

## Investigation of wastewater quality and microbiology from wastewater treatment pond (WWTP) in milenial shrimp farming (MSF) of *Litopenaeus vannamei* Boone (1931) intensive cultivation at Brackish Water Aquaculture Center (BPBAP) Situbondo. Indonesia

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### Abstract

Shrimp *L. vannamei* Boone (1931)) has become a global market product and has become a major species for cultivation. This species, which has many advantages and is intensively cultivated to obtain maximum production. However, wastewater from intensive shrimp culture is known to have a negative impact if directly discharged into the environment, so a wastewater treatment plant (WWTP) is needed before being discharged into the environment. This study aims to investigate the water quality of intensive shrimp cultivation at the Millennial Shrimp Farming (MSF) which is equipped with a waste processor at the Brackish Water Cultivation Center (BPBAP), Situbondo, East Java Province, Indonesia from January to April 2023. Water quality data were collected randomly at 7 data collection times during shrimp maintenance from WWTP wastewater. Water quality is analyzed based on the official applicable method, namely the Indonesian National Standard (SNI) and is carried out at the BPBAP Situbondo Laboratory. The chemical characteristics of shrimp cultivation wastewater based on the intensive system in the millennium intensive farming (IMF) ponds after treatment were obtained still in accordance of range of water quality parameter values during intensive shrimp cultivation systems to environment. However, physical aspects, namely Total Organic Matter (TOM), turbidity and BOD need serious attention. The density of vibrio bacteria, *yellow vibrio* colonies and *green vibrio* colonies in wastewater after treatment still allows for environment. Monitoring the quality of wastewater and wastewater treatment plants needs to be carried out routinely before discard to environment and for the success of shrimp cultivation, as well as maintaining and preserving water quality that is suitable for shrimp life during the maintenance stages.

**Keywords:** Intensive; Culture; Vaname; Waste; Water Quality

### 1. Introduction

Pacific whiteleg shrimp (*L. vannamei* Boone 1931), a highly significant global commodity, has been introduced for aquaculture to many countries outside its native range [1]. The species in Thailand, as introduced to replace *Penaeus monodon* for aquaculture in 1998, and it has represented more than 90 percent of total cultured shrimp production since 2005. The shrimp were able to replace tiger prawns as one of the mainstay commodities for aquaculture in fulfilling fishery exports. Vannamei has a major global commodity [2], with its production exceeding 4,000,000 tonnes in 2016 and with Southeast Asia being an important producer (1,483,935 tonnes) [3]. The world's production of farmed shrimp in 2023 will likely be slightly lower (decrease 0.4 percent) at 5.6 million metric tons (MMT) than in 2022, and

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increase 4.8 percent in 2024 to close to 5.88 MMT. The top five of the shrimp producers in 2023 in order, Ecuador, China, India, Vietnam and Indonesia; the countries have produce 74 percent of global production in 2023 [4] .

Vannamei shrimp has fast growing and can be cultivated under high stocking density and resistant to disease. Shrimp cultivation has grown rapidly both in terms of the development of the number of farming areas and production for export, especially in the last five years. The condition was marked by the introduction of vannamei shrimp to Indonesia in 2001. Penaeid shrimp culture has become a leading export fishery in Indonesia [5]. The Pacific white shrimp (*L. vannamei* Boone 1931) was unofficially introduced to Indonesia in 1999, and received government approval in 2001. By the end of 2007, the shrimp was cultured in over 17 provinces.

Cultivation method to increasing the productivity through intensification has given rise to semi-intensive, intensive and super-intensive cultivation techniques, which are based on the density of the shrimp biomass [6]. Aquaculture productivity has progressively increased over the past 50 years. Development of cultivation method start from conventional of still-water aquaculture and referred to an extensive systems, yields 1121–2242 kg.ha<sup>-1</sup> of fish or shrimp in freshwater or marine systems. In the 1980s, farmers added 1.8–3.7 kW.ha<sup>-1</sup> (1–2 hp.ac<sup>-1</sup>) supplemental aeration to marine shrimp ponds, produce 4483–7846 kg.ha<sup>-1</sup>. The marine systems, flushing of ammonia-laden water and increasing pond aeration to 19–37 kW.ha<sup>-1</sup> (10–20 hp.ac<sup>-1</sup>) allowed shrimp production in excess of 11,209 kg.ha<sup>-1</sup> per cycle with high stocking densities, the amount of feed given will automatically increase. However, an amount of feed will not be eaten, and feed consumed will not be absorbed in internal shrimp system. The highest source of super-intensive shrimp cultivation waste is shrimp feces and leftover feed which can cause the emergence of various viruses and pathogenic microorganisms, and result in endangering to shrimp life.

The intensification method in aquaculture characterized by higher stocking densities and greater inputs of feeds become impact pollution and degradation to environment, including the control of diseases [7]. Super intensive shrimp pond produce waste and can cause an imbalance in the environmental ecosystem, resulting in disease outbreaks [8], The management control of super-intensive technology for 1000 m<sup>2</sup>, the water depth is more significant than 1.8 m, shrimp stocking density (>500 ind.m<sup>-2</sup>), and to increase the productivity and produce a minimal waste load, the method could equipped with clean water reservoirs and a wastewater treatment plant (WWTP) [9], The waste on the shrimp culture of 500 ind.m<sup>-2</sup> stocking density in 1000 m<sup>2</sup> is 50.12 g of total N/kg of shrimp, 15.73 g total P.kg<sup>-2</sup> of shrimp, and 126.85 g C organic.kg<sup>-2</sup> of shrimp. A several time period is needed to decompose the waste by microorganisms and directly causes hoarding, damage to environment and interfering to living organisms in the environment.

The total feed as nutrients is absorbed in body metabolism for commercial aquaculture approximately 30% and the remaining 70% is inedible and becomes waste [8]. The intensive shrimp cultivation contains high Total Suspended Solid (TSS) and Total Organic Matter (TOM), low Dissolved Oxygen (DO), low pH, high Biochemical Oxygen Demand (BOD), Total Nitrogen (TN) and High Total Phosphate (TP) as importance aspects of waste water quality and required threshold limit. The super intensive shrimp cultivation has the potential of high negative impacts if directly disposed of into environment and needs IPAL to treat the waste produced before discharged into environment waters. The aim of the study is to determine the waste load and waste water and sediment produced from BPBAP Situbondo's intensive vannamei shrimp cultivation. The final target is to obtain an effective and efficient technique for the intensive shrimp culture managing conduct to waste processing installations.

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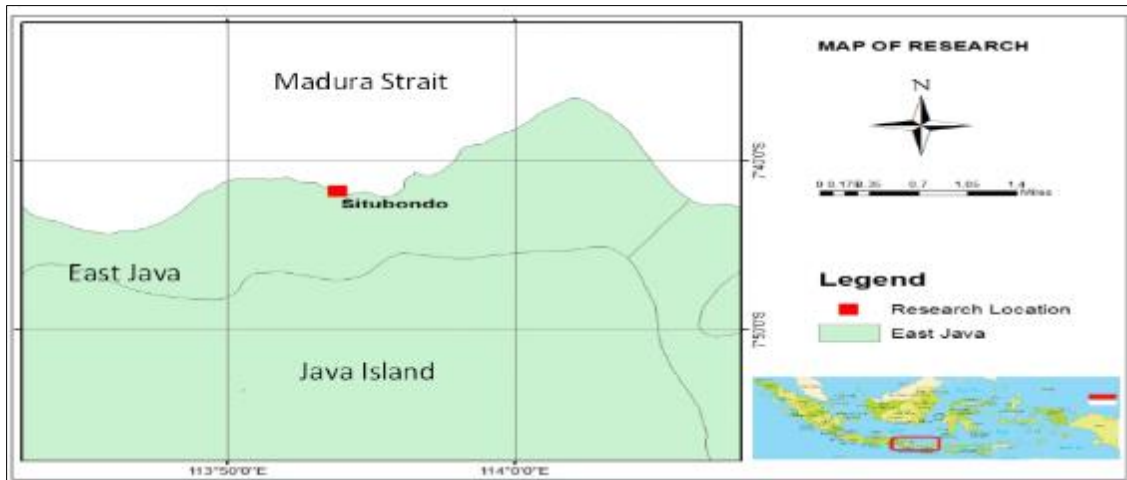
## 2. Material and methods

The research was carried out in intensive technology of white leg shrimp *L. vannamei* Boone (1931) in Millennial Shrimp Farming (MSF) pond at Situbondo Regencies, East Java Province, Indonesia (Figure 1) from January to April 2023.

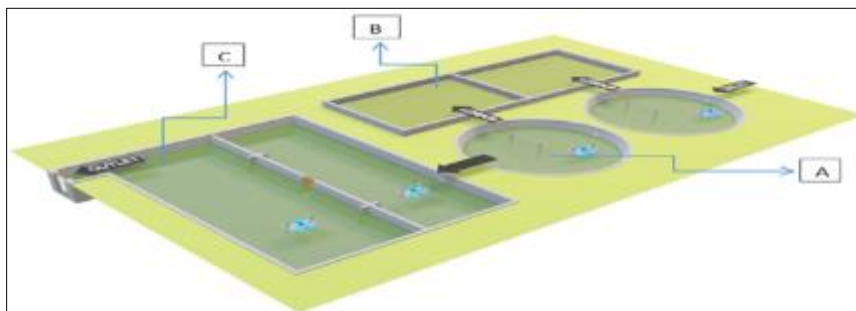
Waste water samples are taken in the morning during replacement from drain pipe outlet water of WWTP. 1 liter of water sample was taken, placed in a tightly closed dark glass bottle system. The main facilities of the MSF pond wastewater treatment plant consist of a sedimentation pond, drying pond and oxygenation pond (Figure 2).

Main facilities vannamei shrimp of MSF ponds wastewater treatment plan A is sediment pond, as the first physical processing stage to reduce TSS through a deposition process. Solid particles will settle, while light particles will float to form foam. Sedimentation ponds reduce TSS content 40 - 60%. The sedimentation ponds equipped with mud draining pumps and the sediment removal is carried out before the thickness reaches 50% of the water depth of the sediment. Pond B is drying pond to collect mud from sedimentation ponds to dry the mud/sediment. This pool is designed automatically every 15 minutes to pump the mud in the sedimentation pool for 9-15 seconds. Oxygenation pond (C) is a waste water storage pond in the final stage. All treated wastewater is channeled and collected. The pond plays a role

in reducing the contents of TSS, TAN, nitrite, nitrate, total N and phosphate. In the pool can be added pro-biotic stage. Aquatic plants/macro-algae such as seaweed, shellfish and herbivorous fish such as tilapia/milk fish can be maintained which function as bio-filters and bio-indicators. Aquatic plants will absorb nutrients and convert into harvest-able biomass. The pool functions to quickly determine the suitability of water processed of WWTP for living organisms and the environment. If the fish in the pond growth normally, the water processed by the WWTP is suitable for aquatic organisms life. Mean while if the fish die, the water processed of WWTP is bad category. Water quality measurements can be carried out as needed.



**Figure 1** Site location of Brackish Water Aquaculture Center (BPBAP) Situbondo, East Java. Indonesia



**Figure 2** MSF pon and Water Water Treatment Plant (WWTP) system, sediment pond (A), drying pond (B) and Oxygenation pond (C)

Water quality analysis is carried out at the BPBAP Situbondo Fish Health and Environmental Laboratory and bacterial analysis is carried out of the total plate count method. The water quality parameters in MSF cultivation, namely Nitrite ( $\text{NO}_2^-$ ), Nitrate ( $\text{NO}_3^-$ ), BOD, TOM, TSS, Phosphate ( $\text{PO}_4$ ), DO, pH, temperature, and salinity. Dissolved Oxygen (DO) is measured using a YSI 550i Dissolved Oxygen meter. The pH was measured using an Eu-tech EC-pH Tester. Water temperature was measured in situ using a digital thermometer, while the salinity was measured using an Atago MASTER-S10 refractometer. The analysis method of water quality is based on Indonesian National Standard (SNI) [10], [11].

### 2.1. Water quality analysis method

Total Ammonia (TAN) is analyzed base on Indophenol blue method [11]. The filtered sample of 25 ml is put into an Erlenmeyer flask. Then 1 ml of 10% Phenol solution is added and homogenized. Then 1 ml of 0.5% Sodium Nitro-preside solution is added and homogenized. Then 2.5 ml of Oxidizing solution is added and homogenized. The Erlenmeyer top tube is closed tightly and placed in a dark room at 22-27 °C of temperature for 1 hour. Measurement based on a spectrophotometer at 640 nm (green-blue) of wavelength, for time perioda wavelength of 24 hours after adding the reagent. The absorbance is plotted on the ammonia standard curve to obtain the total Ammonia concentration value in the

sample. The Oxidizer solution consists of 2 ml of Alkaline Citric solution and 0.5 ml of 5% Sodium Hypo Chloride, which are mixed just before the Ammonia analysis is carried out after stable for a few hours.

Nitrite ( $\text{NO}_2^-$ ) is analyzed a wavelength of based on Sulfanilamide method [10]. 50 ml of the filtered water sample is put into an Erlenmeyer flask, and 2 ml of dye solution is added. After 10 minutes the solution is measured using a spectrophotometer at a wavelength of 543 nm (pink) for 2 hours period. The absorbance obtained is plotted on a Nitrite Standard Curve to obtain the Nitrite concentration. The dye solution is made from 1 g Sulfonamides, 0.1 g NED Dihydrochloride and 10 ml 85% Phosphoric Acid ( $\text{H}_3\text{PO}_4$ ) plus Nitrite-free is distilled water to 100 ml. The solution is stable for 1 month.

Nitrate ( $\text{NO}_3^-$ ) is measured using a test kit and color meter. The color-meter is set up by selecting the program for Nitrate measurement. A total of 10 ml of filtered sample is added to the sample cells with reagent and shaken for 1 minute, then left for 5 minutes. Samples without added reagents are used as turbidity/color blanks. Only then is a reading carried out on the sample to which the reagent has been added.

Phosphate ( $\text{PO}_4$ ) : Phosphate measurements is based on a spectral-photo meter [12] method or a test kit with a colorimeter. Measurement is based on SNI method, 50 ml of the filtered sample is placed in an Erlenmeyer flask. Then add 1-2 drops of Phenol-phthalein indicator, if red, add 5 N Sulfuric Acid ( $\text{H}_2\text{SO}_4$ ) solution until clear. Then 8 ml of the mixed solution is added and homogenized. The solution is measured using a spectral-photo meter at a wavelength of 880 nm (indigo-blue color), after 10 minutes, and in 30 minutes after adding the reagent. The results of the solution absorbance readings are plotted on the Phosphate standard curve to obtain the Phosphate concentration value in the sample. The mixed reagent consists of 4 ml of 5N  $\text{H}_2\text{SO}_4$ , 0.4 ml of Potassium Antimonyl Tartrate solution, 1.2 ml of Ammonium Molybdate solution, and 2.4 ml of Ascorbic Acid solution. The mixed solution is stable for 4 hours, and mix it just before use. If the mixed solution is blue, it was indicate the reagent has been damaged and must be replaced with a new one. Measuring using a test kit, 20 ml of the sample is filtered, 10 ml of reagent is added (test kit), 10 ml is used as a turbidity blank. The colorimeter is prepared by selecting the program for Phosphate measurement. The sample that had been added to the test kit is homogenized and left for 2 minutes. Then, the sample cell containing the sample fluid without reagent is placed in the colorimeter and used as a blank, then the sample with the reagent added is read.

BOD : The water sample is homogenized and aerated until saturation conditions. If the BOD value is too large, dilute with sterile sea water then aerated. After saturation, the water is immediately put into two dark bottles and closed and the first bottle must empty of air bubbles in the bottles when turned upside down. One bottle is analyzed as DO-0 and the other bottle is stored in dark place at a temperature of 20 °C, The sample is analyzed after 5 days as DO-5. DO is analyzed base on Winkle r-tit-ration method [13]. The sample in the dark bottle is unscrewed and immediately added 1 ml of Manganese Sulfate solution and 1 ml of Alkaline Iodide Aide solution with a pipette tip near the surface of the sample liquid, the bottle is closed then homogenized and left for 5-10 minutes. Add 1 ml of reagent for a sample bottle volume of 250-300 ml, for other bottle volumes the addition of reagent is adjusted according to the ratio. Then 1 ml of concentrated Sulfuric Acid is added and homogenized. 50 ml of the mixed solution is taken and tit-rated with 0.025 N Sodium ThioSulfate solution with starch solution indicator until the solution was clear.

TOM is analyzed using the titration method of Potassium Permanganate solution. 25 ml of the homogenized sample is taken and 25 ml of distilled water is added, and put into a beaker. Added 10ml of 0.01 N  $\text{KMnO}_4$  solution and 5 ml of 6N  $\text{H}_2\text{SO}_4$ . Then heated on a hot plate and allowed to boil for 10 minutes. After that, it is removed from the hot plate and 10 ml of 0.01N Oxalic Acid solution is added. Then is titrated using 0.01N  $\text{KMnO}_4$  solution.

TSS : The colorimeter tool is prepared, and a program is selected for TSS measurement. The sample is homogenized, then 25 ml is taken and put into the sample container (cell) of the colorimeter. For blanks, use distilled water which is inserted into the colorimeter sample container (cell) as zero first, then the sample is read. Tissue paper is used to wipe the outside of the sample container (cell) before taking the reading. Water quality recommendation range for shrimp culture is based on [14].

### 3. Results and discussion

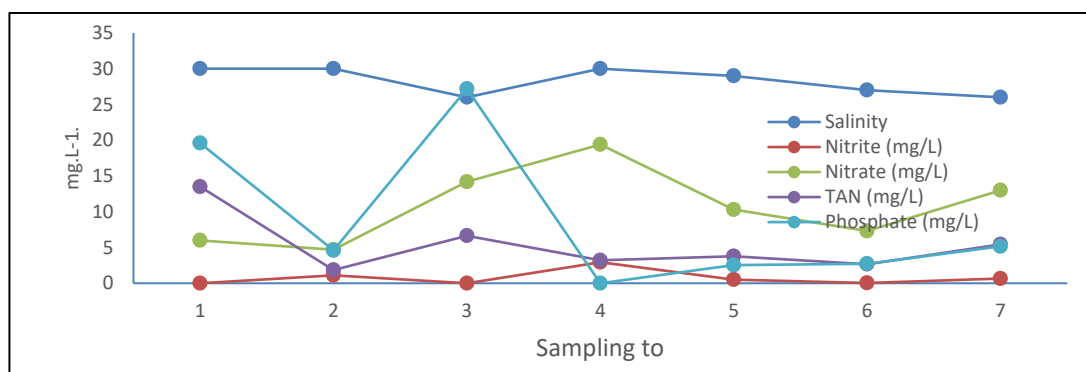
The quality of water in shrimp cultivation media plays a very important role and is the main requirement for success, especially in intensive shrimp cultivation. Intensive shrimp farming methods have been applied and implemented by vannamei shrimp farmers on the beaches of Indonesia, including farmers on the southern coast of Java. In the first year of the shrimp farming, success was achieved and the shrimp farming media water disposal system was directly channeled into the environment without waste treatment. However, cultivation failure occurred after the third year,

which was suspected because the aquatic environment was unable to quickly process to achieve the chemical and biological quality of water that suitable for shrimp growth. Intensive shrimp cultures have impact to environmental in coastal ecosystems, compromising water quality by discharging effluents rich in nutrients and organic matter. The impacts of shrimp farming are often investigated by the unit effect of a farm [15]. Investigation of vannamei shrimp waste water from WWTP was carried out in two production cycles. Water quality data on first cycle was found one data collection. The condition was caused by most of the ponds being attacked of disease, so the shrimp have to be harvested early.

### 3.1. Water quality parameters dynamic

The feed given for the rapid growth of cultivated shrimp must meet the metabolic needs of shrimp growth, and need conversion of the right amount of feed to the weight of individual shrimp or the weight of shrimp mass is required. However, in reality in the field, all feed given is not eaten by shrimp. The main source of waste in intensive shrimp farming is feed [16]. Wastewater samples were taken from the most concentrated wastewater and final waste discharge. The worst wastewater was found with a pH of 6.33 (Table 1) and a pH value was obtained less than 7. These conditions will produce Hydrogen Sulfide ( $H_2S$ ) which is toxic to aquatic organisms, these waste compounds must be immediately removed from the shrimp farming plot. Wastewater with the highest pH value was 7.18 and this high pH is a threat to  $H_2S$ . Nitrite ( $NO_2$ ) in the most concentrated waste conditions was not detected. This condition is caused by the low dissolved oxygen in wastewater which indicates low of nitrogen oxidation process, and binds with hydrogen to form ammonia. Meanwhile, nitrate ( $NO_3$ ) is a nitrogen oxidizer and this compound is more stable than nitrite. At the final disposal of waste of WWTP, the concentration of nitrite increases because oxygen is available for nitrogen oxidation, while the concentration of ammonia decreases as the oxidation process to nitrite. While nitrate decreases slightly due to the influence of the concentration of wastewater which is not too dilute compared to the worst waste conditions.

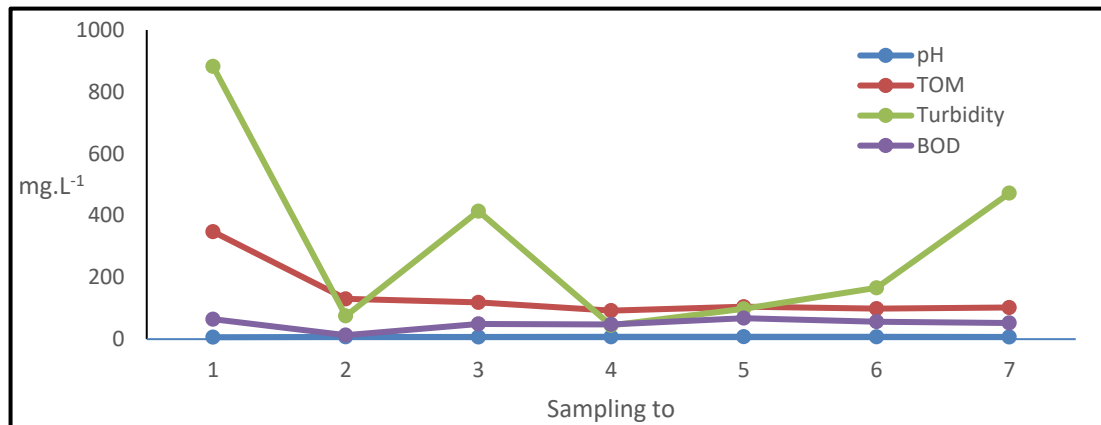
Concentration of Phosphate ( $PO_4^{3-}$ ) in wastewater was found four times greater than those in final waste disposal.



**Figure 3** Chemically wastewater quality discard during *L. vaname* intensive cultivation

The range and average of chemically water quality of wastewater parameters during cultivation of intensive *L. vannamei* cultivation period (Figure 3) was found pH 6.33–7.81 and 7.22, salinity 26–30 ppt and 22,29 ppt, Nitrite ( $NO_2$ ) 0–2.94 and 1,05  $mg.L^{-1}$ , Ammonia (TAN) 1.85–13.5 and 5.23  $mg.L^{-1}$ , Nitrate ( $NO_3$ ) 4.7–19.4 and 10.7  $mg.L^{-1}$ , and Phosphate ( $PO_4$ ) 2.54–27.2 and 10.30  $mg.L^{-1}$ . The chemical parameters are showed conform to water quality standards for intensive *L. vannamei* cultivation. Water salinity describes the total solids in the water after all the carbonates have been converted to Oxides, Chlorides have replaced all the Bromides and Iodides, and all organic matter has been oxidized. Factor influencing of salinity distribution in the waters is the amount of freshwater that enters to marine waters [17]. Commercial aquaculture, approximately of 30% of the total feed given is not consumed byshrimp, and about 25–30% of the feed consumed will be excreted. The amount of N and P present in the feed will be retained in shrimp meat between 25–30%, and the rest is wasted in the aquatic environment [18]. A nitrate is a form of N that is less toxic than nitrite and ammonia, but the compound can be toxic to shrimp at high concentrations. Shrimp exposed to high nitrate concentrations for a long time showed shorter of antennae length, gill abnormalities, and hepatopancreas blisters. Short antennae and gill abnormalities are often considered early clinical signs of declining shrimp health [19]. The concentration of ammonia strongly influences the toxicity of nitrite in the waters; if the concentration of the two variables are high, the compounds are a stressor for shrimp and fish cultured, finally the shrimp and fish are more susceptible to disease [20]. Several cases state that excessive Ammonia concentrations can cause a decrease in the number of blood cells, a decrease in the concentration of oxygen in the blood, a decrease in physical resistance and resistance to disease, and structural damage to various types of shrimp organs. Several research results state that the

accumulation of Ammonia in aquaculture water causes various kinds of damage to aquatic organisms, especially damage to the function and structure of body organs [21].



**Figure 4** Physical discard wastewater quality during *L. vannamei* intensive cultivation

The physical water quality of wastewater parameter range and the average during cultivation of intensive *L. vannamei* cultivation period (Figure 4) were found Total Organic Matter (TOM) 92.27–347.60 mg.L<sup>-1</sup> and 142.109 mg.L<sup>-1</sup>, turbidity 44.6–882 NTU and 307.4 NTU, and BOD 12.9–67.74 mg.L<sup>-1</sup> and 50.11 mg.L<sup>-1</sup>. TOM, turbidity and BOD in concentrated waste were also greater when compared to final disposal, namely 2.5 times, 10 times and 5 times respectively. The need for biological oxygen or Biological Oxygen Demand (BOD) in concentrated waste was obtained actually greater than 64.52 mg.L<sup>-1</sup>, because in the analysis of the Dissolved Oxygen content on the fifth day (DO<sub>5</sub>) the value was found zero even though it had been diluted 10 times. A larger dilution should have been carried out and the sample had run out, the solution could not be carried out with a larger dilution.

Phosphate and nitrite concentrations that associated with total organic matter (TOM) content were obtained exceeding the water quality requirements for intensive vannamei shrimp farming in all three parameters. The recommended phosphate and nitrite parameter standards are <0.1 mg.L<sup>-1</sup>, while the total organic matter parameter is <90 mg.L<sup>-1</sup> [12]. Water quality parameters are important indicators for determining the ecological feasibility status of the aquaculture operational cycle [22]. Stable fluctuations in water quality parameters are positively correlated with productivity during the shrimp farming cycle [23].

The second shrimp production cycle, wastewater samples did not come from the same plot, because the disease attack that caused the plot that was originally used for waste sampling had harvested the previous sample (sample 3). The fourth sampling was taken in another plot. The third sampling of the most concentrated waste was obtained pH slightly above 7, nitrite was not detected, ammonia (TAN) and turbidity was found to be high. The conditions were same as for the first sampling and phosphate was the highest when compared to other sampling. This condition may be suspected to be caused by shrimp metabolism has not absorbed much phosphorus from feed. The impact of these conditions increases dissolved phosphorus in wastewater. These conditions will increase the biological oxygen demand (BOD) because concentrated waste naturally contains more bacteria.

In the next wastewater quality sampling stage, a lower pH was obtained due to the increasing weight of shrimp biomass, waste production will also increase. In this condition, the pH has decreased due to the activity of decomposing bacteria. The decrease in pH is still within the shrimp maintenance range which is still more than 7. At this pH, there is a small chance of the formation of Hydrogen Sulfide compounds which are toxic to shrimp. The addition of groundwater has caused a decrease in salinity to replace the volume of water that is discharged. Nitrogen compounds of nitrite, ammonia (TAN) and nitrate were obtained varyingly. Likewise, total organic matter content (TOM) was obtained varyingly. This condition was different from the decreasing pH condition which increases the phosphate content. The increase in phosphate content is thought to be due to the increase in the amount of feed which then causes an increase in the amount of waste, and phosphate is an indicator. The increase in waste volume from shrimp feces and leftover feed will also cause an increase in water turbidity.

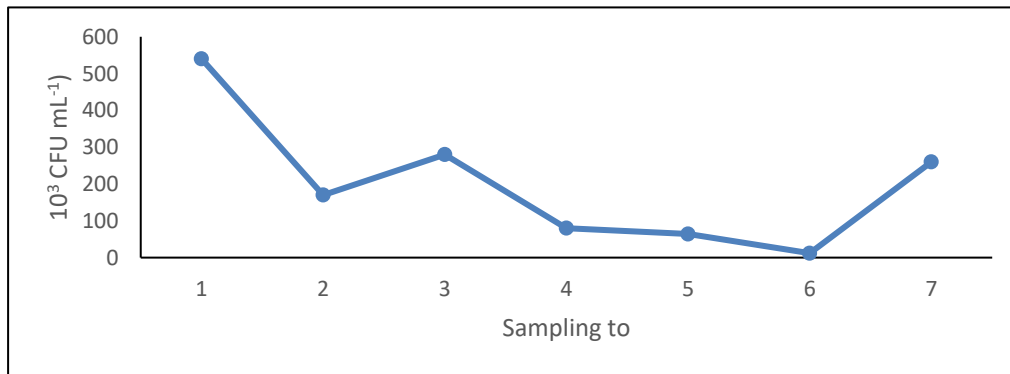
The concentration of biological oxygen demand (BOD) also increased which can be caused by the increase in the amount of waste, so that more oxygen is needed for the waste decomposition process by decomposing organisms in the sixth and seventh samplings carried out on the fifth day, there was no dissolved oxygen in the sample that had been diluted



10 times. The BOD analysis results should have been greater than the two samplings, but because the sample had run out, the analysis could not be carried out with the same high dilution. In the seventh sampling, high biological/bacterial activity was obtained which can be seen from the utilization of oxygen which caused a lack of dissolved oxygen. In the seventh sampling, the Dissolved Oxygen was empty due to a faster decrease compared to the sixth sampling. This condition causes the dissolved oxygen content in sampling seventh<sup>th</sup> to be smaller than the DO in the sixth sampling.

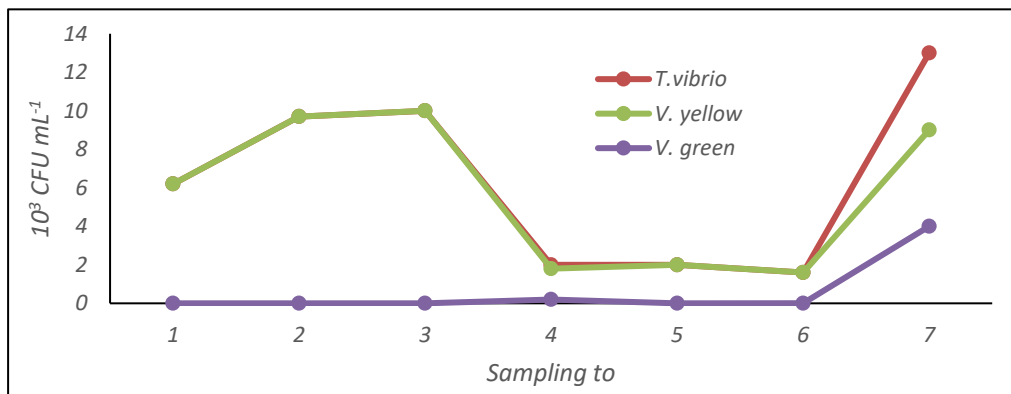
### 3.2. Microbiology parameters

Sampling of microbiology in waste water were done during the rainy season and the density of bacteria in concentrated wastewater was found three times in the final waste, reaching a value of  $100 \times 10^3$  colonies per milliliter ( $\text{CFU mL}^{-1}$ ) (Figure 5).



**Figure 5** Microbiological of *T. bacteria* in WWTP discard water of *L. vaname* intensive cultivation

Meanwhile, the density of vibrio bacteria in concentrated waste was apparently smaller than in waste at the end of disposal. This bacteria colony shows that the types of bacteria in concentrated waste were more non-vibrio types. In both concentrated and final waste, the vibrio colonies that grow are only yellow vibrio colonies, which are not detrimental to shrimp. Vibrio yellow colonies are commonly found in water samples from ponds that use probiotics, which support the shrimp grow. The presence of yellow colony vibrio was actually beneficial for shrimp cultivation



**Figure 6** Microbiological of *T. vibrio*, *V. yellow* and *V. green* in WWTP discard water of *L. vaname* intensive cultivation

The density and the average ( $10^3 \text{ CFU mL}^{-1}$ ) of microbiological aspects during the cultivation *L. vannamei* in intensive pond wastewater obtained bacterial density of 12–540 and  $200.86 \text{ CFU mL}^{-1}$ , presumptive vibrio between 1.6–13 and  $6.36 \text{ CFU mL}^{-1}$ , with yellow vibrio colonies between 1.6–10 and  $5.76 \text{ CFU mL}^{-1}$ , and green vibrio colonies 0–4.0 and  $0.60 \text{ CFU mL}^{-1}$ . The highest bacterial density obtained reached  $100 \times 10^3 \text{ CFU mL}^{-1}$ , and vibrio reached  $10 \times 10^3 \text{ CFU mL}^{-1}$ , which only formed yellow colonies. In these bacterial abundance conditions for intensive shrimp cultivation systems, it is still in the category of safe cultivation for shrimp. Microbiological conditions also vary, the increase only occurs in green vibrio colonies which tend to be detrimental to shrimp cultivation, even reaching  $10^3 \text{ CFU mL}^{-1}$  in seventh<sup>th</sup> sampling. This condition was thought to cause the shrimp to experience a decline in quality, the solution step that the shrimp should be harvested as a precautionary measure against mass shrimp deaths or cultivation failures.

The approach to obtain waste production can be obtained based on the results of proximate analysis of shrimp feed and carapace, nutrient retention, amount of feed, feed conversion ratio, and shrimp biomass production [24]. Based on data on shrimp production of shrimp cultivation in the first production cycle from nine intensive ponds, the average FCR was obtained 1.33. Based on the value that every 1.33 kg of feed given to shrimp will produce 1 kg of shrimp and there was 0.33 kg can not utilized by shrimp or as waste. While in the next production cycle, the average FCR was obtained 1.07, that mean every 1 kg of shrimp produced will produce 0.07 kg of dry waste. Based on the FCR value, if the value of shrimp biomass production is known, then the amount of feed utilized by shrimp can also be known. However, in the absence of proximate analysis results of shrimp feed and carapace, as well as nutrient retention, data cannot be obtained on the amount of waste production.

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#### 4. Conclusion

Wastewater from WWTP discard water of shrimp farming *L. vannamei* Boone, (1931) based on cultivation of intensive system in milenial intensive farming (IMF) was obtained pH, salinity, Nitrite, Nitrate, Ammonia and Phosphate in accordance with the range of water quality parameter values for intensive shrimp farming system. However, physical aspects, namely Total Organic Matter (TOM), turbidity and BOD need serious attention. The density of vibrio bacteria, yellow vibrio colonies and green vibrio colonies in wastewater are worthy of being discharged into the environment that still allows it as a source of water for cultivation activities for the next stage.

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#### Compliance with ethical standards

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##### *Disclosure of conflict of interest*

No conflict of interest to be disclosed

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