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# Performance of Zero Water Discharge (ZWD) System with Nitrifying Bacteria and Microalgae *Chaetoceros calcitrans* Components in Super Intensive White Shrimp (*Litopenaeus vannamei*) Culture

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## Abstract

This research was aimed to study the performance of Zero Water Discharge (ZWD) by using nitrifying bacteria and microalgae *Chaetoceros calcitrans* in super intensive white shrimp (*Litopenaeus vannamei*) culture. The study consists of three consecutive steps: (1) activating and cultivating of nitrifying bacteria and microalgae *C. calcitrans*, (2) conditioning of zero water discharge system, and (3) using ZWD during shrimp culture along with control (a conventional system in which the water was renewed every four weeks and without the addition of nitrifying bacteria and *C. calcitrans*). Based on water quality parameters, low and stable $NH_4^+$ (0.07–0.69 mg/L), $NO_2^-$ (0–3.2 mg/L), $NO_3^-$ (1.04–4.29 mg/L) were obtained in both systems during culture period. Higher feed amount of 1178.28 g in ZWD system compared to the conventional one contributed to a same level of $NH^{4+}$ and $NO_2^-$ level during 90 days culture period. At the end of the period, higher culture performance in terms of total weight (923.38 ± 42.15 g), mean body weight (8.24 ± 0.84 g), survival rate (90.82 ± 2.5%), specific growth rate (7.7 ± 0.11%) and feed conversion ratio (1.27 ± 0.29) was obtained in ZWD, while in control the figures were significantly different: total weight (160.48 ± 6.62 g), mean body weight (5.45 ± 0.28 g), survival rate (27.22 ± 2.09%), specific growth rate (7.24 ± 0.05%), and feed conversion ratio (4.10 ± 0.66). Based on this research, Zero Water Discharge system was able to maintain a stable and an acceptable water quality for shrimp culture. Furthermore, it resulted in better shrimp growth, higher survival rate, as well as lower FCR in high shrimp density.

**Keywords:** Zero Water Discharge system; nitrifying bacteria; *Chaetoceros calcitrans*; *Litopenaeus vannamei*; Super intensive

## Introduction

Crustaceans are sources of high quality protein and its production contributes significantly to the gross national product of Indonesian fishery sector. National production of crustaceans in Indonesia reached up to 639,589 tonnes in 2013 with *L. vannamei* contributed about 60% of the total production (386,314 tonnes) [1]. From January to June 2014, Indonesia shrimp export reached up to 13,000 tons per month (≈ almost 156,000 for a year) [2], with penaeid shrimp as the dominating crustacean commodity, standing at approximately 80% of the total shrimp export [3].

Because of its significant contribution to the Indonesia's economic growth, the increment of shrimp production has become a national policy and recently the production is targeted to reach around 700,000 tonnes. Although the production tends to increase annually, shrimp farm productivity in Indonesia is still a big question mark, since this increment could probably caused by the extensification of shrimp land farming. In Indonesia and other countries (Vietnam, India, Ecuador, China, Malaysia, Philippines) most shrimp farming are still cultured traditionally, using external outdoor earthen pond, with less attention paid on diseases and water quality control [4]. It is not surprising that the system cannot maintain an adequate water quality and might lead to disease outbreak. Later, this condition might contribute to unpredictable shrimp yield production [5].

One major water quality parameter that contributes to these conditions is the accumulation of dissolved N-inorganic compound $NH4^+$ (ammonium) [6]. To overcome this unfavorable condition, farmers generally conduct a frequent water renewal. Unfortunately, due to biosecurity and environmental reasons, water renewal is not considered practically. Furthermore, water renewal method is also known to cause unstable water quality [7].

Many efforts had been conducted to develop and improve a new approach in shrimp rearing strategy (e.g.: probiotic, biofloc, RAS). To this point, the results are still unpredictable in terms of production stability. Recently, the use of Zero Water Discharge by using nitrifying bacteria and *Chlorella* has been applied on cultivation of giant freshwater prawn (*Macrobrachium rosenbergii*) [8]. The application of zero water discharge was reported to help increase survival rate by 10-20%. Based on this promising result, the development of zero water discharge system as an alternative zoo technique in super intensive white shrimp culture needs to be evaluated further.

Zero water discharge provides an improvement for batch system and a modification of green water technology, especially in optimizing the nutrient cycle (inorganic and particulate nitrogen). The ammonium, nitrite, and nitrate level can be maintained at acceptable level by adding nitrifying bacteria and microalgae *Chaetoceros calcitrans*. These microorganisms have a positive effect on increasing water quality. Nitrifying bacteria can convert ammonium to nitrite (*Nitrosomonas*)

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and nitrite to nitrate (*Nitrobacter*) [9], while biomass of microalgae has an important role in nitrate reduction and as food supplement for shrimps [10].

## Materials and Method

### Activation and cultivation of nitrifying bacteria *C. calcitrans*

Culture of nitrifying bacteria was obtained from the Laboratory of Microbiology SITH ITB. Nitrifying bacteria was activated in liquid medium of Winogradsky and scaled up to a volume of 100 L as expressed in Figure 1 [8].

Culture of *C. calcitrans* that were used in this study was obtained from the Laboratory of Aquatic Ecosystem Analysis SITH ITB. *C. calcitrans* was activated in Guillard medium and scaled up to a volume of 40 L as illustrated in Figure 2 [11].

### Acclimatization of white shrimp post larvae

White shrimps PL-10 used in this study were obtained from PT. Suri Tani Pemuka Indramayu, West Java and acclimatized at room temperature (25 ± 1°C) at the Laboratory of Aquatic Ecosystem Analysis, School of Life Sciences and Technology, Institut Teknologi Bandung (SITH ITB), Indonesia to reach the PL-15 stadia.

### Conditioning of zero water discharge system

The study was conducted in cylindrical fiber tank filled with 300 L sterilized and filtered seawater at 30 ppt salinity. As illustrated in Figure 3, ZWD system consists of four compartments: (1) microbial component: nitrifying bacteria and microalgae *C. calcitrans* which play a role in nitrogen cycle of the culture, (2) aeration line to provide and maintain dissolved oxygen level and allow culture homogenization,

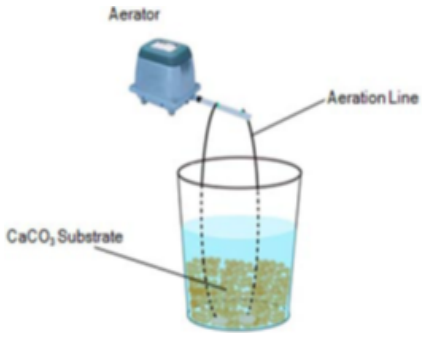


**Figure 1:** The bioreactor used for upscalling the nitrifying bacteria.

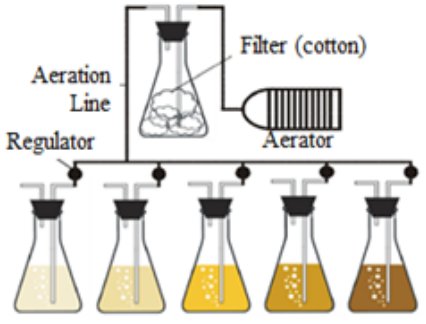


**Figure 2:** Scheme of batch system of *C. calcitrans*.

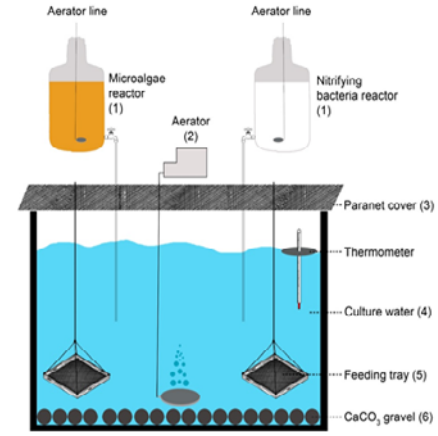


**Figure 3:** Schematic overview of ZWD system for white shrimp culture (*L. vannamei*).

(3) paranet cover to reduce light illumination and enable the system to promote better growth of diatom *C. calcitrans* and nitrifying bacteria, (4) filtered and sterilized culture water as shrimp growth media, (5) feeding tray to control amount of feed delivered daily, and (6) $CaCO_3$ gravel that is used as substrate for nitrifying bacteria colonization and as pH buffering agent.

Before starting the culture, the ZWD system was conditioned by inoculating 10 L of nitrifying bacteria (at $10^6$ CFU/ml) and 12 L of *C. calcitrans* (at $10^6$ cells/ml) into the ZWD systems and total volume is 300 L. The ammonium breakdown capacity of nitrifying bacteria was conditioned by adding 3 g of $NH_4Cl$ (≈ 10 mg/L of ammonium) to the system. The conditioning was performed until the $NH_4^+$ and $NO_2^-$ level decreased to approximately 0 mg/L.

### Treatment implementation

There were two treatments done in triplicate for this research: conventional culture (without any addition of nitrifying bacteria and *C. calcitrans* and 80% of the water volume was renewed once in four weeks) as the control, and ZWD system (with the addition of nitrifying bacteria and *C. calcitrans*). In order to compensate water losses due to siphoning and evaporation, every two weeks six liters (2% of culture volume) new seawater was added into the culture system. After the conditioning, PL-15 shrimp was stocked to the conventional and ZWD culture with stocking density of 120 ind/0.3$m^3$ which refers to the super intensive stocking density (≈400 ind/$m^3$) [12]. Every two weeks, 5 L of nitrifying bacteria and 12 L of microalgae *C. calcitrans* were added at a density of ± $10^6$ CFU/ml and ± $10^6$ cells/ml, respectively, into the ZWD system. The experiment was conducted for 90 days culture period.

### Feeding management

The amount of feed was adjusted weekly based on measurement of mean body weights, estimation of survival rate, and feeding rate (

1). The formula for feeding calculation is :

$$\Sigma \text{ feed (gr)} = SD \times MBW \times FR \times SR$$

where : SD is stocking density (individual/tank), MBW is mean body weight (gr), SR is Survival rate (%), FR is feeding rate (%)

Feed was placed on feeding tray and monitored frequently to

| Mean Body Weight (gr) | Feeding Rate (%) | Survival Rate (%) | Feeding Tray Monitoring Intervals (hours) |
|---|---|---|---|
| <1 | 10.0 | 100 | 3.5 |
| 1-3 | 8.0 | 98 | 3.5 |
| 3-5 | 6.0 | 96 | 2.5 |
| 5–7 | 5.0 | 94 | 2 |
| 7–9 | 4.0 | 92 | 2 |

**Table 1:** Feeding scheme for super intensive of white shrimp culture at 25 ± 1°C.

provide information of daily delivered feed accuracy (Table 1). The size and condition of shrimp can be checked and their consumption rate estimated based on the left-over feed in the tray. The feeding scheme was modified from Tacon [13]. Feed were delivered five times a day at 08:00 am, 11:00 am, 14:00 pm, 17:00 pm and 20:00 pm.

### Physical and chemical water parameters measurement

**Physical parameters:** DO and temperature were measured everyday using a digital meter Hach® 40 qd, and pH level using a pH meter Eutech Instruments. Chemical parameters: ammonium, nitrite, nitrate and orthophosphate were measured twice a week by using Nessler, Diazotation, Nitrate HCl, and Stannous Chloride methods, respectively [14].

### Biological and Microbiological Parameter Measurement

**Biological parameters:** Total weight, mean body weight (MBW), specific growth rate (SGR), survival rate (SR) and feed conversion ratio (FCR) were measured during culture period. The survival rate of the shrimp was calculated by using the following equation [8]:

$$SR=N_t/N_o\times100\%$$

where: SR is survival rate , $N_o$ is initial shrimp number, $N_t$ is final shrimp number, t is culture period (day).

Shrimp specific growth rate was calculated by using the following equation:

$$SGR\ (\%/day)=[\ln (W_2/W_1)/(T_2-T_1)\times100]$$

where: SGR is specific growth rate, $W_1$ is initial body weight (g) at time $T_1$ (day), and $W_2$ is final body weight (g) at time $T_2$ (day)

Microbiological parameters were measured by total bacterial count or total plate count method [15]. Total bacteria was counted every week by plating water sample on nutrient agar (NA) medium, while total *Vibrio* sp. count was conducted every four weeks using thiosulphate citrate bile salt agar (TCBS) medium [16].

## Results and Discussion

### Conditioning of zero water discharge system

During conditioning period, it was observed that nitrifying bacteria was able to convert 10 mg/L of ammonium to 0 mg/L in 5 days (average of breakdown capacity was 2 mg/L per day) as shown in Figure 4. The conversion of ammonium to nitrite can be seen from the increment of nitrite concentration which reached 3 mg/L on day 5 and remained steady until day 15 of culture period (Figure 4). By day 17, the nitrite level had plummeted to nearly 0 mg/L. As a final product of nitrification, nitrate had been accumulated, in which the level was around 60 mg/L by the end of culture period (day 17).

Conditioning process becomes a crucial step prior to shrimp stocking and culture. This step enables ZWD system to convert toxic $NH_4^+$ and $NO_2^-$ into less harmful substances $NO_3^-$. From the result, it can be seen that ammonium oxidizing bacteria was activated on day 5 of conditioning period. However, longer conditioning period of 17 days was taken to activate nitrite oxidizing bacteria. For nitrite oxidizing bacteria, longer activation period is due to slower growth or doubling time of nitrite oxidizing bacteria compared to ammonium oxidizing bacteria [9]. As the second step of nitrification process, $NO_2^-$ oxidation takes place after $NH_4^+$ oxidation reaction. This consecutive reaction also contributes to slower activation process for nitrite oxidizing bacteria since the reaction depends heavily on $NO_2^-$ availability of the first reaction [9].

Even though the nitrification process was able to be achieved during conditioning period, a long conditioning period (17 days) should be considered as a constraint in ZWD system because it will require a longer culture period to run one shrimp production cycle. One alternative solution to overcome this disadvantage is a better activation process of nitrite oxidizing bacteria in stock culture during maintenance, prior to being inoculated into ZWD system.

### Physical and chemical parameters measurement

The range of ammonium, nitrite and nitrate concentrations documented in conventional and ZWD rearing technique are presented in Table 2. Ammonium, nitrite and nitrate concentrations were still in tolerance levels for white shrimp in both rearing strategies [17-19].

The trend for ammonium, nitrite and nitrate concentration during 90 days of culture period is presented in Figure 5. It is noticeable that the ammonium, nitrite and nitrate level tended to slightly increase. In ZWD system, ammonium, nitrite and nitrate levels ranged between 0.07–0.69 mg/L, 0–3.15 mg/L, 1.04–42.9 mg/L, respectively, while in conventional (batch) system, it ranged between 0.20–0.59 mg/L, 0–3.2 mg/L, 1.38–14.17 mg/L, respectively. Based on these levels, all dissolved N-inorganic levels measured during 90 days culture period were not significantly different in both rearing systems ($p>0.05$). In spite of this condition, the breakdown capacity of ZWD system on $NH_4^+$ and $NO_2^-$ levels were higher compared to conventional (batch) system. This is because a higher amount of feed (≈ 1178.28 g) was delivered to ZWD system; around 44% higher than that of conventional system (≈ 656.15 g). This contributed to a higher organic matter accumulation and

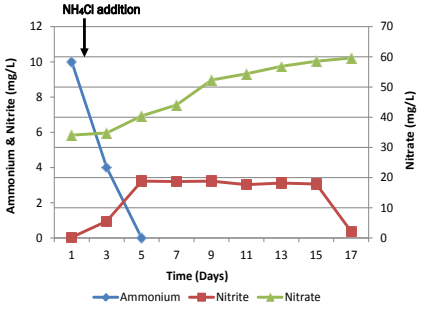


**Figure 4:** Performance of nitrifying bacteria based on ammonium, nitrite and nitrate concentration in ZWD conditioning.

| Parameter | Conventional | ZWD | Limit |
|---|---|---|---|
| Ammonium (mg/L) | 0.20-0.59 | 0.07-0.69 | ≤ 3.95 |
| Nitrite (mg/L) | 0-3.2 | 0-3.15 | ≤ 25.7 |
| Nitrate (mg/L) | 1.38-14.17 | 1.04-42.9 | ≤ 232 |

**Table 2:** Range of ammonium, nitrite and nitrate concentration in conventional and ZWD for 90 days culture period.

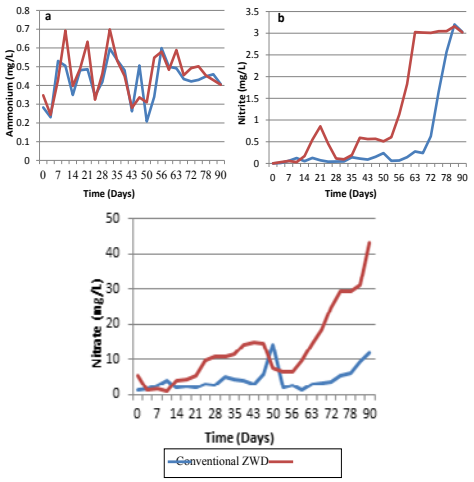


**Figure 5:** (a) Ammonium, (b) nitrite and (c) nitrate levels on conventional and ZWD treatment during 90 days of culture period.

therefore had an effect on higher ammonium ($NH_4^+$) accumulation in the system [20]. Based on [21] estimation, it was assumed that the amount of feed given to shrimp in ZWD for 90 days could produce a total $NH_4^+$ of 55.20 mg/L in shrimp culture which is equivalent to 0.61 mg/L per day. In contrast, total ammonia produced by shrimp for 90 days in conventional system was 30.73 mg/L, which is equivalent to 0.34 mg/L per day (total of 656.15 g feed delivery). From this estimation, it can be stated that the ammonium breakdown capacity with average ammonium conversion rate of 2 mg/L/day was higher in ZWD system than that of conventional culture system.

Better ammonium and nitrite conversion rate were determined by measuring higher nitrate accumulation after 90 days culture period (Figure 5c). At the end of culture period, nitrate level of 42.9 mg/L at ZWD system was significantly higher *($p<0.05$)* compared to conventional system (14.17 mg/L). This level of final nitrification product indicates that ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) performs well in the system. They contributed in maintaining a stable and low toxic $NH_4^+$ and $NO_2^-$ level in culture water, despite the feed delivery was approximately 180% higher in ZWD system.

Besides shrimp productivity, the accumulation of $NH_4^+$ and $NO_2^-$ levels in ZWD should be taken as a serious consideration, since the presence of these toxic substances increases the amount of feed needed for better growth and to avoid shrimp's system failure. Based on this fact, an adequate system conditioning process (Figure 3) becomes a critical step prior to system operation. This conditioning will provide us with certainty on ZWD performance, $NH_4^+$ and $NO_2^-$ breakdown capacity in particular.

Relatively low $NH_4^+$ and $NO_2^-$ levels also probably had an effect on marine diatom *C. calcitrans* inclusion of ZWD system. *C. calcitrans* can uptake both $NO_3^-$ and $NH_4^+$ as N source during assimilation process, thus, the level of $NH_4^+$ and $NO_2^-$ can be maintained at an acceptable level. Keeping the ammonium, nitrite and nitrate in a low and stable level is important for shrimp cultivation. An excessive amount of ammonium might reduce growth, increase oxygen consumption and ammonia-N excretion, alter concentration of hemolymph protein and free amino acid levels, and even cause mortality [22], whereas a high level of nitrite could induce methemoglobin formation; which might result in hypoxia and cyanosis [23] and later contribute to retardation of shrimp growth and death [24].

Predicted microbial loop during 90 days culture period can be described as in Figure 6. In general, nutrient cycle processes that are involved in ZWD systems include nitrogen and carbon cycle. It can be seen that excessive feed input will not only contribute to shrimp growth, but also to the accumulation of organic matter in water, together with shrimp excreted metabolism [25]. The organic matter will then be utilized by heterotrophic bacteria and converted into $NH_4^+$ through ammonification process. Furthermore, $NH_4^+$ ions will be oxidized to form toxic substance $NO_2^-$ by chemolithotroph ammonium oxidizing bacteria and $NO_2^-$ will be converted into less toxic substance $NO_3^-$ by chemolithotroph nitrite oxidizing bacteria, respectively [26]. Finally, $NO_3^-$ as one of macronutrients will be utilized through assimilation process by photoautotrophic organism, microalgae *C. calcitrans*, which serves as shrimp live feed and plays a role in increasing $O_2$ level in water [27-28]. Microalgae biomass also contributes to organic matter increment in water, together with chemolithotrophic and heterotrophic bacteria.

Among three microbial components that have a role in ZWD microbial loop, only two (photoautotrophic and chemolithotrophic bacteria) were applied in this ZWD system, while the heterotrophic component still has not been used yet. However, for further development of the ZWD system, application of functional heterotrophic component will be taken as crucial step. Hopefully, the use of the three components altogether could help stabilizing and lowering the microbial diversity, as well as enhancing the ammonification, nitrification, and assimilation processes in culture water.

In both systems, other physicochemical parameters (i.e. pH, temperature and salinity) range between 7.63-8.8, 25.96-30.63°C and 27.6-38.3 ppt, respectively. These figures are still in tolerance range for white shrimp culture [29-31].

Appropriate culture ZWD condition will create a better environment and growth for the shrimp, even though it might contribute to the decrement of dissolved oxygen budget due to different shrimp survival (90.82% compared to 27.22%) and higher organic input. However, in this system, the DO level decrement was less than 1 mg/L (7.42 ± 0.52 mg/L in conventional and 6.81 ± 0.5 mg/L in ZWD) after 90 days culture period. Despite there was no significant effect of DO level decrement, this result should be taken as a serious consideration in further application and development of ZWD system, especially if the culture process is going to be conducted for a duration of more than 3 months (4 months of normal culture period), in which the shrimp density and the accumulation of organic matter might

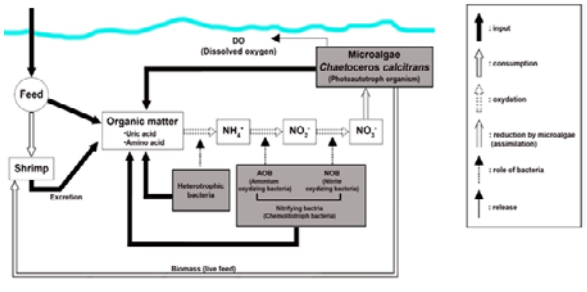


**Figure 6:** Predicted microbial loop of ZWD during 90 days culture period.

reduce the oxygen budget of the system. From the result, it seemed that the dissolved oxygen budget could be covered by oxygen production of microalgae and constant aeration in ZWD system [32].

## Microbiological parameter measurement

Total bacteria counted in conventional and ZWD for 12 weeks culture period is given in Figure 7.

Based on the results, total bacteria count during the trial tended to increase in both rearing system. In ZWD, maximum number of Total Bacterial Count and total *Vibrio* sp. were $1 \times 10^{10}$ CFU/ml and $4.8 \times 10^{2}$ CFU/ml, respectively, while in conventional system the figures stood at $6.7 \times 10^{10}$ CFU/ml and $1.05 \times 10^{2}$ CFU/ml, respectively Table 3. There were no significant differences on Total Bacterial Count and total *Vibrio* sp. obtained during culture period between ZWD and conventional culture system *(p>0.05)*.

It is noticeable that excessive organic matter accumulation in ZWD system did not significantly increase the number of bacteria, despite it has been widely known that the availability of higher organic content could promote the growth of heterotrophic bacteria [33]. The presence of nitrifying bacteria and *C. calcitrans* was well documented in controlling the growth of opportunistic bacteria and indigent *Vibrio* sp. in shrimp culture. Nitrifying bacteria can compete with other bacteria in terms of space and oxygen [34] and marine diatom secrete fatty acids and esters that can act as antibacterial compounds [35]. It is not surprising that the total *Vibrio* sp. in ZWD system was below pathogenicity level of $10^{6}$ CFU/ml [36], even when shrimp culture in ZWD contained higher shrimp density and higher input of feed.

A low total number of bacteria in conventional system are suspected due to regular dilution by water renewal (80 % every four weeks) and also due to less organic matter load of feed and biological wastes (less shrimp density) accumulated during the culture period.

## Biological parameter measurement

Shrimp culture productivity was observed based on mean body weight (MBW), total weight, survival rate (SR), specific growth rate (SGR) and feed conversion ratio (FCR) measurement Table 4.

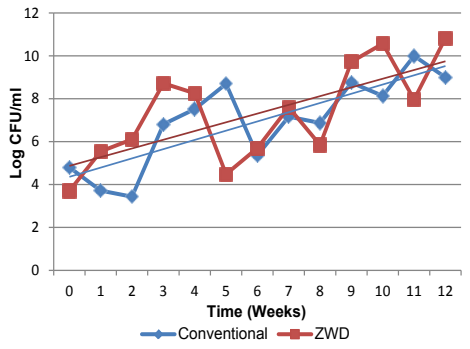


**Figure 7:** Amount of total bacteria in conventional and ZWD for 90 days culture period.

| Treatment | Total Bacteria (CFU/ml) | | Total *Vibrio* sp. (CFU/ml) | |
|---|---|---|---|---|
| | Min. | Max. | Min. | Max. |
| Conventional | $2.7 \times 10^{3}$ | $1 \times 10^{10}$ | $<10^{1}$ | $4.8 \times 10^{2}$ |
| ZWD | $5.05 \times 10^{3}$ | $6.7 \times 10^{10}$ | $<10^{1}$ | $1.05 \times 10^{2}$ |

**Table 3:** Minimum and maximum number of total bacteria and total *Vibrio* sp in conventional and ZWD for 90 days culture period.

| Variable | Conventional | ZWD |
|---|---|---|
| MBW (g) | 5.45 ± 0.28 | 8.24 ± 0.84* |
| SR (%) | 27.22 ± 2.09 | 90.82 ± 2.5* |
| Total Weight (g) | 160.48 ± 6.62 | 923.38 ± 42.15* |
| SGR (%) | 7.24 ± 0.05 | 7.7 ± 0.11* |
| FCR | 4.10 ± 0.66 | 1.27 ± 0.29* |

*Significant difference p<0.05 (α=0.05)

**Table 4:** Biological parameters measured in conventional and ZWD during 90 days of culture period.

| Parameters | ZWD | Conventional |
|---|---|---|
| SR (%) | 90.82 | 27.22 |
| MBW (g) | 8.24 | 5.45 |
| Stocking Density | 12.136 ind/30m³ | 18,348 ind/30m³ |
| Water Consumption (m³) | 52.1 | 78 |
| FCR | 1.27 | 4.10 |
| Total Feed (kg) *(FCR x Total Biomass)* | 127 | 410 |
| Feed Cost (IDR) *(Total Feed x 15,000 IDR/kg)* | 1,905,000 | 6,150,000 |
| Seed Cost (IDR) *(Stocking Density x 35 IDR/Larvae)* | 424,760 | 642,180 |
| Nitrifying Bacteria Culture Cost (IDR) | 30,000 | - |
| Algae Culture Cost (IDR) | 25,000 | - |
| Maintenance Cost (IDR) *(10% of operational cost)* | 240,000 | 200,000 |
| Total Cost (IDR) | 1,624,760 | 6,992,180 |
| *(Feed Cost+Seed Cost+Nitrifying Bacteria Culture Cost+Algae Culture Cost+Maintenance Cost)* | | |
| Total Income (IDR) *(Total Biomass x 50,000 IDR/kg)* | 5,000,000 | 5,000,000 |
| Revenue (IDR) *(Total Income-Total Cost)* | 2,375,240 | -1,992,180 |

*maintenance cost in ZWD system is included algal and nitrifying bacteria culture

**Tabel 5:** Cost and profit estimation to produce 100 kg shrimp using ZWD and conventional batch system.

In ZWD system, better shrimp culture performance was obtained after 90 days culture period. Measurements of biological parameters, including mean body weight (MBW) of 8.24 ± 0.84 g, total weight of 923.38 ± 42.15 g, Survival Rate (SR) of 90.82 ± 2.5%, and specific growth rate (SGR) of 7.7 ± 0.11%, were significantly higher in ZWD than those of conventional culture system (MBW: 5.45 ± 0.28 g, Total Weight: 160.48 ± 6.62 g, SR: 27.22 ± 2.09%, and SGR 7.24 ± 0.05). The FCR in ZWD (1.27) were significantly lower than that of conventional culture system (4.10) *(p<0.05)*. It is of note that the value of two critical culture parameters, SR and FCR, were still in tolerance range of shrimp culture, in which the SR and FCR are expected to be between 51 to 91% and 1.5 to 2.6, respectively [13]. Better culture performance obtained in ZWD culture system was due to better and stable water quality maintained during culture period, even though more feed amount was delivered to support shrimp growth at higher shrimp density.

Beside the advantages of nitrifying bacteria application on water quality ($NH_4$+ and $NO_2^-$), the use of microbial component, marine diatom *C. calcitrans,* has also contributed in creating a favorable condition by providing shading effect and alternative feed for shrimp [37]. Both of these factors have a significant role in reducing cannibalism due to less water visibility (minimize predation during moulting) and as a supplement diet for shrimp.

Besides the information on physico-chemical, microbiological and biological parameters, a cost estimation of shrimp production using both systems also become an important step that needs to be conducted before further application of this new culture approach in aquaculture industry. Estimation was calculated in assumption to produce 100 kg shrimp during 90 days culture period, based on shrimp culture performance (total weight, FCR, MBW, SR) obtained from this research, seed cost, feed cost and water consumption as shown in Table 5.

The calculation was made based on biological parameters obtained during 90 days culture period. Better shrimp growth and survival implied in better and lower FCR in ZWD which resulted in profit by 2,375,240 IDR. Based on this analysis, the use of zero water discharge system for super intensive shrimp rearing technology can improve the profit of the culture compared to the conventional batch system with the same initial shrimp density. This economic advantage was due to better culture performance on SR, MBW, and FCR measured in ZWD system. During culture period, the use of ZWD system also consumed less water volume (52.1 $m^3$) compared to conventional batch system (78 $m^3$) to produce 100 kg of shrimps as shown in Table 4. This condition shows that the use of ZWD system will not only contribute in sustaining a more cost effective system, but also in minimizing water quality disturbance and reducing wastewater release into the environment.

## Conclusion

Based on this research, Zero Water Discharge system can be used as an alternative for shrimp culture since ZWD was able to enhance white shrimp culture performance (water quality, SR, growth and FCR).

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