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Acknowledgement Letter # 358/18

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ACKNOWLEDGEMENT

Reg. No: 358/18 Dated : 16/10/2018

Dear Dr. Gunanti Mahasri,

We acknowledge the receipt of the following articles entitled “The Development of Nanobubble Technology toward Dissolved Oxygen and Presumptive Vibrio Count on the Pacific White Shrimp (Litopenaeus vannamei) Cultivation System.” (Gunanti Mahasri, et al.).

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We hope the article will be published soon.

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Dr. Gunanti Mahasri

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Dr S. Sukumar
Editor, IVJ

Here we attach the manuscript revision of #358/18.
We hope this article will be published immediately.

Thank you very much for your attention and kind consideration.

Best regards

Dr. Gunanti Mahasri

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THE INDIAN VETERINARY JOURNAL

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Oxygen Dissolved Nanobubble Technology Improved the Quality of Pacific White Shrimp Cultivation

The Development of Nanobubble Technology toward Dissolved Oxygen and Presumptive Vibrio Count on the Pacific White Shrimp (*Litopenaeus vannamei*) Cultivation System

Gunanti Mahasri[1*], Sudarno[1] and Ade Irmalia Harifa[2]

[1]Department of Fish and Aquaculture Health Management, Faculty of Fisheries and Marine Universitas Airlangga, Surabaya 60115

[2]Aquaculture, Faculty of Fisheries and Marine Universitas Airlangga, Surabaya 60115

*E-mail: Mahasritot@gmail.com

Abstract

The Pacific White Shrimp (*L. vannamei*) cultivation system using high stocking density gives an adverse impact on the decline in water quality, so the risk of infection increases. One of the causative agents that often infects is *Vibrio* sp. The development of nanobubble technology is expected to improve the quality of the cultivation system by maintaining the balance between the environment, shrimp and pathogens. This study aims to determine the effect of nanobubble technology development on dissolved oxygen and Presumptive Vibrio Count (PVC) in Pacific White Shrimp (*L. vannamei*) cultivation systems. This research was experimental using a Completely Randomized Design (CRD) factorial pattern. Consisting of two factors, namely factor A is the cultivating shrimp with nanobubble and aerator. The B factor is cultivating period which is 0 days, 10 days, 20 days and 30 days. Data analysis used ANAVA (Analysis Of Variance) and continued with Duncan's Multiple Range test. The results of the analysis showed that the use of nanobubble had a significant effect ($p < 0.05$) on dissolved oxygen and PVC. The highest value of dissolved oxygen in Pacific White Shrimp cultivating media during the study was nanobubble treatment on 0 day, which was 11.06 ppm, higher than aerator treatment on 0 day, which was 3.4 ppm. The highest value of PVC *Vibrio* sp. the medium for maintenance of Pacific White Shrimp during the study was aerator treatment on the 30th day, which was 2.88×10^3 (CFU / mL), higher than the nanobubble treatment on the 20th day which was 1.2×10^3 (CFU / mL). The highest value of PVC *Vibrio* sp. in Pacific White Shrimp hepatopancreas during the study was in the aerator treatment 30th day, namely 3.7×10^3 (CFU / g); higher than the nanobubble treatment on 30th day which was 2.53×10^3 (CFU / g). The use of nanobubble technology provides stable and optimal dissolved oxygen for Pacific White Shrimp cultivation and lower PVC value of *Vibrio* sp. in the cultivation system compared to aerators treatment.

Keywords: Nanobubble, Dissolved Oxygen, Presumptive Vibrio Count, Pacific White Shrimp

Corr. author email ID :→

INTRODUCTION

Pacific White Shrimp (*Litopenaeus vannamei*) is one of the leading fisheries products in the fisheries sector (Kaligis, 2015). Intensive and supraintensive Pacific White Shrimp (*L. vannamei*) cultivation systems that use high stocking density provide a high increase in production (Apandi *et al.*, 2016). However, high stocking density can also have an adverse impact on the occurrence of a decrease in water quality. Decreasing water quality in intensive cultivation systems can occur due to high stocking density, leftover feed and feces that accumulate as organic material and poor water quality management. Poor water quality can cause stress on cultivated shrimp, so that the growth of shrimp will be disrupted and reduce the body's resistance to infection and cause death (Diansari *et al.*, 2013). One of the causative agents that often attack shrimp culture is bacteria, especially *Vibrio* sp.

Vibrio sp. is a bacterium that causes vibriosis which is a factor of failure in cultivating shrimp. Infection of *Vibrio* genus bacteria can spread rapidly in intensive cultivation and mortality can reach 100% in aquaculture ponds (Austin and Austin, 2007). According to Juarno *et al.* (2011), shrimp production in Lampung and East Java in 2009 was only 336 thousand tons from the target of 540 thousand tons, deflating from the yields in 2008 of 409.6 thousand tons due to disease attacks. In addition, based on data from the Shrimp Club Indonesia (SCI), the existence of vibriosis outbreaks resulted in SCI intensive shrimp production in which expected to fall by around 30% in the first half of 2015 (Sutanto, 2015).

Vibrio sp. bacterial infection on the shrimp not only caused losses due to high mortality, but also caused serious problems which led to the rejection of the export of shrimp products. In 2005, 2007, and 2009, there were several cases of refusal of exported Pacific White Shrimp by the European Union due to contamination of *V. parahaemolyticus* from frozen shrimp products and *ebi sushi*. Then in 2009 and 2010, there were rejections of Indonesian fish exports by the Chinese for the same reason. The refusal of exported Indonesian fishery products by Taiwan also occured due to the presence of pathogenic *V. parahaemolyticus* (Kusmarwati *et al.*, 2016).

The high loss due to *Vibrio* sp. bacterial infection on Pacific White Shrimp causes the need for better application on cultivation technology. Solubility of oxygen in aquaculture waters is one of the most important parameters in shrimp life because it is related to the respiratory and metabolic systems (Apandi *et al.*, 2016). One of the technologies developed to improve the quality of cultivation is using nanobubble technology. Dissolved oxygen in cultivation with nanobubble systems can be available in a longer time so as to maintain the dissolved oxygen in the waters remains stable (Chiba and Takahashi, 2007).

Oxygen in the nanobubble form will capture solids (pollutants) suspended in liquid and float to the surface. These suspended solids are not similar in size or shape; large bubbles fail to bind solids, but nano bubbles are able to penetrate small cavities in contaminants so that they can wrap up solids and lift them up. Oxygen in water is also needed to decompose organic matter that accumulates in water so there is no increase in ammonia levels which gives a threat to aquatic animals (Apandi *et al.*, 2016).

Characteristics of *Vibrio* sp. is an opportunistic pathogen, meaning that a normal-state organism in a cultivating environment which develops into a pathogen if its environmental conditions and its host deteriorate (Utami *et al.*, 2016). *Vibrio* sp. will dominate due to the utilization of organic material that accumulates. Supito *et al.* (2008) suggested that the dominance and abundance of unstable *Vibrio* sp. in the fishponds indicate risky conditions for shrimp health problems. According to Subaidah *et al.* (2006), mass death usually occurs along with an increase in the population of *Vibrio* sp. bacteria above 10^3 CFU/mL in waters. Leano *et al.* (1998) stated that when the concentration of *Vibrio* sp.on the hepatopancreas has reached a density of 9.0×10^4 CFU/g is enough to make *Vibrio* bacteria become pathogenic. According to Wang *et al.* (2005), the high PVC can be the cause of the decline in shrimp production.

Based on this background, the purpose of this study was to determine the effect of nanobubble technology development on dissolved oxygen and Presumptive Vibrio Count (PVC) on Pacific White Shrimp cultivation systems (*Litopenaeus vannamei*).

METHODOLOGY

Materials and Methods

Tools and materials

The materials used in this study were 240 Pacific White Shrimps sized 7-10 cm with a weight of 4-6 g, fishpond water with salinity of 18-20 ppt, chlorine, nutrient agar media (Merck, Germany), Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar media (Merck, Germany), 0.9% physiological NaCl, powder NaCl, distilled water, 70% alcohol. Other research materials are materials for measuring ammonia levels consisting of distilled water, ammonium chloride (NH_4Cl), phenol solution (C_6H_5OH), sodium nitropruside (C_6FeNa_6O), alkaline citrate solution ($C_6H_5Na_3O_7$), and 5% sodium hypochlorite (NaClO). Material for measuring nitrite consists of a solution of sulfanilamide and a solution of NED Dihydrochloride. The equipment used in this research was 8 pieces of cultivating tub sized 51.5 cm x 38.5 cm x 345 cm, nanobubble generator, pipe, reservoir, filter tub, bioball filter, aerator stone, aeration pump, aerator hose, basin, microtube, hand counter , microscope, DO meter, spectrophotometer, pH meter, thermometer, refractometer, 25 mL measuring cup and 500 mL, oven, 10 mL drop pipette, 2 mL and 10 mL of volumetric pipette, 1 piece of jerry can 10, autoclave (Hirayama, Japan), analytical balance (Ohaus Scout-Pro), cotton, water funnel, 300 mL erlenmeyer, gauze, aluminum foil, binocular microscope, heater and magnetic stirrer (AM4 VUP Scientifica), petri dishes, mortar and stamper, ose loop, vortex (VM-1000), 100 mL glass beaker, tray, sectional set, bunsen, test tube rack, 10 mL test tube, laminar air flow, incubator, 10-100 µL micropipette, 100 µL microtube, microtip, turbidimeter and filter paper.

Research Methods and Design

This research was conducted in the Laboratory of Education of the Faculty of Fisheries and Marine of Universitas Airlangga in August - November 2017. The research

method used in this study was an experimental method. The research design used was a Completely Randomized Design (CRD) factorial pattern. Consisting of two factors, namely factor A is the shrimp is cultivated with nanobubble and aerator. Whereas factor B is cultivated period which is 0 days, 10 days, 20 days and 30 days. Series of preparation are as follows: prepare the 8 pieces of cultivating containers, 1 tendon tub and 1 filter tub. Sterilize the container, prepare the nanobubble generator and aerator. Then, fill the research container with brackish water of 20 liters each. The production of the circuit includes determining the position of the container with the reservoir and the installation of a nanobubble device connected to the treatment circuit. Nanobubble system optimization is conducted to balance the water circulation carried out in the series that has been made. Besides, preliminary research is carried out to obtain the best oxygen content in the cultivation media. Nanobubble generator has a flowrate of 2-16 L / minute while the aerator used is 4 L / minute. Optimization used in this study was for 1 hour in the morning at 06.00 Western Indonesian Time and in the afternoon at 16.00 Western Indonesian Time.

Measurement of Water Quality Parameters (Dissolved Oxygen, Temperature, pH, Nitrite and Ammonia)

Measurement of temperature and dissolved oxygen using DO meter tools and pH using pH meters were carried out every day at 07.00 Western Indonesian Time because at that time there was accumulation of organic matter. Ammonia content measurements were carried out by spectrophotometric method in accordance with SNI 06-6989.30-2005. Ammonia measurement begins by inserting 25 mL of sample water into a 100 mL erlenmeyer. Then add 1 mL of phenol solution, 1 mL of sodium nitropruside and 2.5 mL of oxidizing solution. The addition of all three solutions wasconducted sequentially and must be homogenized. After that, it was incubated at room temperature for one hour. It aims at color formation. After incubation for one hour, then put it in the cuvette on a spectrophotometer and read the absorbance value at a wavelength of 640 nm. Measurement of nitrite content by spectrophotometric method in accordance with SNI 06-6989.9-2004. Nitrite measurement begins by inserting 50 mL of sample water into a 100 mL erlenmeyer. Then, a solution of 1 mL of sulfanilamide acid was added and homogenized and allowed to stand for 8 minutes. Then,1 mL of NED solution was added and homogenized and allowed to stand for 10 minutes. Furthermore, value of nitrite was directly measuredwith a spectrophotometer by looking at the absorbance value at a wavelength of 543 nm.

Preparation of Bacterial Cultured Media

Petri dishes and test tubes used for bacterial culture were sterilized using autoclave, including agar media used for bacterial culture were also sterilized. The process of making a culture medium requires agar powder (Nutrient agar, TCBS agar), so that powder weighing is carried out to match the dosage indicated on the product'spackage and dissolve with distilled water. The appropriate dosage weighing aims to fulfill the media with the right nutrition without any excess or deficiency which can be used in growing bacteria. This is because each media has a composition that has been adapted to the needs of bacteria to grow. Agar Nutrient Media (Merck, Germany) consists of 5.0 tons of meat; artificial extract 3.0 and agar

12.0 (in g / L). Media TCBS agar (Merck, Germany) consists of peptone (casein) 5.0; pepton (meat) 5.0; yeast 5.0; sodium citrate 10.0; sodium thiosulphate 10.0; ox bile 5.0; iron (III) citrate 1.0; thymol blue 0.04; bromthymol blue 0.04 and agar 14.0 (in g / L). The media is heated to dissolve and sterilized using autoclave temperature of 121°C for 15 minutes (except TCBS media so that it is not autoclaved). After the container and media had been sterilized, pour the media into a container of petri dishes and store for 24 hours for the media sterility test. The media that passes the sterility test can be used for bacterial culture (Kusdarwati *et al.*, 2016).

Sampling

The sample used consisted of cultivation media water and Pacific White Shrimp (*L. vannamei*) which were taken from the cultivation tub. Water samples were taken as much as 1 mL which were then dissolved in 9 mL physiological NaCl. Samples of Pacific White Shrimp (*L. vannamei*) were dissected aseptically for their hepatopancreas to be taken and weighed up to 1 g. The hepatopancreas is mashed using mortar and pestle and then dissolved in 9 mL physiological NaCl (Kusdarwati *et al.*, ~~2016).~~

Measuring PVC *Vibrio* sp.

Measurement of Presumptive Vibrio Count (PVC) is a calculation method to determine the number of *Vibrio* bacteria in the sample. In the calculation of PVC, there is a limit of detection (LOD) or dilution limit used. For crustaceans and fish samples the LOD value used is 100 CFU / g (FAO and WHO, 2016). The calculation method was done aseptically on laminar flow, i.e. the samples that had been dissolved in sterile physiological NaCl were taken 100 µL and diluted with a multilevel (dilution 10^{-1}, 10^{-2} and 10^{-3}) using the duplo spread plate method on TCBS agar media. The next step was to incubate the incubator for 18-24 hours at room temperature (28-30 °C) (Letchumanan *et al.*, 2015). After that, the colony that grew on TCBS media was measured and its value was based on SNI 01-2332.3-2006.

Data Analysis

Data analysis of dissolved oxygen and Presumptive Vibrio Count (PVC) in this study used ANAVA (Analysis of Variance) to determine the difference between the treatment given and was further analysed by Duncan's Multiple Range Test (Duncan Multiple Range Test) (Kusriningrum, 2008).

RESULTS AND DISCUSSION

The results of measurements of dissolved oxygen content in the cultivation media of Pacific White Shrimp culture (*L. vannamei*) can be seen in Table I. Statistically, the dissolved oxygen in the cultivation media of Pacific White Shrimp *(L. vannamei*) showed significant differences between nanobubble treatments with aerators ($p < 0.05$). The highest value of dissolved oxygen in the cultivation media of Pacific White Shrimp (*L. vannamei*) during the

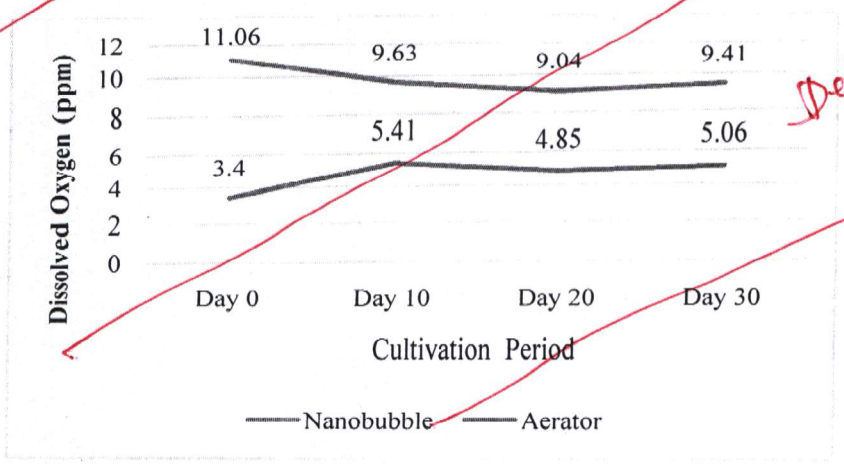
study was nanobubble treatment on 0 day (A1B1) which was 11.06 ppm, higher than the aerator treatment on 0 day (A1B1) which was 3.4 ppm .

Table I. Dissolved Oxygen on the culture media of Pacific White Shrimp (*L. vannamei*) (ppm).

Treatments		Dissolved Oxygen ± SD
Factor A	Factor B	
A1	B1	$11.06^{e} \pm 0.72$
	B2	$9.63^{d} \pm 0.24$
	B3	$9.04^{cd} \pm 0.21$
	B4	$9.41^{c} \pm 0.36$
A2	B1	$3.4^{a} \pm 0.30$
	B2	$5.41^{b} \pm 0.22$
	B3	$4.85^{b} \pm 0.42$
	B4	$5.06^{b} \pm 0.26$

Description: The Notation indicated by superscript letter in the same column shows the comparison between treatments has a significant difference ($P < 0.05$); Factor A: A1: Nanobubble; A2: Aerator; Factor B: B1: 0 day; B2: 10th day; B3: 20th day; B4: 30th day.

Figure 1. Relationships between cultivation period with the dissolved oxygen on the culture media of Pacific White Shrimp (*L. vannamei*)



The graph above showed the dissolved oxygen content in the cultivation media of Pacific White Shrimp (*L. vannamei*) cultivation which was maintained for 30 days using nanobubble and aerator. In the graph, it can be seen that the dissolved oxygen content in the cultivation media of Pacific White Shrimp (*L. vannamei*) cultivation using nanobubble is higher compared to using aerators. The use of nanobubble in the cultivation media of Pacific White Shrimp culture (*L. vannamei*) shows a more stable decrease and it becomes increasing in the end of observation. Meanwhile, the use of aerators shows the unstable changes. The highest dissolved oxygen in the cultivation media of Pacific White Shrimp (*L. vannamei*) cultivation during the study was nanobubble treatment on 0 day that was 11.06 ppm, higher than aerator treatment on the same day which was 3.4 ppm. The dissolved oxygen in the

cultivation media with 0 day to 20th day nanobubble treatment showed a stable decrease and an increase at the end of observation (30th day) was 9.41 ppm. The dissolved oxygen on 20th day was the lowest dissolved oxygen in nanobubble treatment which was 9.04 ppm. The dissolved oxygen content in the maintenance medium in the aerator treatment experienced a fluctuating graph, where there was an increase on the 10th day of 5.4 ppm and a decrease on the 20th day of 4.85 ppm. However, on the 30th day there was a slight increase of 5.06 ppm (Figure 1).

The results of *Vibrio* sp. Presumptive Vibrio Count (PVC) calculation in the cultivation mediaof Pacific White Shrimp (*L. vannamei*) can be seen in Table II. In statistical analysis PVC bacteria *Vibrio* sp. in the cultivation mediaof Pacific White Shrimp (*L. vannamei*) showed a non significant difference between nanobubble treatment with aerator ($p > 0.05$). The highest value of PVC *Vibrio* sp. in the media maintenance of Pacific White Shrimp (*L. vannamei*) during the study was aerator treatment on the 30th day (A2B4) which was 2.88×10^3 (CFU / mL), higher than the nanobubble treatment on the 20th day (A1B3) namely 1.2×10^3 (CFU / mL).

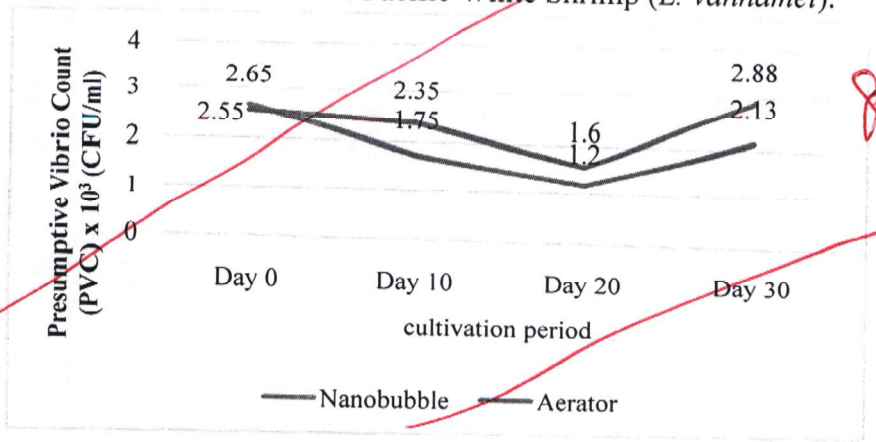
Table II. Presumptive Vibrio Count (PVC) of *Vibrio* sp. bacteria on the cultivation media of Pacific White Shrimp (*L. vannamei*) (Mean ± SD)

Treatments		PVC ± SD
Factor A	Factor B	
A1	B1	$2.65^{c} \pm 0.04$
	B2	$1.75^{abc} \pm 0.13$
	B3	$1.2^{a} \pm 0.31$
	B4	$2.13^{bc} \pm 0.08$
A2	B1	$2.55^{bc} \pm 0.04$
	B2	$2.35^{bc} \pm 0.08$
	B3	$1.6^{ab} \pm 0.22$
	B4	$2.88^{c} \pm 0.11$

Description: The Notation indicated by superscript letter in the same column shows the comparison between treatments has a non significant difference ($P > 0.05$);
Factor A: A1: Nanobubble; A2: Aerator; Factor B: B1: 0 day; B2: 10th day; B3: 20th day; B4: 30th day.

~~Superscript is bearing~~ means bearing different superscripts in a column differ significantly

Count (PVC) in the cultivation media of Pacific White Shrimp (*L. vannamei*).



The graph above showed PVC *Vibrio* sp. in the cultivation media of Pacific White Shrimp (*L. vannamei)* which was maintained for 30 days using nanobubble and aerator. In the graph shows that PVC *Vibrio* sp. bacteria. in the cultivation media of Pacific White Shrimp (*L. vannamei*) using nanobubble is lower when compared to using aerators. The use of nanobubble and aerator in Pacific White Shrimp (*L. vannamei*) cultivation both showed the trend of PVC values of *Vibrio* sp.which is less stable. This showed that there is a fairly high increase in the PVC of *Vibrio* sp. at the end of Pacific White Shrimp (*L. vannamei*) cultivation, which previously experienced a downward trend. The highest value of PVC *Vibrio* sp. in the cultivation media of Pacific White Shrimp (*L. vannamei*) during the study was aerator treatment on 30th day which was 2.8 x 10^3 (CFU / mL), higher than the nanobubble treatment on the same day which was 2.13 x 10^3 (CFU / mL). PVC value of *Vibrio* sp. in the aerator and nanobubble treatment 0 day to 20th day showed a stable decline. The 20th day was the lowest value of PVC *Vibrio* sp., specifically the aerator treatment was 1.6 x 10^3 (CFU / mL) and the nanobubble treatment was 1.2 x 10^3 (CFU / mL). On the other hand, both treatments showed an increase in the 30th day (Figure 2).

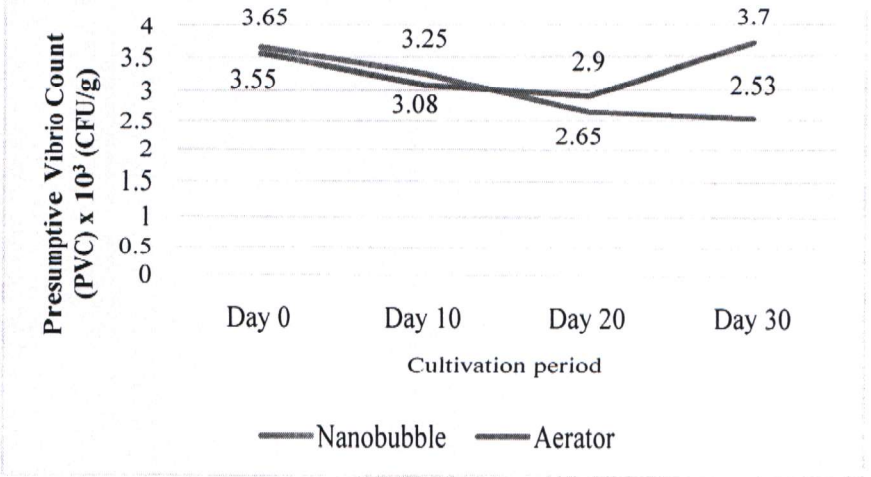
In statistical analysis, PVC *Vibrio* sp.in Pacific White Shrimp (*L. vannamei*) hepatopancreas showed significant differences between nanobubble treatment with aerator ($p < 0.05$). The highest value of PVC *Vibrio* sp. in hepatopancreas of Pacific White Shrimp (*L. vannamei*) during the study was aerator treatment on the 30th day (A2B4) which was 3.7 x 10^3 (CFU / g), higher than the nanobubble treatment on the 30th day (A1B4) which was 2.53 x 10^3 (CFU / g) (Table III).

A2: Aerator; Factor B: B1: 0 day; B2: 10th day; B3: 20th day; B4: 30th day.

A2	B2	$3.08^{d} \pm 0.05$
	B3	$2.9^{bc} \pm 0.02$
	B4	$3.7^{e} \pm 0.03$

Description: The Notation indicated by superscript letter in the same column shows the comparison between treatments has a significant difference ($P < 0.05$); Factor A: A1: Nanobubble; A2: Aerator; Factor B: B1: 0 day; B2: 10th day; B3: 20th day; B4: 30th day.

Figure 3. The relationship between cultivation period and *Vibrio* sp. of Presumptive Vibrio Count (PVC) in Pacific White Shrimps'hepatopancreas (*L. vannamei*) x 10^3 (CFU / g).



The graph above showed PVC *Vibrio* sp. in Pacific White Shrimp (*L. vannamei*) hepatopancreas which was cultivated for 30 days using nanobubble and aerator. The graph showed that PVC *Vibrio* sp. in hepatopancreas of Pacific White Shrimp (*L. vannamei*) using nanobubble is lower than using aerators. The use of nanobubble in Pacific White Shrimp (*L. vannamei*) cultivation showed the trend of PVC *Vibrio* sp. which tends to experience a stable decline, while cultivation using aerator showed a less stable trend. The highest PVC *Vibrio* sp. in the hepatopancreas of Pacific White Shrimp (*L. vannamei*) during the study was in aerator treatment on 30th, namely 30 3.7 x 10^3 (CFU / g), in which the value was higher than observations from 0 day to 20th day. Meanwhile, treatment on 20th day by aerator treatment was the lowest value of PVC *Vibrio* sp. as 2.9 x 10^3 (CFU / g), but it increased in the 30th day. The lowest value of PVC *Vibrio* sp. in Pacific White Shrimps' (*L. vannamei*)

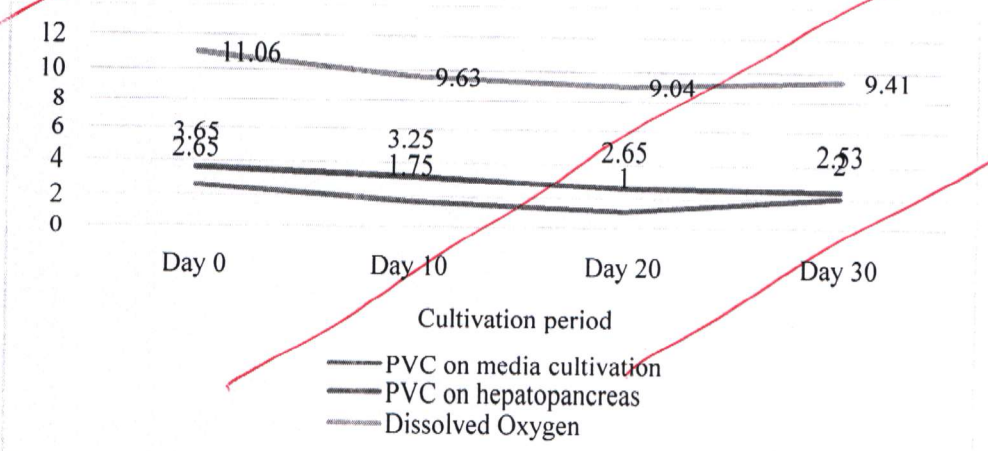
hepatopancreas was in the 30th day of nanobubble treatment, as 2.53 x 10^3 (CFU / g), in which the value showed a stable decrease of the initial PVC value of *Vibrio* sp. on 0 day in nanobubble treatment as 3.65 x 10^3 (CFU / g) (Figure 3).

Table IV. Presumptive Vibrio Count (PVC) *Vibrio* sp.on hepatopancreas, the cultivation media of Pacific White Shrimp (*L. vannamei*), and dissolved oxygen

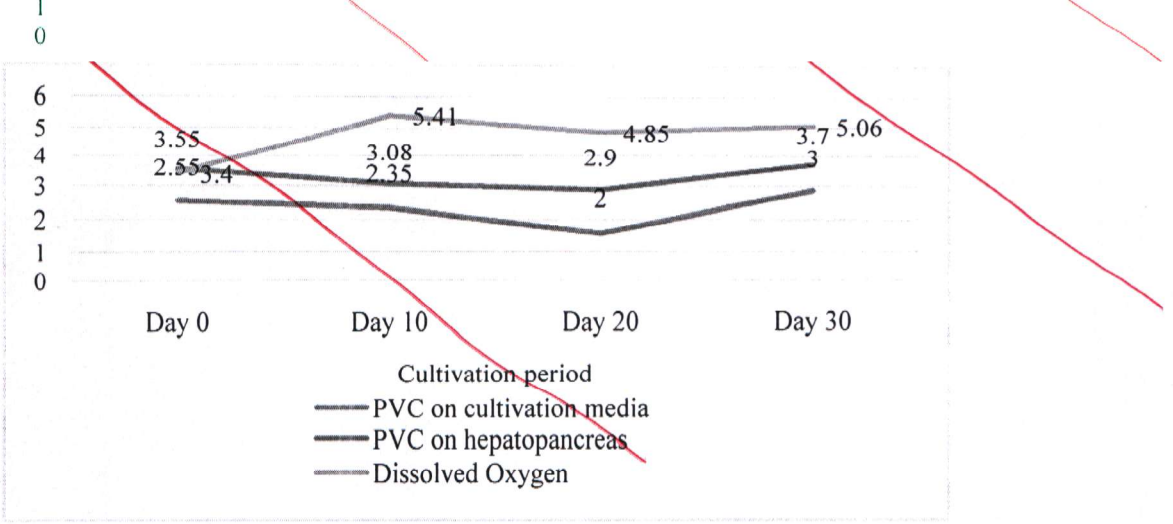
Treatments		Dissolved Oxygen ± SD (ppm)	PVC ± SD	
Factor A	Factor B		Hepatopancreas (x 10^3 (CFU/g))	Cultivation Media (x 10^3 (CFU/mL))
A1	B1	$11.06^{e} \pm 0.72$	$3.65^{de} \pm 0.04$	$2.65^{c} \pm 0.04$
	B2	$9.63^{d} \pm 0.24$	$3.25^{cd} \pm 0.01$	$1.75^{abc} \pm 0.13$
	B3	$9.04^{cd} \pm 0.21$	$2.65^{ab} \pm 0.04$	$1.2^{a} \pm 0.31$
	B4	$9.41^{c} \pm 0.36$	$2.53^{a} \pm 0.03$	$2.13^{bc} \pm 0.08$
A2	B1	$3.4^{a} \pm 0.30$	$3.55^{de} \pm 0.03$	$2.55^{bc} \pm 0.04$
	B2	$5.41^{b} \pm 0.22$	$3.08^{c} \pm 0.05$	$2.35^{bc} \pm 0.08$
	B3	$4.85^{b} \pm 0.42$	$2.9^{bc} \pm 0.02$	$1.6^{ab} \pm 0.22$
	B4	$5.06^{b} \pm 0.26$	$3.7^{e} \pm 0.03$	$2.88^{c} \pm 0.11$

Description: The Notation indicated by superscript letter in the same column shows the comparison between treatments has a significant difference (P <0.05); Factor A: A1: Nanobubble; A2: Aerator; Factor B: B1: 0 day; B2: 10th day; B3: 20th day; B4: 30th day.

Figure 4. Comparison of Presumptive Vibrio Count (PVC) *Vibrio* sp. on the hepatopancreas, Pacific White Shrimps (*L. vannamei*) cultivation media and dissolved oxygen with the cultivation period on nanobubble treatment



The graph above showedthe comparison of PVC *Vibrio* sp.in hepatopancreas, Pacific White Shrimps (*L. vannamei*) cultivation mediaand dissolved oxygen in cultivation media for 30 days using nanobubble. The use of nanobubble in Pacific White Shrimp (*L. vannamei*) cultivation showed the value of PVC *Vibrio* sp.experienced a stable decline. The highest value of PVC *Vibrio* sp. in hepatopancreas and Pacific White Shrimps (*L. vannamei*) cultivation media during the study was on 0 day that was 3.65 x 10^3 (CFU / g) and 2.65 x 10^3



The graph above showed the comparison of PVC *Vibrio* sp. in hepatopancreas, Pacific White Shrimps (*L. vannamei*) cultivation media and dissolved oxygen in cultivation media for 30 days using aerator. The use of aerator in Pacific White Shrimp (*L. vannamei*) cultivation showed the trend of PVC of *Vibrio* sp.as unstable. The highest value of PVC *Vibrio* sp. in hepatopancreas and Pacific White Shrimps (*L. vannamei*) cultivation media during the study was on the 30th day namely 3.7 x 10^3 (CFU / g) and 2.88 x 10^3 (CFU / mL), in which the value occurred at dissolved oxygen of 5.06 ppm. The lowest value of PVC *Vibrio* sp. in hepatopancreas and Pacific White Shrimps (*L. vannamei*) cultivation media during the study were on the 20th day namely 2.9 x 10^3 (CFU / g) and 1.6 x 10^3 (CFU / mL), where the value occurred at dissolved oxygen content of 4.85 ppm (Figure 5).

Water quality in Pacific White Shrimp cultivation media can be seen in Table V. Water quality parameters during the cultivation Pacific White Shrimps (*L. vannamei*) by using aerator and nanobubble were fluctuating. The temperature of the nanobubble treatment was 26 °C to 31.5 °C, while the temperature of aerator treatment was 26 °C to 29.5 °C. Salinity conditions in both treatments were equal to 15-16 ppt. The pH on nanobubble treatment was 5.5 - 8.1 and The pH on aerator treatment was 6.1 - 8.4. Nitrite and ammonia levels in the nanobubble treatment were low, as nitrite 0.0021 - 0.362 ppm and ammonia 0.0485 - 0.6086 ppm. Nitrite and ammonia levels in aerator treatment tend to be higher, as nitrite 0.0144 - 2.7115 ppm and ammonia 0.6130 - 1.58 ppm.

20	15	28,2	7,4	0,0079 – 0,0137	0,0490 – 0,0491
30	16	26,5	7,5	0,0021 – 0,0079	0,0497 – 0,0499

DISCUSSION

The development of nanobubble technology in the Pacific White Shrimp (*L. vannamei*) cultivation system showed higher and stable dissolved oxygen compared to aerator use. The dissolved oxygen in the nanobubble treatment was 9.04 ppm to reach 11.06 ppm while the aerator of dissolved oxygen was 3.4 ppm to 5.41 ppm. The cultivation system using nanobubble had a bubble size that contains oxygen gas in the water which is < 200 nm (Ebina *et al.*, 2013). The size of the bubbles in the larger aerator will not last long in the water so that the oxygen supply will be less stable. The smaller size of nanobubble bubbles will last longer in waters so that oxygen levels in the waters can be available in a longer time and remain stable. The smaller the bubble size, the smaller the buoyancy of the bubble. 1 mm bubble floats at a level of 0.361 feet per second or 3610 times faster than micro bubbles that float at a rate of 0.0001 feet per second (Chiba and Takahashi, ~~2007).~~ The dissolved oxygen in the study was still in the optimal range for shrimp, because shrimp can grow well at minimum oxygen levels ranging from 4 - 6 ppm (Haliman and Adijaya, 2005).

The development of nanobubble technology in the Pacific White Shrimp (*L. vannamei*) cultivation system also showed the Presumptive Vibrio Count (PVC) of *Vibrio* sp. which was lower than using ordinary aerators and the value was still below the safe range. According to Subaidah *et al.* (2006), shrimp mass mortality usually occurs along with an increase in the population of *Vibrio* sp. bacteria. in pond water above 10^3 CFU / mL. Leano *et al.* (1998) stated that when the concentration of *Vibrio* sp. in the hepatopancreas has reached a density of 9.0×10^4 CFU/g, it is enough to make *Vibrio* bacteria pathogenic in nature.

Supito *et al.* (2008) suggested that the dominance and abundance of unstable *Vibrio* sp.on fish ponds indicate riskyconditionsfor shrimp health problems. According to Wang *et al.* (2005), the high value of PVC can be the cause of the decline in shrimp production. *Vibrio* sp. infection in shrimp usually occur through feed and gills, so that the consumption of aquaculture water containing bacteria will continue to bacterial colonization in the digestive system, especially in the hepatopancreas. Hepatopankreas is the main target of several

bacteriain the cultivation of Pacific White Shrimp (*L. vannamei*) using nanobubble showed a was 0.9745 - 1.5535 ppm. Pacific White Shrimp (*L. vannamei*) cultivation using nanobubble on the last day of observation showed that ammonia value was 0.0497 - 0.0499 ppm and nitrite was 0.0021 - 0.0079 ppm. This value was still below the normal range for Pacific White Shrimp, which ammonia levels in Pacific White Shrimp aquaculture ponds are < 0.1 ppm and nitrite content is < 2.5 ppm (WWF-Indonesia, 2014), so the number of *Vibrio* sp. bacteriain the cultivation of Pacific White Shrimp (*L. vannamei*) using nanobubble showed a lower value when compared to Pacific White Shrimp (*L. vannamei*) using aerators.

Nanobubble oxygen will capture solids (pollutants) suspended in the water and lift them to the surface. The suspended solids are not uniform in size or shape, large bubbles fail to bind solids, but nano bubbles are able to penetrate small cavities in contaminants so that they can wrap solids and make them lift to the surface, so that organic matter in the waters will be low and water quality can be maintained (Kilawati and Maimunah, 2014).

Cultivation systems that use propeller aerators have a larger bubble size so that suspended solids will be longer to be decomposed causing anaccumulation of organic matter and a decrease in water quality. Oxygen in water is also needed to decompose organic matter that accumulates in water so there is no increase in ammonia levels which pose a threat to aquatic animals (Apandi *et al.*, 2016). Nitrogen in the form of NH_3 (ammonia) and N_2 gas can be toxic to all aquatic organisms (Mukti *et al.*, 2014). Poor water quality can cause stress on cultivated shrimp so that the growth of shrimp will be disrupted and reduce the body's resistance to infectious diseases and cause death (Diansari *et al.*, 2013).

Characteristics of *Vibrio* sp. is an opportunistic pathogen, that is an normal-state organism in cultivation environment that develops into a pathogen if its environmental conditions and host deteriorate (Utami *et al.*, 2016). Decreasing water quality in intensive cultivation systems can occur due to high stocking density, leftover feed and feces that accumulate as organic material and poor water quality management. *Vibrio* sp. bacteria will dominate due to the utilization of organic material that accumulates.

The development of nanobubble technology can improve the quality of the cultivation system by maintaining a balance between the environment, fish/shrimp and pathogens. Prevention is the most effective action compared to treatment, because prevention is carried out before an attack occurs so that the costs incurred is not too much (Mahasri, 2016).

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ACCEPTANCE LETTER

The following article has been accepted and will be published in **MAY, 2019** issue of Indian Veterinary Journal.

Article No.	Title	Author (s)
358/18	Oxygen Dissolved Nanobubble Technology Improved to Quality of Pacific White Shrimp Cultivation	**Gunanti Mahasri** Ade Irmalia Harifa Sudarno

Sd/-

Managing Editor,
Indian Veterinary Journal

To,

Dr. Gunanti Mahasri,
Department of Fish Health Management,
Faculty of Fisheries and Marine,
Universitas Airlangga, Surabaya 60115
Email : mahasritot@gmail.com

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