Original Research Articles

Effects of biofloc technology on water quality and growth performance of *Macrobrachium rosenbergii*

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This study aimed to evaluate the effects of biofloc technology on the water quality and growth performance of *Macrobrachium rosenbergii*. The experiment was carried out over 165 days at the hatchery complex of the College of Fisheries and Aquatic Sciences, Mindanao State University-Marawi, Philippines. Postlarvae of *M. rosenbergii* (0.011±0.05 g) were stocked in 18 circular tanks (1200 L) consisting of six treatments under biofloc and non-biofloc systems in a completely randomized experimental design with tapioca flour as the carbohydrate source. Commercial pelletized sinking prawn feed with three dietary protein levels, viz., 42, 38, and 35% were chosen as experimental feeds. Results of the present study show that the water parameters were within the optimum range required in the culture of freshwater prawns. However, biofloc technology (BFT) did not influence sediment and water samples' dissolved oxygen, temperature, and pH values. On the other hand, total ammoniacal nitrogen (TAN) and nitrate showed significant variation ($p<0.05$) among treatments, with higher values in the control treatment compared to the biofloc treatments. Moreover, the dietary protein level and carbohydrate addition had no significant effect on soil pH and organic carbon except for the soil total nitrogen value, where higher values were observed in the biofloc treatments. Furthermore, significantly higher mean prawn weight gain, net prawn yield, specific growth rate (SGR), and average daily weight gain (ADG) were recorded in the biofloc treatments compared to non-biofloc treatments. At the end of the culture period, there was 100% survival in all of the tanks. On the other hand, no significant variations were observed in feed conversion ratio (FCR) and protein efficiency ratio (PER) in the control treatment compared to the treatment with biofloc except in tanks using feed of 42% CP under the biofloc system. Therefore, based on the results of the present study, the use of BFT in the culture system of *M. rosenbergii*, helped reduce the protein percentage from 42 to 35, while maintaining the yield.

INTRODUCTION

With an average annual growth rate of 5.3% between 2001 and 2018, aquaculture is the agricultural industry with the fastest growth rates worldwide.[1] Until 2030, this growth is anticipated to continue at a four percent annual rate.[2] This is necessary to address the lack of protein-rich dietary supplies, particularly in developing nations. Yet, there are significant concerns over the viability of a number of the techniques involved, such as the financial and environmental constraints that may limit its development. The industry must develop a technique to improve economic and environmental sustainability for aquaculture to succeed.[3] The current problem facing the aquaculture business is figuring out how to change its current course towards one where development and the environment go hand in hand. Historically, the most popular approach or solution for addressing this pollution has been the continuous replacement of pond water with outside fresh water. A second strategy involves employing various biologically based water treatment methods to remove the majority of the pollutants in the water, like in recirculating aquaculture systems (RAS). However, this method requires a high initial capital outlay. Moreover, RAS operation raises labor and energy costs, making it very expensive for farmers.[4] The development of biofloc technology is a solution for sustainable production

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in light of the expanding viral issues and rising energy expenses. Advanced technology for resolving the aforementioned issues is biofloc technology. The broad consensus is that bacteria and other microbes can cause disease in plants and animals. Nonetheless, the bacterial population may be efficiently utilized with reasonable and scientific control. The rapid expansion of aquaculture required to meet future protein demand will depend on increasing productivity without burdening land and water resources, implementing sustainable technologies that reduce environmental effects, and creating affordable production methods that support long-term economic and social sustainability.

Biofloc technology has become popular in the culture of tilapia, *Penaeus monodon*, and *Litopenaeus vannamei*. Also, it has been successfully applied to shrimp aquaculture in Australia and Indonesia.[5] With zero-exchange shrimp production methods, US researchers have significantly increased stocking densities, achieving harvest biomass levels nearly five times greater than usual intensive pond production rates. This development has dramatically increased interest in the dynamics of regulated shrimp production in systems with low or no interchange.[6] Many shrimp producers are now interested in biofloc technology due to the success stories in Indonesia and the United States. Several shrimp producers in China are also interested in implementing this method. Commercial biofloc studies are being conducted by a group from Brazil. At Setiu, Terengganu, Blue Archipelago is launching a 1,000-ha integrated intensive shrimp farming project for Malaysia. The biofloc technique is not yet widely used in the Philippines. Only white shrimp had been raised using the method. Biofloc technology previously undergone pilot testing in three Mindanao, Visayas, and Luzon farms. Nevertheless, no research has been done on how this technology may affect the Philippines' freshwater prawn culture. The present study represents the first attempt to use BFT in the freshwater prawn culture grow-out in the Philippines with the objective of determine the effects of biofloc technology on water quality and estimating its efficiency in the culture of *M. rosenbergii*.

MATERIALS AND METHODS

EXPERIMENTAL SITE AND DURATION

The study was carried out at the hatchery complex of the College of Fisheries and Aquatic Sciences, Mindanao State University-Main Campus, Marawi City, Lanao del Sur, Philippines over 165 days of culture.

EXPERIMENTAL DESIGN AND TREATMENTS

The experiment consisted of six treatments under the biofloc and non-biofloc systems with different protein levels of feeds. Commercial palletized sinking prawn feed with three dietary protein levels, viz., 42, 38, and 35%, were selected as experimental feeds. Each treatment had three replicates and was randomly assigned to the tank. The rearing units consisted of 18 Fiber Reinforced Plastic (FRP) tanks, each having a 1200L capacity. The C/N ratio of the treatments was calculated using the formula of Avnimelech.[7] The system was maintained for 165 days without any water exchange. Water loss due to evaporation was compensated by adding dechlorinated water as per requirement.

BFT CULTURE TANK SYSTEM

All tanks were provided with heater sand and filled with water with a depth of 60 cm. All tanks were provided with 4 air-stone hoses type of diffuser system throughout the experiment to increase bio flocculation rate and were kept for one week for dechlorination. Biofloc water was prepared before stocking, which involved adding commercially available probiotics (Biomate), tapioca, and feeds by following the manufacturer's instructions. After one week, all tanks were stocked with freshwater prawns at $15/m^2$.[8] Before stocking, water and sediment parameters and the initial weight of the organism were recorded.

EXPERIMENTAL ANIMAL

Postlarvae of *M. rosenbergii* were purchased from BFAR-NFDC, Dagupan City, Pangasinan, Philippines. The seeds were transported in oxygenated double-layered polythene bags. Postlarvae were reared for two (2) weeks in fiberglass tanks for acclimatization before stocking them in the experimental tanks. Stocking was done early in the morning.

CARBON SOURCES AND FEEDING

Tapioca flour was selected as the carbohydrate source. The product was acquired from the local market. The amount of carbohydrates added to the BFT system was computed using the formula of Avnimelech.[9] Commercial feed at 15% of initial weight (1-90 days) was used to feed the freshwater prawns, calculated and adjusted by estimating the monthly sampled mean biomass. The ration was divided and distributed twice a day between 0900 and 1000 hours and between 1700 and 1800 hours, both in similar portions. Following the first feeding, pre-weighed carbohydrate source was supplied to the tanks fed with 42, 38, and 35% protein feed, respectively, by mixing it with water obtained from the corresponding culture tanks in a glass beaker and poured directly into the water column.[9] The carbon source was added one hour (1) after feeding. The feeding rate and carbon source added were adjusted after every sampling.

MONITORING OF THE PHYSICO-CHEMICAL AND ENVIRONMENTAL PARAMETERS OF WATER AND SEDIMENT

Water quality parameters such as temperature, dissolved oxygen, pH, and nitrite were measured in situ using the YSI instrument at 9:00 am on a daily basis. Total ammoniacal nitrogen and nitrite of water samples were measured using the water test kits. Water samples were collected using a water sampler from three locations of each tank and pooled together. Sediment samples were collected from three locations using PVC pipes. Sediment and water samples were collected on a bi-weekly basis between 9:00 and 10:00 in the morning. Water and sediment samples were brought to a laboratory for the analysis of nitrate-N ($N0_3$-N), organic carbon, and total nitrogen, respectively.

DETERMINATION OF GROWTH AND YIELD PARAMETERS OF *M. ROSENBERGII*

The effects of BFT to *M. rosenbergii* at different protein levels were assessed based on growth parameters such as:

Mean weight gain (g) = Mean final weight - Mean initial weight

Net prawn yield (g/m^2) = Total biomass at harvest - total biomass at stocking

Specific growth rate (SGRd-1) = (Log final weight (g) - log initial weight (g) / rearing period in days

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

Protein efficiency ratio (PER) = Live weight gain (g) / Protein intake (g)

Average daily weight gain (ADG) (g) = (Final mean weight (g) – Initial mean weight (g) / rearing period in days

Survival rate (%) = 100 × (Number of prawns stocked - Number of prawns died) / Number of prawns stocked

STATISTICAL ANALYSIS

All non-repeatedly measured variables such as prawn growth, yield, FCR, SGR, PER, and survival rate were analyzed using One-way analysis of variance (ANOVA) to determine any significant differences. Duncan's Multiple comparison was used to compare means between groups. Water and sediment quality parameters were analyzed using Two-way ANOVA. All data were considered significant at $p<0.05$ level.

RESULTS

As shown in **Table 1**, the mean values of the water quality parameters such as temperature and water pH, DO and nitrite showed no significant difference ($p>0.05$) observed among the treatments. Based on the results of the present study, the water parameters were within the optimum range required in the culture of freshwater prawns.[8] Optimum water quality requirements for the grow-out of giant freshwater prawns are follows; water temperature 28-31℃, pH 7-8.5, and dissolved oxygen 3-7 ppm. In the present study, these parameters are within the optimum range. Water pH value in the present study was in the range of 7.0-7.3, which is very near to the prescribed optimum value. However, BFT did not show any influence on the dissolved oxygen, temperature, and pH values both in sediment and water samples. On the other hand, ammonia and nitrate, showed significant variation ($p<0.05$) among treatments, with higher values in the control treatment compared to the biofloc treatment. The treatment with carbohydrate addition showed a significant reduction ($p<0.05$) in inorganic nitrogen production in water and sediment. The treatment 35% CP in non-biofloc treatment showed significantly ($p<0.05$) higher water TAN concentrations while lower values were reported from treatment 38% and 42% CP from biofloc treatment. The results revealed that the addition of carbohydrates to the water column is effective in reducing ($p<0.05$) the TAN and nitrite-N levels during the rearing period. Nevertheless, the dietary protein level and carbohydrate addition had no significant effect on soil pH, and organic carbon except for the soil total nitrogen value, where higher values were observed in the biofloc treatment.

The effect of biofloc technology on weight, prawn yield, SGR, FCR, PER, ADG, and survival of *M. rosenbergii* in indoor trials using different %CP feeds for a duration of 165 days are shown in **Table 2**. Significantly higher mean prawn weight gain was observed in the biofloc treatments added with carbohydrate source with 42% CP having the highest mean prawn weight gain of 27.51±3.03 g followed by 38 and 35% CP with 24.58±2.79 g and 23.46±0.16 g, respectively. On the other hand, significantly lower mean prawn weight gain was obtained in the non-biofloc system, with 35% CP having the lowest mean prawn weight gain of 15.89±0.87 g. Consequently, higher net prawn yield was observed in the biofloc treatments, with 42% CP having the highest net prawn yield of 407.25±26.20 g, followed by 38 and 35% CP with 368.63±24.11 g and 351.86±1.38 g, respectively. Nevertheless, a significantly lower net prawn yield was observed in the non-biofloc tanks, with 35% CP having the lowest net prawn yield of 238.40±7.50 g.

Furthermore, SGR in the biofloc tanks shows higher values in biofloc tanks with 42% CP having the highest SGR of 2.42±0.02 g/day and 35% CP under the non-biofloc treatment having the lowest SGR of 2.26±0.01 g/day. The average daily weight gain in the biofloc systems shows better results than the non-biofloc system, with 42% CP having the highest ADG of 2.91±0.19 g. On the other hand, no significant difference was observed in FCR in all treatments. Furthermore, a 100% survival rate was observed in all treatments for non-biofloc and biofloc systems. In the present study, feeds with three levels of protein were examined. Growth parameters were found to be better in biofloc systems fed with 42% CP and more or less the same in the tanks fed with 38 and 35 %CP. On the other hand, lower growth parameters were obtained in the non-biofloc system.

Consequently, significantly better results were observed in treatments fed with 35% CP combined with biofloc than in treatments fed with 42% CP alone. With this, adding carbohydrates reduces the need for dietary protein concentration. From the present study, it is clear that there is no compromise in the growth parameters of prawns grown in the BFT-applied tanks fed with lower protein.

DISCUSSION

This study aimed to determine the effects of biofloc technology on water quality and to estimate its efficiency in the culture of giant freshwater prawns. In the present study, all the water parameters were within limits required in the culture of freshwater prawns, with lower values in the biofloc systems compared to the non-biofloc treatments.[8] The accumulation of dissolved nitrogen, especially ammonium,

Table 1. Physico-chemical characteristics (mean ± SD) of the rearing water both for the biofloc and non-biofloc tanks during the culture of giant freshwater prawn, *Macrobrachium rosenbergii,* using different CP% feeds for 165 days.

WATER PARAMETERS	TREATMENT					
	Control			With Biofloc		
	35% CP	*38% CP*	*42% CP*	*35% CP*	*38% CP*	*42% CP*
Temperature	29.68±0.19^{a}	29.69±0.10^{a}	29.61±0.08^{a}	29.59±0.08^{a}	29.41±0.02^{a}	29.61±0.06^{a}
pH	7.0±0.00^{a}	7.0±0.00^{a}	7.0±0.00^{a}	7.3±0.02^{a}	7.3±0.01^{a}	7.3±0.02^{a}
DO (mg/l)	7.15±0.09^{a}	7.21±0.09^{a}	7.35±0.07^{a}	7.31±0.02^{a}	7.34±0.13^{a}	7.48±0.05^{a}
Nitrite (mg/l)	1.96±0.03^{a}	1.81±0.10^{a}	2.25±0.02^{a}	2.79±0.17^{a}	0.97±0.82^{a}	1.07±0.71^{a}
TAN (mg/l)	0.65±0.01^{c}	0.60±0.02^{bc}	0.61±0.02^{c}	0.29±0.17^{ab}	0.21±0.08 ^{a}	0.23±0.08 ^{a}
Nitrate (mg/l)	3.37±0.08 ^{d}	3.4±0.08 ^{d}	3.31±0.05^{c}	3.28±0.04^{c}	3.02±0.02^{a}	3.06±0.02^{b}
SEDIMENT PARAMETERS						
pH	6.4±0.08^{a}	6.5±0.02^{a}	6.53±0.10^{a}	6.8±0.03^{a}	6.6±0.03^{a}	6.73±0.02^{a}
Organic Carbon	0.21±0.02^{a}	0.2±0.02^{a}	0.23±0.08^{a}	0.2±0.03^{a}	0.23±0.02^{a}	0.26±0.03^{a}
Total-Nitrogen	0.102±0.01^{a}	0.121±0.04^{ab}	0.11±0.08^{ab}	0.204±0.10^{b}	0.195±0.3^{b}	0.11±0.28^{a}

Values with the same superscripts are not significantly different at $p< 0.05$.

Table 2. Effects of biofloc technology on weight, prawn yield, SGR, FCR, PER, ADG, and survival (mean ± SD) of *Macrobrachium rosenbergii* (0.011 g) in indoor trials using different %CP feeds for 165 days.

PARAMETERS	TREATMENT					
	Control			With Biofloc		
	35% CP	*38% CP*	*42% CP*	*35% CP*	*38% CP*	*42% CP*
Mean prawn weight gain (g)	15.89±0.87^{a}	17.13±1.92^{ab}	16.29±0.89^{ab}	23.46±0.16^{bc}	24.58±2.79^{c}	27.51±3.03^{d}
Net prawn yield (g/m^{2})	238.40±7.50^{a}	256.95±16.66^{a}	244.32±7.70^{a}	351.86±1.38^{bc}	368.63±24.11^{bc}	407.25±26.20^{c}
FCR	3.14±0.07^{a}	2.90±0.14^{a}	2.96±0.10^{a}	3.24±0.24^{a}	3.34±0.24^{a}	3.28±0.12^{a}
SGR (g/day)	2.26±0.01^{a}	2.28±0.02^{a}	2.27±0.01^{a}	2.38±0.02^{b}	2.39±0.02^{b}	2.42±0.02^{b}
PER	0.91±0.02^{b}	0.91±0.04^{b}	0.81±0.03^{ab}	0.89±0.07^{b}	0.80±0.06^{ab}	0.73±0.03^{a}
ADG (g)	1.70±0.05^{a}	1.84±0.12^{a}	1.75±0.05^{a}	2.51±0.01^{b}	2.63±0.17b^{c}	2.91±0.19^{c}
Survival rate (%)	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Values with the same superscripts are not significantly different at $p< 0.05$.

due to the addition of food and organisms raised at high density is one of the key issues in intensive shrimp culture systems, impacting their food absorption, growth, and survival rates. Ammonia-N and organic carbon levels rose in response to dietary protein content, according to Cavalli et al.[10] However, as demonstrated in the current study, nitrogenous component concentrations were decreased in both water and sediment with the aid of biofloc technology and the addition of a carbohydrate source. The same result was observed in the study of Deng et al.[11] on the effect of different carbon sources on water quality, microbial community, and structure of biofloc systems, wherein they evaluated the water quality in zero-exchange bioflocs and reported low concentrations of ammonia, which resulted from the synthesis of new cells by heterotrophic bacteria. Their study showed a significant decrease in TAN concentration in biofloc systems compared to the control treatment. The research mentioned above also showed a decrease in TAN concentration. Shrimps exposed to high ammonium levels appear to have decreased disease resistance. According to Zhu and Chen,[12] autotrophic nitrifying bacteria remove ammonia at a rate high enough to keep water quality at a level that does not cause fish mortality. As observed in the present study, the toxic ammonia is transformed into less toxic nitrite and nitrate. According to Mallasen et al.,[13] nitrate is not a limiting factor for giant river prawn larviculture, so the high nitrate in the present study has no significant implications.

Results of the growth parameters in the present study showed significantly higher values in the biofloc system compared to the control. This finding is in agreement with the result obtained in the study of Xu and Pan[14] in which they conducted a 30-day feeding experiment to investigate the effects of promoted bioflocs on growth performance, feed utilization, digestive enzyme activity, and whole-body composition of *Litopenaeus vannamei* juveniles in zero-water exchange culture tanks. Their results showed that the promoted bioflocs significantly improved shrimp growth performance and feed utilization. The growth (in terms of final weight, weight gain, and specific growth rate) of the shrimp in both bioflocs treatments was significantly higher than that obtained in the control. Furthermore, in the present study, 35% CP in combination with biofloc has significantly higher mean prawn weight gain, net prawn yield, SGR, and ADR compared to treatments using only high protein CP (42%) and without the combination of biofloc. The dietary protein's nutritional value affects shrimp growth. About 60% of the cost of production on extensive, semi-intensive, and intensive farms is accounted for by feed. As a result, efforts have been made to lower the cost of feed by using less expensive, highly nutritious ingredients or by improving the animals' ingestion and assimilation of feeds. The level of protein in the diet must be optimized because it is a costly component of the fish diet.[15] Various research findings have emphasized the significance of conserving protein by adopting less expensive energy sources, such as lipids and carbohydrates.[16,17] Lipids and carbohydrates are less expensive energy sources than proteins.[15] The biofloc production depends critically on the carbon sources and their nutritive values.[18,19] In the present study, tapioca powder was used as a carbohydrate source, which was then utilized by the bacteria as food and produced microorganisms' protein via the system's subsequent absorption of nitrogen.[20] Based on the present study's results, tapioca's application as a carbohydrate source has been successful.

It has been shown that biofloc technology can eliminate toxic metabolite compounds like ammonia through bacterial metabolism. The animal ingesting flocculated microbial protein effectively makes up for the feed's lower protein content by converting more N inputs into harvestable products. Therefore, based on the results of the present study, the use of BFT in the culture system of giant freshwater prawn, *M. rosenbergii*, helped reduce the protein percentage from 42 to 35 without compromising the yield. Farmers can adopt farming using feeds with 35% protein with BFT application.

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REFERENCES

1. *The State of World Fisheries and Aquaculture 2020. Sustainability in Action*. FAO; 2020. doi:10.4060/ca9229en

2. Nova Scotia DFA. *Aquaculture Strategy: Creating Sustainable Wealth in Rural and Coastal Nova Scotia*. Dept. of Fisheries and Aquaculture; 2012.

3. Megahed ME. 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*. 2010;5:119-142.

4. De Schryver P, Crab R, Defoirdt T, Boon N, Verstraete W. The basics of bio-flocs technology: The added value for aquaculture. *Aquaculture*. 2008;277(3-4):125-137. doi:10.1016/j.aquaculture.2008.02.019

5. Taw N. Biofloc technology expanding at white shrimp farms biofloc systems deliver high productivity with sustainability global aquaculture advocate. 2010;May/June.

6. Browdy CL, Venero JA, Stokes AD, Leffler J. Superintensive bio-floc production technologies for marine shrimp Litopenaeus vannamei: technical challenges and opportunities. In: *New Technologies in Aquaculture: Improving Production Efficiency, Quality and Environmental Management*. Elsevier; 2009:1010-1028. doi:10.1533/9781845696474.6.1010

7. Avnimelech Y. *Biofloc Technology - A Practical Guide Book*. The World Aquaculture Society; 2009.

8. New MB. *Farming Freshwater Prawns: A Manual for the Culture of the Giant River Prawn (*Macrobrachium Rosenbergii*)*. Vol 428. FAO; 2002.

9. Avnimelech Y. Carbon/nitrogen ratio as a control element in aquaculture systems. *Aquaculture*. 1999;176(3-4):227-235. doi:10.1016/s0044-8486(99)00085-x

10. Cavalli RO, Wasielesky W Jr, Franco CS, Miranda FC. Evaluation of the short-term toxicity of ammonia, nitrite and nitrate to *Penaeus paulensis* (CRUSTACEA; DECAPODA) Brood stock. *Brazilian Archives of Biology and Technology*. 1996;39(3):567-575.

11. Deng M, Chen J, Gou J, Hou J, Li D, He X. The Effect of different carbon sources on water quality, microbial community and structure of biofloc systems. *Aquaculture*. 2018;482(January):103-110. doi:10.1016/j.aquaculture.2017.09.030

12. Zhu S, Chen S. An experimental study on nitrification biofilm performances using a series reactor system. *Aquacultural Engineering*. 1999;20(4):245-259. doi:10.1016/s0144-8609(99)00019-9

13. Mallasen M, Valenti WC, Ismael D. Effects of nitrate concentration on larval development of the giant river prawn, *Macrobrachium rosenbergii*. *Journal of Applied Aquaculture*. 2004;14(3-4):55-69. doi:10.1300/j028v14n03_05

14. Xu WJ, Pan LQ. Effects of bioflocs on growth performance, digestive enzyme activity and body composition of juvenile *Litopenaeus vannamei* in zero-water exchange tanks manipulating c/n ratio in feed. *Aquaculture*. 2012;356-357:147-152. doi:10.1016/j.aquaculture.2012.05.022

15. Gumus E, Ikiz R. Effect of dietary levels of lipid and carbohydrate on growth performance, chemical contents and digestibility in rainbow trout (*Oncorhynchus mykiss* Walbaum, 1792). *Pakistan Veterinary Journal*. 2009;29(2):59-63.

16. García Gallego M, Bazoco J, Akharbach H, Suárez MD, Sanz A. Utilization of different carbohydrates by the European eel (*Anguilla anguilla*). *Aquaculture*. 1994;124(1-4):99-108. doi:10.1016/0044-8486(94)90365-4

17. Okoye FC, Eyo AA, Aminu NG. Growth of tilapia, *Oreochromis niloticus* hybrid fingerlings fed lipid-based diets. In: Eyo AA, ed. *Fish Nutrition and Fish Feed Technology*. ; 2001:53-58.

18. Hollender J, van der Krol D, Kornberger L, Gierden E, Dott W. Effect of different carbon sources on the enhanced biological phosphorus removal in a sequencing batch reactor. *World Journal of Microbiology and Biotechnology*. 2002;18(4):359-364. doi:10.1023/a:1015258308460

19. Oehmen A, Yuan Z, Blackall LL, Keller J. Short-term effects of carbon source on the competition of polyphosphate accumulating organisms and glycogen accumulating organisms. *Water Science and Technology*. 2004;50(10):139-144. doi:10.2166/wst.2004.0629

20. Avnimelech Y. Minimal discharge from intensive fish ponds. *World Aquaculture*. 1998;29:32-37.