ABAH BIOFLUX

Animal Biology & Animal Husbandry International Journal of the Bioflux Society

Small-scale production of biofloc using various carbon sources for the freshwater culture of tilapia, *Oreochromis* sp.

Christopher M. A. Caipang, Han X. Choo, Zemin Bai, Huilin Huang, Clara M. Lay-Yag, Joel Lim

[1]School of Applied Science, Temasek Polytechnic, Singapore. Corresponding author: C. M. A. Caipang, cmacaipang@yahoo.com

Abstract. The use of various carbon sources on the production of biofloc and its effects on water quality, microbial population and fish yield in freshwater tilapia, *Oreochromis* sp. culture was evaluated in glass aquaria. Two biofloc treatments (wheat flour biofloc and corn flour biofloc) and one control were managed in 20 L glass aquaria indoor tanks. Biofloc was produced in the glass aquaria for a month and the carbon sources and commercial feed (crude protein content 35%) were added at a carbon:nitrogen (C:N) ratio of 16:1. Moderate aeration was provided in all aquaria to keep the flocs in suspension and to provide ample amount of dissolved oxygen. One month after biofloc production, five tilapia fingerlings (average weight of 2.5 g) were stocked in each aquarium and reared for another 15 days. The fish were fed commercial pellets at 6% body weight and the carbon sources were added to the treatment groups in order to maintain the C:N ratio of 16:1. The levels of total suspended solids (TSS) and biochemical oxygen demand (BOD) as well as the total heterotrohic bacteria and bacilli populations were significantly higher in the treatment groups than in the control. There was an observed variability in the levels of ammonia, nitrite and nitrates between the treatments and the control during studies. Fish survival was 100% in all groups. No significant differences in the average weights of tilapias were observed in all groups, although the tilapias that were reared in the biofloc groups have lower FCR than the control. This was clearly evident in the tilapias that were reared in biofloc using wheat flour as carbon source. Taken together, the results of this study showed that tilapias are able to utilize the bioflocs as food; thus, can augment the nutritional requirements of the fish. As a carbon source for biofloc production, wheat flour could be potentially used to maintain the stability of biofloc in freshwater tilapia aquaculture.

Key Words: Aquaculture, bacteria, BFT, phytoplankton, water quality.

Introduction. Biofloc technology (BFT) when applied in an aquaculture, setting focuses on the more efficient use of nutrient inputs with limited or zero water exchange. The main underlying principle of BFT is to recycle nutrients by maintaining a high carbon/nitrogen (C/N) ratio in the water. This high C/N ration in the rearing water triggers the growth of heterotrophic bacteria that convert ammonia into microbial biomass (Avnimelech 1999). The microbial biomass will further aggregate with other microorganisms and particles suspended in the water forming what has been called “biofloc”, which eventually can be consumed *in situ* by the cultured animals or harvested and processed as a feed ingredient (Avnimelech 1999, 2007; Crab et al 2007; De Schryver et al 2008; Kuhn et al 2009, 2010). With this principle, BFT is therefore considered as a promising system for a sustainable and environmentally friendly aquaculture system, and has been applied both at laboratory and commercial scale for various aquaculture species such as tilapia (Avnimelech 2007; Azim & Little 2008; Crab et al 2009), shrimp (Burford et al 2004; Hari et al 2006), sturgeon and snook (Serfling 2006). In spite of the benefits of BFT on water quality in aquaculture, there have been only a few studies conducted to validate which among the carbon sources can maintain continuous and stable bioflocs in freshwater culture system.

The objective of the present study was to determine the effects of various carbon sources, namely wheat flour and corn flour on biofloc production and water quality in the culture of freshwater tilapia using glass aquaria. In addition, the effects of these various carbon sources on fish yield were also assessed.

Material and Method

Glass aquaria and experimental design*.* The experiment was carried out in small glass aquaria (20 L capacity) at the Aquaculture Workstation of Temasek Polytechnic. Two treatments (with 3 replicates) and one control (with 3 replicates) were compared, namely, biofloc produced using wheat flour as carbon source (Treatment 1 - Carbon A), biofloc produced using corn flour as carbon source (Treatment 2 - Carbon B) and aquaria with no flour added served as control. The biofloc production in glass aquaria was conducted for a period of 30 days.

At the start of the experiment, each glass aquarium was filled with 1 liter of freshwater that was obtained from the koi ponds of Temasek Polytechnic. The volume was scaled up to 18 L using chlorine-free freshwater and added with 100 mL of *Chlorella* sp. at a density of approximately 10^8 cells mL^{-1}. Subsequently, Vitagen, a commercial yoghurt drink that contained *Lactobacillus* sp., a known probiotics, was cultured in MRS broth overnight. The following day 100 mL of the broth (cell density of 10^7 mL^{-1}) was added to all aquaria. In all treatment groups, flour was added at a carbon:nitrogen (C:N) ratio of 16:1 (Crab et al 2012) daily for 1 week, every 2 days on the second week and every 4 days on the 3rd week until the end of the experiment. Commercial feed pellets were added at 0.5 g every 2 days. For the control aquaria, 0.5g of feed was added daily in each aquarium for 30 days. During biofloc production, water exchange was carried out once a week in the treatment groups at 20% of the water volume, while in the control the same volume of water was changed every 3 days.

After a month of biofloc production in glass aquaria, five tilapia fingerlings with average body weight of approximately 2.5 g were stocked in each aquarium. The treatment groups were fed with commercial feed pellets every 2 days, while flour was added daily at a C:N of 16:1. The control group was fed daily with commercial feed pellets. Feeding rate was fixed at 6% B.W. of the total fish weight in all groups. Feeds were given every morning at 9:00 AM to all aquaria. For the control group, 20% of the water volume was changed every 2 days while for the treatment groups, the same volume of water was changed every 10 days or whenever the total suspended solids exceed 500 mg L^{-1}.

Monitoring the water quality parameters*.* Water temperature and pH were determined daily in the morning using YSI 556 multiparameter system to ensure pH and temperature were within suitable range for tilapia culture. Total ammonia-nitrogen (TAN), nitrite (NO_2-N) and nitrate (NO_3-N) from the water samples were determined using commercial kits and a benchtop spectrophotometer (DR3900) following the procedures of the manufacturer (Hach Company, Colorado, USA). Total suspended solids (TSS) and biochemical oxygen demand (BOD) were analyzed every 10 days using the procedures described in APHA (1989). After filtering water (25 or 50 mL), the pre-dried and weighed filter paper containing suspended materials was dried in an oven until constant weight. The dried samples were weighed to 0.01 mg using a Mettler AC 100 balance and the TSS TSS was calculated from the weight differences. For BOD determination, water samples in each aquarium were collected, saturated with air and filled in two BOD bottles (1 light and 1 dark). The initial oxygen concentration in the light BOD bottles was determined using a commercial kit (Hach Company). The dark bottles were firmly closed, wrapped with black plastic and placed in a dark room at 25-27°C for 5 hours. At the end of this time, the dissolved oxygen level was determined again and BOD was calculated in mg L^{-1}.

Bacterial count*.* Total heterotrophic bacteria and bacilli counts from the water were determined every 10 days. Water samples were serially diluted and 100 μL of the various dilutions were plated onto both nutrient agar plates and MRS agar plates. Each dilution was performed with 2 replicates. The plates were then incubated at 28ºC for 2 days and bacterial counts were expressed as $\log_{10}$ colony forming units (CFU) mL^{-1}.

Fish growth parameters. At the end of the experiment, harvesting was done and the weight of individual tilapia was recorded. Average body weight (ABW) and feed conversion rate (FCR) were calculated using the following equations:

Average body weight (ABW) = Total weight of fish in the tank (g) / Total number of fish in the tank

Feed conversion ratio (FCR) = Dry weight of feed given (g) / Weight gain (g)

Statistical analyses. The values of the different water quality and fish yield parameters were expressed as Means + SD. The fish yield parameters in the different groups were compared using one-way ANOVA. If the main effects were significant, differences among the treatments were tested with Tukey's multi-comparison test of means. The analyses were run at 5% significance level using Systat.

Results and Discussion. The average water temperature and pH were within the suitable range (3.7-11 for pH, and 22-29°C for temperature) for tilapia culture except for the decline in pH nearing the end of the experiment.

The TSS readings in both treatments (Carbon A and B) were higher compared to the controls throughout the experiment (Figure 1). BOD readings corresponded to the TSS values and were also higher in both treatments as compared to the control (Figure 2). These results were also in conjunction with those of Azim & Little (2008), where TSS and BOD levels in BFT treatments (Carbon A and B) were significantly higher compared to control.

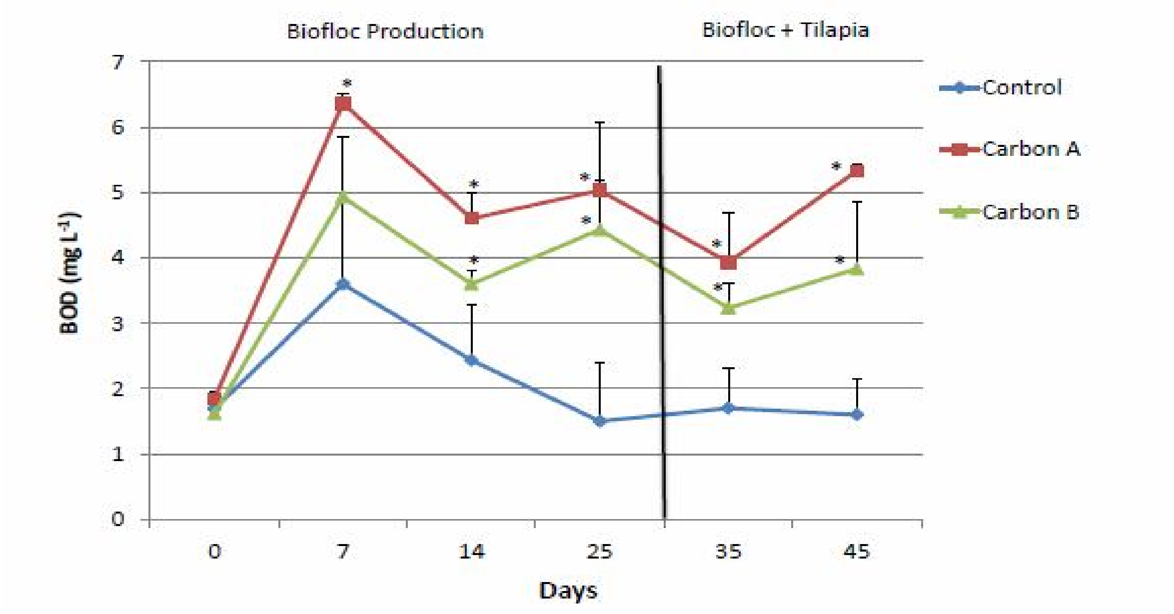

Figure 1. Biochemical oxygen demand (BOD) in glass aquaria with and without biofloc. Asterisk at each time point indicates significant difference at $p<0.05$.

Dissolved inorganic nitrogen (TAN, NO_2-N, NO_3-N) concentrations throughout the experiment period are shown in Figures 3-5, respectively. At the start of the experiment, TAN levels in BFT treatments remains elevated in the treatment aquaria compared to the controls. After day 30, TAN concentration in the controls began to increase above the TAN concentration in the treatments. Nitrite concentrations in the treatments remains lower compared to the controls before the addition of tilapia on day 30. After day 30, NO_2 concentration increased sharply before decreasing on the last sampling date. Throughout the experiment, NO_3 concentration in both treatments remains lower compared to the controls.

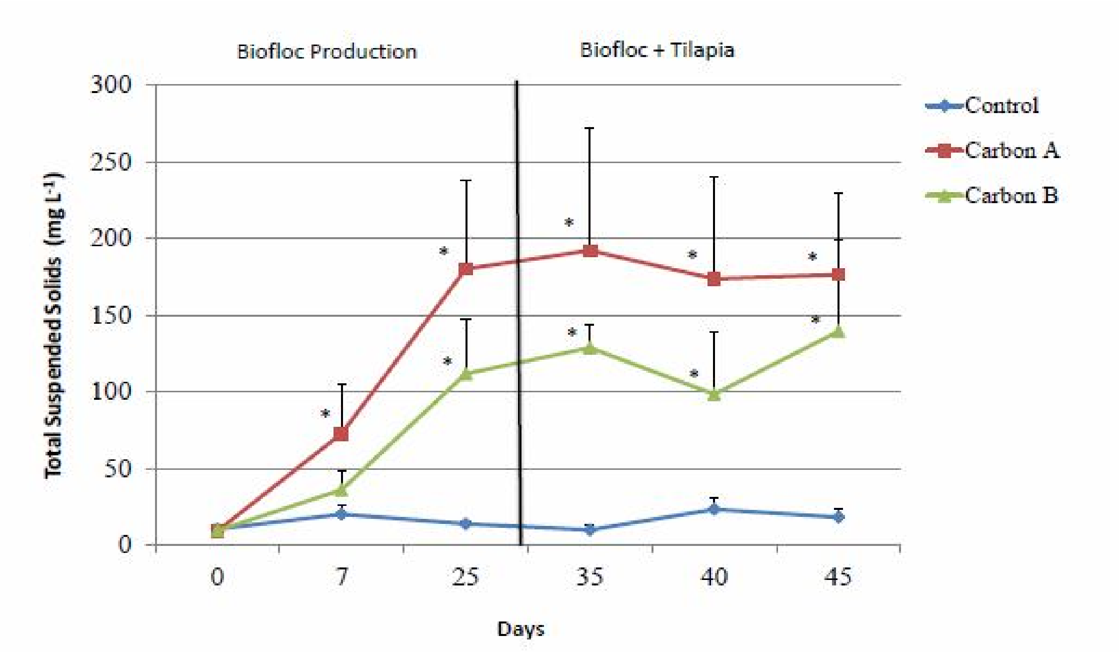

Figure 2. Total suspended solids (TSS) in glass aquaria with and without biofloc. Asterisk at each time point indicates significant difference at $p<0.05$.

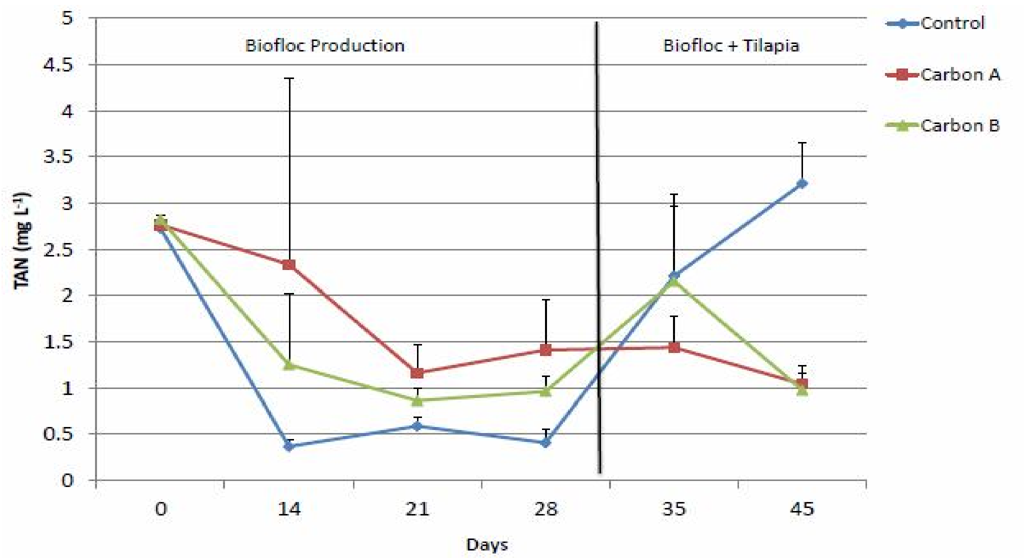

Figure 3. Total ammonia-nitrogen (TAN) in glass aquaria with and without biofloc. Asterisk at each time point indicates significant difference at $p<0.05$.

Based on the results obtained, higher TAN concentration in the treatment aquaria during biofloc production (before day 30) could be due to the less frequent water exchange compared to the controls, which lead to organic matter build-up and higher rate of mineralization, which increase the TAN concentration in the treatments. After tilapia was added on day 30, TAN concentration remains stable while the TAN concentration in the controls increased above the concentration of the treatment tanks. This could be due to the increase in ammonia excretion by the tilapia fingerlings, along with the lack of organic carbon to convert inorganic nitrogen into bacterial biomass (Megahed 2010). During biofloc production, nitrite and nitrate concentrations in the controls remain higher compared to the treatment tanks. These results may suggest that nitrification was most likely occuring in the controls, resulting in higher concentration of inorganic nitrogen species. Another possibility is the daily addition of commercial feeds in the control group in contrast with every alternate day feeding in the treatment groups. The uneaten feeds could also contribute to higher nitrogenous waste in the control group.

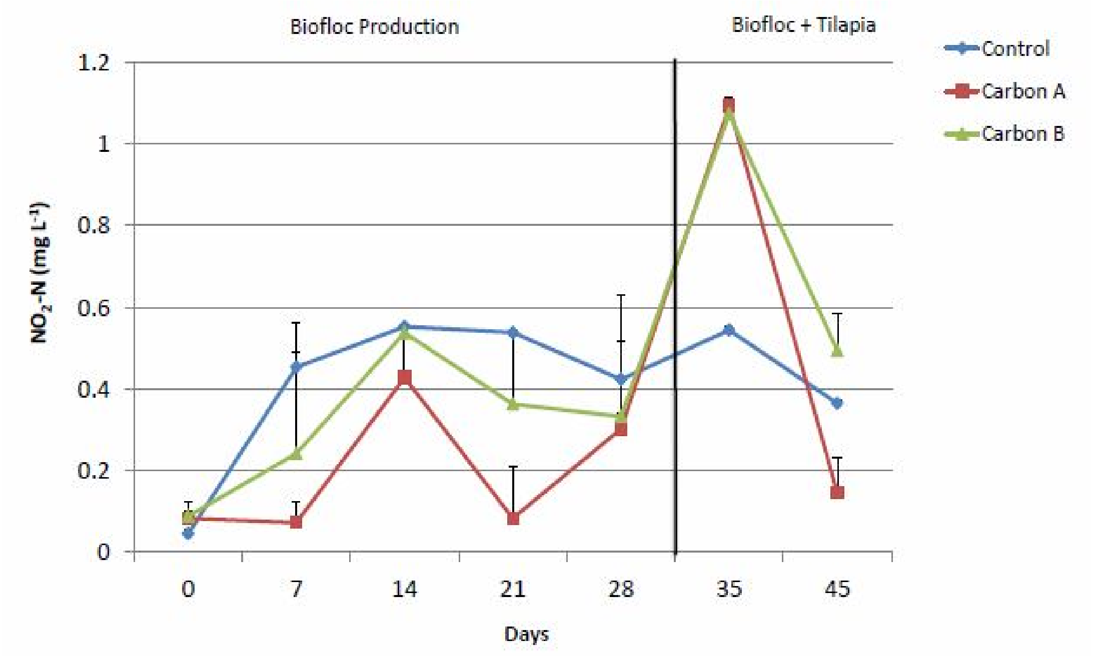

Figure 4. Nitrite-N (NO_2-N) in glass aquaria with and without biofloc. Asterisk at each time point indicates significant difference at $p<0.05$.

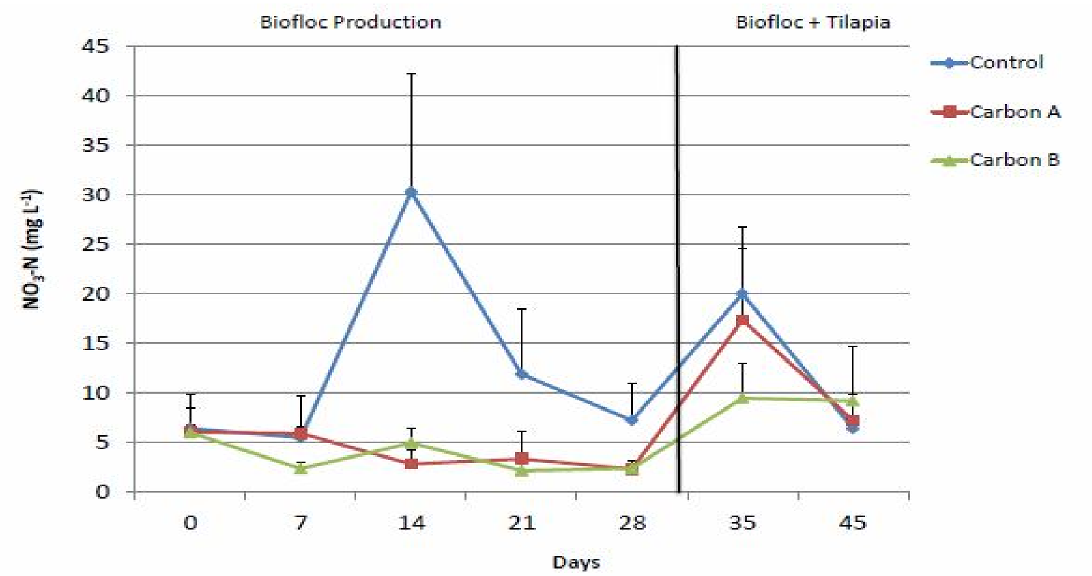

Figure 5. Nitrate-N (NO_3-N) in glass aquaria with and without biofloc. Asterisk at each time point indicates significant difference at $p<0.05$.

There was higher population of heterotrophic bacteria (Figure 6) and bacilli (Figure 7) in the treatment groups compared with the control. This is possibly due to the increased production of bacteria in these aquaria upon addition of the carbon sources. This is supported by Avnimelech (1999) where maintaining high C:N ratio in a system could stimulate the growth of heterotrophic bacteria. In the total bacteria count, the readings for wheat flour treatment were consistently higher as compared to the control group. However for the corn flour treatment, the bacteria count was similar to the control group in three samplings (1st, 2nd and 4th sampling) and only increasing above the control group during the 3rd sampling. This indicates that wheat flour could be a better carbon source in maintaining stable growth of the bacterial population growth in comparison with the corn flour. The high population of bacilli in all groups at the beginning of sampling indicated the growth of *Lactobacillus* sp., during the addition of the broth containing a commercial yoghurt drink. Succeeding samplings in all groups showed a sharp drop, which is an indication that the conditions of the rearing waters of tilapia may not be the best growth medium of the probionts although they can still be viable. However, the population of the bacilli remained stable in the wheat flour biofloc group, and this could likely explain that wheat flour has the potential of supporting and maintaining bacilli in a BFT system. The presence of high populations of bacilli in a BFT system needs to be carefully examined in future studies in terms of their contributions towards the beneficial effects of biofloc in aquaculture.

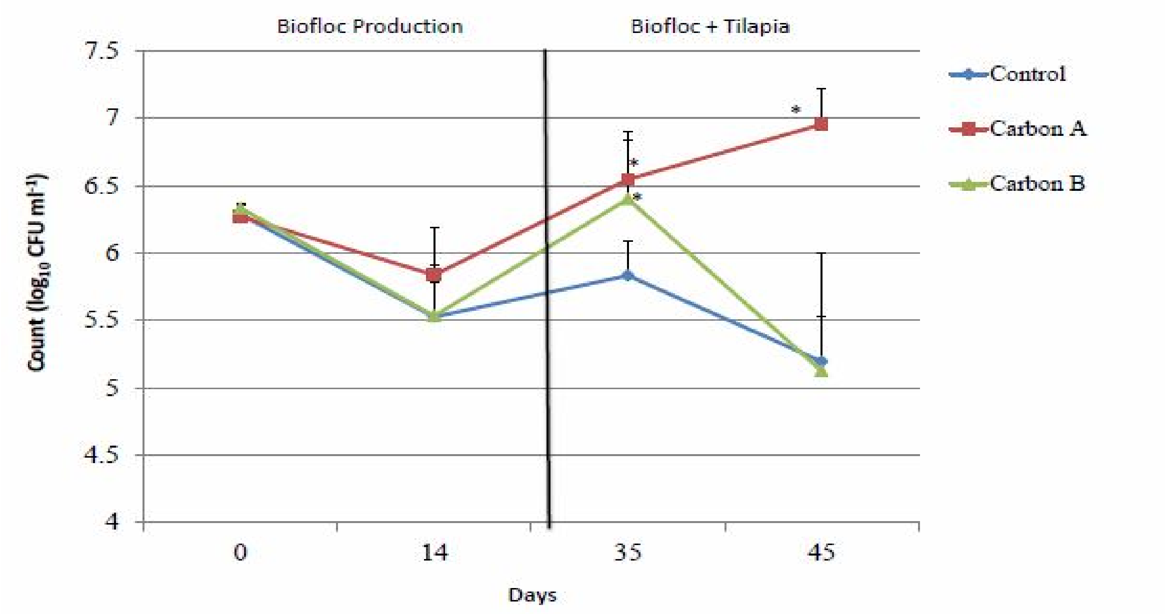

Figure 6. Heterotrophic bacterial counts in the rearing water of glass aquaria with and without biofloc. Asterisk at each time point indicates significant difference at $p<0.05$.

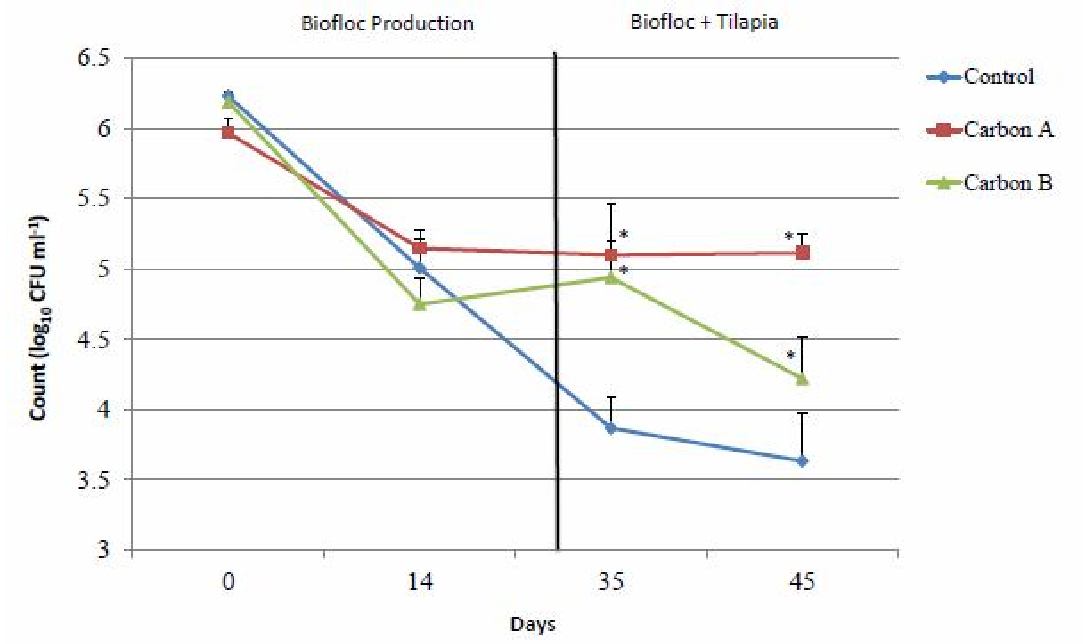

Figure 7. Bacilli population in the rearing water of glass aquaria with and without biofloc. Asterisk at each time point indicates significant difference at $p<0.05$.

At the end of the experiment, the tilapia fingerlings in each aquarium were weighed and the growth parameters of each treatment (controls, wheat flour and corn flour) were determined. The average weight of tilapia in the biofloc groups and the in control are shown in Figure 8. There were no significant differences in the average weights of tilapias that were grown in either biofloc or without biofloc, although the average weight of tilapias in the corn flour (Carbon B) biofloc group was lowest.

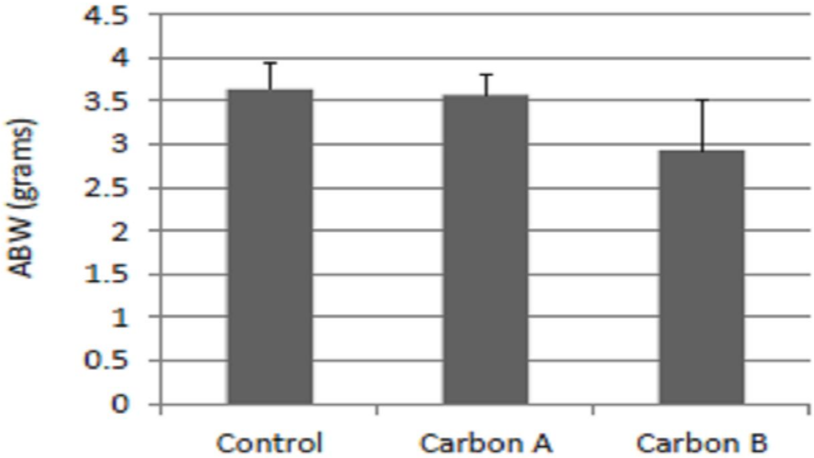

Figure 8. Average body weights of tilapias reared in glass aquaria with and without bioflocs.

On the other hand, the FCR of tilapias reared in the wheat flour (Carbon A) biofloc was the lowest among the groups (Figure 9).

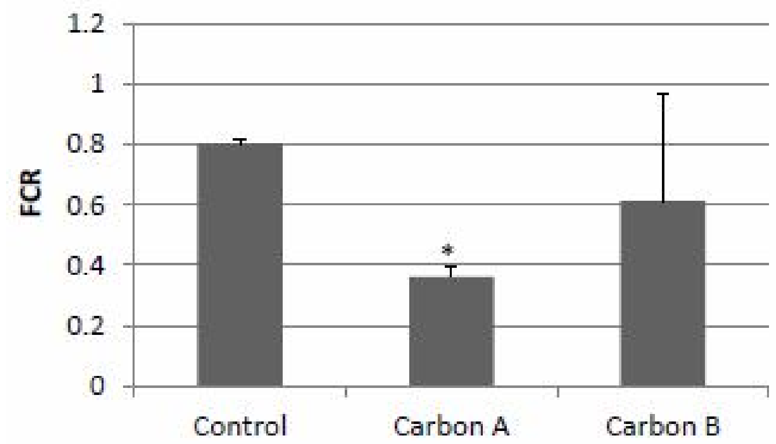

Figure 9. Feed conversion rate (FCR) tilapias reared in glass aquaria with and without biofloc. Asterisk above column bar indicates significant difference at $p<0.05$.

Based on the results, bioflocs especially those produced using wheat flour as carbon source can augment the nutritional requirements contributed by commercial feeds as shown by the comparable effects on fish weight between the biofloc treatment groups and the control (given with commercial feed). This could indicate the utilization of biofloc by the tilapia as food. As was reported by Avnimelech (2007) that biofloc could be taken in by Mozambique tilapia using stable nitrogen isotope labeling technique. However, the use of wheat flour for the production of biofloc has a positive effect on FCR. This difference in the feed conversion efficiency could be related to the nutritional content of the bioflocs that are produced using different types of carbon sources. Ekasari et al (2014) demonstrated such differences exist in the nutritional content of bioflocs that are produced by different carbon sources and the results of the present study corroborated the earlier findings

Tilapias that were reared in the biofloc groups have lower FCR compared with the control. The results obtained are in conjunction with the results of Azim & Little (2008), where the FCR values obtained was also significantly higher in the control tanks as compared to the biofloc treatments. The results further confirm that biofloc is utilized by tilapia as food. FCR in the corn flour treatment is higher compared to the wheat flour treatment, further indicating nutritional differences in the biofloc between the two treatments. Future research on the effect of the two carbon source on the nutritional quality of biofloc is needed.

Conclusions. Using glass aquaria, it was evident that biofloc clearly contributed to the growth and production of tilapia. The nutritional quality of biofloc was sufficient to augment the growth requirements of herbivorous and omnivorous fish species including tilapias. This was shown by the comparable growth rates of tilapias reared in BFT with those fish fed entirely with commercial feed pellets. In glass aquaria, the water quality of the biofloc groups were variable indicating that stability of various parameters could be affected by the amount of bioflocs that are being produced. However, the production and potential use of heterotrophic bacteria using fish waste could potentially improve water quality in the BFT systems, thus it would result in lower nutrient discharge and increase nutrient retention thereby ensuring future sustainability of aquaculture systems relying on BFT. A clear understanding on the various microbiological aspects, characterization of biofloc, and possible manipulation of microbial community is crucial towards the successful design and implementation of such technology in aquaculture.

Acknowledgements. This paper is part of the Major Student's Project (Optimization studies of biofloc technology (BFT) in freshwater tilapia culture: effects on water quality and fish growth) of Choo H. X. The authors gratefully acknowledge the funding support provided by the School of Applied Science, Temasek Polytechnic.

References

APHA (American Public Health Association) - American Water Works Association, and Water Pollution Control Association, 1989 Standard methods for the examination of water and wastewater. 17^{th} ed., American Public Health Association, Washington, DC, USA.

Avnimelech Y., 2007 Feeding with microbial flocs by tilapia in minimal discharge bioflocs technology ponds. Aquaculture 264:140–147.

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

Azim M. E., Little D. C., 2008 The biofloc technology (BFT) in indoor tanks: water quality, biofloc composition, and growth and welfare of Nile tilapia (*Oreochromis niloticus*). Aquaculture 283:29-35.

Burford M. A., Thompson P. J., McIntosh R. P., Bauman R. H., Pearson D. C., 2004 The contribution of flocculated material to shrimp (*Litopenaeus vannamei*) nutrition in a high-intensity, zero exchange system. Aquaculture 232:525–537.

Crab R., Defoirdt T., Bossier P., Verstraete W., 2012 Biofloc technology in aquaculture: beneficial effects and future challenges. Aquaculture 365-357:351-356.

Crab R., Kochva M., Verstraete W., Avnimelech Y., 2009 Bio-flocs technology application in over-wintering of tilapia. Aquaculture Engineering 40:105–112.

Crab R., Avnimelech Y., Defoirdt T., Bossier P., Verstraete W., 2007 Nitrogen removal techniques in aquaculture for a sustainable production. Aquaculture 270:1–14.

De Schryver P., Crab R., Defoirdt T., Boon N., Verstraete W., 2008 The basics of bio-flocs technology: the added value for aquaculture. Aquaculture 277:125-137.

Ekasari J., Angela D., Waluyo S. H., Bachtiar T., Surawidjaja E. H., Bosier P., De Schryver P., 2014 The size of biofloc determines the nutritional composition and the nitrogen recovery by aquaculture animals. Aquaculture 426-427:105-111.

Hari B., Madhusoodana K. B., Varghese J. T., Schrama J. W., Verdegem M. C. J., 2006 The effect of carbohydrate addition on water quality and the nitrogen budget in extensive shrimp culture systems. Aquaculture 252:248–263.
Kuhn D. D., Boardman G. D., Lawrence A. L., Marsh L., Flick G. J., 2009 Microbial floc meals as a replacement ingredient for fish meal and soybean protein in shrimp feed. Aquaculture 296:51–57.
Kuhn D. D., Lawrence A. L., Boardman G. D., Patnaik S., Marsh L., Flick G. J., 2010 Evaluation of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp, *Litopenaeus vannamei*. Aquaculture 303:28–33.
Megahed E. M., 2010 The effect of microbial biofloc on water quality, survival and growth of the green tiger shrimp (*Penaeus semisulcatus*) fed with different crude protein levels. Journal of the Arabian Aquaculture Society 5:9-14.
Serfling S. A., 2006 Microbial flocs. Natural treatment method supports freshwater, marine species in recirculating systems. Global Aquaculture Advocate 34–36 June 2006.

Received: 30 May 2014. Accepted: 01 July 2015. Published online: 02 July 2015.
Authors:
Christopher Marlowe Arandela Caipang, School of Applied Science, Temasek Polytechnic, Singapore, 529757, e-mail: cmacaipang@yahoo.com
Han Xuan Choo, School of Applied Science, Temasek Polytechnic, Singapore, 529757, e-mail: choohanxuan@live.com.
Zemin Bai, School of Applied Science, Temasek Polytechnic, Singapore, 529757, e-mail: baizemin@tp.edu.sg
Huilin Huang, School of Applied Science, Temasek Polytechnic, Singapore, 529757, e-mail: huilin@tp.edu.sg
Clara M. Lay-Yag School of Applied Science, Temasek Polytechnic, Singapore, 529757, e-mail: claramly@tp.edu.sg
Joel Lim, School of Applied Science, Temasek Polytechnic, Singapore, 529757, e-mail: 1204436H@student.tp.edu.sg

How to cite this article:
Caipang C. M. A., Choo H. X., Bai Z., Huang H., Lay-Yag C. M., Lim J., 2015 Small-scale production of biofloc using various carbon sources for the freshwater culture of tilapia, *Oreochromis* sp. ABAH Bioflux 7(1):103-111.