc pellets for the feeding trials were made by putting 1 g of the floc into plastic eppendorf holders that were frozen at -20°C for future use.

Aerobic flocs

Anaerobic flocs

Anoxic flocs

Fig 3: A presentation of color difference in the three states of harvested bioflocs.

3. Data Analysis and Statistics

Measurements of biofloc development results were analyzed by ANOVA with repeated measures with reactor type as the main factor and day and day period as sub factors. In the analyses, day period was nested in day. Prior to ANOVA data was checked for normality using Shapiro-Wilk test and homogeneity of variance using Mauchly's sphericity test. 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 development and generation from the aerobic, anaerobic and anoxic systems

Mean values of five parameters measured; pH, temperature, redox potential (ORP), nitrate concentration and oxygen saturation in the three reactors and outcomes of ANOVA are presented in Table 1. There was a significant interaction effect among the reactors and sampling time as shown in the graphs for all the parameters except oxygen saturation since it was measured in the aerobic reactors only.

Table 1: Mean values of parameters measured and sampling time in the different reactors.

Parameters:	Means Tukey test					
	Reactors			Day period		Interaction
	Aerobic	Anaerobic	Anoxic	Morning	Afternoon	Reactor X day*
pH	8.58 [a]	7.73 [b]	8.24 [ab]	8.2	8.1	***
Temperature°C	25.58 [b]	28.44 [a]	28.09 [a]	27.32	27.43	***
ORP (mV)	155.33 [a]	-260.46 [c]	59.99 [b]	-13.18	-16.89	***
NO_3 (mg/L^{-1})	196.09 [a]	13.71 [b]	164.09 [a]	121.72 [b]	127.52 [a]	***
Oxygen Sat (mg/L^{-1})	8.29			8.14 [b]	8.44 [a]	
Oxygen Sat (%)	104.64			103.68 [b]	105.57 [a]	

Table 1: Mean values of parameters measured and sampling time in the different reactors. * 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. *$P<0.05$;**$P<0.01$; ***$P<0.001$.

4.1.1 PH

In this study aerobic pH levels remained stable for the first 12 days. From then onwards until the end of the experiment the values kept varying at an average of 8.5 and significantly different from the pH in the anaerobic and anoxic reactors ($P<0.05$). Anaerobic reactors had a lower pH 7.7 while in the anoxic reactors pH value increased steadily with values ranging from 7.0 to 8.6 and a mean anoxic pH of 8.2 Fig 4. There were no significant differences in the time of the day when the sampling was done ($P>0.05$).

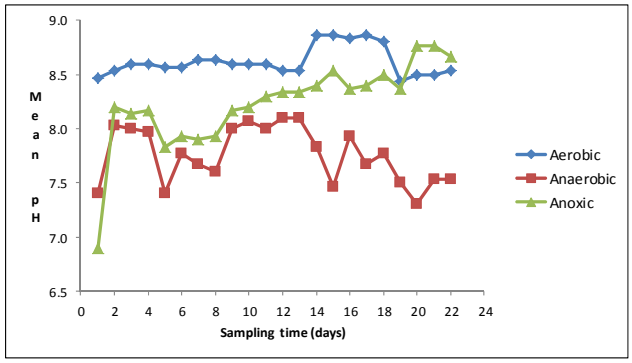

Fig 4: Average daily pH in aerobic, anaerobic and anoxic reactors. No standard errors given.

4.1.2 Temperature

Immersion heaters set at 28°C were used to regulate temperature during the experiment. Temperature in the aerobic reactors mean of 25.5°C was significantly different from both anaerobic and anoxic reactors which had a mean of 28.4°C ($P<0.05$) Fig 5. Results of ANOVA showed that there were no any significant differences in the time of the day when sampling was done ($P>0.05$).

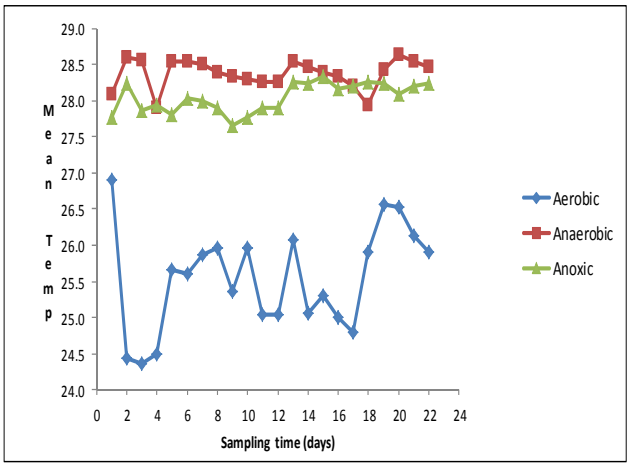

Fig 5: Average daily temperature in aerobic, anaerobic and anoxic reactors. No standard errors given.

4.1.3 Redox potential (ORP)

Redox potential differed significantly among all the reactors in this study (P<0.05) Table 1. Aerobic reactors had a positive mean redox potential of 155.3 mV. Anaerobic reactors reached complete anaerobic conditions with a redox potential of -344 mV after 13 days. The anaerobic reactors had a negative mean redox of -260.46 which reduced further towards the end of the experiment Fig 6. Anoxic reactors had a positive mean redox of 59.9. Also in this parameter results of ANOVA showed that there were no significant differences in the time of the day when the sampling was done.

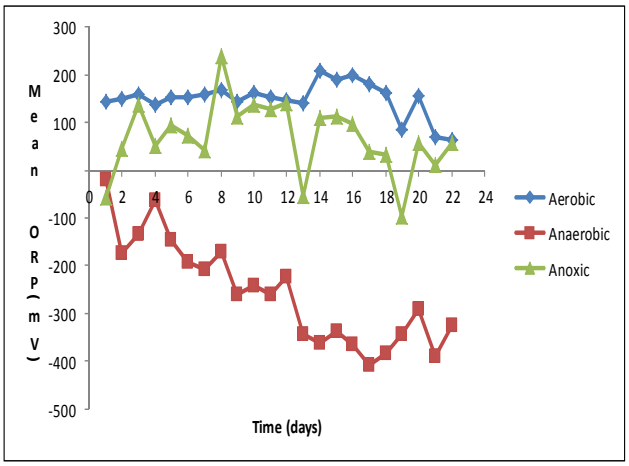

Fig 6: Average daily redox potential in aerobic, anaerobic and anoxic reactors. No standard errors given.

4.1.4 Nitrate concentration

Nitrate concentration was significantly different in the anaerobic reactors (p<0.05) as compared to aerobic and anoxic reactors. Aerobic reactors had the highest mean nitrate concentration of 196.09 mg/l. NO_3 concentration was relatively low in the anoxic reactors with a mean of 164.09 mg/l while in anaerobic reactors the lowest concentration of 13.71 mg/l was observed. From the 8th day to the end of the experiment the nitrate concentration in anaerobic reactors was zero Fig 7. Results of ANOVA showed significant difference in the time of the day when sampling was done with high nitrate levels being recorded in the afternoon.

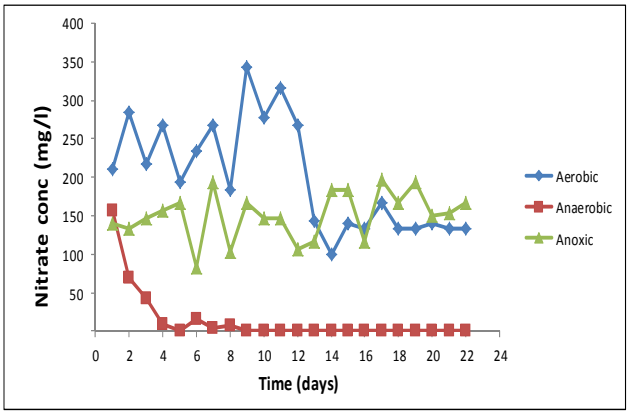

Fig 7: Average daily nitrate concentration in aerobic, anaerobic and anoxic reactors. No standard errors given.

4.1.5. Oxygen Saturation

Oxygen saturation was measured only in the aerobic reactors the mean recorded percent saturation was 104.64. The values were high at the start of the experiment and reduced gradually from day 10 to the end of the experiment Fig 8. Results of ANOVA show that there were significant differences in the time of the day that the sampling was done (P<0.05) with higher values being recorded in the afternoon.

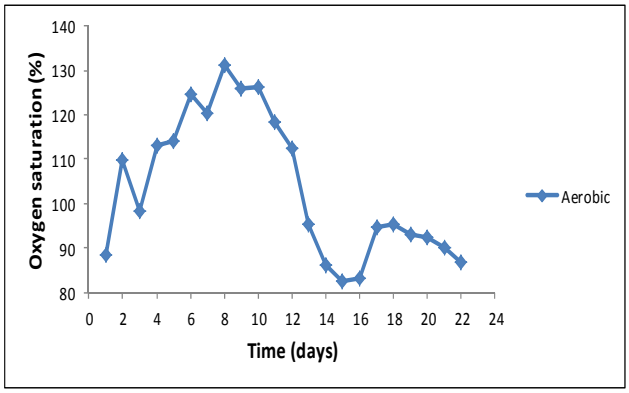

Fig 8: Average daily oxygen saturation in aerobic reactors. No standard errors given.

5. Discussion

5.1 Evaluation of biofloc development from the aerobic, anaerobic and anoxic conditions.

5.1.1 PH

The variation of pH often provides a good indication of the ongoing biological reactions. PH fluctuations affect enzyme systems responsible for microbial activity thus it should be considered in design and operation of biological treatments. In aerobic reactors nitrification was taking place causing biological conversion of ammonia to nitrate nitrogen. Nitrifying bacteria are aerobic in nature requiring free dissolved oxygen. The nitrification process lowers the pH which causes reduction in growth of nitrifying bacteria. A decline in pH under aerobic conditions can also be attributed to an increase in dissolved carbon dioxide associated with decomposition of sludge [22, 23]. Reported that under aerobic conditions, half of the decomposed organic carbon in sludge treatments is incorporated in bacteria biomass and the other half mineralized to CO_2. The optimum pH range is between 7.5 and 8.5. Below pH of 6 nitrification stops [24]. Observed aerobic pH was within the optimum range with a mean of 8.5.

During anaerobic digestion the acid genesis step lowers pH hence the shifts in curves observed in Fig 4. The pH decline in these reactors was also higher most probably due to the accumulation of volatile fatty acids [25]. At the same time methane forming bacteria consume the volatile acids and alkalinity is produced which increases and stabilizes the pH [26]. [23]. Also reported that anaerobic decomposition is a multi-step process and less bacteria biomass and more CO_2 is produced even though some carbons are incorporated in the intermediate fermentation products.

In anoxic reactors denitrification was the main process taking place consuming nitrates which were introduced by addition of sodium nitrate. This lead to a considerable increase in pH as observed in Fig 4. Anoxic respiration is said to occur with the transfer of nitrate ions (NO_3^-) to the digester with sludge or by addition of nitrate-containing compounds such as sodium nitrate ($NaNO_3$) that increases digester alkalinity [26, 27]. Further reported that denitrification is an alkalinity producing process performed by denitrifying facultative bacteria which use either dissolved oxygen or nitrate as oxygen source for metabolism and oxidation of organic matter.

5.1.2 Temperature

In the present study a temperature control of 28°C was put in place in all the reactors. The main reason was to fix the biofloc development process between treatments and provide a favorable environment for microbial activity. The observed low temperature in the aerobic reactors might be because these reactors were not covered while the anaerobic and anoxic reactors were wrapped in plastic during the entire experiment period Fig 5. [26]. Stated that maintenance of a steady state temperature range should be practiced with anaerobic digesters. This has shown to favor the acid forming bacteria that produce fatty acids that are used by methane forming bacteria. In addition, the diversity of bacteria found in biological treatments has been shown to vary with temperature [28].

5.1.3 Redox potential

Aerobic zones are rich in dissolved oxygen concentrations, therefore dissolved oxygen are more efficient controls. In the present study redox potential measurements were only done to ensure positive millivolts potential. Anaerobic treatments achieved completely anaerobic condition with average redox potential of approximately -350 mV after the 13th day Fig 6. To break down phosphate of the bacterial cell mass removed as sludge, [26] stated that one should look for ORP values less than -300 mV that will ensure adequate electron donors for respiration of phosphate consuming bacteria and for growth of obligate anaerobic bacteria. In anoxic reactors the fluctuations in ORP might have been caused by the fresh sludge that was added every day and the NaNO3 at irregular time intervals. The ORP in anoxic zone were between a range of -100 and 100 mV indicating fairly neutral state for denitrification to take place. [29]. Showed that in anoxic condition a transitional state from oxidation to reduction is favorable to the hydrolysis and acidification of waste water with a fitting ORP of –100 mV to +50 mV.

5.1.4 Nitrate concentration

Nitrification is an aerobic process and therefore only occurs in oxygen rich environments. Two steps are involved; Nitrosomonous bacteria converting ammonia to nitrite and Nitrobacter bacteria finishing conversion of nitrite to nitrate. If the ammonia and nitrite oxidizing are in equilibrium, nitrite levels stay low while nitrate levels increase gradually. In the present study the amount of NO_3 was expected to increase with time because denitrification was not able to occur in the complete aerobic system. However the nitrate concentration showed a shifting profile with a decreasing tread towards the end of the experiment. A possible explanation of the lack of nitrification might be competition with heterotrophic bacteria for NH_4 or substrate inhibition mechanism produced by high organic matter loads or ammonia concentrations. Space competition between heterotrophic and nitrifying bacteria might be another reason that suppressed the nitrification process [30].

Anaerobic zones are always free of dissolved oxygen and nitrate. This was observed in our experiment with nitrate levels close to zero from day 8 onwards. This provided optimal conditions in the anaerobic reactors since presence of nitrate ions (NO_3^-) inhibit methane forming bacteria [30].

In the anoxic reactors daily addition of sludge added a lot of easy reducible organic matter from which total ammonium nitrogen (TAN) was released. This allowed a small chance of nitrification to take place. Anaerobic ammonium oxidation (Anammox), a route considered for denitrification might have been a major contributor of anoxic denitrification [31]. Addition of sodium nitrate contributed to nitrate concentration levels an average of 164 mg/l^{-1}. It is believed that the addition of nitrate during anoxic conditions offers a source of oxygen for certain microorganisms present in waste water. In addition, nitrate presence reduces levels of hydrogen sulphide, the main cause of odor and toxicity in waste water [32, 33].

5.1.5 Oxygen saturation

Continuous aerating contributed to the high oxygen saturation levels in the aerobic reactors. However daily feeding of the reactors with fresh sludge that had high ammonia levels might have contributed to the shifting profile and decreasing trend of oxygen concentration.

6. Conclusion

The findings of this study demonstrated the biofloc development process in aerobic, anaerobic and anoxic conditions. The assessment parameters; pH. Temperature, redox potential, nitrate concentration and oxygen for the aerobic state should be properly maintained to achieve the desired results. In the present study the culture conditions were within optimum range that made the development process a success. The made flocs were intended to be used for growth studies with Nile tilapia and is subject of further research.

7. Acknowledgement

This research was financially supported by the Netherlands Fellowship Program. The authors are grateful to the technical staff of Wageningen University, Aquaculture and fisheries group for their kind help and contribution to this research.

8. References

1. Wasielesky WJr, Atwood H, Stokes A, Browdy CL. Effect of natural production in a zero exchange suspended microbial floc based super-intensive culture system for white shrimp *Litopenaeus vannamei.* Aquaculture 2006; 258:396-403.
2. Avnimelech Y. Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture 2007; 264:140-147.
3. Kuhn DD, Boardman GD, Lawrence AD, Marsh L, Flick GJJ. Microbial floc meal as a replacement ingredient for

fish meal and soybean protein in shrimp feed. Aquaculture 2009; 296:51-57.
4. Avnimelech Y. Bio-filters: the need for an new comprehensive approach. Aquac. Eng 2006; 34(3):172-178.
5. Hari B, Kurup BM, Varghese JT, Schrama JW, Verdegem MCJ. The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 2006; 252(2-4):248-263.
6. Ponis E, Robert R, Parisi G. Nutritional value of fresh and concentrated algal diets for larval and juvenile Pacific oysters (*Crassostrea gigas*). Aquaculture 2003; 221(1-4):491-505.
7. Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J. Biosci. Bioeng 2006; 101(2):87-96.
8. Wang JK. Conceptual design of a microalgae-based recirculating oyster and shrimp system. Aquac. Eng 2003; 28(1-2):37-46.
9. Avnimelech Y, Mokady S, Schroeder GL. Circulated ponds as efficient bioreactors for single-cell protein production. Israeli Journal of Aquaculture-Bamidgeh 1989; 41(2):58-66.
10. McIntosh PR. Changing paradigms in shrimp farming: IV. Low protein feeds and feeding strategies. Global Aquaculture Advocate 2000; 3:44-50.
11. Velasco M, Lawrence AL, Neill WH. Development of a static-water eco assay with microcosm tanks for post larval *Penaeus vannamei*. Aquaculture 1998; 161:79-87.
12. Hargreaves JA. Photosynthetic suspended-growth systems in aquaculture. Aquacultural Engineering 2006; 34:344-363.
13. Dempster P, Baird DJ, Beveridge MCM. Can fish survive by filter-feeding on microparticles? Energy balance in tilapia grazing on algal suspension. Journal of Fish Biology 1995; 47:7-17.
14. Jime´nez-Montealegre R, M. Verdegem, J. Zamora, J. Verreth. Organic matter sedimentation and resuspension in tilapia, *Oreochromis niloticus*, ponds during a production cycle. Aquacultural Engineering 2002; 26:1-12.
15. Al-Hafedh Y, Alam A, Alam M. Performances of plastic bio filter media with different configurations in a water recirculation system for the culture of Nile tilapia, *Oreochromis niloticus*. Aquacultural Engineering 2003; 29:139-154.
16. Metcalf Eddy. Wastewater engineering: treatment and reuse, 4th editio. McGraw-Hill, New York, New York, USA, 2003.
17. Dickson MW. Pilot-scale cultivation of micro algae as an ingredient for fish feeds in Zambia. Aquaculture research 2008; 18(2):109-120.
18. Wong MH, Hung KM, Chiu ST. Sludge- grown algae for culturing aquatic organisms: Part ll. Sludge grown algae as feeds for aquatic organisms. Environmental Management, 1996; 20, 3 / Mayo de, 1996.
19. Craig S, Helfrich LA. Understanding Fish Nutrition, Feeds and Feeding (Publication 420–256). Virginia Cooperative Extension, Yorktown (Virginia), 2002, 4.
20. De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W. The basics of bio-floc technology: the added value for aquaculture. Aquaculture 2008; 277:125-137.
21. Avnimelech Y, Ritvo G. Shrimp and fish pond soils: processes and management.Aquaculture 2003; 220:549-567
22. Milstein A. Water quality and freshwater fish culture intensification: the Israeli example. Aquaculture and Fisheries Managemen 1993; 24:715-724.
23. Henze M, Harremoes P, Jansen J, Arvin E. Wastewater treatment, biological and chemical processes. Third edition. Springer Verlag, Berlin, Germany, 2002, 430
24. Focht DD, Chang AC, Nitrification and denitrification processes related to wastewater treatment. Adv. Appl. Microbiol 1975; 19:153-186.
25. Vidal G, Carvalho A, Mendez R, Lema JM. Influence of the content in fats and proteins the anaerobic biodegradability of dairy wastewaters. Bioresource Technology 2000; 74:231-239.
26. Gerardi MH. Waste water Bacteria, wastewater microbiology Ed1. John Wiley and sons New Jersey, 2003, 188.
27. Glass C, Silverstein J. Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. Water Res 1998; 32(3):831-839.
28. Azim ME, Wahab MA, Van Dam AA, Beveridge MCM, Verdegem MCJ. Optimization of fertilization rate for maximizing periphyton production on artificial substrate and the implication for periphyton based aquaculture. Aquat. Res 2001; 32:749-760.
29. Shen YL, Wang BZH. Hydrolysis-acidogenosis process and its application and study. J. Harbin University Civil Eng. Archit 1999; 32(6):35-38.
30. Wijeyekoon S, Mino T, Satoh H, Matsuo T. Effects of substrate loading rate on biofilm structure. Water Research 2004; 38:2479-2488.
31. Mulder A, Van de Graaf AA, Robertson LA, Kuenen JG. Anaerobic ammonium oxidation discovered in a denitrifying fluidized bed reactor. FEMS Microbiol Ecol 1995; 16:177-184.
32. Ting OP. Wastewater quality changes under anoxic conditions: A study on palm oil mill effluent (POME) addition in pressure sewer as external nitrate source. MSc Thesis. University Teknologi Malaysia, 2004.
33. Abdul-Talib S, Hvitved-Jacobsen T, Vollertsen J, Ujang Z. Anoxic transformations of wastewater organic matter in sewers process kinetics, model concept and wastewater treatment potential.; *Wat. Sci. Tech* 2002; 45(3):53-60.