Profiles of *Vibrio* and heterotrophic bacteria in the intensive Vanamee shrimp culture using bioremediation technique in Karawang

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Abstract. *Vibrio* sp. group is one of the parameters of the health level of a shrimp culture. The aim of this study is to learn the population of vibrio and heterotrophic bacteria in Vanamee shrimps' cultivation under the use of probiotic bacteria. The profile analysis of the *Vibrio* sp. and heterotrophic bacteria were done in the Vanamee shrimp culture with implementation of selected bacteria as bioremediation agents in Balai Layanan Usaha Budidaya Perikanan, Karawang. The studies were conducted in 110 days using the culture density of 60 shrimps/m². Three ponds of 2,500 m² namely A5, A7 and B5 ponds were set up for the observation and sample-taking for nine times. The *Vibrio* sp. and heterotrophic bacteria were analyzed using a plate-count technique with Thiosulphate Citrate Bile-salt Sucrose (TCBS) agar and Sea-Water Complete (SWC) media respectively. The shrimp’s growth and survival rate, and the water quality parameters were also monitored periodically. The result showed that *Vibrio* sp. population were fluctuated. The population was between 1.4 - 29.0 x 10² CFU/mL. The result showed that *Vibrio* sp. population were fluctuated. The highest *Vibrio* population was 10³ CFU/mL while the highest profile of heterotrophic bacteria around 10⁵ CFU/mL in average. The shrimp weight in final day of culture showed positive increase with the average body weight of 22.34±0.68 g/indv, while the average daily growth rate was 0.31±0.01 % g/day and the average of survival rate in the final experiment was 58.42±1.50%. The water quality was fluctuated by increasing days of culture. The temperature of water during culture period ranged between 26.46 ± 1.87°C to 26.97 ± 0.87°C, with the pH value ranged from 7.31 ± 0.36 to 7.59 ± 0.28 and the salinity ranged between 24.23 ± 43.87 to 24.61 ± 4.15 ppt.

Keywords: *Vibrio*, heterotrophic bacteria, *L. vannamei*, water quality.

1. Introduction
The increasing of the world population in the last few decades have lead to the increasing in food demand, especially protein sources. Aquaculture is one of the potential sectors that can fill up the
world's protein needs amidst the stagnation of production from the capture fisheries sector. Until 2016, the total food supply produced by aquaculture reached more than 140 million tons, while capture fisheries was less than 80 million tons [1]. Increased production in aquaculture is mostly done through intensive cultivation systems. In an intensive aquaculture system, the amount of organic matter both from feed residues and metabolic waste of the culture system has also increased [2]. The organic matter that accumulates in rearing water has been potential in increasing the rate of aerobic metabolism of the bacteria and plankton [3].

*Vibrio* sp. population is one of the parameters used to determine the health status of shrimp culture. These microorganisms can cause serious losses in shrimp culture throughout the world. Members of the genus *Vibrio*, including *V. parahaemolyticus* and *V. harveyi* have been described as the main pathogenic species in shrimp and are responsible for most of the larval deaths [4-6]. These bacteria cause serious infections, called vibriosis, to the shrimps and leads to the decline of the shrimp production, and growth rates in surviving individuals, thus having a negative impact on the overall financial efficiency of the business [4]. Vibriosis is commonly acting as a secondary infection, especially when shrimps under the stress. Bacterial infections are related to stress due to high density, malnutrition, parasitic infections, high organic matter, low oxygen, and extreme temperature fluctuations [7-8].

The physico-chemical-biology status of the rearing water of shrimp is an important aspect in shrimp culture. Persistent or recurrent infections may be due to poor water quality and low water exchange rates. The use of microbes in shrimp farming successfully helps in maintaining the quality of environmental conditions during the culturing period [9-11]. The quality of good culture media will have a positive impact on the production process and is expected to improve the performance of animal growth.

In aquaculture, the use of microbes to improve water quality has been known as probiotics. Probiotics are expected to have beneficial effects through the production of inhibitory compounds, competition for chemicals or available energy, competition for adhesion sites, enhancement of the immune response, and improvement of water quality [7]. That bacteria can inclusively break down organic wastes and play roles as waste and sediment bioremediators through nitrification and denitrification [3]. A study combined both bacteria EM-1 and microalgae (*Chlorella* sp.) as bioremediator agent of aquaculture wastewater in reducing ammonia and phosphorus. The result showed that symbiotic of microalgae and bacteria EM-1 have a higher removal percentage and removal rate on phosphorus than conventional microalgae bioremediation [6]. Another study reported that *Bacillus subtilis* was able to utilize dissolved inorganic nitrogen waste 1.17 times greater than the controls in the study [5]. The use of various types of bacterial strains has been reported could eliminate organic waste and metal elements such as Cu, Zn, and Fe in a bioreactor [8]. *Paenibacillus* spp. and *Bacillus cereus* were also reported effective in inhibiting the shrimp larval pathogens, like *Vibrio* spp. and *V. harveyi* both in vitro and in vivo [9]. *Bacillus subtilis* strain is a kind of microbes that could produce a variety of extracellular enzymes and antimicrobial peptides [12-13]. The secretion of these substances may be beneficial for controlling pathogenic bacteria and for the improvement of the rearing water quality. Considering culture conditions, bacteria with probiotic properties can be helpful in terms of shrimp health and wastewater management. This study was conducted to evaluate the profile of *Vibrio* and heterotrophic bacteria in intensive *Vanname* shrimp culture using the bioremediation technique.

2. Materials and Methods

2.1. Materials

This research is an observational-descriptive on the mass shrimps’ production on the three different ponds, and we did not perform any comparison, since each pond was handled differently in the probiotic addition, such as the amount of probiotics given, the frequencies, and water change. The commercial probiotic products that used in this study were *Thiobacillus* spp., *Bacillus* sp., and *Lactobacillus* spp. with a dosage of 1-2 kg/ha for two weeks. While the experimental shrimp post-
larvae were *Litopenaeus vannamei* from Balai Layanan Usaha Produksi Perikanan Budidaya (BLUPPB), the Ministry of Marine Affairs and Fisheries, Karawang Regency, West Java, Indonesia. The feed used in this study was a commercial feed with 40% protein content.

### 2.2. Methods and culture conditions

Prior to the start of the experiment, the three ponds were allowed to dry to eradicate possible sources of disease. The sediments were removed, then the wall and pond bottom were cleaned and repaired. The brackish water is pumped into the pond through fine filtration systems. Some of the materials used for the water treatment were chlorine at 10 ppm-30 ppm, *crustacide* (Nuvet Plus) 1.5 ppm, and saponin 25 ppm. The ponds then left for a week. The water pond was also treated with minerals and probiotic before stocking. Each pond were using four-pedals wheels. Water maintenance was carried out for 14 days before stocking. Shrimp fry stocked with a density of 60 individuals/m² and maintained for 100 days. Feeding was carried out 4 times a day by using commercial feed, while the feeding rate used refers to SNI 7772: 2013 [14]. Probiotics powder that consisted of *Lactobacillus*, *Thiobacillus*, and *Bacillus* (total cell density at $10^9$ CFU/mL) were given with a dose at 1-2 kg/ha of the pond once a week. This experiment was conducted for 100 days in a continually aerated system. Like water level and feeding rate, the maintenance of the shrimps was conducted based on the procedure of shrimp’s culture refers to SNI 7772: 2013 [14]. The water level was maintained at a depth of 100 cm throughout the culture period by pumping water into the pond, the feeding rate was routinely changed depends on shrimp’s weight.

### 2.3. Bacterial quantification

Shrimp rearing water were collected from each pond. The quantification of bacterial cells population was carried out by the serial dilution method (10-fold dilution), followed by plating on agar medium. Bacteriological media used to calculate heterotrophic bacteria count (HBC) in this research was SWC (Sea-Water Complete) agar (5 g bacto peptone, 1 g yeast extract, 3 mL glycerol, 18 g bacto agar, 750 mL seawater, and 250 mL distilled water). Moreover, selective media Thiosulphate Citrate Bile-salt Sucrose (TCBS) agar was used to calculate presumptive *Vibrio* count (PVC).

### 2.4. Biological performance and water quality analysis

Biological parameters were observed to see the survival rate and development of shrimp growth during the maintenance period. Biological parameters were observed included survival rate (SR), weight gain (WG) and average daily growth rate (ADG) [10], and calculated by using the following equation:

\[
\text{Survival rate (SR)} = \frac{N_t - N_0}{100} \tag{1}
\]

\[
\text{Weight gain (WG)} = W_t - W_0 \tag{2}
\]

\[
\text{Average daily growth rate (ADG)} = \frac{(W_t - W_0)}{t} \times 100 \tag{3}
\]

where, $W_0$ and $W_t$ are the initial weight (g) and final weight (g) of shrimps, $N_0$ and $N_t$ are initial and final number of shrimp, and $t$ represents culture time in days. The temperature was measured by a thermometer, power of hydrogen (pH) was measured by pH meter, and salinity was measured by refractometer.

### 2.5. Data analysis

Data were collected by using Ms. Excell 2013 and presented as descriptive statistics.

### 3. Results

The abundance of *Vibrio* of the rearing media can be observed in figure 1.
The result showed that, generally, the vibrio population shown existence of two peaks of maximum populations. The total presumptive *Vibrio* on day-0 of culture was detected less than 10 CFU/mL in all ponds. After 29 days of maintenance, the *Vibrio* population was increased with the highest density recorded on pond A7. Later, its population was declined until it reached day-53. After that, it increased until day-82 of observation. The population declined when it reached day-102 of observation. The highest vibrio population was detected in pond A5, followed by pond A7, and B5 as the lowest. Maximum vibrio population found in pond A7 occurred in day-29 of observation (2.9 x 10^3 CFU/mL), while in pond B5, the maximum vibrio population was recorded in day-43, with the amount of vibrio population of 1.9 x 10^3 CFU/mL (figure 1).

The observation of the heterotrophic bacteria’s density (figure 2) shown a similar profile with the *Vibrio* bacteria group; there were two peaks of the maximum population during the cultivation period, but the peaks were in a different timelines. The total heterotrophic bacteria’s density (THB) on day-0 of culture was detected less than 1000 - 2000 CFU/mL in all ponds. After 53 days of maintenance, the heterotrophic bacteria’s population was increased with the highest density recorded on pond A7. Later, the population was declined until day-75 observation, then increased until day-82 of observation with the maximum population of 1 x 10^5 CFU/mL in pond A7. On day-102 observation, the population was declined again. The highest bacteria population was detected in pond A7, followed by A5, and pond B5 has the lowest bacteria population. The maximum heterotrophic bacteria population in pond A7 occurred in day-82, with the amount of population of 1.06 x 10^5 CFU/mL. While in pond A5, the maximum population occurred in day-53 was 6.65 x 10^4 CFU/mL, and in pond B5, the maximum bacteria heterotrophic population was 6.1 x 10^4 CFU/mL on day-55.
Figure 3. (a) Weight gain; (b) average daily growth rate; and (c) survival rate (SR) of white shrimps *Litopenaeus vannamei* during culture period with bioremediation technique.

Figure 4. Water quality profile of the white shrimp (*Litopenaeus vannamei*) a) temperature, b) pH, and c) salinity during culture period.

The shrimps’ weight gain can be observed in figure 3a. The shrimp weight in final day of culture showed a positive increase with the average body weight of 22.34 ± 0.68 g/indv, while the average daily growth rate (figure 3b) was 0.31 ± 0.01 % g/day. The average survival rate until the end of the maintenance was 58.42 ± 1.50%.
The water quality parameter that observed in the culture period include the temperature, pH, and salinity (figure 4). The water temperature during culture period ranged between 26.46 ± 1.87°C to 26.97 ± 0.87°C (figure 4a), with the pH value ranged from 7.31 ± 0.36 to 7.59 ± 0.28 (figure 4b). Meanwhile, the salinity ranged between 24.23 ± 43.87 to 24.61 ± 4.15 ppt (figure 4c).

4. Discussion
In an intensive shrimp’s culture with high stocking-density cultivation, a strict-control of water quality is necessary, and this condition will determine the succession of the shrimps’ quality of life. This can be caused by the high waste in the form of feces or feed that left unconsumed and accumulated on the water system. Several parameters for water quality that needed to be taken care of are as follow: pH, dissolved oxygen, temperature, salinity, and heterotrophic bacteria populations, and Vibrio bacteria. On the shrimp cultivation, disease that easily occurred commonly caused by Vibrio sp. [11]. This type of bacteria can grow faster if the amount of organic materials in the water is increasing. When the Vibrio is higher than other bacteria, that could cause a decline in survival rate in the stocking and development phase [15].

The use of microbe had been reported to give an advantage in aquaculture, both for increasing the immunity and the quality of the water quality of the aquatic organisms. The controlling of the water quality is necessary in order to maintain the parameters qualities are still optimal for the aqua biotics’ growth and to inhibit the growth of potentially pathogenic microbes for shrimps’ maintenance [16]. This caused by the existence of the pathogens would bring a direct impact towards the biotics’ living [17]. In this study, the utilization of microbes was intended as bioremediation and bio-control agents towards Vibrio bacteria.

One of the control efforts in the purification process in order to degrade the cultivation’s waste is to use bioremediation bacteria that will degrade organic waste and toxic metabolites from incomplete degradation process. Aside from those, bacteria also maintain the number of bacteria that cause disease through the competition system in food usage and inhibit the pathogenic bacteria, usually from Vibrio sp. group and heterotrophic bacteria.

The Vibrio population until day-40 shown an increase and a fluctuated value until the maintenance time ended (figure 1), nevertheless the population was still below the limit. According to SNI 7772:2013 [14] concern limit for Vibrio bacteria that might damage shrimp cultivation is 10³ CFU/mL. The handling methods from each pond that affected the Vibrio populations were water exchange, different feed loading, and water quality. Water exchange was done in phytoplankton blooming time, a decrease in shrimps’ health condition, and the high amount of organic matter due to uneaten feed and metabolic waste. Those processes often performed in order to save the shrimps or the production target. The estimation of weight the shrimp’s populations and growth regularly for feed management, also appeared to be the reason of the fluctuating density of Vibrio in this study. According to Alfiansah et al [18] shrimp pond management might affect the water quality as well as bacterial communities.

In this experiment, regularly solid waste discard and maintaining the number of feed loading were likely to have affected the density of bacteria in shrimp’s pond. Alfiansyah et al [18] mention that the oxygen demand for decomposition of organic matter might be increase due to feed loading, and the dissolved oxygen might become depleted at the bottom of the shrimp’s pond. The water exchange and siphoning the sludge regularly which were conducted in this study might help to decrease organic matter in the shrimp ponds. Alfiansyah et al [18] explained that good water management such as discarding sludge, maintaining pH, and salinity, might avoid abrupt water quality change and could minimize the organic matter and anaerobic area which are very suitable nutrient and condition for the potential pathogenic bacteria to multiply such as Vibrio.

Another explanation related to the density of Vibrio in this study was the capability of Bacillus spp to inhibit Vibrio proliferation. Study by Ravi et al [9] showed that Paenibacillus spp. and Bacillus cereus were also reported effective in inhibiting the shrimp larval pathogens, like Vibrio spp. and V. harveyi both in vitro and in vivo. Zhang et al [19] found that Bacillus have the capability to inhibit the
growth of pathogenic _Vibrio_, because of _Bacillus_ strains is a kind of microbes that could produce a variety of extracellular enzymes and antimicrobial peptides [12-13]. The secretion of these substances may be beneficial for controlling pathogenic bacteria like _Vibrio_ and for the improvement of the rearing water quality.

Tompo [20] has been studied of the population of _Vibrio_ sp. isolated from water and sediment in semi-intensive ponds. The result showed that the population of heterotrophic bacteria in the sediment pond were in the range of \(10^6\) to \(10^{12}\) CFU/mL while the population of bacteria in water was in the range of \(10^3\) to \(10^9\) CFU/mL. Also reported that the populations of _Vibrio_ sp. in the pond sediment for all treatments during the study was in the range of \(10^3\) to \(10^5\) CFU/mL and in aqueous media was still in the range of \(10^3\) to \(10^5\) CFU/mL for all treatments. Meanwhile, founded that the populations of _Vibrio_ sp. in the pond on enlargement water of whiteleg shrimp ( _Litopenaeus vannamei_ ) was still in the range of 0.1-0.7 \(x\) \(10^6\) CFU/mL [21]. In general, bacterial populations found in both sediment and water pond on the media were still in a safe range for the growth of cultured organisms [21].

The low _Vibrio_ bacteria population in our research might be affected by the usage of bioremediation bacteria that had been added in this cultivation and the water sterilization process in the pond preparation process. _Bacillus_ bacteria that added in the cultivation works as the competitor for food usage and releases extracellular enzymes that inhibit _Vibrio_ bacteria’s growth. The concentrations of total presumptive _Vibrio_ below the limit may indicate that the _Thiobacillus_ spp., _Bacillus_ spp., and _Lactobacillus_ spp. administered could be proliferated in shrimp and contributed to control the abundance of _Vibrio_. Yudiati et al [12] also reported that the addition of probiotic in Vaname shrimp culture also dropped the total _Vibrio_ population and total ammoniac amount from organic materials.

Vaseeharan and Ramasamy [22] reported in their study that _V. harveyi_ could be inhibited by _Bacillus subtilis_ BT23 type bacteria in a co-culture of _in vitro_ experiment. The antagonistic ability would increase alongside the increase of the _B. subtilis_ BT23 concentration given. This showed that the concentration of the antagonist agent is affecting the _V. harveyi_ growth. _Bacillus subtilis_ also possess the ability to inhibit several _Vibrio_ species such as _V. anguillarum_, _V. vulnificus_, _V. alginolyticus_, _V. harveyi_, dan _V. Parahaemolyticus_, and _V. Campbelli_ [17; 23]. _Bacillus_ members are known for possessing anti-microbe components such as bacitracin and polymyxin that inhibit other microbes’ growth [18,24]. Several studies stated that _Bacillus_ could produce bioactive lipopeptide component with high spectrum such as fengicyn, iturin, bacillomycins, mycosubtilin [25] and surfactin [26]. Another possibility is that the existence of competition mechanism for the nutrients [14] and volatile components [27].

Heterotrophic bacteria population (figure 2) in the cultivation pond shown a low population at the beginning of the maintenance, occurred in almost all cultivation pond. The high population is shown in pond A7 on day-53 and day-82. Overall, the highest density of heterotrophic bacteria was around \(10^5\) CFU/mL, while the lowest was around \(10^4\) CFU/mL during this experiment. This might be caused by high amount of left-over feed and feces. Heterotrophic bacteria use organic compound as energy source in metabolism process, added by electron source from oxygen compound.

According to de Paiva-Maia et al [28] the abundance of microorganisms is a function of the substrate supply. The uneaten feed and metabolic waste (feces) which consist of protein, carbohydrate, lipid, and was produced everyday by the shrimps in this experiment might promote the substrate availability for heterotrophic bacteria to proliferate. The increase of heterotrophic bacteria growth could also be explained by Lu et al. [5] that resulted _Bacillus_ was able to metabolize dissolved inorganic nitrogen waste [5]. Another possibility of the high concentration of heterotrophic bacteria found in this study was probably related to the administration of the _Thiobacillus_ spp., _Bacillus_ sp., and _Lactobacillus_ spp regularly every week. This treatment can cause an increasing of total bacteria in the water. Meanwhile, the addition of several materials such as chlorine 30 ppm, _crustaside_ (Nuvet Plus) 1.5 ppm, and saponin 25 ppm in the preparation of the pond caused a decline in the population of the heterotrophic bacteria in the first cultivation times. Furthermore, the drying process and reversing the base of the cultivation area were done. The longer drying time was expected to increase the
number of dead bacteria and eliminate the toxic metabolites compounds in the sediment. After it reached day-53, a significant increase in bacteria population was seen, yet it decreased again until day-75.

Leonard et al. [29] found that the bacteria heterotrophic in a marine fish farm sea bass (Dicentrarchus labrax) with a recirculating water system was typical of the marine environment such as: Pseudomonas, Oceanospirillum, Marinobacter, Paracoccus and Erythrobacter, which are two genus of Vibrionaceae, one strain of Vibrio and one strain of Aeromonas. Tompo [20] reported that the limit value of heterotrophic bacteria population in the shrimp cultivation system is $10^4$ CFU/mL water. This group of bacteria helps degenerate the remains from the organic feed that wasted inside the pond. The low heterotrophic bacteria population might also cause by the usage of disinfectant in preparation process or the ponds’ maintenance. Because of those reasons, the used of probiotic is still necessary. During the addition process, aside from the number of bacteria added, the frequency and cultivation pattern are also an important parts. In the intensive cultivation system with high stocking-density and loading nutrients, the higher number of probiotic bacteria is necessary.

A study by Zhang et al. [30] showed that water quality such as ammonium, chemical oxygen demand and total nitrogen have effects on the composition of bacterial communities in L. vannamei aquaculture water. Based on the results of the Pearson Correlation test, Jeana et al. [31] found that the relationship between organic matter with a total bacterium in intensive shrimp cultivation with semi-biofloc system indicates the linear positive correlation, the greater the value of organic matter the greater the total value of bacteria. Total bacteria at intensive shrimps with semi-biofloc system obtained in the research were generally between $2.0 \times 10^3 - 2.4 \times 10^5$ (CFU/mL) [31]. The addition of the probiotics can increase the population of heterotrophic bacteria as well as the population of the phytoplanktons, that will lead to improving the environmental quality of the sediment and water in grow-out ponds stocked with Litopenaeus vannamei in a closed recirculation system [28].

The parameters of water quality such as pH, temperature, and salinity of the Vaname shrimp cultivation ponds were in a good condition, although a low pH fluctuation was found (about 6.38 and 7.92). The water exchange and siphoning the sludge regularly during this experiment might help to decrease organic matter in the shrimp ponds. Boyd and Tucker [32] mentioned that the concentration of organic matter can cause the changing of pH in aquaculture ponds. The fluctuation of pH is not good for shrimps’ growth since optimal pH value for Vaname shrimp growth is between 7.5–8.5. Low pH condition will affect shrimps’ metabolism and physiological process. Low water pH might be caused by soil acidity, water source, and rainwater [33]. Meanwhile, the fluctuation of salinity during the cultivation was found between 16 to 30 ppt. It is widely known in aquaculture that the percentage of water exchange, the number of feed loading, the number of surviving shrimp, the organic matter concentration, and the algae blooming were quite different for each pond even if they have the same treatments and operating procedure during the cultivation. These differences might cause the differences in water quality and make the fluctuation of water quality parameters like organic matter, pH, and salinity in this study. The fluctuating of salinity in this study might also be affected by salinity concentration and volume of the water supply from the reservoir during the water exchange. However, the range of water quality value was considered good for shrimps’ growth. The Vaname shrimp will grow in a well and optimal condition in 15–25 per mile salinity [34] and temperature ranged between 24.4 – 28.3 °C [35]. The water quality in the maintenance time was known still on the range that can be tolerated by the shrimps.

The shrimps’ weight gain in all ponds showed a positive increase with the average of survival rate reached $58.42 \pm 1.50\%$. Three ponds showed a relatively good result. Ochoa-Solano and Olmos-Soto [35] reported that the genus Bacillus produces extracellular enzymes for breaking down various carbohydrates, lipids, and proteins into smaller units. Meanwhile, Talpur et al. [36] reported that the addition of Lactobacillus plantarum as a probiotic increase the activity of shrimp amylase and protease enzymes. Xie et al. [37] mentioned that increasing the activity of these two types of enzymes will directly increase the digestive process of nutrients such as carbohydrates and protein in the feed which will contribute to supporting shrimp growth and can reduce the FCR ratio during the production
The succession of culture with the survival rate mentioned before could be caused by the role of probiotic added in the cultivation itself. The shrimps’ weight gain showed a positive increase with survival rate values that reached 57.33 – 60.13 %. All of the three ponds shown a relatively good result. Bachruddin et al [38] stated that the live survival rate in vaname shrimps’ cultivation was approximately 50-59%, with the weight of 4.7-7.4 gram/individuals, and until the shrimps reach 60 days of age. The succession of the stocking with survival rate mentioned before could be caused by the role of probiotic added in the cultivation itself. Aside from that, the shrimp growth showed a positive value when the shrimp were 60 days of age, it weights 11.5 g/individuals. While the other experiment of the probiotic usage showed a mean weight result of 9.38 gram/individuals [39] and 6.31 gram/individuals when the shrimp were 90 days of age [40].

5. Conclusion
The bioremediation technique has been giving a stable impact towards Vibrio and heterotrophic bacteria population, yet this technique could give a well daily growth rate (0.31 ± 0.01 % g/day) and survival rate (58.42 ± 1.50 %) for the shrimps. The growth rate could be maintained until 110 days of age with the mean weight of 27.5 g/individuals approximately. In view of Vibrio, heterotrophic bacteria and shrimp growth over the rearing period, these three kinds of bioremediation bacteria (Thiobacillus spp., Bacillus sp., and Lactobacillus spp.) is proposed to be regularly applied. Further bioremediation technique is necessary for every shrimps’ cultivation work and the search of the right type and composition will bring an excellent results. Moreover, regular monitor of the organic matter and bacterial abundance and maintaining the water quality is suggested to support the growth and the effectiveness of bacteria performance.

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