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An Appraisal of the Feasibility of Tilapia Production in Ponds Using Biofloc Technology: A review

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Abstract: The continuous expansion of aquaculture sector has no doubt triggered debate on environmental issues and has accelerated global demand for fishmeal and fish oil in equal measure. In the recent past, scientists have described bioflocs and periphyton technology as self sustaining biotechnology units capable of purifying aquaculture waste water and manufacturing fish food simultaneously. Based on literature evidence, the authors wish to show potential of biofloc technology (BFT) towards improving yield, safety and economic sustainability of cultured tilapia. In addition to fish feed dilemma currently threatening aquaculture sector, this paper describes the concept and dynamics of BFT as possible solution. Tilapia productivity and added values have also been addressed. The BFT works on the basis of recycling nutrients by maintaining high C/N ratio, which stimulates heterotrophic bacteria that converts ammonia into microbial biomass making it possible for protein to be eaten twice (in feed and microorganism) by cultured fish. Although bioflocs contain adequate protein, lipid, carbohydrates and fatty acids, they also ensure biosecurity thus protecting fish from variety of disease infections. Tilapia growth rates of up to 0.3 $g.day^{-1}$ yielding up to 300 $mt.ha^{-1}$ has been reported in well managed bioflocs ponds. Also, significant reduction of fish feed by up to 20% has been reported thus lowering total production cost in biofloc aided ponds. Even though the exact mechanisms influencing bioflocs are unknown, the paper describes biological, chemical and physical interactions as possible dynamics shaping microbial communities in bioflocs. The bioflocs technology is inexpensive thus making

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it the forgotten asset for present and future aquaculture. In this respect, authors suggest that a strategy to have a predominance of easily digestible bacteria containing energy rich compounds should be of particular interest in promoting BFT in aquaculture sector.

Key Words: Bio-floc technology, Fish food, periphyton, C/N ratio

Overview of global aquaculture and tilapia production

The rapid growth of global aquaculture industry cannot be over emphasised. Within the last 50 years, aquaculture has grown from being almost negligible to fully comparable with capture fisheries in terms of global food production. The growth in the sector has been due to the new technological advancements in fish production e.g. hybridization, genetical engineering, formulated diets and the biofloc technology (BFT) in various culture systems including ponds, cages, tanks and recirculation systems (FAO, 2012). However, the rate of aquaculture growth is not uniform worldwide. Whereas the continental Asia has become the global giant in aquaculture production, Africa is yet to report significant quantities of aquaculture products in global scale despite availability of enormous natural resources (FAO, 2010).

The stagnating capture fishery has shifted the heavy responsibility of increasing fish supply to aquaculture sector (Avnimelech, 2007). A fivefold increase in aquaculture production is needed within the next five decades to maintain current aquatic food consumption level (Avnimelech *et al.*, 2009). Therefore, new sustainable technologies such as biofloc and periphyton become relevant. The technologies are cost effective, environmentally sustainable and support sustainable of aquaculture (Naylor *et al.*, 2000; Avnimelech, 2009).

Today, tilapia, which is also popular as 'aquatic chicken' has become the shining star of aquaculture with many farms beginning, others expand as consumption rate increases across the globe (Fitzsimmons, 2005; FAO, 2012). Annual global production of cultured tilapia has continued to increase in recent years (FAO, 2010).

Fish feed accounts for over 50 % of the total cost of fish production (Craig and Helfrich, 2002). Therefore sustainability of aquaculture depends on feed source and management. Major source of fishmeal being the ever diminishing capture fishery, the sustainability of the aquaculture sector is questioned (Naylor *et al.*, 2000). Most available fish feeds have a feed conversion ratio (FCR) of 3, therefore to produce 1 kg live weight fish, 1-3 kg dry weight feed is needed (Naylor *et al.*, 2000). The sustainability of the aquaculture industry cannot be achieved unless progressive reduction of wild fish inputs into fish feed is addressed (Naylor *et al.*, 2000; Liti *et al.,* 2005; Munguti *et al.,* 2009).

So far, nutrition research has concentrated on the replacement of animal protein by plant proteins (Liti *et al.,* 2006) but the palatability of many plant materials is hindered by presence of anti-nutritional factors and low bioavailability (Francis *et al*., 2001).

Earthen ponds will remain the major aquaculture production system especially in developing nations for a long time. Consequently, initiatives geared towards developing nutrition strategies such as bioflocs and periphyton that maximize the contribution of natural and supplemental feeds in ponds would help to expand aquaculture production. In this regard, this paper reviews the concept and nutritional capacity of biofloc technology that has contributed to the success of tilapia production in biofloc aided ponds.

The concepts of biofloc technology

The scientific and practical concept of biofloc technology was conceived independently around mid 1990s by Hopkins *et al*. (1993) and by Avnimelech *et al*. (1994). BFT is an aquaculture system which centres on a more efficient use of nutrient input with minimal or zero water exchange (Avnimelech, 1999; Widanarni *et al.,* 2012). The main principle of BFT is to recycle nutrient by maintaining a high carbon / nitrogen (C: N) ratio in the water in order to stimulate heterotrophic bacterial growth that converts ammonia into microbial biomass (Avnimelech, 1993, 1999). In fact, the microbial biomass yield per unit substrate of heterotrophic bacteria is about 0.5 g biomass $C.g^{-1}$ substrate C used (Eding *et al.,* 2006). Bacterial growth increases when carbon source such as wheat bran or cellulose is sprayed on the surface of pond water with continuous aeration at an optimum C: N ratio of 15:1 (Chamberlain and Hopkins, 1994; De Schryver *et al.,* 2008)). However, continuous addition of carbon sources may elevate concentrations of total ammonium nitrogen (TAN) and nitrite-nitrogen (NO_2-N) to critical levels in BFT ponds (Avnimelech, 1999). Timmons *et al*. (2002) reported that even low concentrations of TAN and NO2-N are toxic to most aquaculture species and therefore, control of ammonia and nitrite to low concentration while keeping water exchange minimal is vital. To date, scientists are yet to document standards for carbon sources for BFT aided extensive culture systems. In this regard, pond dynamics and carbohydrate requirement to minimize TAN in such ponds needs further evaluation. Nonetheless, about 360 g $C.m^{-3}day^{-1}$ is sufficient amount of organic carbon needed for nitrogen assimilation in an intensive tilapia aquaculture pond stocked at 50 $kg.m^{-3}$ (Crab, 2010).

Microbial proteins are produced in ponds when organic matter added as manure or feed is decomposed by microbial organisms under both aerobic and anaerobic conditions (Azim and Little, 2008). The process of decomposing organic matter under aerobic condition is faster than anaerobic condition (Reddy and Patrick, 1975) and leads to the production of new bacterial cell, amounting to 40-60% of the metabolized organic matter (Avnimelech, 1999). Though the heterotrophic production is important, it is limited by the need for constant aerobic conditions, a process that many fish farmers cannot regularly afford.

The number of bacteria in biofloc ponds can be between 10^6 and 10^9 mL^{-1} of floc plug, which contains between 10-30 mg dry matters making the pond a biotechnological industry (Avnimelech, 2007). As a by-product, bacteria produce between 60-600 $kg.ha^{-1}day^{-1}$ of protein for fish (Avnimelech, 1999). However, identification of bacteria in aquaculture is still challenging as many bacteria species cannot be cultured in laboratory conditions. Of late, BFTs have received attention for tilapia cultivation because they promote high production, control water quality and recycle feed simultaneously in the same unit (Little *et al.,* 2008). The addition of the 'periphyton loop' in aquaculture ponds can be accomplished by simple modification such as addition of static substratum (poles) to the pond to maximise benefits (Azim *et al.,* 2005).

Dynamics of bioflocs in aquatic ecosystems

Although the exact mechanisms that engineer microbial communities to flocculate are unknown (Crab, 2010), promoting floc formation in activated sludge systems could be significant for application in BFT. Within the floc's matrix, Crab (2010) suspected a combination of physical, chemical and biological interactions, leading to formation of dense cultures of microbial communities. Possible factors may include temperature, pH, turbulence, dissolved oxygen and organic loading rate, among others.

The factor of temperature cuts across many aspects in the BFT matrix. Temperature directly influences the amount of dissolved oxygen in the water (Boyd, 1998) and metabolism in both microbial community and the cultured species, which determines fish growth aspects (Boyd, 1998). Adjusting water temperature in BFT ponds is tricky because of additional costs especially in outdoor ponds. In most cases, temperature is left at the mercy of climatic conditions to determine the optimum operation temperature and this may affect the species to be cultured. Meanwhile, scientists are investigating the optimum relationship between temperature and floc morphology. Wilen *et al*. (2000) observed high rate of deflocculation of flocs at lower temperature (4°C) compared to higher temperatures of 18-20°C, probably due to a decrease of the microbial activity within the flocs. Krishna and Van Loosdrecht (1999) reported that temperatures of 30-35°C encourage bulking of the sludge due to the excessive production of extracellular polysaccharides. Crag (2010) proposed that an intermediate water temperature of 20-25°C could be best to obtain stable flocs in BFT set ups.

Notably, scientists concur that changes in pH directly influences the stability of both bioflocs present and cultured fish in the ponds (Mikkelsen *et al.,* 1996). Indeed the pH is the fulcrum that shifts that balance between toxic and non toxic ammonia in aquatic ecosystems. Toxic ammonia has lethal effects on fish welfare in the pond (Boyd, 1998). The pH is an environmental stressor leading to effects on physiological functioning of some fishes (Portz *et al.,* 2006). pH is a difficult parameter to control in any given biofloc system (Crab, 2010) probably due to different chemical and biological processes in BFT units.

The quality of aeration device may determine the mixing intensity and floc size distribution. Thus creates equilibrium between the rate of aggregation and the rate of breakage (Chaignon *et al.,* 2002). This probably is the most challenging factor to many farm managers especially in developing countries where power availability is not guaranteed all the time.

Dissolved oxygen (DO) concentration in the water directly depends on quality of mixing device and the prevailing temperature (Boyd, 1998). Crab (2010) noted that the DO level is not only essential for the metabolic activity of microbial cells within aerobic flocs but also influence floc structure. Whereas, Wilen and Balmer (1999) observed a trend towards larger and more compact flocs formation at higher DO concentrations, Crab (2010) reported presence of a higher amount of filamentous bacteria at DO levels below 1.1 mg $O_2.L^{-1}$ because filamentous bacteria are better competitors during anoxic conditions.

Basing on the findings of Crab (2010), the type of organic carbon source in biofloc ponds not only influence flocs nutritional quality but also affect water quality parameters such as dissolved oxygen levels due to aerobic microbial metabolism. Hence fish oxygen requirement is crucial information before deciding which carbon source to use. The mode of application of organic carbon can be either as additional organic carbon source (e.g. glucose, acetate, and glycerol) or by changing the feed composition thus increasing its organic carbon content (Avnimelech, 1999). Crab (2010) advised that the use of low-value organic carbon sources e.g. glycerol could enhance benefits derived from BFT initiatives.

At low substrate levels, filamentous bacteria tend to have an advantage over non filamentous bacteria due to higher surface area to volume ratio (Crab, 2010). The mode of application and quantity of organic carbon to be supplied are still subjects of research as BFT environments are different from each other. While some scientists prefer application of small amounts continuously, others prefer large doses at regular intervals (Crab, 2010).

Bioflocs as food for tilapia

Moav *et al*. (1977) recognized the contribution of microbial metabolism to fish nutrition. Together with Shroeder (1978), they defined the concept of heterotrophic food web where fish can be fed directly or indirectly on primary producers and also have a chance to feed on bacteria degrading residues present in the pond. In the recent past, biosynthesis and utilization of single cell proteins (SCP), which are a group of microorganisms including unicellular algae, fungi, bacteria, cyanobacteria, and yeast by tilapia within culture systems has attracted the attention of researchers (El-Sayed, 1999). For instance, Avnimelech (2007) confirmed the biofloc uptake by Mozambique tilapia using stable nitrogen isotope labelling technique. A chronological account of biofloc as food for fish has been advanced by Avnimelech (1999, 2007, 2011). The major components making bacterial cells are proteins and the C: N ratio of most microbial cells is about 4-5 (Avnimelech, 1999). In a pond system rich with organic substrates consisting of mostly carbon, the bacteria are forced to utilise any little nitrogen from the water to synthesis the mostly needed protein for growth (Avnimelech, 1999). In the end, the bacteria continue to assimilate total ammonia nitrogen (TAN) and produce more microbial proteins thus enable recycling of unused feed protein (Avnimelech, 1999). The microbial biomass eventually aggregates with other algae, bacteria and suspended particles to form bioflocs, which can be consumed by the fish or harvested and processed as a feed ingredient (Avnimelech, 1999; Avnimelech 2007; De Schryver *et al.,* 2008; Kuhn *et al.,* 2009).

Avnimelech (1999) discovered that single cell proteins produced using cheap carbon and nitrogen sources, can partially replace expensive commercial protein sources in aquaculture feeds. In addition, Avnimelech *et al*. (1999) found no significant difference in terms of growth between tilapia fed with single cell proteins and those fed on 30% protein rich diet hence microbial proteins can be successfully used as protein sources by different tilapia species.

As farm managers come to terms with the unravelling reality of biofloc technology in the current aquaculture industry, the question of what quantity of biofloc is meaningfully significant has to be addressed. To approximate the quantity of microbial protein in a given pond, Avnimelech (1999) suggested two ways basing on some critical assumptions as shown below:

- ✓ Fish excretes about 75％ of nitrogen applied in the feed.
- ✓ about 50% of this nitrogen is converted to microbial protein (the rest settle as sludge or denitrified)
- ✓ 37.5% of any amount of crude protein in fish diet is thus converted to microbial protein

Based on the assumptions stated above, a tilapia pond fed by 30% crude protein feed at a daily rate of 100 g.m^{-3} would generate about 11 g.m^{-3} of microbial protein daily. Further to this, Avnimelech (1999) reported a second method by evaluating the amount of suspended protein per m^{3} of pond water. In other analysis (not reported in this paper), the bacterial biomass is made up of about 61% crude protein (Avnimelech, 2009). Therefore pond water containing 200 mg.L^{-1} (200 g.m^{-3}) of suspended bioflocs would approximately contain 120 g.m^{-3} of crude protein, which Avnimelech (2009) equates to about 4 daily feeding ratios for a pond stocked at 5 kg.m^{-3} with tilapia. Moreover, unlike the non biofloc ponds, fish in bioflocs treated ponds feed continuously because microbial production is a continuous process (Avnimelech, 2009). Protein utilization rises from 15-25% in conventional ponds to 45％ in BFT ponds probably due to recycling of protein hence making BFT perfect self feeding biotechnology machines (Avnimelech, 1999). As Avnimelech (2009) put it, 'protein is eaten twice' (in feed and in microorganism).

Nutritional quality of bioflocs

Many authors concur that a properly maintained BFT can manufacture sufficient protein to maintain significant fish growth although minimal supplementary feeding is obligatory (De Schryver and Verstraete, 2009; Crab *et al.,* 2010b; Ekasari *et al.,* 2010). According to Azim and Little (2008), bioflocs that contain more than 38% protein, 3％ lipid, 6％ fibre, 12％ ash and 19 k J g^{-1} energy (on dry matter basis) is considered appropriate for tilapia production. However, Webster and Lim (2002) reported that bioflocs containing 50％ crude protein, 4％ fibre, 7％ ash and 22 kJ.g^{-1} energy (on dry matter basis) are better for herbivorous/omnivorous fishes including tilapia. Recently, Widanarni *et al.* (2012) did a proximate analysis on a floc sample collected from tilapia BFT system, which was stocked between 25-100 fish m^{-2} and fed at 32％ crude protein diet with a C/N ratio of 15 and found 40-50％ crude protein (Figure 1). Indeed these values are far much better than most commercial pellet feeds used in aquaculture farms today. Jauncey (2000) reported that 25-30% crude protein is just sufficient for larger tilapia diets. In terms of lipid content, Widanarni *et al.* (2012) found between 10–25％, again much higher than 5-12%, which is the optimum dietary lipid requirement for tilapia (Lim *et al.*, 2009) suggesting that the lipid content of biofloc could be sufficient to meet lipid requirements of tilapia in BFT ponds. Nevertheless, to achieve maximum growth, a level of 12% crude lipid is recommended (Chou and Shiau, 1996). De Silva and Anderson (1995) discouraged fibre and ash contents exceeding 12% in fish diets because the increase in fibre content would consequently result in a decrease of the quantity of usable nutrient in the diet.

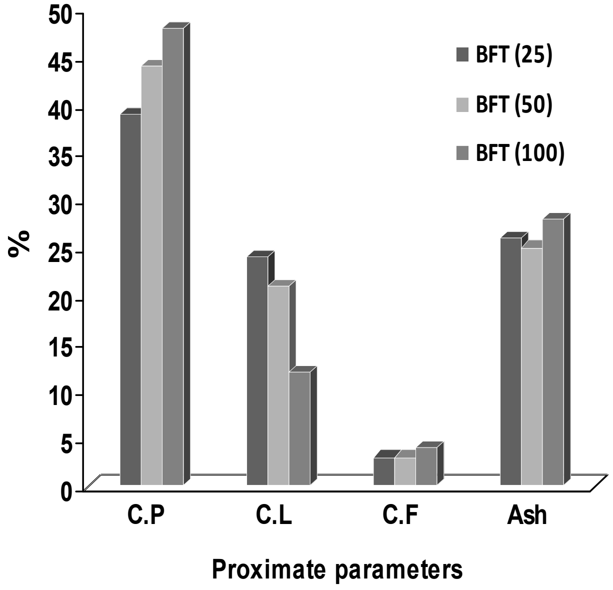

Fig. 1: Proximate parameters of bioflocs collected from BFT treatment at different fish density; BFT (25) = 25 fish/m^2, BFT (50) = 50 fish/m^2, BFT (100) = 100 fish /m^2 CP= crude protein; CL=Crude lipid; CF=crude fat. (Adapted from Widanarni *et al.*, 2012)

Although bioflocs show an adequate protein, lipid, carbohydrate and ash content for use as an aquaculture food, more research is needed on their amino acid, vitamin and fatty acid composition (Azim *et al.,* 2008). Regarding the composition of the Poly-Unsaturated Fatty Acids (PUFAs) in the bio-flocs, Ekasari *et al.* (2010) found that bioflocs using glycerol as the carbon source contained more n-6 PUFAs than those supplied with glucose at 0 and 30 g.l^{-1} salinity level after 18 days (Figure 2). Azim and Little (2008) analysed essential fatty acids of biofloc system fed with high (35% CP) and low (24% CP) fed tanks. They found 27-28% poly-unsaturated, 28-29％ mono-unsaturated, 30-35％ saturated fatty acids and 10–12％ unknown peaks (Table 1) similar with findings of Tacon *et al.* (2003).

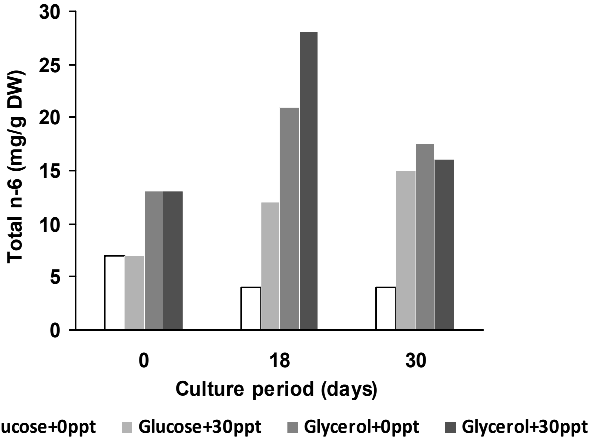

Fig. 2: Total n-6 PUFAs of bioflocs grown on glucose and glycerol as a carbon source and salinity of 0 and 30 ppt at different periods of incubation time (Adapted from Ekasari *et al.*, 2010)

Vitamins are also essential factor for the growth and survival of aquaculture species and as such, Anupama (2000) reported that bacteria and algae could contain some vitamins and minerals. However, Crag (2010) did not detect significant amount of vitamin C content bioflocs and recommended supplementation of vitamin C in BFT. Different organic carbon sources not only stimulate specific microbial community but also nutritional properties (Crab, 2010). An analysis of biochemical composition and the energy contents of the bioflocs supplied with different carbon sources revealed high

Tab. 1: Fatty acid composition (% lipid) of biofloc in high and low protein fed tanks (adapted from Azim and Little, 2008)

Fatty acid	35% CP Feed	24% CP Feed
C14:0	2.48	2.02
C15:0	0.77	0.70
C16:0	19.10	17.88
C18:0	6.24	7.27
C20:0	1.44	0.87
C22:0	1.31	1.06
C24:0	0.33	0.40
Total saturated	34.92	30.20
16:1n-9/n-7	7.74	7.15
18:1n-9	8.51	10.08
18:1n-7	11.05	11.28
20:1n-11/n-9	0.80	0.74
20:1n-7	0.00	0.13
Total monounsaturated	28.10	29.38
18:2n-6	15.38	16.68
20:2n-6	0.55	0.50
20:3n-6	0.40	0.46
20:4n-6	3.55	3.11
22:4n-6	0.34	0.59
22:5n-6	3.28	4.47
Total n-6 PUFA	23.50	25.81
18:3n-3	0.65	0.73
18:4n-3	0.06	0.05
20:3n-3	0.00	0.02
20:4n-3	0.00	0.06
20:5n-3	0.46	0.39
22:5n-3	0.00	0.09
22:6n-3	0.74	0.77
Total n-3 PUFA	1.91	1.38
16:2	0.00	0.02
16:3	1.32	0.80
16:4	0.03	0.08
Total PUFA	26.76	28.09
Unknown	10.22	12.33
Total	100.00	100.00
Total lipid％	3.16	3.23

protein, energy and lipid content in glucose bioflocs while high ash content was found in the acetate biofloc as starch based bioflocs yielded more carbohydrates (Crab, 2010) (Table 2).

Tab. 2: Nutritional qualities of the bioflocs.

	Glucose	Starch	Acetate
Crude protein (% DW)	40 ± 6	21 ± 3	19 ± 8
Crude lipid (% DW)	41 ± 9	17 ± 1	21 ± 11
Ash (% DW)	5 ± 2	3 ± 2	20 ± 10
Carbohydrate (% DW)	14 ± 17	59 ± 6	40 ± 13
Gross energy (kJ/g DW)	27 ± 2	21 ± 1	19 ± 1

Samples were taken over a 20 days period on regular time intervals (4 days) and the reactors were stable in time. Every carbon treatment was performed in triplicate (In courtesy of Crab, 2010)

Tilapia production using BFT ponds

Many authors consider BFT as a more sustainable and environmentally friendly aquaculture system, which has been tried both at laboratory and commercial scale for various aquaculture species including tilapia (Avnimelech, 2007; Azim and Little, 2008; Crab *et al.*, 2009). There is general consensus that application of BFT can improve the production performance of tilapia (Avnimelech, 2007; Kuhn *et al.*, 2009). Therefore, information concerning parameters of bioflocs and their influencing factors should be important in the development of BFT and aquaculture at large. The feeding habit of tilapia allow for grazing on attached periphyton as well as filter feeding on bioflocs suspended in the water column. Earthen pond system presents a perfect environment for BFT because the interaction of water with natural pond bottom encourages faster colonization by microbial community. In deed most of the tilapias are known to utilize *in situ* produced food particles including suspended bacteria (Figure 3) (Beveridge *et al.*, 1989; Beveridge and Baird, 2000).

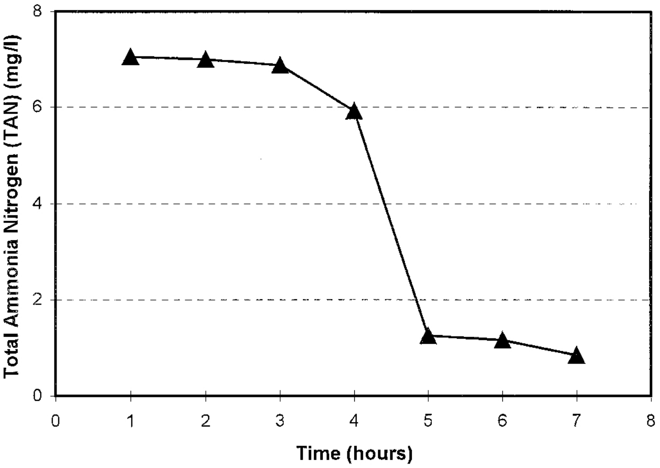

Fig. 3: Changes in TAN concentration in a suspension of pond bottom soil (2% dry soil) following the addition of glucose (TAN / glucose ratio of 1/20) (in courtesy of Avnimelech, 1999)

According to Avnimelech (2007), up to 200-300 tons.ha^{-1} biomass of tilapia can be harvested in well managed ponds with BFT. In a 50 day study, Crab *et al.* (2009) found an average daily growth rate of 0.27-0.29 g per fish for the hybrid tilapia (*O. niloticus x O. aureus*) stocked at a size of 50 and 100 g respectively in biofloc technology of tilapia during winter season. The stocking density was 20 kg.m^{-3} while C/N ratio was 20. Therefore, the BFT seems to be a practical solution for over-wintering of tilapia. To evaluate the effect of BFT on production performance of mixed sex red tilapia (*Oreochromis* sp.) at different stocking densities (25, 50 and 100 fish.m^{-3}), Widanarni *et al.* (2012) performed a 14 weeks study in 3 m^3 concrete tanks using 77 g fish. Molasses was added on BFT treatments as the organic carbon source at a C/N ratio of 15. Widanarni *et al.* (2012) found highest total yield in non BFT tanks whereas the highest survival was observed in BFT treatment with lowest density. Widanarni *et al.* (2012) attributed the observation to increased reproductive capacity of tilapia in BFT units thus tilapia yield may decline because most energy obtained from quality biofloc food could be directed to gonadal development at the expense of growth. Therefore, monosex male tilapia culture should be preferred. Besides, the suitability of molasses as carbon source in BFT ponds needs special scientific investigation.

The studies of Avnimelech *et al.* (1994) evaluating feed uptake and response to additional carbohydrates with tilapia hybrids (*O. niloticus x O. aureus*) in circular 50 m^2 pond at a density of 10 kg.m^{-2} yielded interesting results (Table 3). Fish were fed with either high protein diet of 30% (CN =11.1; conventional pond) or low protein diet of 20% (CN =16.6, the BFT treatment). The daily feed addition was 2% body weight in the conventional non BFT pond and 2.6% in the BFT treated pond to cover carbohydrates needed for the microbial ammonium conversion. Fish growth was superior in the 20% protein BFT treatment,

Tab. 3: Fish grown and yield coefficients of tilapia fed with conventional pellets in 2 pond experiments for 51 days in courtesy of Avnimelech *et al.* (1994)

	Treatments	
	Conventional control (30%) protein	BFT Carbon enriched 20% protein
Feed C:N ratio	11.1	16.6
Fish weight g/fish		
Initial weight	112	112
Final weight	193	218
Daily gain	1.59	2.0
Mortality (%)	14.6	10.3
Feed conversion coefficient	2.62	2.17
Protein conversion coefficient	4.38	2.42
Feed cost coefficient (US$/kg fish)	0.848	0.583

most likely due to the lower toxic inorganic nitrogen concentration as they were converted to microbial proteins. Lower Food Conversion Ratio (FCR) and Protein Conversion Ratio (PCR = protein in feed/protein in fish) was recorded in the BFT pond suggesting that protein utilization was higher in BFT compared to conventional non BFT pond. The active recirculation of proteins by microorganisms is credited for the increased protein utilization in fish. This is definitely positive information to farm managers who may even aim at increasing further recycling of proteins. Avnimelech *et al.* (1994) observed significant reduction of feed price in biofloc ponds (Table 3) hence lowering cost of production. Due to the fact that proteins are the most expensive ingredients of feed (Liti *et al.*, 2005) its reduction in fish farming systems is sweet news to all farmers.

In south califonia USA, Farrell (2006) grew *Tilapia mozambica* using BFT technology ponds with a daily water exchange rate of 5% in conjunction with excessive sludge drainage where tilapia biomass of 20 kg.m^{-2} was obtained. The 50 m^2 BFT ponds were fed with 20% protein pellet. Farrell (2006) obtained an average daily fish growth was 2.7 g while the pond expenses in BFT were lower than those in the conventional high water exchange pond by US $ 0.4/kg.

By using BFT in tilapia production, scientists can theoretically calculate the potential savings on food making BFT ideal for adoption. Indeed, Avnimelech (2011) reported that feed rations in biofloc tilapia systems can be lowered to at least 20% compared to conventional non BFT systems levels and still achieve better results. Assuming an average food conversion ratio (FCR) of 2.2 and 30% protein content diet (Kang'ombe *et al.*, 2007), a non BFT set up would require 2.2 kg of feed to produce 1kg of fish. With 30% of the feed being protein and 25% of the feed is actually taken up by the fish (Piedrahita, 2003), this leads to protein uptake of about 0.165 kg protein per kg fish produced. This resembles the protein content of 14-17% on wet fish biomass for tilapia as reported by Hanly (1991) almost two decades ago. In contrast, a system with bioflocs will have part of the feed recycled into flocs, which are fish food. Hence reduction of the conventional feed quantity is possible (Crag, 2010).

Assuming that 75% of feed is not taken up by the fish, this amount can be recycled into bioflocs. In case of complete conversion into bioflocs and that the fish will consume 25% of the bioflocs; the protein uptake through the bioflocs will be around 0.056 kg protein per kg fish multiplied by amount of conventional feed added (Crag, 2010). This direct and indirect uptake of commercial feed, leads to a total uptake of the feed by the fish by a factor of 1.75 higher than in a tilapia culture units without application of bioflocs technology (Crag, 2010).

Biofloc systems and water quality in ponds

Bioflocs technology has become a sustainable technique used in aquaculture to maintain good *in situ* water quality. This biological phenomenon happens through the development and control of dense heterotrophic microbial bioflocs by adding carbohydrate to the water (Avnimelech *et al.*, 1989; Avnimelech, 1999; Crab *et al.*, 2007; Crab *et al.*, 2009). However, research has shown that the capacity of the BFT to control the water quality in the culture system and the nutritional properties of the flocs are influenced by the type of carbon source used to produce the flocs (Crab, 2010).

According to Avnimelech and Ritvo (2003), only about 25% of the feed nutrients are converted into harvestable products hence contributing to high nitrogen residues in aquaculture water, especially total ammonia nitrogen (TAN), which is the sum of both ammonia and ammonium. In this regard, there is no doubt that the rapidly expanding intensive aquaculture all over the world contribute adverse environmental impacts as substantial amounts of wastewater, comprising wasted feed and faecal matter are contained in aquaculture the effluents (Read and Fernandes, 2003).

The main nitrogen pathways that naturally remove ammonia nitrogen in aquaculture systems include photoautotrophic removal by algae, autotrophic bacterial conversion of ammonia-nitrogen to nitrate-nitrogen, and heterotrophic bacterial conversion of ammonia-nitrogen directly to microbial biomass (Ebeling *et al.*, 2006). Therefore, development of dense heterotrophic microbial flocs in ponds can accelerate the biological 'cleaning' of organic and inorganic wastes in ponds (Avnimelech, 2005; Azim *et al.*, 2003a).

The periphyton community also play significant role in water quality control in aquatic systems. They consist of attached aquatic biota on submerged substrates and harbours algae, bacteria, fungi, protozoa, zooplankton and other invertebrates (Azim and Wahab, 2005). Given adequate light of up to about 0.5 meter depth in the water, periphyton can perform high rates of photosynthesis and autotrophic production (Craggs *et al.*, 1996; Vermaat, 2005). In this process, periphyton entraps organic detritus, removes nutrients from the water column and helps controlling the dissolved oxygen concentration, suspended solids and the pH of the surrounding water (Azim *et al.*, 2002; Dodds, 2003). Periphyton has an average C/N ratio of 10 (Azim and Asaeda, 2005) and nitrogen assimilation capacity of about 0.2 $gNm^{-2}day^{-1}$. From this it is clear that one needs a large surface, which allows periphyton growth, to treat intensive aquaculture wastewater. Since aquaculture contributes largely to their adjacent ecosystem, every effort focused on reducing negative impact is crucial to the sustainability of both *in situ* and surrounding environment.

Avnimelech (1999) demonstrated the effect of adding carbohydrates on the immobilization of TAN in a laboratory experiment consisting of a sediment suspension amended with ammonium about 10 $mg.l^{-1}$ and glucose at a concentration 20 times higher than that of the TAN. He found that almost all the added ammonium disappeared over a period of about 2 hours, following a short lag period (Figure 3).

Bioflocs as biosecurity in aquaculture systems

So far, the concept of green water technology in aquaculture development has been received with varied perceptions. With the bad publicity that aquaculture has received in regards to mishandling of antibiotics, scientists have continued to explore safe ways of arresting pathogenic infections in aquaculture facilities without using antibiotics (Naylor *et al.*, 2000). According to Defoirdt *et al.* (2011), most antibiotics are no longer effective in treating bacterial disease because of resistance developed to them by bacteria. Crab *et al.* (2010b) reported that biofloc technology could be a possible alternative to fight pathogenic bacteria in aquaculture facilities. Some group of bacteria in bioflocs have been found to conduct quorum quenching, which is the disruption of bacterial cell-to-cell communication (quarum-sensing) with small signal molecules (Defoirdt *et al.*, 2008). In fact, Defoirdt *et al.* (2004) reported that disruption of bacterial cell-to-cell communication mechanism prevents expression of virulence factors thus creating a disease free environment. Studies of Lezama-Cervantes and Paniagua-Michel (2010) on the role of biofloc in shrimp production revealed that primary production and promotion of *in situ* microbial populations, as is the case in biofloc technology, are beneficial for shrimp. In deed this can be extended to tilapia culture as well. However, the exact mechanism of the protective action of bioflocs and its selective action needs further investigation.

Recently, scientists have hypothesised possibilities of immunostimulatory features of the bioflocs leading to enhancement of the immunity of fish to provide broad-based resistance towards many infections (Crab *et al*., 2012). According to Wang *et al*. (2008), existing immunostimulants are group of live and synthetic compounds including bacteria and bacterial products, complex carbohydrates, nutritional factors, animal extracts, cytokines, lectins and plant extracts. Therefore bioflocs might also contain immunostimulatory compounds since biofloc technology deals with bacteria and bacterial products. However, the contribution of each bacterium to fish welfare is still not well understood.

Advantages and disadvantages of biofloc technology

Freshwater scarcity is for sure becoming global concern due to high human population growth. Use of biofloc technology encourages water conservation. Significant reduction of inorganic nitrogen accumulation; increased utilization of protein feed and reduction of feed expenditure in biofloc mediated systems have been reported in biofloc systems (Avnimelech, 1999, 2009; Kuhn *et al*., 2009; Crab *et al*., 2010, 2012). The BFT approach can decrease fish oil and fishmeal utilization in aquaculture by providing high quality cheap alternative protein source to the cultured organism. The biofloc technology provides opportunity to produce high fish yield all year-round, and the flexibility to locate production facilities (tanks) near large markets to deliver a fresher, safer product and lower transport cost (Schneider *et al*., 2005). The fact that there are no cases of fish escape to the environment, biological pollution is thus prevented. Being more independent from the external environment due to increased levels of control, BFT systems improved hygiene, disease risk management, lower feed quantity supplied and thus overall cost of production (Summerfelt, 2006).

However, the entire biofloc process is completely dependent on the availability of sunlight (Azim and Asaeda, 2005) and therefore maximum nitrogen uptake is limited to light sufficiency. The task of harvesting the periphyton is laborious making the application of the periphyton treatment technique in the intensive aquaculture sector expensive. Nevertheless, the technique may be significant in smaller extensive aquaculture systems in developing countries.

The biofloc technology is not yet fully predictable and can therefore be risky to implement at farm level in developing countries (Crab *et al.*, 2012). It is not easy to convince farmers to implement the technique, since the concept of biofloc technology goes in against common wisdom that water in the pond has to be clear (Avnimelech, 2009). Release of polluted effluents from bioflocs also pause challenges as environmentalists are keen to prevent such happenings. Some microbial community in the bioflocs may turn to cause diseases to the cultured fish. The most challenging issue is the experience and technical knowledge regarding management of biofloc technology and the economic benefits that goes with it.

Conclusions and recommendations

BFT offers aquaculture a sustainable tool to simultaneously address its environmental, social and economical issues concurrent with its growth. Microbial flocs developing in bio-flocs technology (BFT) ponds are potential food source for fish. Indeed, the BFT can be considered as a self sustaining biotechnology machine as it maintains water quality *in situ* and manufacture food concurrently thereby becoming the neglected asset in aquaculture industry. As the cost of fish feed continue to rise, BFT could be the solution. It is because of the stress free environment created by the bioflocs that enhance quick growth rate of disease free fish in BFT aided aquaculture systems.

Nevertheless, further investigations should be focused on some aspects of the BFT such as management of the biofloc production and the health effects of bioflocs to cultured animals. The microbiological characterization in BFT needs special scrutiny to unravel scientific myths surrounding probiotic organisms in the microbial community of the bioflocs. Indeed this could solve the problems of antibiotic use in aquaculture sector. BFT is certainly the bright idea for farmers in semi arid areas in developing countries because less water is used. The government, NGOs and researchers should join hands to improve livelihood in such areas through BFT initiatives.

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