



UNIVERSITI PUTRA MALAYSIA

***IMPROVEMENT OF BIOFLOC SYSTEM FOR POST LARVAL REARING
OF GIANT FRESHWATER PRAWN (*Macrobrachium rosenbergii* de
Man, 1879)***

MD. EILIOUS HOSAIN

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By

MD. EILIOUS HOSAIN

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

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DEDICATIONS

To

My Parents
My Siblings
My late and alive Grandparents
My late elder brother Md. Shahadat Hosain



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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Doctor of Philosophy

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MD. EILIOUS HOSAIN

August 2021

Chairperson : Associate Professor S M Nurul Amin Harmuj Ali Sarker, PhD
Faculty : Agriculture

Giant freshwater prawn, *Macrobrachium rosenbergii*, is a commercially important crustacean, which is being cultured throughout the South-East Asia. The production of *M. rosenbergii* has been declined owing to lack of adequate post larvae (PL) production, non-effective farming system and diseases that cause high economic losses. Biofloc technology (BFT) can be an alternative culture system as it has been proven a viable culture system that improves water quality, provides live feeds and health care of culture species as well as increases the production of many fishes, prawns and shrimps. Little report is published on prawn productive performance in biofloc technology system (BFT) so far. This study was conducted to investigate the growth, survival and proximate composition of *M. rosenbergii* PL in BFT. Therefore, five experiments were conducted on nursery phase of *M. rosenbergii* PL to identify the suitable carbon source, C-N ratio, salinity and floc volume, and influence of copepod addition in BFT under completely randomized design of each treatment with triplicate. The first experiment tested five carbon sources *i.e.*, wheat bran, rice flour, maize starch, molasses and palm kernel cake for biofloc. The PL growth was similar ($P > 0.05$) among five carbon sources. PLs survival (88.66%) was higher ($P < 0.05$) in maize starch and followed by rice flour (73.0%), molasses (68.33%), wheat bran (61.0%) and palm kernel cake (56.33%) treatments. The better FCR (2.21) and the highest lipid content (1.98% dry weight) were obtained in maize starch bioflocs than other four carbon sources. The water quality parameters *viz.* temperature, pH, dissolved oxygen and ammonia did not differ ($P > 0.05$) among five different carbon treatments. The second experiment examined the effects of four C-N ratios of 10, 15, 20, 25 and clear water system as control. Specific growth rate (SGR) was similar between the control ($9.29\% \text{ d}^{-1}$) and the CN-20 ($9.47\% \text{ d}^{-1}$) and CN-25 ($9.34\% \text{ d}^{-1}$) treatments which were significantly higher than that in the C-N10 treatment ($8.03\% \text{ d}^{-1}$). A higher survival (87.34%) was obtained in the C-N ratio 20 when compared to control (80.33%) and other C-N ratios groups, but no significant differences among five treatments were observed. The best FCR (2.65) was observed in the C-N ratio 20 treatment. Biofloc crude protein content was higher in the C-N ratio 20 than C-N ratio 10, 15 and 25 groups. The third experiment was conducted to compare the

performance of BFT under four different salinities (0, 5, 10 and 15‰). Better growth of PLs were found in 10‰ and 15‰ saline water biofloc system. A higher survival (85.66%) of *M. rosenbergii* PL was obtained in 15‰ biofloc system and followed by 10‰, 5‰ and freshwater BFT. The best FCR was found in 15‰ and 10‰ salinity groups. Ciliate and rotifer abundances were higher in 15 ‰ saline water biofloc system than 10‰, 5‰ and freshwater biofloc system. A lower density of *Vibrio* spp. remained in 15‰ biofloc system than in 10‰ system. The fourth experiment investigated the effects of different floc volume of 2-5, 7-10 and 12-15 ml L⁻¹ as well as zero-exchange BFT (control). A higher ($P < 0.05$) prawn survival (82.33%) was obtained in 2-5 ml L⁻¹ floc treatment and followed by 7-10, 12-15 ml L⁻¹ and zero-exchange BFT treatments. *Vibrio* spp. density was lower ($P < 0.05$) in 2-5 ml L⁻¹ floc treatment than 12-15 ml L⁻¹ and zero-exchange BFT. The fifth experiment was conducted to evaluate the effects of copepod addition in BFT under three treatments *i.e.*, clear water system as control, BFT with or without copepods addition. A higher final weight (117.23 mg), weight gain (107.82 mg), SGR (8.40 % d⁻¹), and survival (94.46%) of PLs were found in BFT with copepod addition treatment ($P < 0.05$) than BFT without copepod and control. The best FCR (1.08) and crude protein content (66.91%) in PLs were significantly higher ($P < 0.05$) in the copepod addition BFT than without copepod addition BFT and control. Significantly lower ammonia was found in two BFT groups than control. Overall, the study suggested that maize starch as carbon source; C-N ratio of 20, salinity range of 10-15 ‰, floc volume 2-5 ml L⁻¹ and addition of copepod for the better growth and survival of *M. rosenbergii* PL in the nursery phase using BFT. Thus, the biofloc based nursery system could be implemented in commercial venture of *M. rosenbergii* industry to enhance the productivity and profit.

Keywords: Giant freshwater prawn, post larvae, biofloc system, carbon source, C-N ratio, biofloc volume, nursery operation

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

**PENAMBAH-BAIKAN SISTEM TEKNOLOGI BIOFLOK UNTUK
PENTERNAKAN PASCA-REGA UDANG GALAH
(*Macrobrachium rosenbergii* de Man, 1879)**

Oleh

MD. EILIOUS HOSAIN

Ogos 2021

Pengerusi : Profesor Madya S M Nurul Amin Harmuj Ali Sarker, PhD
Fakulti : Pertanian

Udang galah, *Macrobrachium rosenbergii*, adalah krustasea komersial penting yang dikultur di serata Asia Tenggara. Pengeluaran *M. rosenbergii* telah berkurangan disebabkan pengeluaran pasca-rega (PL) yang tidak mencukupi, sistem penternakan yang efektif serta penyakit yang menyebabkan kerugian besar. Teknologi bioflok (BFT) boleh menjadi sistem kultur alternatif setelah terbukti sebagai sistem penternakan yang berdaya maju yang meningkatkan kualiti air, menyediakan makanan hidup dan penjagaan kesihatan spesies yang dikultur serta meningkatkan pengeluaran ikan dan udang. Setakat ini hanya sedikit laporan yang diterbitkan mengenai prestasi pengeluaran udang dalam teknologi bioflok (BFT). Kajian ini dijalankan untuk menyelidik pertumbuhan, kemandirian dan komposisi proksimat bagi PL *M. rosenbergii* dalam sistem BFT. Oleh yang demikian, lima eksperimen telah dijalankan pada fasa nurseri PL udang untuk mengenal pasti sumber karbon, nisbah C-N, kemasinan, isipadu flok yang sesuai, dan pengaruh pertambahan kopepod dalam BFT di bawah susun atur rawak sepenuhnya bagi setiap rawatan dengan tiga replikat. Eksperimen pertama menguji lima sumber karbon iaitu dedak gandum, tepung beras, kanji jagung, molas and kek isirong sawit. Pertumbuhan PL udang adalah sama ($P > 0.05$) di antara lima sumber karbon. Kemandirian PL (88.66%) adalah lebih tinggi ($P < 0.05$) pada rawatan kanji jagung diikuti tepung beras (73.0%), molas (68.33%), dedak gandum (61.0%) dan kek isirong sawit (56.33%). FCR yang lebih baik (2.21) dan kandungan lipid tertinggi (1.98% berat kering) telah diperolehi pada bioflok kanji jagung berbanding dari empat sumber karbon yang lain. Parameter kualiti air seperti suhu, pH, oksigen terlarut dan amonia tidak berbeza ($P > 0.05$) di antara lima rawatan karbon yang berlainan. Eksperimen kedua mengkaji kesan empat nisbah C-N iaitu 10, 15, 20, 25 dengan sistem air jernih sebagai kawalan. Kadar pertumbuhan spesifik (SGR) adalah sama di antara kawalan (9.29% d⁻¹) dan rawatan CN-20 (9.47% d⁻¹) dan rawatan CN-25 (9.34% d⁻¹) serta ketara lebih tinggi dari rawatan C-N10 (8.03% d⁻¹). Kemandirian yang lebih tinggi (87.34%) telah diperolehi pada C-N nisbah 20 berbanding dengan kawalan (80.33%) dan kumpulan C-N yang lain tetapi tiada perbezaan ketara di antara kelima-lima rawatan. FCR terbaik

(2.65) telah dikesan pada rawatan C-N nisbah 20. Kandungan protin kasar bioflok adalah lebih tinggi pada C-N nisbah 20 berbanding kumpulan C-N nisbah 10, 15 and 25. Eksperimen ketiga telah dijalankan untuk membandingkan prestasi BFT pada empat kemasinan berbeza (0, 5, 10 dan 15%). Pertumbuhan PL yang lebih baik telah didapati pada sistem bioflok air payau 10% dan 15%. Kemandirian yang lebih tinggi (85.66%) bagi PL *M. rosenbergii* telah diperolehi pada sistem bioflok 15% diikuti oleh system BFT 10%, 5% dan air tawar. FCR terbaik telah berlaku pada kemasinan 15% dan 10%. Penyahan amonia yang dikeluarkan lebih tinggi pada sistem bioflok 10 dan 15% kemasinan. Kepadatan siliat dan rotifer adalah lebih tinggi pada sistem biofloc 15% berbanding sistem bioflok 10%, 5% dan air tawar. Ketumpatan *Vibrio* spp. yang lebih rendah didapati dalam sistem bioflok 15% berbanding sistem 10%. Eksperimen keempat mengkaji kesan isipadu flok yang berlainan bagi 2-5, 7-10, dan 12-15 ml L⁻¹ serta BFT penukaran sifar (kawalan). Kemandirian udang (82.33%) yang lebih tinggi ($P < 0.05$) telah didapati pada 2-5 ml L⁻¹ rawatan flok dan diikuti oleh 7-10, 12-15 ml L⁻¹ dan rawatan BFT penukaran sifar. Ketumpatan *Vibrio* spp. ($P < 0.05$) adalah lebih rendah pada rawatan flok 2-5 ml L⁻¹ berbanding 12-15 ml L⁻¹ dan BFT penukaran sifar. Eksperimen kelima telah dijalankan untuk menilai kesan penambahan kopepod pada BFT dalam tiga rawatan iaitu sistem air jernih sebagai kawalan, BFT dengan atau tanpa penambahan kopepod. Berat akhir yang lebih tinggi (117.23 mg), penambahan berat (107.82 mg), SGR (8.40% d⁻¹), dan kemandirian (94.46%) bagi PL telah didapati pada BFT dengan rawatan penambahan kopepod berbanding BFT tanpa kopepod dan kawalan. FCR terbaik (1.08) dan kandungan protein kasar (66.91%) pada PL adalah ketara lebih tinggi ($P < 0.05$) dengan penambahan kopepod berbanding tanpa penambahan kopepod BFT dan kawalan. Amonia yang ketara lebih rendah telah dikesan dalam kumpulan dua BFT berbanding kawalan. Keseluruhannya, kajian ini mengesyorkan kanji jagung sebagai sumber karbon, nisbah C-N 20; tahap kemasinan 10-15%, isipadu flok 2-5 ml L⁻¹ dan penambahan kopepod adalah disarankan bagi pertumbuhan dan kemandirian yang lebih baik bagi fasa nurseri PL *M. rosenbergii* menggunakan BFT. Jadi, sistem nurseri udang menggunakan bioflok boleh diterap dalam pelaburan komersial industri udang galah untuk meningkatkan produktiviti dan keuntungan.

Kata kunci: Udang galah, pasca rega, sistem bioflok, sumber karbon, nisbah C-N, isipadu bioflok, operasi nurseri

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This thesis was submitted to the Senate of the Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

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LIST OF ABBREVIATIONS

Ammonia-N	Ammonia-Nitrogen
ANOVA	Analysis of variance
AOAC	Association of Official Analytical Chemists
BF	Biofloc
BFT	Biofloc technology
BFT-C	Biofloc technology with copepods
BFZ	Zero exchange biofloc system
BOD	Biological oxygen demand
CFU	Colony forming unit
CHO	Carbohydrate
C-N	Carbon to nitrogen
COD	Chemical oxygen demand
CON	Control
CS	Culture system
DO	Dissolved oxygen
DIN	Dissolved Inorganic Nitrogen
DW	Dry weight
FAO	Food and agriculture organization
FCR	Food conversion ratio
FW	Final weight
IBSrs	Integrated biofloc system of <i>L. venameii</i> and red seaweed
I-AQUAS	International Institute of Aquaculture and Aquatic Sciences
IW	Initial weight

RF	Rice flour
MO	Molassess
MRS	De Man, rogosa and sharpe agar
min	Minutes
Nitrite-N	Nitrite-nitrogen
Nitrate-N	Nitrate-nitrogen
MS	Maize strach
P	Period
PKE	Palm kernel Expelar
RAS	Recirculating aquaculture system
SD	Stocking density
SE	Standard error
SR	Survival rate
Sal	Salinity
SGR	Specific growth rate
SVI	Sluudge volume index
TB	Total bacteria
TCBS	Thiosulphate citrate bile salt sucrose
TSA	Tryptone soya agar
TSS	Total suspended solid
UPM	Universiti Putra Malaysia
USA	United State of America
VSS	Volitile suspended solid
WB	Wheat bran

WG	Weight gain
YSI	Yellow Spring Instruments
g	Gram
m	Meter
L	Liter
ml	milileter
mg	Miligram
ppm	Parts per million
°C	Degree celsius
%	The percent sign
<	The less than sign
>	The greater than sign

CHAPTER 1

INTRODUCTION

1.1 Background

The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879), is a commercially important crustacean freshwater aquaculture species in tropical and subtropical countries (New, 2002). It lives in freshwater environment which is linked to brackish waters, because of larval survival, development and metamorphosis requires the brackish waters (10-12‰) in natural environment or in commercial hatcheries (David *et al.*, 2016; New, 2010). The *M. rosenbergii* shows a wide range of distribution worldwide and inhabiting in estuaries, rivers, lakes, ponds and ditches also native to Southeast Asia, South Pacific countries, northern Oceania, and western Pacific islands (De Grave *et al.*, 2008; FAO, 2019; New, 1990, 1995; New and Nair, 2012).

In Malaysia, *M. rosenbergii* is commonly known as udang galah and annual production was 619.0 and 414.92 tonnes in 2010 and 2019, respectively (Anon, 2019; Banu and Christianus, 2016; Farook *et al.*, 2019). The global production of *M. rosenbergii* in 2016 and one decade ago were 0.23 and 0.22 million tonnes showing slow increment production. The *M. rosenbergii* is famous for lean protein, healthy fat include omega-3 fatty acids, many vitamins and minerals, low in calories and beneficial for human health though some high in cholesterol (Bhavan *et al.*, 2010; Cavalli *et al.*, 2001; De Silva, 2016; Muralisankar *et al.*, 2017). Therefore, prawn aquaculture industries require sustainable development to boost up production for the food security of growing populations.

Currently, biofloc technology (BFT) has recognized an alternative successful aquaculture system for finfishes and crustaceans culture, which minimize water usages and pollutants emission (Ballester *et al.*, 2017; Luo *et al.*, 2014; Dauda, 2019). BFT is an environment friendly, zero to minimal water exchange aquaculture system. This system proliferates heterotrophic organisms and requires organic carbon source supplementation to maintain carbon nitrogen ratio. This technology removes hazardous nitrogenous compound, provides on station live feeds, augment productivity and enhances performance in terms of growth, survival, feed conversion ratio, and protein efficiency ratio (Ahmad *et al.*, 2017; Dauda *et al.*, 2018). The BFT also increases the immunity and robustness to pathogens (Ahmad *et al.*, 2017). It ensures beneficial nutritional compounds such as protein, lipid, carbohydrate, vitamins, minerals as well as some bioactive and derivative compounds, such as organic acids, polyhydroxy acetate and polyhydroxy butyrate to many culture animal during nursery phase, grow-out and broodstock rearing (Ahmad *et al.*, 2017; Cardona *et al.*, 2016; Crab *et al.*, 2010; Dauda *et al.*, 2018).

1.2 Problem statement

The production of *M. rosenbergii* has not been increased in last decade when compared to white leg shrimp (FAO, 2019). Some studies have been identified the reasons of declining the prawn production, which are inadequate post larvae (PL) or advanced PL production in hatcheries, diseases, lack of effective culture system, climate change and natural disasters (Schwantes *et al.*, 2009; Sin and Shapawi, 2017; Sultana *et al.*, 2017; Farook *et al.*, 2019). For example, the lack of stable PL nursery techniques, unavailability of PL as well as feeds are the main obstacle for prawn farming in several countries such as Bangladesh, India, Malaysia, Thailand, Philippines etc. (Schwantes *et al.*, 2009; Banu and Christianus; 2016; Tambalque III *et al.*, 2015; Ghosh *et al.*, 2017; Sultana *et al.*, 2017; Jayasankar, 2018).

There are several types of nursery operation system for *M. rosenbergii* that include earthen ponds, cages/hapas, flow through, recirculation aquaculture system (RAS) and green water static systems (Lober and Zeng, 2009; Coyle *et al.*, 2010). Each of these have different drawbacks. For example, flow through system is often cheaper than RAS, however, it requires a greater amount of high quantity of water (Forster and Slaski, 2010; Losordo *et al.*, 2004). There has been succeed of PLs production with using green water culture where microalgae enrich zooplankton and maintains water quality (Lober and Zheng, 2009), but require to maintain live microalgae. An alternative system could be biofloc technology, which can be reduced water usages and economically viable as well as it is recognized as an environment-friendly aquaculture system which can potentially increase fish and crustacean production (Taw, 2010; Dauda, 2019). The novelty of the present study is to find out suitable carbon source, C-N ratio, salinity preference, optimum floc volume and impact of copepod addition in BFT for culturing of *M. rosenbergii* in nursery phase.

1.3 Objectives and hypotheses of the study

1. To investigate the effect of different carbon sources and carbon to nitrogen ratios on water quality, floc volume; growth, survival and proximate composition of *M. rosenbergii* post larvae in biofloc system

Null hypothesis (*H*₀): Different carbon sources and C-N ratios do not have any influence on nursery culture of *M. rosenbergii* post larvae in BFT system.

Alternative hypothesis (*H*_a): Nursery culture of *M. rosenbergii* post larvae will be influenced by different carbon sources and C-N ratios in BFT system.

2. To compare the effect of different salinities on growth, survival and proximate composition of *M. rosenbergii* post larvae in biofloc system

Null hypothesis (*H*₀): Four different salinity at 0‰, 5‰, 10‰ and 15‰ will not impact on the nursery culture of *M. rosenbergii* post larvae in BFT system.

Alternative hypothesis (*Ha*): Nursery culture of *M. rosenbergii* post larvae will be impacted by different salinity in BFT system.

3. To determine the effect of different floc volume on growth and survival of *M. rosenbergii* post larvae in biofloc system

Null hypothesis (*Ho*): Different floc volumes level based biofloc systems will not influence on nursery culture of giant freshwater prawn post larvae.

Alternative hypothesis (*Ha*): Nursery culture of *M. rosenbergii* post larvae will be influenced by different floc volumes level based biofloc systems.

4. To evaluate the effect of copepod addition on survival, growth and biochemical composition of *M. rosenbergii* post larvae in biofloc system.

Null hypothesis (*Ho*): Periodical copepod addition will not impact on nursery culture of giant freshwater prawn post larvae BFT system.

Alternative hypothesis (*Ha*): Nursery culture of *M. rosenbergii* post larvae will be influenced by periodical copepod addition in BFT system.

1.4 Overall hypothesis of the study

Null hypothesis (*Ho*): There is no effect of biofloc technology system on culture of *M. rosenbergii* during nursery phase.

Alternative hypothesis (*Ha*): Culture of *M. rosenbergii* will be influenced in biofloc technology system during nursery phase.

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