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Performance of zero water discharge (ZWD) system with nitrifying bacteria *B. megaterium* and microalgae *C. calcitrans* components in super intensive Pacific white shrimp *Litopenaeus vannamei* culture at low salinity

Kinerja sistem zero-water discharge (ZWD) dengan komponen bakteri nitrifikasi, *B. megaterium* dan mikroalga *C. calcitrans* pada budidaya udang putih *Litopenaeus vannamei* superintensif besalinitas rendah

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ABSTRACT

This research aimed to obtain the performance of super intensive white shrimp rearing using zero water discharge (ZWD) system. This study consisted of four steps, (1) activation and cultivation of nitrifying bacteria, microalgae *C. calcitrans* and *B. megaterium*; (2) acclimatization of Pacific white shrimp PL10 with 30 g/L of salinity and decreasing salinity at 2–3 g/L/day; (3) conditioning of ZWD system; (4) white shrimp rearing in 400 L of tank for ten weeks. The experiment used three treatments, (a) shrimp reared without any addition of microbial agent with water exchange conducted every week as much as10–20% of total rearing volume as control (K); (b) ZWD systems with the applications of nitrifying bacteria, (*C. calcitrans* and *B. megaterium*) without water discharge (P1); and (c) ZWD system with the application of microalgae *C. calcitrans* and *B. megaterum* without water discharge (P2). According to the results, application of nitrifying bacteria, microalgae *C. calcitrans* and *B. megaterium* were able to improve the performance of ZWD system performance of white shrimp rearing at low salinity. In addition, the ZWD system was also able to increase the growth rate and survival rate of shrimp when it compared to control. The best rearing performance was found in ZWD system with application of microalgae *C. calcitrans* and *B. megaterium*.

Keywords: *Litopenaeus vannamei*, ZWD, low salinity, microalgae, nitrification bacteria.

ABSTRAK

Penelitian ini bertujuan untuk mengetahui kinerja budidaya udang putih super intensif bersalinitas rendah menggunakan sistem *zero water discharge* (ZWD). Penelitian ini terbagi dalam tiga, yaitu (1) aktivasi dan kultur bakteri nitrifikasi, mikroalga *C. calcitrans* dan *B. megaterium*; (2) aklimatisasi udang putih PL10 salinitas 30 g/L dan penurunan salinitas 2–3 g/L/hari; (3) pengondisian dari sistem ZWD; (4) pemeliharaan udang putih selama 10 minggu di bak bervolume 400 L. Penelitian ini menggunakan tiga perlakuan ; (a) perlakuan kontrol tanpa penambahan mikroba dan pergantian air setiap minggu sebanyak 10–20% (K) ; (b) sistem ZWD dengan bakteri nitrifikasi, mikroalga *C. calcitrans* dan *B. megaterium* tanpa pergantian air (P1); (c) sistem ZWD dengan mikroalga *C. calcitrans* dan *B. megaterum* tanpa pergantian air (P2). Berdasarkan hasil yang didapat, aplikasi bakteri nitrifikasi, mikroalga C. *calcitrans* dan *B. megaterum* mampu meningkatkan kineja sistem ZWD pada budidaya udang putih *L. vannamei* bersalinitas rendah. Selain itu, aplikasi bakteri nitrifikasi, mikroalga *C. calcitrans* dan *B. megaterum* pada sistem ZWD juga mampu meningkatkan laju pertumbuhan dan sintasan udang putih dibanding dengan kontrol. Kinerja pemeliharaan terbaik dijumpai pada sistem ZWD dengan aplikasi mikroalga *C. calcitrans* dan *B. megaterum*.

Kata kunci: bakteri nitrifikasi, *Litopenaeus vannamei*, mikroalga, salinitas rendah, ZWD

INTRODUCTION

White shrimp (*Litopenaeus vannamei*) is an endemic species of West Pacific ocean, from Peru to Mexico. The white shrimp was first introduced to Asia in 1978−1979, but began its commercial production in 1996 in Taiwan and China, then spread to Southeast and South Asia. In 2008, the worldwide production of paneid shrimp reached up to 3.399.105 tons and *L. vannamei* contributed 2.259.183 tons or 67% of total production(Liao & Chen, 2011). In 2015, the overall production all around the globe was estimated at 3.6 million tons and Indonesia contributed 16.5% or about 600.000 tons (GOAL, 2013).

The production of white shrimp in Indonesia recently is using a closed system, either traditional, semi-intensive, and intensive. According to Zhang *et al.* (2015) and Verdegem (2013), a closed system of aquaculture is potentially enrich the nutrient content in the rearing container. In closed aquaculture system, waste management in the rearing media was overcome using water exchange; but unfortunately, it may increase the nitrogen and phosphorus content which potentially triggers eutrophication in aquatic environment.

This eutrophication problem has been tried to be overcome in several ways, such as biofloc, recirculation aquaculture system (RAS), and zero water discharge. Application of certain technology might reduce water usage during the rearing period, depress pathogenic bacteria, improve water quality, and provide feed supplement (De Schryver *et al*., 2008; Martin *et al*., 2010; Zhao *et al*., 2012). The production enhancement of white shrimp was also done by expanding farm area. Recently, the central production of white shrimp only centered in coastal area which the salinity around 30 g/L. Biologically, the white shrimp has a wide tolerance for salinity fluctuation around 0.5−40 g/L (Gao *et al.,* 2012), so that white shrimp culture activity might be done on wider rage area, not only in coastal area.

One of the applicable alternative on low salinity white shrimp rearing is zero water discharge (ZWD). The zero water discharge is an aquaculture system without water discharge. New water is only added to replace water loss due to evaporation. In this system, the microbe role is quite essential, especially chemoautotroph and heterotroph microbes, to control nutrient cycle, water quality, feed supplement supply, and inhibit pathogenic bacteria (Zhao *et al*., 2012; Suantika *et al.,* 2015). The ZWD system is also applicable by increasing heterotroph bacteria in nutrient assimilation process using carbon addition in C/N ratio manipulation to control inorganic N in culture media (Nootong *et al*., 2011; Gao *et al.,* 2012; Panjaitan, 2010).

According to the description above, this study used low salinity for white shrimp rearing using zero water discharge through microbes manipulation (nitrification bacteria, microalgae, *Bacillus megaterium,* and *Chaetoceros calcitrans*). This study was aimed to evaluate zero water discharge performance by manipulating microbial loop in maintaining water quality, reducing pathogenic bacteria population (*Vibrio* sp.), and supporting the white shrimp growth in low salinity.

MATERIALS AND METHODS

Activation and culture of nitrification bacteria *B. megaterium* and microalgae *C. calcitrans*

Bacillus megaterium was cultured in nutrient broth (NB) media with dosage of 8 g for 1 L aquades, while *C. calcitrans* was cultured in Gullard media which contain mineral, phosphate, silicate, and natrium citrate in 1 L seawater with ratio 1 mL : 1 mL: 1 mL: 1 mL. Meanwhile, the media that was used to culture nitrification bacteria was *Nitrosomonas winogradsky* + $CaCO_3$ 0.5%.

White shrimp acclimatization

The study used PL 10 phase of white shrimp fry which brought from PT. Suri Tani Pemuka, Indramayu, West Java. The white shrimp fries were acclimatized for seven days in fiber tank filled with 300 L of seawater at salinity of 30 g/L. During the acclimatization, the white shrimp fry was fed using micropellet (40% protein content) about 10% of the fry biomass. The feeding frequency was four times a day, started at 8.00, 12.00, 17.00 and 21.00. Then the salinity was decreased from 30 g/L to 2 g/L in 10 days with decreasing speed of 2−3 g/L each day. When salinity reached 4 g/L, the white shrimp fry was moved into 600 L nursery pond with 2 g/L of salinity level. The nursery phase was done 2−3 weeks to adapt the experimental shrimp at the low salinity level to minimize mortality before they were moved into the to grow-out pond.

The rearing system of white shrimp using zero water discharge

The zero water discharge system consisted of several components. Those components were listed below :

1. Tank sized 0.5 m^2 (water volume 400 L)
2. Rearing media (salinity 2–3 g/L)
3. $CaCO_3$ gravel which was sunk to the bottom of the tank and cover it all.
4. Nitrification bacteria (10^6 CFU/mL) to control nitrification process
5. *C. calcistrans* (10^6 cells/mL) as nitrate/orthophosphate absorbent and live feed and oxygen source.
6. *B. megaterium* (10^7 CFU/mL) to control nutrient assimilation
7. Aeration pump to supply oxygen
8. A 100 Watt heater to maintain the water temperature around 26 ± 1°C
9. A siphon for cleaning organic material from the tank bottom.

The zero water discharge system scheme is shown in Figure 1.

Experimental design

This study was designed using complete randomized design consisted of three treatments and three replications. The treatments were control (K) (without bacteria and microalgae *Chaetoceros calcitrans* addition and water discharge), treatment 1 (P1) (nitrification bacteria, *Bacillus megaterium,* and *Chaetoceros calcitrans* addition, no water discharge), and treatment 2 (P2) (*Bacillus megaterium* and *Chaetoceros calcitrans* addition, no water discharge). The stock density was 160 ind/tank equal to 400 ind/m^3 and the initial weight was 0.07 ± 0.046 g. During a 10-weeks of rearing, the white shrimps were fed using commercial feed contained 40% protein three times a day about 10–5% of shrimp biomass. The uneaten feed and feces were siphoned out twice a week.

Physical and chemical water quality measurement

The water quality parameter consisted of dissolved oxygen, temperature, and pH were measured using Eutech digital instrument, whereas salinity was measured using SCT meter (accurate 0.1 g/L). Ammonium, nitrite, and nitrate were measured using HACH spectrophotometer (Nessler method) with 420, 520, 275, and 220 nm of wavelength (Rice *et al*., 2012). Dissolved oxygen, water temperature, pH, and salinity were measured twice a week, while nitrite, nitrate, and ammonium were measured once a week.

Microbiology and biology observation of white shrimp

The water sample was taken each week to observe the amount of heterotroph bacteria, whereas the *Vibrio* sp. colony was observed twice a week using serial dilution method and spread plate technique (Cappucino & Sherman, 2011). The media that was used to observed the *Vibrio* sp. bacteria colony was thiosulphate citrate bile salt agar (TCBSA). The media was selective for *Vibrio* sp., while the heterotroph bacteria was cultured in nutrient agar (NA). To count the bacteria colony on the culture media, the following equation was applied (Cappucino & Sherman, 2011):

$$\text{Total bacteria count (CFU/mL)} = \sum \text{colony} \times \frac{1}{\text{dilution factor}} \times \frac{1}{\text{mL sample}}$$

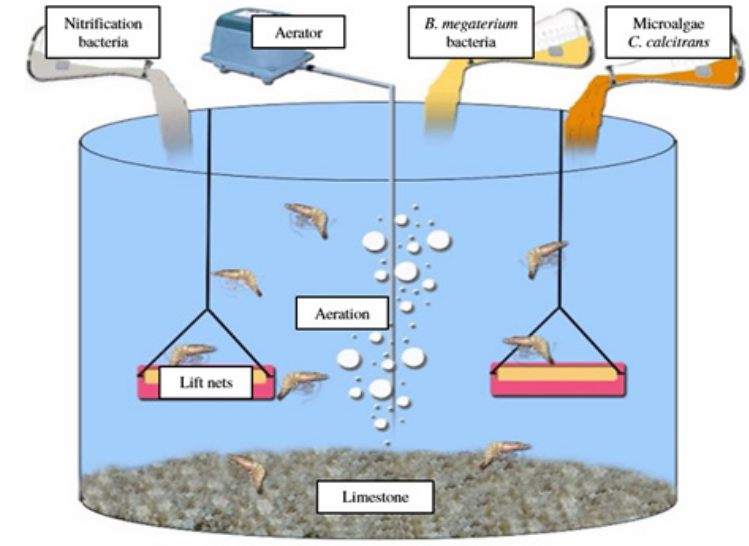

Figure 1. Grow-out scheme of white shrimp using zero water discharge

The observed biological parameters of white shrimp, growth rate, survival rate, feed conversion ratio, final weight, and biomass, were calculated using the following equation:

Survival rate (%)

$$SR\ (\%) = \frac{Nt}{No} \times 100$$

Specific growth rate (%/day)

$$SGR = \frac{\ln Wt - \ln Wo}{t} \times 100$$

Biomass

$$Biomass = D \times W$$

Feed conversion ratio

$$FCR = \frac{F}{Wt + D - Wo}$$

Note :

N_0 : Initial population (ind)
N_t : Final population (ind)
W_t : Final weight (g)
W_0 : Initial weight (g)
t : Rearing period (day)
D : Stocking density (ind/tank)
$\hat{W}$: Average weight (g/ind)
F : Total feed (g)
Wt : Final weight (g)
D : Deceased weight (g)
W_0 : Initial weight (g)

Data analysis

All the data were analyzed statistically using one way ANOVA test and descriptively using SPSS 17 to evaluate any differences on each treatment. The physical and chemical water quality parameters were analyzed using ANOVA, while the total count of heterotroph and *Vibrio* sp. were analyzed descriptively.

RESULTS AND DISCUSSIONS

Conditioning zero water discharge system

During the accustoming stage, the nitrification bacteria was able to oxidize ammonium from 9.84 ± 1.22 mg/L to 1.71 ± 0.43 mg/L on the fourth day of accustoming the ZWD system, so the ammonium oxidation rate was around 2.03 mg/L per day. The ammonium oxidation become nitrite was seen from the increasing of nitrite concentration from 0.68 ± 0.61 mg/L to 16.52 ± 1.77 mg/L on the fourth day and became 1.00 ± 0.78 mg/L after 16 days. The final product of nitrification from this ZWD system was nitrate. A nitrate accumulation at the end of the study reached up to 31.51 ± 1.52 mg/L (Figure 2).

Conditioning of ZWD system was a crucial step before the rearing activity started. The nitrification bacteria was able to oxidize NH_4^+ and NO_2^- which was toxic for aquatic organism, into NO_3^- which was less toxic. Result showed that ammonium oxidation was faster than nitrite oxidation. It was suspected caused by the growth of *Nitrosomonas* sp. which played an essential role in ammonium oxidation, was faster (±8 hours) than the *Nitrobacter* sp. (±12 hours) which plays crucial role in nitrite oxidation. After 72 hours, population of *Nitrosomonas* sp. was eight times over the *Nitrobacter* sp. (Gerardi, 2002).

Even though the nitrification process was achieved during the conditioning stage, however, a 16-day of conditioning required more considerations in zero water discharge system due to a longer period to rear white shrimp in

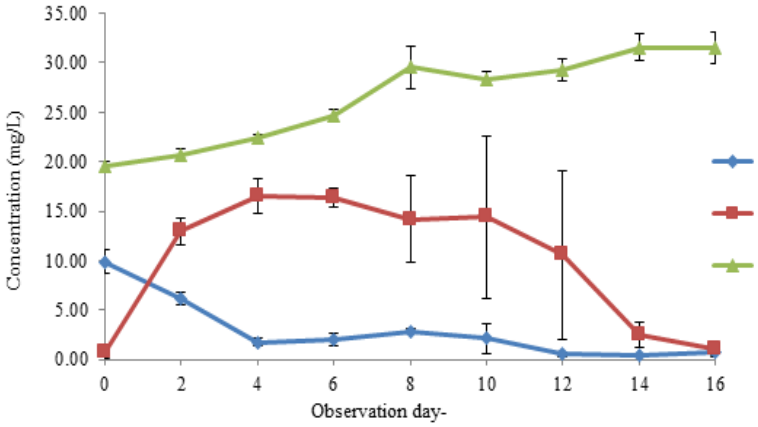

Figure 2. The nitrification bacteria performance in oxidizing ammonium and nitrite in ZWD system.

Table 1. Average value of physical-chemical water quality

Physical-chemical water quality parameter	Treatment	Range	Average ± deviation standard
Dissolved oxygen (mg/L)	Control (K)	6.24 – 8.89	7,.37 ± 0.70[a]
	Treatment 1 (P1)	6.03 – 10.41	7.45 ± 1.00[a]
	Treatment 2 (P2)	5.90 – 10.84	7.64 ± 1.17[a]
pH	Control (K)	7.67 – 8.49	8.01 ± 0.24[b]
	Treatment 1 (P1)	7.21 – 8.33	7.70 ± 0.32[a]
	Treatment 2 (P2)	7.64 – 8.54	7.94 ± 0.24[ab]
Ammonium (mg/L)	Control (K)	0.90–3.69	2.44 ± 0.78[a]
	Treatment 1 (P1)	1,.33–3.82	2.43 ± 0.76[a]
	Treatment 2 (P2)	0.90–4.66	2.98 ±1.03[a]
Nitrite (mg/L)	Control (K)	0.73–25.99	8.29 ±9.36[b]
	Treatment 1 (P1)	0.11–6.65	2.19 ±1.64[a]
	Treatment 2 (P2)	0.61–16.09	5.32 ±5.25[a]
Nitrate (mg/L)	Control (K)	13.44–43.34	24.04 ± 10.04[a]
	Treatment 1 (P1)	31.30–58.72	41.76 ± 7.87[b]
	Treatment 2 (P2)	16.13–38.77	22.04 ± 6.84[a]

Note: Different superscripts in the same column showed significant difference statistically (P<0.05); (K = without bacteria and microalgae addition with 10–20% v/v of water discharge; P1 = nitrification bacteria, *B. megaterium*, and *C. calcitrans* addition, without water discharge; P2 = *B. megaterium* and *C. calcitrans* addition, without water discharge)

one single production cycle. One of the solution to overcome this weakness was to optimize the activation of nitrification bacteria for oxidating ammonium and nitrite. It might be preferred to culture the bacteria in a stock before inoculate to the ZWD system.

Physical and chemical water quality parameter

The average range of DO and pH during 10 weeks of rearing period is shown in Table 1. The dissolved oxygen changes was affected by aeration and respiration rate of white shrimp, whereas pH was affected by excretion rate and decomposition process of organic material. According to statistical analysis, the dissolved oxygen concentration was not significantly different (P>0.05), while pH level showed significant difference (P<0.05) between P1 and K treatment, but it did not significantly different with P2.

Dissolved oxygen concentration during 10 weeks of rearing period was still in an optimal range to support white shrimp growth. According to Boyd (2010), dissolved oxygen concentration should be 3 mg/L or more to maintain feed consumption and proper growth. Moreover, the study by Chakravarty *et al.* (2016) stated that dissolved oxygen in the white shrimp rearing environment was range between 4.4–8.6 mg/L. Meanwhile, pH level in overall was quite stable, even though statistically it showed significant difference, but it was still tolerable for white shrimp. According to Utojo (2008), a normal pH level for white shrimp was around 7.5–8.5. A stable pH level could achieved by $CaCO_3$ addition on the bottom of the tank as pH buffer and substrate for nitrification bacteria, so that the pH level drop due to nitrification and decomposition could be avoided.

Although there was no significant difference in NH_4^+ concentration, however the NH_4^+ concentration in P2 and P1 were higher than that of control. It was caused by a higher feed amount in P1 (654.80 g) and P2 (695.26 g) than control (429.70 g). The differences in the given feed amount caused the different in growth and survival rate (Table 2). The higher feed amount, the organic material accumulation will be higher as well, so that NH_4^+ will be accumulated (FAO, 2014).

The nitrite concentration in the control, P1, and P2 treatment were 0.73–25.99 mg/L, 0.11–6.65 mg/L, and 0.61–16.09 mg/L, respectively. During the culturing period, nitrite concentration showed an increase at second to fourth week in the K and P2 treatment, while the P1 treatment tended to be more stable. Statistically, nitrite concentration was significantly different among treatments (P<0.05). In P1 treatment, nitrite concentration was quite more stable because of the nitrification bacteria addition, so that the NO_2^- and NO_3^- oxidation was run normally. Similar result did not occur in P2 and K treatment, which NO_2^- was increasing until

the fourth week. The NO_2^- increase presumably due to lack of nitrification bacteria in the K and P2 treatment, therefore the nitrification process was going slowly.

The final step of nitrification process is nitrate (NO_3^-), which is the final product of ammonium and nitrite oxidation. NO_3^- concentration during the rearing period of white shrimp in low salinity showed escalation tendency from the beginning to the end of the study. It presented that the nitrification process was occurred in each treatment because the nitrification bacteria is categorized as opportunist bacteria. The highest NO_3^- concentration was found in P1 treatment (31.30–58.72 mg/L), then followed by treatment K (13.43–43.34 mg/L) and P2 (16.13–38.77 mg/L). Statistical analysis showed that the nitrate concentration was significantly different among treatment ($P<0.05$). Nitrification bacteria addition in treatment P1 created a high level of NO_3^- indicated that the nitrification process was going swiftly compared to the K and P2 treatment. The accumulation of NO_3^- was also indicated that the microalgae *C. calcitrans* was poorly developed and nitrate assimilation by *C. calcitrans* was also depressed.

In term of microbial-based aquaculture, microbe is literally play essential roles in controlling N-inorganic and water quality improvement. Those were affected by several factors, such as microbe species, feed protein content, C/N ratio, and other abiotic factor. Gerardi (2002) stated that nitrification bacteria utilization in aquaculture system to oxidize ammonium and nitrite were affected by alkalinity, pH, temperature, salinity, organic and inorganic material, substrate, and light intensity. Meanwhile, *Bacillus megaterium* or heterotroph bacteria utilization in controlling inorganic nitrogen in aquaculture system was strictly affected by feed protein content and C/N ratio manipulation through nitrogen addition (Nootong *et al*., 2011; Bosma &Verdegem, 2011).

Even though ammonium was increase at certain week, rose beyond the optimal range approximately 2.44–3.95 mg/L (Lin & Chen, 2001), it was still below the lethal concentration (LC) 50 after 48 hours in salinity of 10 mg/L which was 39.72 mg/L (2.09 mg/L of unionized ammonia-N). The pH level also affected ammonium toxicity in white shrimp. In Table 1, the overall range of pH level was around 7.2–8.5 which was below 9. The pH level <9 is considered harmless because it did not convert ammonium NH_4^+ to NH_3 which certainly toxic to white shrimp (Gonzalez-Felix, 2007).

Nitrite is a toxic compound to white shrimp other than ammonium so that it is necessarily controlled to lower the nitrite in rearing media. The nitrite concentration in control and P2 treatment passed beyond the tolerable range of low salinity white shrimp rearing which was >6.1 mg/L. According to Furtado *et al.* (2016), nitrite concentration over 2.5 and 10 mg/L at salinity of 8 and 24 mg/L was considered feasible for white shrimp. Meanwhile nitrate is a nitrogen compound which is less toxic compared to ammonium and nitrite. The tolerable range of nitrate for white shrimp rearing was 177 mg/L at salinity of 23 g/L (Furtado *et al*., 2014).

Heterotroph and *Vibrio* sp. bacteria population dynamic in low salinity of white shrimp rearing

The population dynamics of heterotroph bacteria in low salinity of white shrimp culture is shown in Figure 3. The total amount of heterotroph in K, P1, and P2 treatment were 10^2–10^5 CFU/mL, 10^1–10^5 CFU/mL, 10^3–10^5

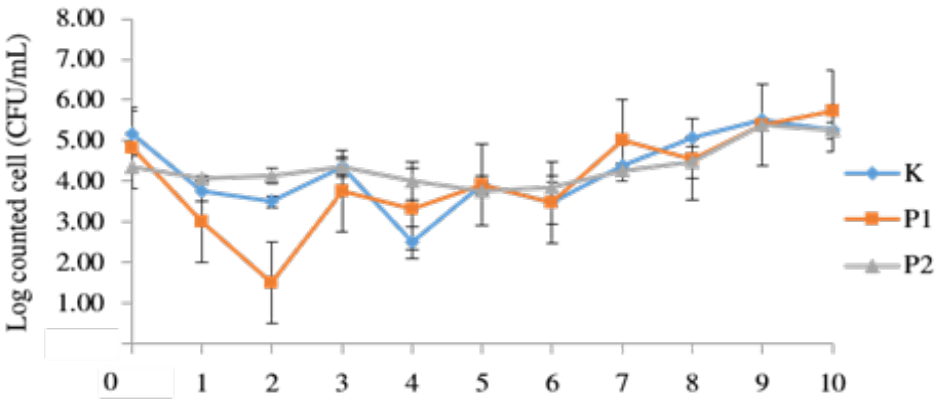

Figure 3. Population dynamics of heterotroph at low salinity of white shrimp culture. K = without bacteria and microalgae addition with 10–20% v/v of water discharge; P1 = nitrification bacteria, *B. megaterium*, and *C. calcitrans* addition, without water discharge; P2 = *B. megaterium* and *C. calcitrans* addition, without water

Table 2. Final weight, specific growth rate, survival rate, biomass, and feed conversion ratio of white shrimp at low salinity.

Parameter	K	P1	P2
Final weight (g/ind)	2.28 ± 0.21[a]	3.35 ± 0.51[b]	3.67 ± 0.20[b]
Specific growth rate (%/day)	4.97 ± 0.31[a]	5.52 ± 0.21[b]	5.65 ± 0.08[b]
Survival rate (%)	73.96 ± 10.28[a]	83.96 ± 7,19[ab]	94.79 ± 2.81[b]
Biomass	267.63 ± 11.48[a]	446.60 ± 36.67[b]	555.97 ± 37.01[c]
Feed conversion ratio	1.61 ± 0.07[b]	1.47 ± 0.08[b]	1.25 ± 0.07[a]

Note: Different superscript in the similar row indicates statistically significant difference (P<0.05) . K = without bacteria and microalgae addition with 10–20% v/v of water discharge; P1 = nitrification bacteria, *B. megaterium*, and *C. calcitrans* addition, without water discharge; P2 = *B. megaterium* and *C. calcitrans* addition, without water discharge

CFU/mL, respectively. At the end of the culture, there was an increase on heterotroph bacteria population, but the statistical analysis showed no significant difference (P>0.05). At the end of the culture, heterotroph bacteria population reached up to 10^5 CFU/mL. It was assumed that there was nutrient accumulation from feces and uneaten feed which is acted as carbon and nitrogen source to support heterotroph bacteria growth (Castillo-Soariano *et al*., 2013).

The population dynamics of *Vibrio* spp. at low salinity white shrimp culture is presented in Figure 4. Total population of *Vibrio* spp. in K, P1, and P2 treatment were 10^1–10^3 CFU/mL, 10^1–10^3 CFU/mL, 10^1–10^3 CFU/mL, respectively. At the end of the culture, population of *Vibrio* spp. decreased in P1 and P2 treatment, but instead increased in K treatment. Based on statistical analysis, the population of *Vibrio* spp. in each treatment was not significantly different (P>0.05).

Total population of *Vibrio* spp. for each treatment were in the similar range (10^1–10^3 CFU/mL). The number of population was below quorum sensing and pathogenic number was >10^4 CFU/mL. It was presumably caused by the low salinity (2–3 g/L). On the contrary, *Vibrio* spp. was considered to survived at higher salinity (>10 g/L) which was in line with Abraham and Debasis (2009) who stated that *Vibrio* spp. existence was strictly affected by salinity variation. A lower salinity (4–9 g/L) that potentially depressed *Vibrio* spp. Was about ≤10^3 CFU/mL, while higher salinity tended to increase *Vibrio* spp. population up to 10^4–10^5 CFU/mL.

Vibrio spp. is a threatening pathogen and mainly found in marine aquaculture species, such as crustaceans and molluscs. The *Vibrio* spp. is frequently pathogenic and causes huge loss in marine culture species. Vibriosis is a common disease caused by *V. harveyi*, *V. parahaemolyticus*, *V. alginolyticus*, *V. anguillarum*, *V. vulnificus*, and other species. The other disease caused by *Vibrio* spp. is white gut disease (WGD) which inhibits white shrimp growth (Chatterjee & Haldar, 2012; Gunalan *et al*., 2014).

Biological parameter of white shrimp

The measured biological parameters (biomass, specific growth rate, survival rate, final weight, and feed conversion rate) were shown in Table 2.

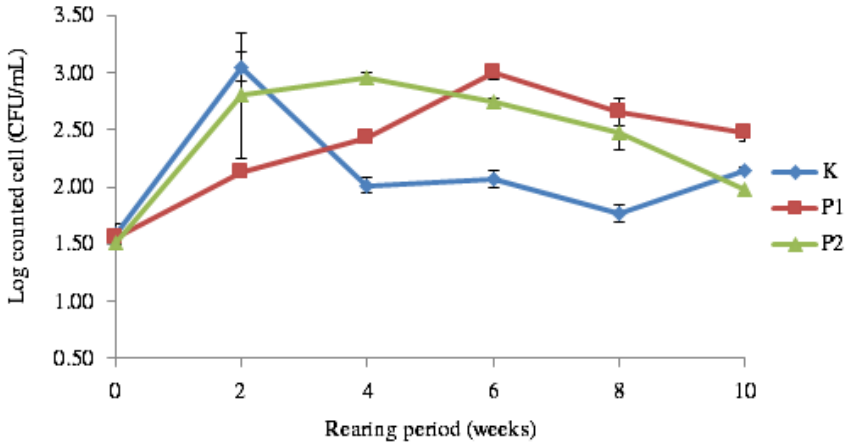

Figure 4. Population dynamic of *Vibrio* at low salinity white shrimp culture. K = without bacteria and microalgae addition with 10–20% v/v of water discharge; P1 = nitrification bacteria, *B. megaterium*, and *C. calcitrans* addition, without water discharge; P2 = *B. megaterium* and *C. calcitrans* addition, without water discharge.

According to statistical analysis, biomass, specific growth rate, and final weight were significantly different (P<0.05).

The P2 treatment resulted higher biomass, specific growth rate, and final weight. The ZWD performance on white shrimp rearing at low salinity reached the highest performance in P2 treatment. It was assumed that the combination between *Bacillus megaterium* and *C. calcitrans* stimulated floc formation. The floc formation was seen through water color changes that became brownish green, while the K and P1 treatment was quite clear. The floc formed in P2 treatment could acted as additional feed and nutrition for supporting shrimp growth.

According to Choo and Caipang (2015), water color changing from dark brownish-green to greenish-brown influenced by feed amount showed that there was a transition from microalgae to bacteria as biofloc component. Even though P1 treatment was also added *Bacillus megaterium* and *C. calcitrans,* unfortunately, the microalgae production was not optimal. It was because of the microalgae reduced the direct light intensity, then nitrification bacteria growth was inhibited and decreased nitrification rate. Beside organic material, light is one of the inhibiting factor in nitrification process (Gerardi, 2002; Merbt *et al.,* 2012; Vergara *et al*., 2016).

Generally, white shrimp reared at low salinity resulted in lower growth compared to white shrimp reared at higher salinity (30 g/L). Suantika *et al.* (2015) described that white shrimp reared using zero water discharge at salinity of 30 g/L and stocking density of 400 ind/m^3 produced 8.24 ± 0.84 g in final weight and specific growth rate 7.7±0 11 %/day. Compared the previous statement, the present study produced almost 3.67 ± 0.20 g in final weight and specific growth rate 5.65 ± 0.08 %/day.

Low growth of white shrimp reared at low salinity was assumed due to enormous energy demand in ion and amino acid osmolarity in body tissues for osmoregulation process. It was in line with Shinji *et al.* (2012) who stated that white shrimp reared at salinity 3 g/L would synthesized certain amino acid (L-serine and lysine) which suspected to regulate osmoregulation process. Roy *et al.* (2010) also presented that rearing media modification using magnesium and potassium fertilizer was further effective compared with feed modification technique in producing high growth and survival rate, and stable osmoregulation. Therefore, a major consideration must be placed on feed modification technique using certain amino acid and also utilization of several particular minerals to support white shrimp rearing at low salinity.

Production performance using zero water discharge is not only observed through the growth parameter but also the feed conversion ratio and survival rate. The feed conversion ratio and survival rate of present study is presented in Table 2. Satistical analysis showed that feed conversion ratio and survival rate showed were significantly difference among treatments (P<0.05). The lowest FCR was found in P2 treatment (1.25 ± 0.07), followed by P1 (1.47 ± 0.08), and K (1.61 ± 0.07). The highest survival rate was seen in P2 (94.79 ± 2.81), followed by P1 (83.96 ± 7.19%) and K (73.96 ± 10.28%).

The FCR value is essential in white shrimp rearing because it indicates feed amount to produce 1 kg of white shrimp biomass. The lower FCR value the more profitable because the highest cost component in shrimp culture is feed. In another study using zero water discharge at the densitity of >200 ind/m^3, resulted FCR 1.2−1.5 (Maia *et al*., 2016; Browdy *et al*., 2014), that were still in line with the present study (FCR 1.25−1.47). In addition, survival rate value also determines the superiority of the ZWD application in practice. The study using ZWD technology at low salinity resulted higher survival rate (84−95%) compared with that of conventional system (74%).

CONCLUSION

In conclusion, P2 treatment (*B. megaterium* and microalgae *C. calcitrans* addition) presented better result in supporting growth, survival, and feed conversion ratio of white shrimp at low salinity. The ZWD system in P2 treatment resulted final weight 3.67 g; specific growth rate 5.65%/day; survival rate 94.79%; and feed conversion ratio 1.25.

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