

Biofilm Technology in the Production of *Macrobrachium rosenbergii*: an Appraisal of Feasibility - a Short Review

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Abstract: The COVID-19 pandemic has negatively impacted the global economy and society. World Health Organization (WHO) reported that as of early July 2023, the virus has infected more than 690 million individuals and has resulted in more than 6.9 million deaths worldwide. This study aims to investigate spatial epidemiological factors of COVID-19 in the Middle East and North Africa (MENA) region. By employing various spatial modeling techniques, this study establishes that multiscale geographically weighted regression (MGWR) is the best-fitted model, with the lowest residual sum of squares (11.22) and the lowest Akaike's Information Criteria (AIC) value (58.41), explaining 84.3% of the variance ($R^2=0.843$). Our study finds that population density, total vaccination doses, unemployment, and GDP per capita are critical factors associated with COVID-19 in the MENA region. These valuable insights provide policymakers and public healthcare experts with the information needed to develop targeted interventions that can mitigate risk factors related to the COVID-19 pandemic.

Keywords: Giant freshwater prawn, *Macrobrachium rosenbergii*, biofilm, water quality.

1. Introduction

Giant freshwater prawns (*Macrobrachium rosenbergii*) are dominant crustaceans identified for their size and culinary appeal. Found in freshwater environments spreading Asia, Africa, and the Americas, these prawns may grow up to an outstanding size, with some individuals reaching lengths of over a foot (Wowor et al., 2007). Due to their high market demand and fast growth, massive freshwater prawns which are distinguished by their striking blue-green tone and outstanding claws are a popular choice in aquaculture. They play a vital role in the global seafood industry due to their tolerance for various conditions and their adaptability in aquaculture systems (Ezekiel et al., 2018).

Aquaculture is a key to sustainable protein production that provides sufficient opportunity to reduce poverty and hunger while promoting the efficient use of natural resources. The aquaculture sector is expanding rapidly in most countries while contributing immensely to global food production. In 2019, the aquaculture sector in Malaysia has achieved significant growth, with a presentation of 411,782 metric tonnes valued at RM3.304 billion. This accounts for 22% of the overall national fisheries

production. Compared to 2018, the sector has demonstrated positive performance, with a notable increase of 5.19% in quantity and 8.10% in value (DOFM, 2019). Aquaculture is a rapidly expanding food production sector recognized for its ability to provide a sustainable solution for attaining global food security (Subasinghe et al., 2009).

The aquaculture industry has expanded due to the advancement of modern aquaculture technologies including hybridization, genetic engineering, and biofloc technology, applied in various culture systems, along with the introduction of new aquatic species (Ogello et al., 2014). Apparently, brackish water culture plays a significant role in the aquaculture production, contributing around 75% of the total output. Amongst the brackish water species, seaweed production represented 46% with a quantity of 188,111 metric tonnes and a value of RM65.86 million. Other species, such as shrimp, fish, and shellfish, accounted for 29% to the sector's production, yielding 119,069 metric tonnes worth RM2.46 billion. Meanwhile, freshwater aquaculture commodities made up the remaining 25% of the total production, amounting to 104,602 metric tonnes valued at RM780 million (DOFM, 2019).

The aquaculture industry plays a central role in improving fish supply. An increase in aquaculture productivity is required to fulfil the present demand for aquatic food (Boyd et al., 2022). Thus, sustainable technologies, such as biofilm and periphyton, have become relevant. These technologies are cost-effective, ecologically friendly, and contribute to the long-term viability of aquaculture.

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2. The Production of *M. rosenbergii*

The giant freshwater prawn, *Macrobrachium rosenbergii* (de Man, 1879), is native to the tropical and subtropical regions of the Indo-West Pacific, spanning from India through Southeast Asia to Papua New Guinea (Jee, 1998; De Grave et al., 2013a). In 1961, Shao-Wen Ling made a significant discovery by demonstrating the ability of *M. rosenbergii* larvae to survive in brackish environments (Banu et al., 2016). Meanwhile, Takuji Fujimura and his team successfully innovated the mass breeding techniques for commercial-scale hatchery production of *M. rosenbergii* post-larvae (PL). Their findings opened up new possibilities for the advancement in freshwater prawn farming. In recognition of their remarkable contributions, the World Aquaculture Society honoured Shao-Wen Ling and Takuji Fujimura with life membership in 1974 and 1979, respectively (Banu et al., 2016). These two individuals were celebrated as the "Fathers" of giant freshwater prawn farming due to their pioneering work and significant achievements in the field.

Apart from that, Malaysia has natural environments such as lakes, streams, ponds, rivers, estuaries, and coastal areas that provide an excellent environment for aquaculture growth. Freshwater prawns hold a crucial ecological significance within river ecosystems, as emphasized by Murphy and Austin (2005). Being omnivorous scavengers, they actively feed on algae, decaying organisms, and other detritus present in the river ecosystem. The giant freshwater prawn stands out as a highly valuable aquaculture commodity. Its significant economic worth is attributed to its high protein content, alluring taste, and attractive appearance, making it sought-after and esteemed in the industry (Funari & Shen, 2022). This may be observed in the global prawn production record of 551 farms producing 233,989 tonnes in 2016 which valued at RM 2.4 billion (Banu et al., 2016). Consequently, the aquaculture sector offers Malaysia an alternative strategy to enhance local food security while simultaneously boosting export revenue (Banu et al., 2016).

The production of giant freshwater prawns through pond extension requires a lot of significant additional water and land area, both of which are minimal resources. However, by improving pond production per unit of land area, the stocking densities, water usage, feed, and fertilizer use will be increased. This also results in increased waste production, which is not a sustainable method of managing the aquaculture sector (New et al., 2002). Additionally, intensive aquaculture requires significant investment and technical expertise, which are not accessible to all aquaculture farmers. Using resources from agricultural systems and optimizing natural food are essential to increase aquaculture's total nutritional retention (Azim et al., 2007).

At present, giant freshwater prawn farming can be carried out using either the monoculture system (monoculture), also known as single farming, or the polyculture system, also known as integrated farming (New et al., 2002). There are still many unpolluted freshwater resources in our country, particularly in the states of Pahang, Negeri Sembilan, Terengganu, Sabah, and Sarawak, where these areas are strategic areas for giant freshwater prawn breeding and growth (Banu et al., 2016).

However, a few aspects should be considered before the giant freshwater larvae are introduced into the farm. Firstly, the pond's size and depth depend on the number of farmed prawns (New et al., 2002). It will result in reduced size and survival of prawns if ponds are too small. This is also due to the natural behaviour of the prawns that cannibalize each other. The second factor to consider is the timing and technique of pond drying. As the best and optimum practice, ponds should be exposed to the sun's heat to eliminate predators. In the pond's ecosystem, fish and eels are observed to be persistent predators of crustaceans if they are present. The sun plays an essential function in accelerating the decomposition of organic materials, such as human waste, leaves, and dead grass. New et al. (2002) recommended that the pond drying procedure should be longer than two weeks for optimal ecosystem health.

3. The Concept of Biofilms

Biofilm is a complex community of microorganisms that forms on various submerged surfaces in water. The complexity of aquatic biofilms is affected by variables, including the surface type and the environment (Davey et al., 2000). While all biofilms share common characteristics, their composition can differ. Typically, bacterial cells adhere to surfaces, producing multicellular biofilm structures. However, other microorganisms, such as fungi, protozoa algae, and viruses are also present in biofilms. These microorganisms interact within the biofilm, contributing to its overall complexity and enhancing its functionality (Donlan et al., 2002).

Biofilm formation is a dynamic process where bacteria initially attach to surfaces, followed by the gradual integration of other microorganisms, developing into a community. Algae can contribute nutrients to biofilm through photosynthesis, while fungi provide structural integrity (Funari & Shen, 2022). Viruses and protozoa are also members of the biofilm community and influence its dynamics. It is essential to comprehend the complexity of biofilms because they have implications in numerous disciplines, such as environmental science, medicine, and industrial processes. Biofilms can have both positive and negative impacts such as water filtration and biofouling problems. Research on biofilms enhances the understanding of their features in various environments and supports the development of effective strategies to minimize their negative impacts. Biofilms are composed of germs, extracellular enzymes, and particles that are bound with each other by a polymeric matrix (Romani et al., 2016). Most of the extracellular polymeric compounds (EPS) are consist of polysaccharides. These substances enable biofilms to stay anchored, adhering to surfaces through a three-dimensional polymer network that temporarily stabilizes biofilm cells in place (Flemming et al., 2010).

Biofilms, consisting of microorganisms that firmly sticking to surface areas and enclosed in a polysaccharide matrix, are resistant to mild cleaning method. Furthermore, the biofilm matrix may include non-cellular elements, such as corrosion particles, mineral crystals, sediment bits, or clay, and blood components, depending on the particular environmental conditions under which the biofilm is formed (Donlan et al., 2002).

Biofilms can live in various surfaces, such as living cells, medical tools, commercial pipe systems, and natural aquatic environments.

Biofilm formation is a continuous procedure affected by environmental stimuli. Initially, the cells accumulate and develop into microcolonies through division and recruitment. Eventually, they grow and envelop themselves into an extracellular matrix. Within this matrix, complex and diverse interactions occur, facilitating nutrient absorption (Hall et al., 2004). In aquatic biofilms, various trophic interactions happen including protozoa preying on bacteria and algae, rotifers consuming protozoa, bacteria and detritus, nematodes feeding on algae, and metazoans grazing on biofilm. These interactions may also result in structural modifications of the biofilm (Majdi et al., 2011).

Biofilm is an excellent source of both autotrophic and heterotrophic biomass because it is attached to various phytoplankton and zooplankton. In addition, fish and prawns can derive more energy from affixed biomass than planktonic forms. Anand et al. (2013) reported generating a wide variety of biofilm components, including 37 taxa of algae belonging to the Bacillariophyceae class (13 genera), Cyanophyceae (10 genera), Chlorophyceae (11 genera) and Euglenophyceae (3 genera), as well as five genera of zooplankton belonging to the Rotifer (3) and Crustacea (2) families.

4. Bacterial Attachment, Biofilm Formation and Growth Mechanisms

The growth of biofilms is influenced by various physical, chemical, and biological mechanisms. The adhesion process involves cells attaching to a surface, while communication refers to the interconnection of cells with each other. These attachment mechanisms determine both the adhesive and cohesive characteristics of the biofilm (Garrett et al., 2008; Zheng et al., 2021). According to Flemming et al. (2010), biofilm development usually involves 4 phases: attachment, microcolony formation, maturation with cellular differentiation, and finally, detachment or diffusion. In biofilms, microorganisms generate fimbriae, curli, flagella adhesion to healthy proteins, and capsules to firmly adhere to a surface.

Bacterial adherence is the first stage of biofilm development, which involves transporting microbes to a substrate's surface. The adsorption of macromolecules from the surface of a biomaterial, whether natural or synthetic, facilitates this procedure. Therefore, a "conditioning layer" is formed, in which the fluid creates an inorganic or organic layer on the solid surface, modifying its physical or chemical properties (Garrett et al., 2008). Before microorganisms adhere on a surface, a conditioning layer makes it easier for bacterial cells to adhere onto that surface. Consequently, reversible connections are formed by non-specific communications at the molecular level in between surface structures of the bacteria such as flagella, fimbriae, pili, and substrate (Kreve et al., 2021). Furthermore, the conditioning layer may act as a resource of nutrients for the adhering microorganisms. Bacteria can form biofilms on surfaces that have been subjected to these preparatory fluids (Kishen & Haapasalo,

2010).

The first non-specific adhesion of microorganisms to the substrate surface makes up the second phase of biofilm development. This adhesion process is facilitated by electrostatic attraction, covalent or hydrogen bonding, and the development of bridges between bacteria and substrate (Huang et al., 2011). Initially, communications between bacteria and substrate might be weak. Nevertheless, as time passes, these interactions intensify, causing the development of permanent bacterial adhesion. Bacterial surface structures play a crucial role in forming bridges between the bacteria and the conditioning film (Zhao et al., 2023).

As demonstrated by Fathollahi and Coupe (2021), various environmental factors influence bacterial attachment to the underlying surface including pH, temperature, fluid flow rate, and nutrient availability. According to Rolfe et al. (2012), the species or strain of bacteria and the growth phase (log or stationary phase) can also influence the adherence. In addition, substrate-related factors such as substrate's physical and chemical properties, also influence bacterial adhesion (Kishen & Haapasalo, 2010).

The third stage involves the precise binding of microorganisms to the substrate, in which molecules on the surface of bacterial cells known as adhesins or ligands, connect with receptors on the substrate (Straub et al., 2019; Berne et al., 2018; Belas, 2014). This targeted bacterial adhesion is less susceptible to the effects of surrounding environmental conditions (Kreve & Reis, 2021). The process of bacterial adhesion to a substrate is dynamic and occurs over time (Dunne, 2002). The reversible and irreversible interactions that occur during the initial stage of bacterial attachment occur rapidly, typically lasting from a few seconds to a few minutes. In contrast, the observed interactions in the subsequent second and third stages are longer, spanning from several hours to multiple days. The time required for this process depends on the specific type of bacteria and the current environmental conditions (Floyd et al., 2017). Consequently, this process creates *in vitro* models that provides enough time for bacteria-substrate interaction while optimizing the environmental conditions (Guzmán-Soto et al., 2021). The formation and maturation of biofilm structure happen after the bacterial adhesion (Kishen & Haapasalo, 2010).

5. The Production *M. rosenbergii* Using Biofilm Technology

Cannibalism was observed in high-density rearing systems by (Romano & Zeng, 2017), who found high mortality levels among cultured shrimps. Cannibalism also increases among newly moulted individuals. Introducing substrates into a culture system is one of the most effective strategies for increasing survival by reducing cannibalism to a minimum level. It can provide shelter from other predators besides serving as a biofilm substrate.

Consistent findings from previous studies (Table 1) have demonstrated an inverse relationship between stocking density and shrimp production, as noted by Wasielesky et al. (2001).

Khattoon et al. (2007) observed the presence of post-larvae within PVC-coated pipes in their study, indicating the potential function of these pipes as protective shelters during moulting stages. This result led to a noteworthy increase in the survival rates (51%–

60%) compared to the control group (37%). Furthermore, during the nursery phase of *Litopenaeus vannamei*, Sandifer et al. (1987) documented a notable survival rate (24%) in tanks equipped with fiberglass window screens.

Table 1. Culture organisms that use substrate to promote the growth of biofilm.

Species	Substrate type	Response	Water quality	Reference
1. Pink shrimp (<i>Farfantepenaeus paulensis</i>)	Flexible PVC tubes ranging 0.5 to 15 cm in length and 0.5 cm in diameter.	Reduced exportation of phosphorus by 33% and created higher outputs of nitrate and nitrite.	Ammonium level was stable and low, ranging from 5.94 to 16.09 mM.	Thompson et al. (2002)
2. Rohu (<i>Labeo rohita</i>)	Sugarcane (<i>Saccharum officinales</i>), palm leaf (<i>Borassus flabellifera</i>), coconut (<i>Cocos nucifera</i>), and bamboo (<i>Bambusa bambos</i>).	The specific growth rate was higher with sugarcane, palm, coconut, and bamboo (2.0%, 1.72%, 1.99%, and 1.62%, respectively), compared to without substrate (1.42%).	The level of ammonia was 52.4 mg/L in the control group compared to sugarcane, palm, coconut, and bamboo (78.2%, 88.9%, 69.9%, and 67.6%, respectively).	Keshavanath et al. (2012)
3. Common carp (<i>Cyprinus carpio</i>)	Sugarcane (<i>Saccharum officinales</i>), palm leaf (<i>Borassus flabellifera</i>), coconut (<i>Cocos nucifera</i>), and bamboo (<i>Bambusa bambos</i>).	The specific growth rate was higher with sugarcane, palm, coconut, and bamboo (2.0%, 1.9%, 2.0%, and 1.4%, respectively), compared to without substrate (1.4%).	The level of ammonia was 52.4 mg/L in the control group compared to sugarcane, palm, coconut, and bamboo (78.2%, 88.9%, 69.9%, and 67.6%, respectively).	Keshavanath et al. (2012).
4. White leg shrimp (<i>L. vannamei</i>)	Polyethylene mosquito screen (1 mm mesh size).	The final biomass and survival of shrimp in the tank with substrate reached 314%, with a survival rate of 93.9±2.4%.	There are no significant differences in ammonia and nitrite levels.	Schveitzer et al. (2013).
5. Giant freshwater prawn (<i>Macrobrachium rosenbergii</i>)	Bamboo kanchi (periphyton substrates).	The addition of substrate produced 660 kg ha ⁻¹ in 120 days compared to just 463 kg ha ⁻¹ with the same culture period without substrate.	The ammonia level was low (0.038 mg/L) in the culture pond compared to the culture tank without substrate.	Asaduzzaman et al. (2008).
6. Whiteleg shrimp (<i>Litopenaeus vannamei</i>)	Artificial substrate (Aquamats™).	No specific results on the growth.	Ammonia and nitrites remained between 0.17-0.19 mg L ⁻¹ and 0.10-0.11 mg L ⁻¹ .	Audelo- Naranjo et al. (2012).

7.	Giant freshwater prawn (<i>Macrobrachium rosenbergii</i>)	T1-no substrate (control), T2-hollow PVC pipe, T3-polyethylene, and T4-black coloured nylon netting.	Final weights of post-larvae (PLs) in treatments T3, T4, and T2 were 2.70%, 31.54%, and 21.05%, respectively, compared to T1 (without substrate). The specific growth rate of PLs were 5.04%, 5.19%, and 5.18% in treatments T2, T3, and T4, respectively, compared to T1 without substrate (4.72%).	Total ammonia was maintained between 0.01 to 0.15 mg L ⁻¹ .	Mamun et al. (2010).
8.	Giant tiger prawn (<i>Penaeus monodon</i>)	Polyvinyl chloride pipes (PVC).	The specific growth rate of post-larvae increased by 28% in the substrate.	The level of ammonia nitrogen was lowest (0.03 mg L ⁻¹) compared to other groups.	Khatoun et al. (2007).
9.	Asian stinging catfish (<i>Heteropneustes fossilis</i>)	Sugarcane bagasse.	The specific growth rate in sugarcane bagasse and supplemental feed group recorded 2.01%, followed by sugarcane bagasse alone (1.99%).	The ammonia level was maintained in the range of 6.7–6.9 mg L ⁻¹ .	Radhakrishnan & Sugumaran (2010).

6. Nutritional Quality of Biofilms

Fish meal and oil are important elements used as feed ingredients in poultry, aquaculture, and swine farming (El-Sayed, 2020). Aquaculture production heavily relies on wild-caught fish due to the essential role of fish meal and oil in the diets of numerous aquaculture species, as highlighted by Barlow (2003). Within the aquaculture sector, approximately 5–6 million tonnes of low-value or bycatch fish are directly utilized as feed, either in unprocessed form or as components of farm-made feeds. Additionally, from 1992 to 2006, there was a notable increase in the overall consumption of fish meal and fish oil in aquafeeds, with quantities more than tripling from 0.96 to 3.06 million tonnes and from 0.23 to 0.78 million tonnes, respectively (FAO, 2009).

Furthermore, in various aquaculture practices (intensive, semi-intensive, or extensive), the utilisation of fish meal requires 2 to 5 times more fish protein to nourish the farmed species adequately (Naylor et al., 2000). Consequently, recent research focused on developing feeds that require less fish meal supply, oil or expensive ingredients, including protein substitution to plant proteins. In addition, Naylor et al. (2000) mentioned that extensive and traditional aquaculture systems commonly refrain from using significant amounts of fish meal. Instead, they introduce nutrient-rich substances into the water to stimulate the growth of algae and other natural organisms that serve as food sources for the farmed fish. This condition contributes to the development of the biofilm technology, particularly for both intensive and semi-intensive aquaculture systems.

Previous studies have revealed that the nutritional composition of biofilms is generally compatible with fish's dietary requirements. The protein content of the biofilm ranged from 23%–30%, the lipid content from 2%–9%, the NFE content from

25%, and the ash content from 16%–42%. These findings suggest biofilms with high nutritional value could be used as food supplements for fish and shrimp cultures. According to Siddhartha et al. (2015), *Penaeus monodon* juveniles require 35% to 40% protein and up to 10% lipids in their diet.

However, the recommended protein requirement for Indian significant carps is 30% of their body weight (Mahavadiya et al., 2021). Biofilms are recognized to be a high-quality source of protein (Ghosh et al., 2022). Therefore, biofilms are associated with improved growth in both fish and shrimps (Anand et al., 2013). Other than just providing macronutrients, microalgae and heterotrophic bacteria also act as immune boosters, growth enhancers, sources of bioactive compounds, and dietary stimulants (Ju et al., 2008; Kuhn et al., 2010; Xu et al., 2012), all of which can improve the growth performance of cultured shrimps.

7. Biofilm Impacts on Water Quality

Nitrification in aquaculture systems is pivotal in determining water quality by regulating nitrogen dynamics. The primary nitrogen source in aquaculture is derived from feed, which can substantially influence the natural nitrogen cycle, particularly in intensive or semi-intensive aquaculture settings (Kumar et al., 2017). Excessive nitrogen from the feed undergoes conversion into inorganic nitrogen compounds, particularly ammonia, posing a health risk to the cultured organisms. However, nitrifying bacteria within biofilm formations significantly mitigates ammonia levels by facilitating the conversion of nitrite to nitrate. Additionally, certain heterotrophic communities within the biofilm directly utilize inorganic nitrogen. Achieving a balanced carbon-to-nitrogen ratio in substrate-based treatments enhances water quality and substantially reduces ammonia nitrogen

concentrations (Azim et al., 2002a; Kumar et al., 2017).

The incorporation of biofilms in the rearing of *Catla catla* fingerlings resulted in a significant enhancement of water quality, as demonstrated by Pradeep et al. (2003). Within freshwater ponds, introducing biodegradable substrate contributes to improved water quality, thereby creating a more conducive environment for fish growth. The biofilm treatment exhibits significantly lower ammonia levels than a conventional aquaculture system (Keshavanath et al., 2012). Apparently, use of non-biodegradable substrates in brackish water ponds reduces ammonia and nitrite nitrogen levels, stimulating the growth of *L. vannamei* (Kumar et al., 2017). Nitrifying bacteria and microalgae create opportunities to attenuate detrimental nitrogen compounds in water, while nitrate generated through the nitrification process serves as a growth-promoting nutrient for microalgae (Thompson et al., 2002).

Maintaining appropriate alkalinity and pH levels is essential for ensuring the health and welfare of cultured animals as these factors significantly influence water quality. The addition of substrate to *L. vannamei* culture resulted in significant improvements in the pH and alkalinity levels of the culture system throughout the culture period. Notably, the pH was consistently maintained within the range of 7.4–8.4, while the alkalinity ranged from 88 to 230 mg/L as CaCO₃ (Zhang et al., 2015).

Incorporating photosynthetic organisms into biofilms alters the pH and alkalinity levels of the culture system, potentially increasing or decreasing the acidity and alkalinity levels (Kumar et al., 2017). Several studies have consistently shown improved water quality when employing biofilm-based systems with diverse substrate materials (Asaduzzaman et al., 2010; Anand et al., 2013). The biofilm and submerged substrate also reduce water turbidity by trapping organic matter or facilitating its accumulation (Van Dam et al., 2002). Some researchers also found lower dissolved oxygen (DO) levels in substrate-installed tanks (Anand et al., 2019).

8. Biofilms and Probiotics in The Production of *M. rosenbergii*

Probiotics are living microorganisms that improve gastrointestinal microbiota and the overall health of the receiving hosts when supplied in adequate amounts (Ringo, 1999; Hagiwara et al., 1994). Probiotics may also be components of microbial cells added to feed or culture water to benefit the host (Verschuere et al., 2000). They can be used separately or together with prebiotics or other immunostimulants. Applying probiotics is recommended as an environmentally friendly strategy for disease prevention in aquaculture (Servin, 2004). Several studies have extensively examined the utilization of probiotics in aquaculture, emphasizing their wide-ranging benefits such as improved growth performance, increased disease resistance, enhanced immune responses, better regulation of gut microbiota, water quality improvement through bioremediation, and

augmentation of nutrient levels in zooplankton populations (Azad et al., 2019). In aquaculture, probiotics are introduced through water treatments or feed supplements. Enrichment of zooplankton with probiotics by encapsulation allows bacteria to remain alive and even multiply on the live feed. As a result, enriched live feeds can efficiently provide probiotics to the hosts (Vijayan et al., 2006; Rahiman et al., 2010).

The growth effects of probiotics on fish and crustaceans have been investigated in multiple studies. Seenivasan et al. (2016) assessed the impacts of three probiotic strains (*Bacillus subtilis*, *Lactobacillus sporogenes*, and *Saccharomyces cerevisiae*) that incorporated into the diet of *M. rosenbergii* post-larvae and examined its survival and growth parameters over 60 days. The results indicated probiotics diets on *M. rosenbergii* produced higher weight gain and growth rates than the control. In a study conducted by Sumon et al. (2018) observed that the administration of probiotics (specifically *Clostridium butyricum*) resulted in substantial growth stimulation and enhanced enzyme production (including protease, amylase, and lipase) in *M. rosenbergii*. Additionally, Ghosh et al. (2016) and Azad et al. (2018) reported 30% enhancement in the growth and productivity of *M. rosenbergii* when supplemented with a mix of commercial probiotics, which were Zymetin (*Bacillus mesentericus*) and Super PS (*Rhodobacter* sp. and *Rhodococcus* sp.). The research showed that the combination is superior to the addition of each probiotic individually. On top of that, particular probiotics can develop biofilms in the digestion system, supplying an obstacle versus microorganisms. These probiotic biofilms can enhance the survival and function of probiotics in the digestive tract, thus preserving a healthy microbiota and promoting health.

Probiotics in the form of biofilms might raise survival rates and function. When probiotics develop a biofilm in the intestinal tract, it can give a protective environment that enables them to sustain the harsh conditions of the digestion tract, such as the acidic environment of the stomach. This condition suggests that more probiotics might reach the intestinal tracts in active and excellent state, thereby boosting their advantageous impacts (Amara & Shibl, 2015; Salas-Jara, 2016). Specific probiotic strains can produce substances that encourage the development of valuable biofilms by various probiotics or intestine microorganisms, cultivating a more varied and robust gut microbiota (Gao et al., 2022).

Generally, the capability of probiotics to form biofilms includes the significant improvement on the health of the farmed species. Nevertheless, the particulars of these impacts might differ depending on the particular probiotic species and the strains employed. Further research is essential to comprehensively understand these interactions. Consequently, the most effective approach for harnessing their health-promoting benefits in prawn culture, specifically associated with biofilm technology can be determined.

9. Conclusions and Recommendations for Future Research

Biofilm technology has several advantages, including controlling water quality, on-site feed production, and other possible additional properties. Biofilm technology provides a sustainable aquaculture approach in addressing its environmental, social, and economic concerns while supporting its growth.

Further research is required to comprehend the complexity of the biofilm, encompassing microbial relationships, physiology of gut health, and immune interactions in giant freshwater prawns. The management of biofilm and water quality monitoring are somewhat complex. Knowledge and skills still need to be improved and addressed to support the successful adoption of this technology on a commercial-scale production. Additionally, future research is required particularly on the disease resistance in *M. rosenbergii* with the potential positive effects of biofilm technology. This advancement shall enable the aquaculture development while promoting social responsibility and environmental concerns.

10. Declaration

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Authors' contribution

Sow Cyn Shieng conducted literature reviews and wrote the original manuscript. Keng Chin Lim assisted in manuscript preparation and revision. Fatimah Md Yusoff and Norulhuda Ramli contributed to the manuscript revision. Murni Karim supervised the content, revision, and proofreading of the manuscript. All authors confirmed the final draft of the manuscript.

Competing interests

The authors declare that they have no competing interests.

Ethical considerations

The authors declare and confirm that the manuscript is original, has no misconduct, has never been published in another journal, and written only to be published in this journal.

11. References

Amara, A. A., & Shibl, A. (2015). Role of Probiotics in health improvement, infection control and disease treatment and management. *Saudi pharmaceutical journal*, 23(2), 107-114.

Anand, P. S., Kohli, M. P. S., Roy, S. D., Sundaray, J. K., Kumar, S., Sinha, A., ... & kumar Sukham, M. (2013). Effect of dietary supplementation of periphyton on growth performance and digestive enzyme activities in *Penaeus monodon*. *Aquaculture*, 392, 59-68.

Anand, P. S., Balasubramanian, C. P., Christina, L., Kumar, S., Biswas, G., De, D., ... & Vijayan, K. K. (2019). Substrate based black tiger shrimp, *Penaeus monodon* culture: Stocking density, aeration and their effect on growth performance, water quality and periphyton development. *Aquaculture*, 507, 411-418.

Anand Pss, Kumar S, Panigrahi A, Ghoshal Tk, Dayal Js, Biswas G, Sundaray Jk, De D, Ananda, Raja gallic, b., (2010). Where do fishmeal and fish oil products come from? An analysis of the conversion ratios in the global fishmeal industry. *Marine policy* 34, 815–820.

Asaduzzaman M, Wahab MA, Verdegem MCJ, Benerjee S, Akter T, Hasan MM, Azim ME (2008). Effects of addition of tilapia *Oreochromis niloticus* and substrates for periphyton developments on pond ecology and production in C/N-controlled freshwater prawn *Macrobrachium rosenbergii* farming systems. *Aquaculture* 287(3-4):371-380.

Audelo-Naranjo, J. M., Voltolina, D., & Romero-Beltrán, E. (2012). Culture of white shrimp (*Litopenaeus vannamei* Boone, 1931) with zero water exchange and no food addition: an eco-friendly approach. *Latin american journal of aquatic research*, 40(2), 441-447.

Avnimelech Y. (2007) Feeding with Microbial Floccs by Tilapia in Minimal Discharge Bioflocs Technology Ponds. *Aquaculture*, 264: 140-147.

Azad, M. A. K., Islam, S. S., Sithi, I. N., Ghosh, A. K., Banu, G. R., Bir, J., & Huq, K. A. (2019). Effect of probiotics on immune competence of giant freshwater prawn *Macrobrachium rosenbergii*. *Aquaculture research*, 50(2), 644-657.

Azim Me, Verdegem Mcj, Khatoon Hm, Wahab A, Van Dam Aa, Beveridge Mcm (2002a). A Comparison Of Fertilization, Feeding And Three Periphyton Substrates For Increasing Fish Production In Freshwater Pond Aquaculture In Bangladesh. *Aquaculture* 212:227-243

Azim, M. E., & Little, D. C. (2007). Intensifying aquaculture production through new approaches to manipulating natural food. *CABI Reviews*, (2006), 23-pp.

Barlow, S.M. (2003). 'Fish meal', *Encyclopedia of Food Sciences and Nutrition*, pp. 2486–2491. doi:10.1016/b0-12-227055-x/00479-x

Banu, R., & Christianus, A. (2016). Giant freshwater prawn *Macrobrachium rosenbergii* farming: a review on its current status and prospective in Malaysia.

Belas, R. (2014) 'Biofilms, flagella, and mechano sensing of surfaces by bacteria', *Trends in Microbiology*, 22(9), pp. 517–527. doi: 10.1016/j.tim.2014.05.002.

- Berne, C. et al. (2018) 'Bacterial adhesion at the single-cell level', *Nature Reviews Microbiology*, 16(10), pp. 616–627. doi:10.1038/s41579-018-0057-5.
- Boyd, C. E., McNevin, A. A., & Davis, R. P. (2022). The contribution of fisheries and aquaculture to the global protein supply. *Food security*, 14(3), 805-827.
- Daniels, C.L., Merrifield, D.L., Ringø, E. And Davies, S.J. (2015) Probiotic, Prebiotic and Synbiotic Applications for The Improvement of Larval European Lobster (*Homarus Gammarus*) Culture. *Aquaculture* (In Press).
- Davey, M. E., & O'toole, G. A. (2000). Microbial biofilms: from ecology to molecular genetics. *Microbiology and molecular biology reviews*, 64(4), 847-867.
- De Grave S (2013a) *Macrobrachium lepidactylus*. The IUCN Red List of Threatened Species 2013: e.T198341A2521772. <https://doi.org/10.2305/IUCN.UK.2013-1.RLTS.T198341A2521772.en> (accessed 02 November 2018)
- DOFM. (2018 (and earlier issues)). Annual Fisheries Statistics and earlier issues (2018). Retrieved from <https://www.dof.gov.my/index.php/pages/view/82>
- DOFM. (2018). Annual Fisheries Statistics 2018. Retrieved from <https://www.dof.gov.my/index.php/pages/view/3929>.
- DOFM (Department of Fisheries Malaysia). 2019. Annual Fisheries Statistics-2019. Department of Fisheries Malaysia, Kuala Lumpur, Malaysia.
- Donlan rm (2002). Biofilms: microbial life on surfaces. *Emerg. Infect. Diseases* 8(9):881-890. Food and agriculture organization of the United Nations fao and the sdgs. Indicators: measuring up to the 2030 agenda for sustainable development. [Http://www.fao.org/3/a-i6919e.pdf](http://www.fao.org/3/a-i6919e.pdf), 2017; 39.
- Dunne, W.M. (2002) 'Bacterial adhesion: Seen any good biofilms lately?', *Clinical Microbiology Reviews*, 15(2), pp. 155–166. doi:10.1128/cmr.15.2.155-166.2002.
- El-Sayed, A.-F.M. (2020) 'Nutrition and feeding', *Tilapia Culture*, pp. 135–172. doi:10.1016/b978- 0-12-816509-6.00007-0.
- Ezekiel, B.B.; Firuza, B.M.; Mohammad, L.A.; Subha, B. Analysis of factors for determining suitable site for Giant Freshwater Prawn (*Macrobrachium rosenbergii*) farming through the local knowledge in Negeri Sembilan of Peninsular Malaysia. *Pertanika J. Soc. Sci. Humanit.* 2018, 26, 2867–2882.
- FAO (Food and Agriculture Organization of the United Nations). (2009). The state of world fisheries and aquaculture 2008. Effect of aquaculture on world fish supplies. *Nature* 405, 1017–1024.
- Fathollahi, A. and Coupe, S.J. (2021) 'Effect of environmental and nutritional conditions on the formation of single and mixed-species biofilms and their efficiency in cadmium removal', *Chemosphere*, 283, p. 131152. doi: 10.1016/j.chemosphere.2021.131152.
- Flemming, H. C., & Wingender, J. (2010). The biofilm matrix. *Nature reviews microbiology*, 8(9), 623-633.
- Floyd, K.A., Eberly, A.R. and Hadjifrangiskou, M. (2017) 'Adhesion of bacteria to surfaces and biofilm formation on medical devices', *Biofilms and Implantable Medical Devices*, pp. 47–95. doi:10.1016/b978-0-08-100382-4.00003-4.
- Fruh, D., Norf, H., And Weitere, M. (2011). Response Of Biofilm-Dwelling Ciliate Communities to Enrichment With Algae. *Aquat. Microb. Ecol.* 63, 299–309.
- Funari, R., & Shen, A. Q. (2022). Detection and characterization of bacterial biofilms and biofilm- based sensors. *ACS sensors*, 7(2), 347-357.
- Garrett, T. R., Bhakoo, M., & Zhang, Z. (2008). Bacterial adhesion and biofilms on surfaces. *Progress in natural science*, 18(9), 1049-1056.
- Gao, J., Sadiq, F. A., Zheng, Y., Zhao, J., He, G., & Sang, Y. (2022). Biofilm-based delivery approaches and specific enrichment strategies of probiotics in the human gut. *Gut Microbes*, 14(1), 2126274.
- Ghosh, A.K., Bir, J., Azad, M.A.K., Hasanuzzaman, A.F.M., Islam, M.S. & Huq, K.A. (2016). Impact of commercial probiotics application on growth and production of giant freshwater prawn (*macrobrachium rosenbergii* de man, 1879). *aquaculture reports*, 4: 112-117.
- Ghosh, S. et al. (2022) 'Engineered Biofilm: Innovative next gen strategy for quality enhancement of fermented foods', *Frontiers in Nutrition*, 9. doi:10.3389/fnut.2022. 808630.v
- Guzmán-Soto, I. et al. (2021) 'Mimicking biofilm formation and development: Recent progress in in vitro and in vivo biofilm models', *iScience*, 24(5), p. 102443. doi: 10.1016/j.isci.2021.102443.
- Hagiwara, A. et al. (1994) 'Increased sexual reproduction in *Brachionus plicatilis* (Rotifera) with the addition of bacteria and Rotifer extracts', *Journal of Experimental Marine Biology and Ecology*, 181(1), pp. 1–8. doi:10.1016/0022-0981(94)90100-7.
- Hall-Stoodley, L., Costerton, J.W., And Stoodley, P. (2004). Bacterial Biofilms: From the Natural Environment To Infectious Diseases. *Nat. Rev. Microbiol.* 2, 95–108.

- Huang, R., Li, M. and Gregory, R.L. (2011) 'Bacterial interactions in dental biofilm', *Virulence*, 2(5), pp. 435–444. doi:10.4161/viru.2.5.16140.
- Jee Ka (1998) Farming of Giant Freshwater Prawn. In *Aquaculture Practices in Malaysia: Malaysian Fish, Society Occasional Publication*.
- Keshavanath P, Manissery JK, Bhat AG, Gangadhara B (2012). Evaluation of four biodegradable substrates for periphyton and fish production. *J. Appl. Aquac.* 24:2012.
- Khatoun H, Yusoff Fm., Banerjee S, Shariff M, Mohamed S (2007). Use Of Periphyticcyano bacterium And Mixed Diatoms Coated Substrate for Improving Water Quality, Survival and Growth Of *Penaeus Monodon Fabricius* Post larvae. *Aquaculture* 271:196-205.
- Kishen, A., & Haapasalo, M. (2010). Biofilm models and methods of biofilm assessment. *Endodontic Topics*, 22(1), 58-78.
- Kreve, S. and Reis, A.C. (2021) 'Bacterial adhesion to biomaterials: What regulates this attachment? A Review', *Japanese Dental Science Review*, 57, pp. 85–96. doi: 10.1016/j.jdsr.2021.05.003.
- Kuhn Dd, Lawrence Al, Boardman Gd, Patnaik S, Marsh L, Flick Gj (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: 303: 28-33.
- M.A.K. Azad, S.S. Islam, I.N. Sithi, A.K. Ghosh, G.R. Banu, J. Bir, K.A. Huq, (2019). Effect of probiotics on immune competence of giant freshwater prawn *Macrobrachium rosenbergii*, *Aquaculture. Res.* 50 (2) (2019) 644–657
- Mahavadiya, D., Jungi1, D. and Dharmesh, S. (2021) Feeding and Nutritional Requirements of Indian Major Carps: A Review, *Feeding and Nutritional Requirements of Indian Major Carps: A Review*. Available at: <https://ijasr.forexjournal.co.in/papers-pdf/7.pdf> (Accessed: 10 June 2023).
- Majdi, N., Traunspurger, W., Boyer, S., Mialet, B., Tackx, M., Fernandez, R., Gehner, S., Ten-Hage, L., And Buffan-Dubau, E. (2011). Response Of Biofilm Dwelling Nematodes to Habitat Changes in The Garonne River, France: Influence Of Hydrodynamics And Microalgal Availability. *Hydrobiologia* 673, 229–244.
- Mamun, M. A. A., Hossain, M. A., Hossain, M. S., & Ali, M. L. (2010). Effects of different types of artificial substrates on nursery production of freshwater prawn, *Macrobrachium rosenbergii* (de Man) in recirculatory system. *Journal of the Bangladesh Agricultural University*, 8(2), 333-340.
- M.G. (2018). Growth performance, digestive enzyme activity, and immune response of *Macrobrachium rosenbergii* fed with probiotic *Clostridium butyricum* incorporated diets. *Journal of King Saud University-Science*, 30(1): 21-28.
- Murphy, N. & Austin, C. (2005). Phylogenetic relationships of the globally distributed freshwater prawn genus *Macrobrachium* (Crustacea: Decapoda: Palaemonidae): biogeography, taxonomy, and the convergent evolution of abbreviated larval development. *Zoologica Scripta*, 34(2): 187- 197
- New, M.B. (2002). Farming freshwater prawns: a manual for the culture of the giant river prawn (*Macrobrachium rosenbergii*).
- Ogello, e. O., musa, s. M., aura, c. M., abwao, j. O., & munguti, j. M. (2014). International journal of aquatic science an appraisal of the feasibility of tilapia production in ponds using biofloc technology: a review. 5(1), 21–39.
- Oser bl (1959). An integrated essential amino acid index for predicting the biological value of proteins. In: albanese aa (eds) protein and amino acid nutrition. Academic press, new york, pp. 281-295.
- Radhakrishnan, M. V., & Sugumaran, E. (2010). Effect of sugarcane bagasse on growth performance of *Hereropneustes fossilis* (Bloch.) fingerlings. *Journal of Experimental Sciences*, 1(1).
- Ringo, E. (1999) 'Intestinal microflora of fish larvae and fry', *Aquaculture Research*, 30(2), p. 73. doi:10.1046/j.1365-2109.1999.00302. x.
- Rolfe, M.D. et al. (2012) 'LAG phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation', *Journal of Bacteriology*, 194(3), pp. 686–701. doi:10.1128/jb.06112-11.
- Romano, N., & Zeng, C. (2017). Cannibalism of decapod crustaceans and implications for their aquaculture: a review of its prevalence, influencing factors, and mitigating methods. *Reviews in Fisheries Science & Aquaculture*, 25(1), 42-69.
- Romani, A. M., Guasch, H., & Balaguer, M. D. (2016). Aquatic biofilms: ecology, water quality and wastewater treatment. Caister Academic Press. Sandifer Pa, Hopkins Js, Stokes Ad (1987). Intensive Culture Potential of *Penaeus vannamei*. *J. World Aquac. Soc.* 18:94-100.
- Salas-Jara, M. J., Ilabaca, A., Vega, M., & García, A. Biofilm forming *Lactobacillus*: new challenges for the development of probiotics. *Microorganisms*. 2016; 4 (3).

- Schweitzer R, Arantes R, Baloi MF, Costodio PS, Arana LV, Seiffert WQ, Andreatta ER (2013). Use of artificial substrates in the culture of *Litopenaeus vannamei* (Biofloc system) at different stocking densities: Effects on microbial activity, water quality and production rates. *Aquacult. Eng.* 54:93-103
- Seenivasan, C., Radhakrishnan, S., Muralisankar, T. & Bhavan, P.S. 2016. Effects of probiotics on survival, growth, and digestive enzymes activities in freshwater prawn *Macrobrachium rosenbergii* (De Man, 1879). *Proceedings of the Zoological Society*, 69(1): 52-60.
- Servin, A.L. (2004) 'Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens', *FEMS Microbiology Reviews*, 28(4), pp. 405–440. doi: 10.1016/j.femsre.2004.01.003.
- Siddhartha Pati, A.C. and Dash BP (2015) 'A study on the growth of juveniles of tiger prawn, *Penaeus Monodon* (fabricius) under different photoperiods', *Journal of Aquaculture Research & Development*, 6(12). doi:10.4172/2155-9546.1000385.
- Straub, H. et al. (2019) 'Bacterial adhesion on soft materials: Passive physicochemical interactions or active bacterial mechanosensing?', *Advanced Healthcare Materials*, 8(8), p. 1801323. doi:10.1002/adhm.201801323.
- Subasinghe, R., Soto, D., & Jia, J. (2009). Global Aquaculture and Its Role in Sustainable Development. *Reviews In Aquaculture*, 1(1), 2-9. Doi:10.1111/J.1753- 5131.2008.01002.X
- Sumon, M.S., Ahmmed, F., Khushi, S.S., Ahmmed, M.K., Rouf, M.A., Chisty, M.A.H. & Sarower,
- Sun, Y.-Z., Yang, H.-L., Huang, K.-P., Ye, J.-D. And Zhang, C.-X. (2013) Application of Autochthonous *Bacillus* Bioencapsulated in Copepod To Grouper *Epinephelus Coides* Larvae. *Aquaculture* 392–395, 44–50.
- Thompson FI, Abreu Pc, Wasielesky W (2002). Importance Of Biofilm for Water Quality and Nourishment in Intensive Shrimp Culture. *Aquaculture* 203:263-278.
- Tidwell JH, Coyle SD, Schulmeister G (1998). Effects of added substrate on the production and population characteristics of freshwater prawns (*Macrobrachium rosenbergii*) to increasing amounts of artificial substrate in ponds. *J. World Aquac. Soc.* 31:174-179.
- Van Dam Aa, Beveridge Mcm, Azim Me, Verdegem Mcj (2002). The potential of fish production is based on periphyton. *rev. fish biol. fish.* 12:1-31.
- Verschuere, L. et al. (2000) 'Probiotic bacteria as biological control agents in Aquaculture', *Microbiology and Molecular Biology Reviews*, 64(4), pp. 655–671. doi:10.1128/mnbr.64.4.655-671.2000.
- Wasielesky Wj, Poersch Lh, Bianchini A (2001). Effect of stocking density on pen reared pink shrimp
- farfantepenaeus paulensis* (pérez-farfante, 1967) (Decapoda, Penaeidae). *Nauplius* 9:163-167.
- Wowor, D., & Ng, P. K. (2007). The giant freshwater prawns of the *Macrobrachium rosenbergii* species group (Crustacea: Decapoda: Caridea: Palaemonidae). *The Raffles Bulletin of Zoology*, 55(2), 321-336.
- Xu wj, pan lq, sun xh, huang j (2012). Effects of bioflocs on water quality, growth and digestive enzyme activities of *litopenaeus Vannamei* (boone) in zero-water exchange tanks. *Aquacult. Res.* <http://dx.doi.org/10.1111/j.1365-2109.2012.03115.x>
- Zhang, K. et al. (2015) 'Effect of using sodium bicarbonate to adjust the ph to different levels on water quality, the growth and the immune response of shrimp *litopenaeus vannamei* reared in zero-water exchange biofloc-based culture tanks', *Aquaculture Research*, 48(3), pp. 1194–1208. doi:10.1111/are.12961.
- Zhao, A., Sun, J., & Liu, Y. (2023). Understanding bacterial biofilms: From definition to treatment strategies. *Frontiers in cellular and infection microbiology*, 13, 1137947.
- Zheng, S., Bawazir, M., Dhall, A., Kim, H. E., He, L., Heo, J., & Hwang, G. (2021). Implication of surface properties, bacterial motility, and hydrodynamic conditions on bacterial surface sensing and their initial adhesion. *Frontiers in Bioengineering and Biotechnology*, 9, 643722