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Use of Nitrifying Bacteria for Promoting Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man) Nursery Phase in Indoor System

Suantika G*, Turendro OR and Situmorang ML

Microbial Biotechnology Research Group, School of Life Sciences and Technology, Institute Teknologi Bandung, Indonesia

Abstract

This research aims to determine the effect of periphytic biofilter in static culture of giant freshwater prawn *Macrobrachium rosenbergii* de Man through the application of nitrifying bacteria in 50 l containers equipped with $CaCO_3$ as substrate for the nitrifying bacteria and bamboo shelter as a substrate for the periphytic attachment. This experiment was carried out by following three consecutive stages: (1) inoculation of nitrifying bacteria into the culture at the concentration of 10^5 CFU.mL^{-1}, 24 h before the prawn Post Larvae age 40 days (PL_{40}) was stocked; (2) stocking of prawn PL_{40} into four treatment groups with different number of bamboo shelter: without shelter (0) at the prawn density of 10 individuals per container, and with addition of 2, 4, or 8 shelters, at the prawn density of 30 individuals per container; and (3) measurement of biological, physical, chemical and microbiological parameters of water quality. Following 60 days culture period, optimum performance was obtained in the culture with 4 shelters with the prawn final body weight, length, and total biomass of 1.00 ± 0.04 g, 4.91 ± 0.03 cm, and 63.17 ± 4.27 g, respectively. The observed water quality parameters (level of ammonium, nitrite, nitrate, dissolved oxygen, pH and temperature) were within the tolerance level for prawns and were relatively stable during the culture period. Moreover, a stable microflora community was observed, with the abundance of periphytic bacteria in the culture water was between 10^2 and 10^5 CFU.mL^{-1}. Five bacterial isolates were obtained from the water samples and periphytic culture on bamboo substrate samples. Based on the overall results, the use of periphytic biofiltration technology through the application of nitrification bacteria can potentially increase the stability of physical-chemical parameters of water quality and microflora community, supporting the growth and survival of prawn *M. rosenbergii* in nursery phase.

Keywords: Biofiltration technology; Nitrifying bacteria; *Macrobrachium rosenbergii* de Man; Nursery phase

Introduction

Giant freshwater prawn (*Macrobrachium rosenbergii* de Man) is one of the freshwater aquaculture commodities that grow significantly during the last decade [1]. The high growth was significantly supported by several advantages attributes to the commodity such as: (1) more resistance to the water quality and diseases compared to the penaeid shrimp, (2) less complicated in term of rearing technology and protocol, and (3) able to be cultured at wider area in inland ponds.

From economic point of view, the increase demand of freshwater prawn product contributes to the development of rearing technology, productivity improvement, and expansion of the industry, including hatchery, nursery, and grow out stage. Even though several improvements have been made in order to improve the quality and quantity of the products, there are still many disadvantages attribute to its culture starting from larviculture, nursery, and grow out [2].

In Asian hatcheries including Indonesia, one of the critical stages for further industrialization of this sector is the high unpredictability on the survival and growth of the prawn during nursery phase. During this stage the prawn culture mostly conducted in earthen ponds with very low rate of water make up and using untreated surface water. Besides, the culture is conducted without any consideration of ambient factors that negatively affecting growth and survival of the larvae such as water quality, diseases control, and predator control [3]. It is not surprising that under this unpredictable and stressful culture condition PL-Juvenile productivity during the nursery phase is usually very low and takes a longer culture period to reach Post Larvae age 40 days (PL_{40})/ juvenile stage (1.5-2.0 g). Recently, juvenile production efficiency of the nursery phase was about 15% or equivalent with 14 individuals.m^{-2}.

Several improvements have been made in order to improve the performance of juvenile production, such as the use of shelter technology [2,4]. However, the use of the technology was not able to significantly improve the quantity of the juvenile produced. By using this apartment technology, the stocking density of 20 individuals.m^{-2} and survival percentage of 80% were obtained during the laboratory scale trial. Since the application of this technology was aimed only to provide an expansion of the vertical territories for PL to avoid cannibalism without any consideration on the improvement and manipulation of the water quality, further improvement of the nursery stage either on quality or quantity by manipulation of the microbial loop in order to ensure a wider vertical territory and improvement of water quality still remaining as an optimistic opportunity.

Based on the rearing condition above, the development of a biofiltration technology combined with the application of the three-dimensional territory substrate adapted for the need of the commercial operation was conducted in order to create a better and more stable water quality and at the same time enable to provide a wider territory horizontally and vertically.

Materials and Methods

A) *Macrobrachium rosenbergii* de Man Post Larvae 40 days (PL_{40})

All experiments were performed using *Macrobrachium rosenbergii*

***Corresponding author:** Suantika G, Microbial Biotechnology Research Group, School of Life Sciences and Technology, Institute Teknologi Bandung, Jl. Ganesa 10 Bandung 40132, Indonesia, Phone: +62 22 2511575; Fax: +62 22 253 4107; E-mail: gsuantika@sith.itb.ac.id

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de Man Post Larvae 40 (PL_{40}) with average initial weight and length was 0.19 g and 2.14 cm, respectively. The PL_{40} origin was from a commercial hatchery at the java North Sea coastal area, Sukamandi-West Java, Indonesia.

B. Nitrifying bacteria

Nitrifying bacteria as a component of biofiltration agent and periphytic component that used for the trial was cultured and scaled up in a batch bioreactor system [5] (Figure 1). The nitrifying bacteria was isolated and purified from a commercial freshwater hatchery at Sukabumi, West Java, Indonesia and reared in the Laboratory of Microbiology, School of Life Sciences and Technology, Institut Teknologi Bandung, Indonesia.

C. Experimental set up

Rearing cages were placed in laboratory representing four treatments of the trial. At treatment with two shelter (treatment "2") the rearing cage at indoor rearing tank was equipped with two 3-dimensional cubical bamboo frame (each 72 dm^3 in volume; Figure 2), which occupied ± 20% of the total culture volume. At treatment with four shelter (treatment "4"), the rearing cage at the indoor rearing tank was equipped with four 3-dimensional cubical bamboo frame which occupied ± 40% of the total culture volume. At treatment with eight shelter (treatment "8"), the rearing cage at the indoor rearing tank was equipped with eight 3-dimensional cubical bamboo frame which occupied ± 60% of the total culture volume. For control (treatment "0"), the rearing cages were run without any addition of 3-dimensional cubical bamboo frame. The trial was conducted by using three replicates per treatment during 60 days of culture period.

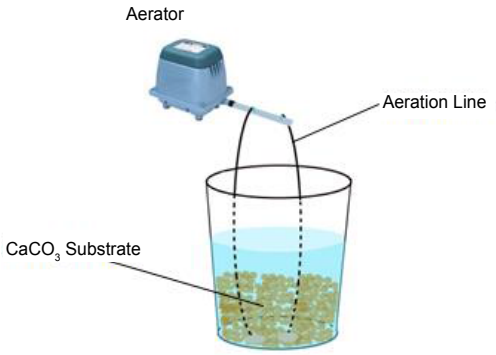

Figure 1: Bioreactor of nitrifying bacteria.

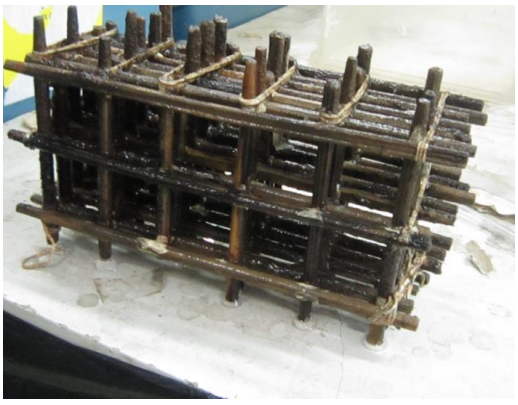

Figure 2: Three-dimensional cubical bamboo frame shelter.

Nitrifying bacteria were inoculated into each rearing tanks at the concentration of 10^5 $CFU.mL^{-1}$, 24 h prior prawn PL_{40} stocking, in order to manipulate the microbial loop especially in breaking down the ammonium accumulation level in the culture through a nitrification process and at the same time to uptake the nitrate accumulation as the end product of the nitrification. Bacterial addition was conducted once every two weeks during 60 days of culture period.

Initial stocking density was arranged differently among the treatments. At the 0 shelter, prawn stocking density was 10 individuals, while at treatment with 2, 4, and 8 shelters, the stocking density were 30 individuals (~200% stocking density increment). This difference in stocking density between treatment and control refers to the study conducted by Suantika et al. [5]. The prawn reared in container with 50 l water volume capacity as illustrated in Figure 3 for 60 days.

During the trial, PL was fed with an artificial diet at the feeding level of 5% of the total body weight. Feed was delivered three times a day at 09.00 am, 12.00 am, and 03.00 pm by spreading the feed homogenously into the rearing frames manually.

D) Sampling and counting

Several prawn and culture water quality parameters including biological, physical, chemical, and microbiological parameters were measured during the trial.

E) Biological parameters

Growth, length, survival percentage (SP), and total final biomass were measured at the beginning of culture period (day 0) and at the end of culture period (day 60). Measurement was conducted on all stocked PL and harvested PL. PL growth was calculated by using the following equation:

$$SGR\ (\%\ day)=[Ln\ (W2_{/}W_{1)}/(T2\text{-}T_{1)} \times 100]$$

Where:

SGR=Specific Growth Rate

W_1=Initial live body weight (gram) at time T_1 (day)

W_2=Final live body weight (gram) at time T_2 (day)

The survival percentage of the PL during the trial was conducted by using the following equation:

$$SP=Nt/No \times 100\%$$

Where:

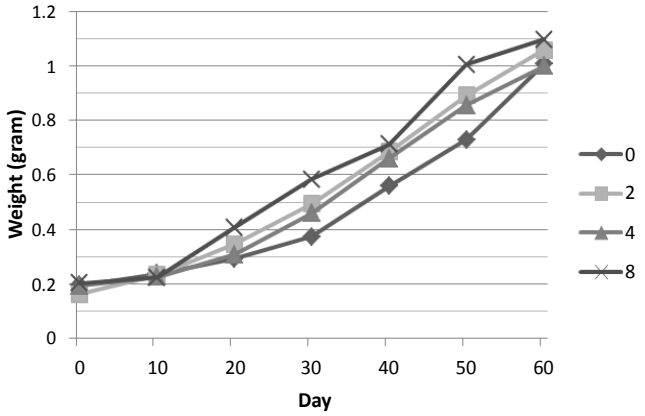

Figure 3: Schematic diagram of prawn culture system.

SP=Survival percentage

No=Initial individual quantity

Nt=Final individual quantity

Physio-chemical parameters

Physical (temperature, dissolved oxygen, pH) and chemical (ammonium, nitrite, and nitrate) parameters of the culture water were measured daily and once per four days, respectively, during the culture period. Concentration of ammonium (NH_4^+), nitrite (NO_2^-), and nitrate (NO_3^-) were measured using a HACH Spectrophotometer by following the Nessler-, Diazotation-, and Nitrate HCl method at the wavelengths of 425, 371 and 275, 220 nm, respectively [6].

F) Bacterial sampling

Microbiological analyses were performed on a weekly basis by sampling 10 mL water from each rearing tank, and following the methods for serial dilution and total plate count (TPC). 100 µL samples from the serial dilution were plated in triplet on Nutrient Agar (NA) (Difco, USA). The plates were incubated at 25°C and bacterial counts performed after 24 h [7,8].

G) Statistical analysis

All data were statistically checked using one-way analysis of variance (ANOVA). Significant differences among mean values ($P<0.05$) were tested using Duncan's multiple range test.

Results and Discussion

In general, the application of nitrifying bacteria and the use of 3-dimensional bamboo shelter positively affected the culture performance of giant freshwater prawn (*M. rosenbergii*) culture at the nursery phase. This new approach on rearing strategy enable the batch system to provide a more appropriate culture conditions both in water quality and culture carrying capacity improvements. In term of water quality, the application of nitrifying bacteria that has important note on nitrogen cycle especially in reducing accumulation of toxic inorganic nitrogen into nitrate, contribute significantly in maintaining a low and stable ammonium and nitrite level in the water. This condition significantly affecting the prawn culture performance including growth, SP, total biomass (Figures 4 and 5).

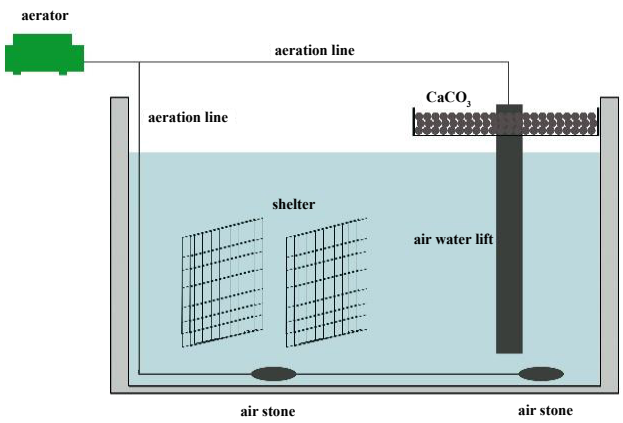

Figure 4: Average individual body weight during 60 days of culture period.

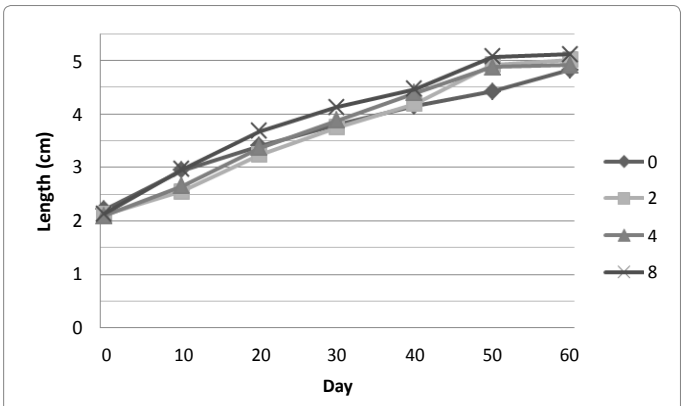

Figure 5: Average individual body length 60 days of culture period.

Treatment	Biomass	SGR	SP
(Shelter)	(g. tank^{-1})	(%BW.day^{-1})	(%)
0	38.77 ± 0.716^{a}	0.027 ± 0.001^{a}	100 ± 0^{a}
2	50.48 ± 4.194^{ab}	0.031 ± 0.002^{a}	82.22 ± 0.03^{a}
4	63.17 ± 4.227 ^{b}	0.027 ± 0.005^{a}	98.89 ± 0.01^{a}
8	67.94 ± 1.605 ^{b}	0.028 ± 0.002^{a}	94.44 ± 0.01^{a}

Note: Different letters in the same column show significant difference ($P<0.05$).

Table 1: Prawn biomass and SGR (Mean ± St.Dev) during 60 days of culture period.

The use of nitrifying bacteria enables the culture system to provide culture with physical – chemical parameter in the tolerance range for the prawn, especially for ammonium, nitrite, and nitrate (Table 1). The measured water quality parameters are illustrated in Figures 6 and 7. Ammonium concentration of 0.1 ppm is known to cause problem to the prawn culture [5,9]. Nitrite level remains under 1 ppm during the culture period at all treatments, while highest nitrate level remains under 60 ppm. The stability of N inorganic level in the culture water is the aim of aqua culturist to create more predictable culture conditions since the toxicity of the compound can cause problem to the culture performance. The nitrifying bacteria that was added affects the culture by lowering and stabilizing ammonium and nitrite concentration. Nitrate concentration starts to become stable after day 24, due to the occurrence of natural microalgae at the culture system.

One consideration of continuous intoxication process in culture system is probability of acidification occurred. This acidification contributes to the gradual decrease of pH level in the water, therefore it will also affect the prawn growth in the culture system. However, in the trial pH level remain relatively stable at the level 7.5–8 during six weeks' culture period. This stable pH level occurred due to high buffering capacity by addition of $CaCO_3$ in the system. The $CaCO_3$ also used as substrate for nitrifying bacteria [10]. Temperature and dissolved oxygen level during the culture period ranged between 23–26°C and 5–8 ppm, respectively, and was still in tolerance range for *M. rosenbergii* [9,11]. All the physio-chemical parameters during the culture period were also in the ideal values according to Emerenciano et al. [12].

Beside its effect on pH buffering, $CaCO_3$ is also needed by prawn for growth, specifically during the molting process [13]. Other advantages of nitrifying bacteria addition into prawn culture are to suppress the growth of pathogenic bacteria in the culture system. Based on Suantika et al. [10] the use of nitrifying bacteria in zero water discharge contribute to inhibiting the growth of *Vibrio* sp. which causes vibriosis on prawn.

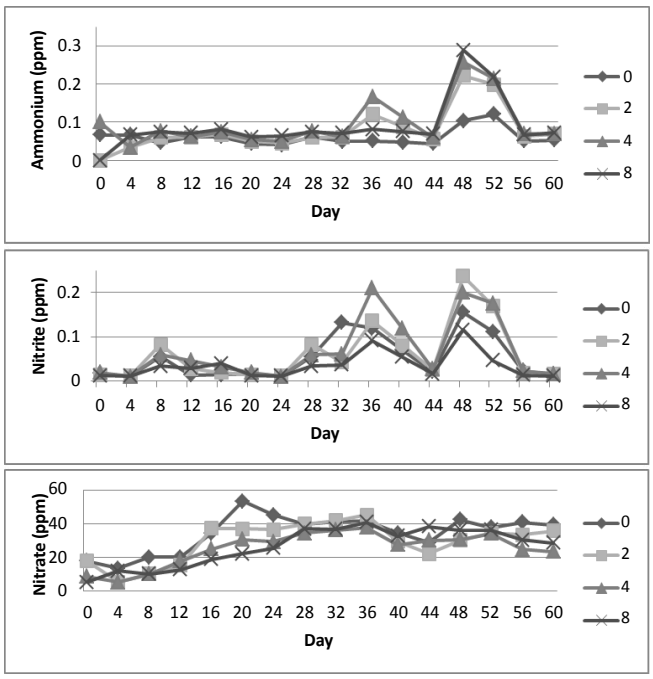

Figure 6: Chemical parameters during 60 days culture period.

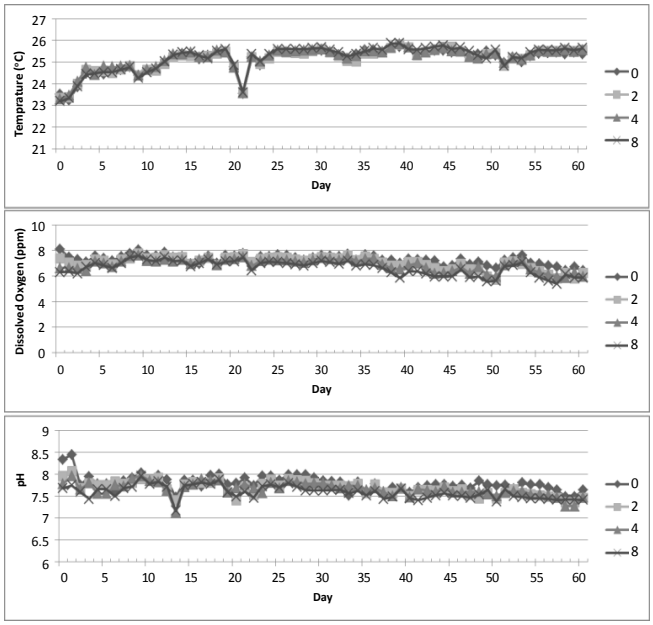

Figure 7: Physical parameters during 60 days culture period.

Survival percentage at the end of the culture period is shown in Table 2. Highest mean of survival (100%) was achieved in the control, followed by treatment 4 shelters (98%), treatment 8 shelters (94%), and treatment 2 shelters (82%). This difference in prawn survival between treatments was not significant ($P > 0.05$). Meanwhile, treatment 8 shelter and 4 shelters obtained a significantly highest total biomass per tank of 67.94 ± 0.05 g and 63.17 ± 4.23 g ($P<0.05$), respectively, compared to the control with the lowest total biomass per tank of 38.77 ± 0.72 gram (Table 2). Treatment 2 shelters also obtained a higher total biomass per tank (38.77 ± 0.72 g) compared to the control, but the difference was not significant ($P > 0.05$). There were no significant differences observed in SGR between all treatments (Table 2).

In this study, even though the use of nitrifying bacteria can improve the prawn culture condition, critical issue related to the prawn cannibalistic behavior was observed during the culture period, which may lead to decreased culture performance. Correspondingly, based on research conducted by Paul et al. [14], stocking density had impacted to the final individual body weight, total biomass, SGR, and survival of prawn culture, in which the lower density pond had a higher value compared to the higher density pond. By the application of nitrifying bacteria and use of three-dimensional bamboo shelter we could decrease these effects.

From the result presented in Table 2, addition of three-dimensional bamboo shelter contributed to higher stocking density of the culture and survival percentage of the prawn. It was shown that the prawn growth in the system with higher stocking density had no difference compared to the system with lower stocking density; moreover, the highest total biomass of 67.94 ± 1.60 g was obtained in the system with shelter addition. These results suggested that the enlargement of territorial area through shelter addition reduced the prawn stress due to the higher stocking density as well as prawn mortality due to cannibalism, as also reported in Suantika et al. [5] where the shelter addition is able to provide more territorial area both horizontally and vertically.

Conclusion

The addition of nitrifying bacteria combined with shelter can enhance growth and performance of Giant Freshwater Prawn (*Macrobrachium rosenbergii* de Man). The overall results of the study showed that treatment III results in better prawn growth and performance compare to control ($p<0.05$). At the end of the study physical and chemical parameters during 60 days of prawn culture period are stable in the tolerance range for *M. rosenbergii* to live. From the biological parameter prawn culture with eight shelters gives the highest biomass compared to others.

During the experiments, the increase of nitrate concentration and ammonium can be controlled and maintained by addition of nitrifying bacteria and microalgae. Thus, addition of higher volume cubical cages

Parameter	Treatment (Shelter)			
	0	2	4	8
Ammonium	0.060 ± 0.022[a]	0.078 ± 0.057 [a]	0.096 ± 0.063 [a]	0.090 ± 0.068 [a]
Nitrite	0.053 ± 0.005 [a]	0.062 ± 0.067 [a]	0.068 ± 0.069 [a]	0.035 ± 0.030 [a]
Nitrate	34.238 ± 11.247[b]	29.271 ± 11.878 [ab]	25.165 ± 10.045 [a]	26.261 ± 11.592 [ab]
pH	7.800 ± 0.173[c]	7.680 ± 0.161 [b]	7.660 ± 0.160 [b]	7.591 ± 0.147 [a]
Temperature	25.120 ± 0.550 [a]	25.125 ± 0.543 [a]	25.170 ± 0.530 [a]	25.201 ± 0.602 [a]
DO	7.297 ± 0.378 [c]	7.009 ± 0.502 [b]	6.879 ± 0.475 [b]	6.642 ± 0.557 [a]

Note: Different letters in the same column show significant difference ($P<0.05$).

Table 2: Water quality parameter (Mean ± St.Dev) during 60 days culture period.

results in higher prawn growth and performance, while the addition of nitrifying bacteria and microalgae can maintain water quality with increases of stocking density up to 100%.

Acknowledgements

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References

1. Sugiyama S, Staples D, Funge-Smith S (2004) Status and Potential of Fisheries and Aquaculture in Asia and the Pacific, RAP Publication 25.
2. Gunawan (1998) Proses Pembenihan Udang Galah Macrobrachium rosenbergii dalam Sistem Budidaya terkontrol. Limnology Research and Development Centre LI.
3. Suantika G (2001) Development of a Recirculation System for The Mass Culturing of The Rotifer Brachionus plicatilis. Ph.D Thesis in Applied Biological Science. Universiteit Gent, Belgiu.
4. Ali F (2008) Udang Galah: Dongkrak Produksi dengan Apartemen Bambu. Agrina 4: 91.
5. Suantika G, Astuti DI, Aditiawati P, Sasmita PG (2009) Development of Zero-Water Discharge Technology and Nitrifying Bacteria Application in Nursery Phase of The Giant Freshwater Prawn (Macrobrachium rosenbergii de Man). World Aquaculture 2009. Veracruz, Mexico.
6. American Public Health Association, American Water Works Association, Water Pollution Control Federation, & Water Environment Federation (1915) Standard methods for the examination of water and waste water. American Public Health Association.
7. Cappucino JG, Sherman N (2005) Microbiology: A Labolatory Manual. New York: Benjamin Cummings.
8. Madigan MT, Martinko JM (2006) Brock Biology of Microorganism. New Jersey: Pearson Prentice Hall, Pearson Education, Inc.
9. Boyd C, Zimmermann S (2010) Grow-out Systems-Water Quality and Soil Management. In: Freshwater Prawns Biology and Farming (New MB, Valenti WC, Tidwell JH, D`Abramo LR, Kutty MN (eds.)): 239-253.
10. Suantika G, Astuti DI, Arief RR, Rusni M, Turendro OR (2012) Use of Zero Water Discharge Technology through the Application of Nitrifying Bacteria and Textile Vertical Substrate in Grow-Out Phase of Macrobrachium Rosenbergii De Man. Journal of Aquaculture Research & Development 3: 139.
11. Tidwell JH, D'Abramo LR, Coyle SD, Yasharian D (2005) Overview of recent research and development in temperate culture of the freshwater prawn (Macrobrachium rosenbergii De Man) in the South Central United States.
12. Emerenciano MGC, Luis RMC, Marcel MP, and Anselmo MB (2017) Biofloc Technology (BFT): A Tool for Water Quality Management in Aquaculture. Water Quality.
13. New MB, Wagner CV, Tidwell JH, D'Abramo LR, and Kutty MN, (eds.), (2010) Freshwater Prawns Biology and Farming. Oxford, UK: Wiley-Blackwell.
14. Paul PAR, Mosharraf H, Sherazul I, Subrata M, Monjurul H (2016) Effect of Stocking Density on the Growth and Production of Freshwater Prawn (Macrobrachium rosenbergii). International Journal of Fisheries and Aquaculture Sciences 6: 77-86.