Test the effectiveness of *Porphyridium aerugineum* as *Vibrio* controller in shrimp ponds

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Test the effectiveness of *Porphyridium aerugineum* as *Vibrio* controller in shrimp ponds

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Abstract. Bacterial disease control in shrimp ponds was currently taking a more environmentally friendly approach. Thwas study aims to find out the effectiveness of *Porphyridium aerugineum* as a controller of Vibrioswas in ponds. This research was carried out at with two treatments with three replications. The first treatment was the administration of whole-cell microalgae *P. aerugineum* and the second treatment was control without microalgae administration. Tiger shrimp was stocked at the stadia PL 12 (density of 8 shrimp fry/m2). The study used 6 ponds measuring 10m x 25m with a water depth of 80cm. Microalgae was given once a week. A sampling of pond water and sediment was carried out once in two weeks. The results showed that the addition of wholecell microalgae *P.aerugineum* in ponds can reduce the ratio of total Vibrio bacteria and total plate count in ponds water but not in pond sediments. In ponds fed microalgae P.aerugineum, shrimp survival rate and production were higher than those of in controls. The results of thwas study showed that the adminwastration of microalgae as an additional supplement in ponds gave better results compared to the control.

1. Introduction

Microalgae are one of the most interesting microorganisms in the field of biotechnology because they have so many benefits for the life of mankind, for example the content of macromolecules in microalgae biomass which has been widely used as an alternative energy source to replace fossil fuels such as biodiesel, lipids and bioethanol as well as for sources of raw materials for health products [1–3]. Unicellular microalgae are a promising source of antibacterial alternatives. One type of microalgae that has the potential to produce chemical compounds that function as antibacterials was *Phorphyridium* sp. known to contain phenols, phenol compounds, alcohols and aldehydes that act as antibacterials [4].

Research to find out the ability of microalgae as an antiviral has been conducted by [5] screened extracts from 10 marine microalgae for inhibition of 2 enveloped rhabdoviruses of significant economic importance, in aquaculture, the African Swine Fever Virus (ASFV), which affects over 50 species of freshwater and marine fwash, including salmonid fwash, and in pig production sector, the Viral Hemorrhagic Septicemia virus (VHSV). Only the aqueous extracts from *Chlorella autotrophica, Ellipsoidon sp., and Porphyridium cruentum* showed an important antiviral action, possibly attributed

to sulfated polysaccharides, which accounted for c.15%, 15%, and 30% of the total soluble exopolysaccharides, respectively.

Antiviral sulfated polysaccharides from several species of red microalgae mainly conswast of xylose, glucose, and galactose; they are unusually stable when exposed to extreme pH and temperature [4,6]. The cell wall sulfated polysaccharide of the red microalga *Porphyridium* sp. exhibited impressive antiviral activity against HSV type 1 and 2 both in vitro cell culture and in vivo rats and rabbits [4]. These authors showed that *Porphyridium* sp. polysaccharides had a direct effect on the HSV-1 cycle, and that the polysaccharides significantly inhibited viral infection, possibly the result of a tight binding between HSV-1 particles and *Porphyridium* sp. polysaccharides.

Antibacterial compounds from microalgae were recently reviewed, attesting the variety of compounds with biological activities and the diversity of microalgal species screened and studied [2,6]. Phycobiliproteins from *Porphyridium aerugineum* contain phycocyanin, which was reported to inhibit the growth of the (G+) *S. aureus* with a MIC of 7 μg/mL [7]. In *Porphyridium.* cruentum, phycoerythrin was reported as an antibacterial agent with a MIC of 0.29 μg/mL against *S. aureus* [7], and 1% of extracellular sulfated polysaccharides (EPS) presented activity against *E. coli* and S. *aureus* [8].

This study aims to determine the effectiveness of microalgae *Phorphyridium aerugineum* as a vibriosis control in ponds.

2. Method

This study used 6 pond plots each measuring 250 m^2 with a water depth of 80 cm containing tiger shrimp (*Penaeus monodon*) measuring PL 12 as many as 8 larvae/ m^2 with a water volume of 200,000 L. After 7 days of shrimp stocking, experiment was carried out with the addition of wholecell microalgae *Phorpyridium aerugenum* with a density of about 10^6 cells / mL as much as 20 L per pond plot. The parameters observed in this study were bacterial population and TBV/TPC ratio. Sampling for observations of bacterial populations and water quality was carried out every two weeks. Observation of water causity was carried out in two ways, namely in situ field testing (temperature, salinity, DO, pH) and testing in the laboratory ex situ conswasting of (NH3-N, NO3-N, NO2-N, and the percentage of TBV / TPC ratio) with a two-week time interval, Observation of White Spot Syndrome Virus (WSSV) was carried out at the beginning of the study, observation of shrimp vitality was carried out at an interval of one month.

2.1. Pond preparation

The implementation of the study began with the drying of the pond which was carried out ± 2-3 days which aimed to increase the pH of the soil and evaporate the toxins in the previous cultivation pond. Furthermore, probiotic RICA 1 was a non-pathogenic probiotic bacterium, served to eradicate pathogen and kill or break the chain of pathogens that are still available at the bottom of the pond after drying. The next step was to carry out calcification which was useful for improving the pH of the base soil of the pond. In addition to improving acidity it also served as a desinfectant and as a provider of nutrients (phosphorus) that plankton need. Then to grow natural feed (Phytoplankton) in the pond then the next step was to add SP 36 fertilizer and urea.

2.2. Preparation of pond's water

Before the water was flowed to the pond, seawater was first pumped from the drilled well to the reservoir using a spiral suction hose then deposited ± 3 days for filtering and then treated with the addition of chlorine as much as 15-30 ppm and stirred using a pinwheel which functions as a disinfectant on water media to control bacteria and viruses. Furthermore, the water medium was flowed through the pipe.

2.3. Preparation of shrimp larvae

The animal test used was a windu shrimp fry (*Penaeus monodon*) that measures 12 PL and was free from disease. To ensure that the fry used are free from Vibriosis and Virus dissease, sampling was carried out for testing biological parameters, namely the detection of AHPND (Hepatopancreatic necrosis disesase) and WSSV (White Spot Syndrome Virus) molecularly using PCR (Polymerase chain reaction). AHPND and WSSV Parameter Testing was conducted at RIBAFE's Fish and Environmental Health Laboratory. Testing was performed 2 times at the beginning of the study and the end of the study.

2.4. Test parameter

The data collection technique in thwas study was carried out by the experimental method. Using a Complete Randomized Design (RAL) with 2 treatments and 3 replications. Treatment 1. Maintenance of tiger shrimp with the addition of wholecell microalgae *Porphyridium aerugineum*. Treatment 2. Control without the addition of microalgae. The parameters observed in this study were: Percentage ratio between common bacteria (TPC) and *Vibrio* bacteria (TBV). Observation of bacterial populations and water quality parameters was carried out once every two weeks. The calculation of the *Vibrio* bacterial population used the petri dish method with *Vibrio* selective media (TCBSA), while for general bacteria it uses TSA media. The observed water quality parameters consist of in-situ parameters (temperature, pH value, DO, and salinity) and parameters analyzed in the Laboratory (NH3-N, NO3-N, NO2-N and Phosphat). Administration of microalgae wholecell was carried out once a week. *P.aerugineum* was cultured in the plankton laboratory of RIBAFE Maros. Observations of the microalgae population were carried out every two weeks by taking as much as 100L of pond water and sheathed with plankton net and given lugol preservatives to be taken to the plankton laboratory for observation. Calculation of Total Bacteria (TPC and Total Vibrio (TBV) The number of cells in each ml or gram was determined using the following equation:

$$\text{Cfu/Ml} = \frac{T}{Q} \times \frac{1}{S} \times \frac{1}{V} \quad (1)$$

Variable:
T: Number of colony grown on petri dishes
Q: Number of plate used
S: The amount of dilution used
V: The number of sample volumes inoculated to agar media

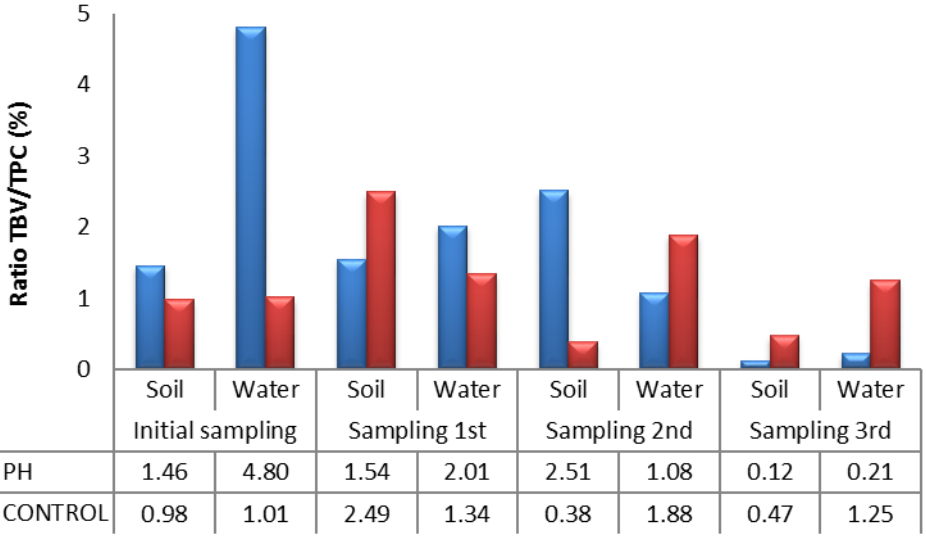
3. Result and Discussion

3.1. Ratio of TBV/TPC percentage

In the treatment with the addition of *P.aerugineum* showed a significant decrease in the TBV / TPC ratio at the end of the study period, especially in pond water. In the 1st sampling, the value of the TBV/TPC ratio in pond water was 2.01%, then the 2nd sampling became 1.08% and in the last sampling it became 0.21%. The improvement in the value of this ratio was thought to be the influence of the provision of *P.aerugienum* wholecell directly into the pond every week. As was known, the *P.aerugineum* microalgae cycle reaching the peak of growth was on the 5th day, so the schedule of adminwastration of this microalgae at the time of its growth reaches the peak. Phycobiliproteins from *Porphyridium aerugineum* contain phycocyanin, which was reported to inhibit the growth of the (G+) S. *aureus* with a MIC of 7 μg/mL [7]. In *Porphyridium. cruentum*, phycoerythrin was reported as an antibacterial agent with a MIC of 0.29 μg/mL against S. *aureus* [7].

The average ratio of total bacteria, both pond water and pond sediment (soil) obtained in this study was still at a very safe level for the organisms being cultivated, where the average ratio

produced was less than 10%, the percentage ratio of TBV / TPC both water and pond sediment if more than 10% was harmful to farmed shrimp.



	Soil	Water	Soil	Water	Soil	Water	Soil	Water
	Initial sampling		Sampling 1st		Sampling 2nd		Sampling 3rd	
PH	1.46	4.80	1.54	2.01	2.51	1.08	0.12	0.21
CONTROL	0.98	1.01	2.49	1.34	0.38	1.88	0.47	1.25

Figure 1. Ratio of TBV/TPC Percentage.

3.2. The density of P. aerugineum in pond water

The results of the identification of the density of plankton *Porphyridium aerugineum* in tiger shrimp farming ponds obtained plankton density between 40,000 sel/ mL to 692,000 sel /mL (Fig. 2). The highest density of *P.microalgae* was processed at the 2nd sampling for all tests on treatment with the addition of microalgae. In the 3rd sampling, there was a decrease in the density of *P.aerugineum* for all treatment tests with the addition of *P.aerugineum* microalgae while in the control treatment there was no *P.aerugineum* microalgae for all samplings carried out. The highest value of plankton density in tiger shrimp farming ponds was obtained in the 2nd PH 3 sampling pond of 692 ind/L and the lowest plankton density value was obtained in PH 1 sampling 1 pond of 40,000 ind /L.

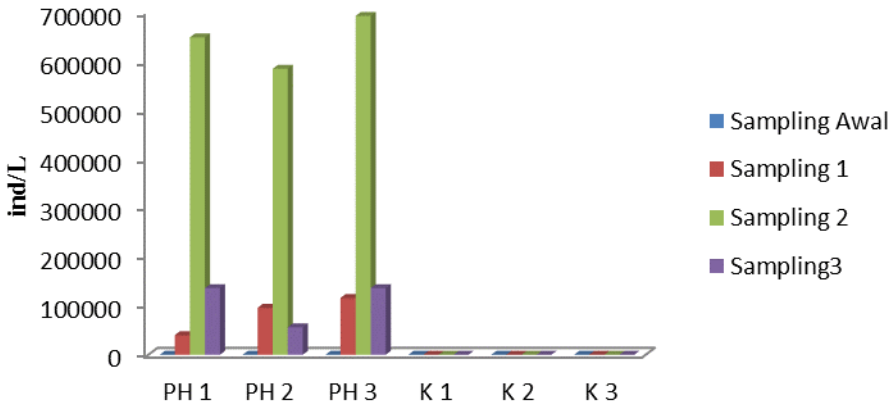


Figure 2. Density of *P.aerugineum* in Pond's water.

3.3. Tiger shrimp vitality (Penaeus monodon)

The results of weighing the vitality (weight) of tiger shrimp (*Penaeus monodon*) obtained an average of 3.13gr to 7.47 grams. In the first sampling, the weight of shrimp for treatment with the addition of microalgae was on average 3.47gr heavier than the control which was only 3.13 g. In the 2nd

sampling, the average weight of shrimp was obtained for treatment with the addition of microalgae of 7.47g while for the control treatment, the average weight of shrimp was obtained at 6.33 g. These results (Figure. 3) showed a positive influence of microalgae adminwastration on the addition of tiger shrimp mass raised even though in principle *P.aerugineum* was not intended as feed but as a vibriosis control.

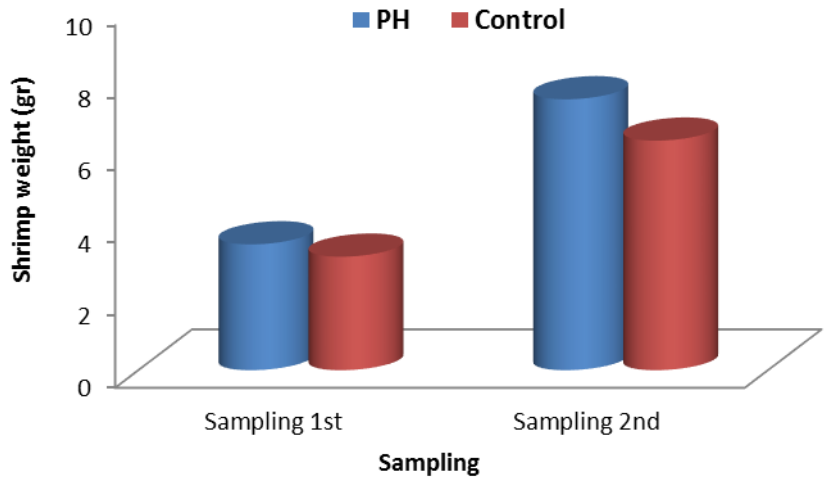


Figure 3. Weight shrimp curve.

3.4. Physical chemistry parameters

In this study, no *Vibrio* bacteria was found fluorescent. This showed that the water of the aquaculture pond was still in a safe condition for the cultivated organisms (tiger shrimp). Naturally, *Vibrio* bacteria is available in ponds (seawater, brackwash water). But it can not be eliminated, it can only be controlled or inhibited. Vibrio bacteria live peacefully as long as there was no significant change in water quality.

The existence of *Vibrio* sp. in aquaculture waters is also influenced by physical and chemical parameters. Chemical parameters affecting the abundance of *Vibrio* bacteria are temperature, dissolved oxygen and organic matter in the cultivation medium. As stated by [9,10] that physical parameters and unfavorable chemical parameters are the cause of the abundance of the number of *Vibrio* sp bacteria. on tiger shrimp rearing water.

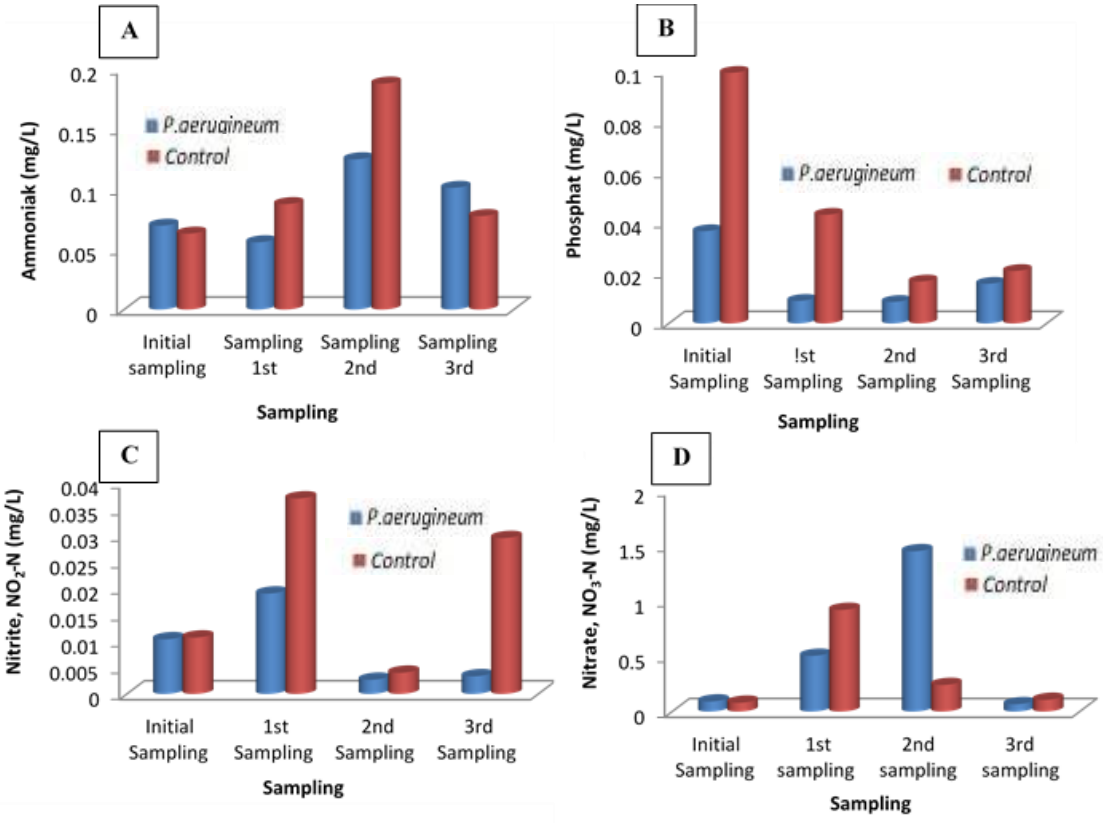


Figure 4. Shrimp pond's water quality. A. Ammonia, B. Phosphat, C. Nitrite and D. Nitrate.

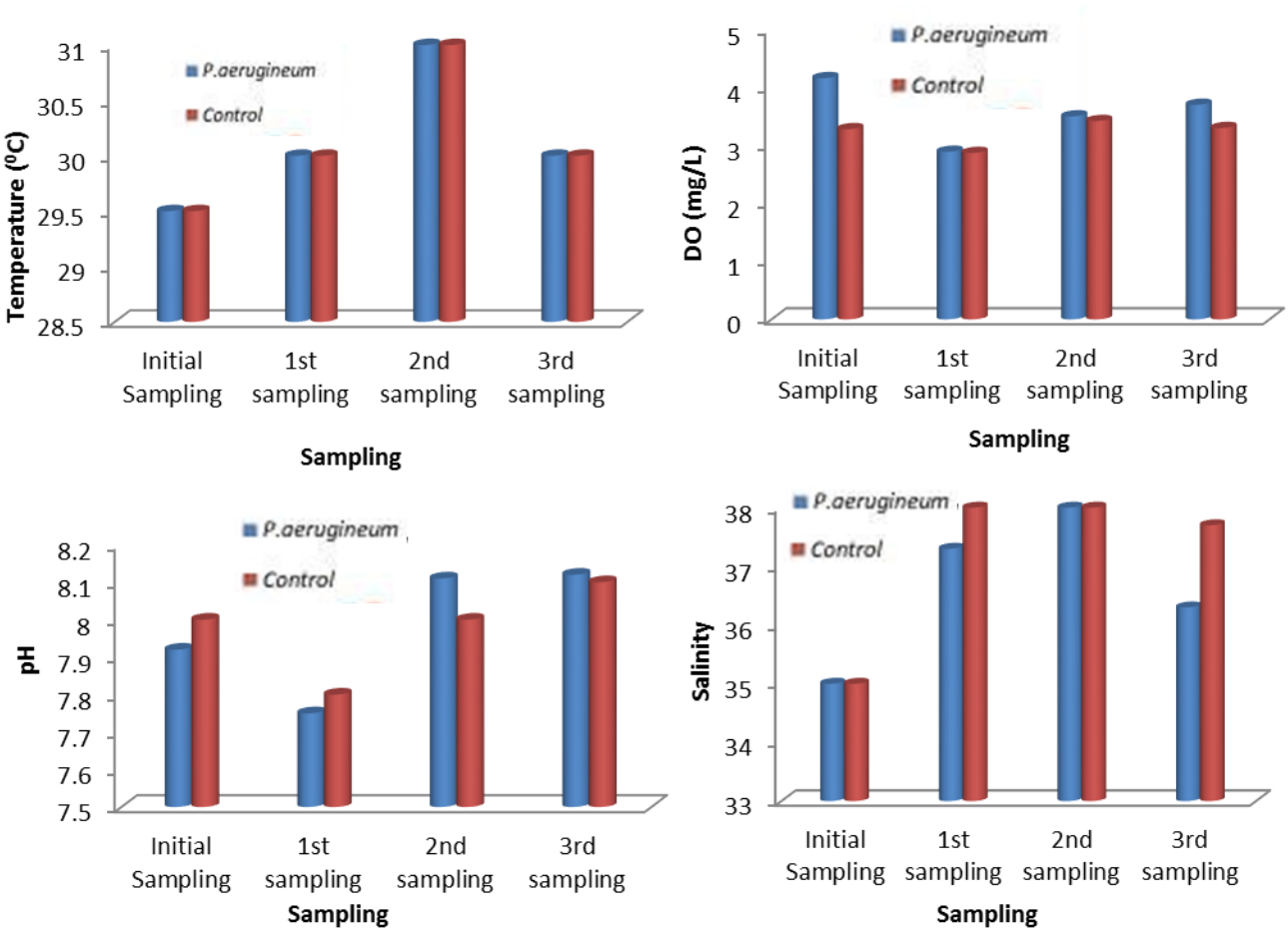


Figure 5. Shrimp pond's water quality. A. Temperature B. Dwassolve Oxygen C. pH and D. Salnity.

In the process of cultivation, there are three stress-provoking factors in shrimp. Namely, pathogens, the environment, shrimp [11]. The biggest provoking factor was stress caused by environmental factors. The next stress-causing factors in shrimp, ranging from metabolic processes that produce feces and are decomposed to the nitrification process. Then water parameters such as Dwassolved Oxygen (DO), Hydrogen Potential (pH), to temperature. Based on several research results, it was known that fluctuations in Nitrite content in ponds greatly affect stress in shrimp.

In general, the pH of water was neutral, the pH of seawater in aquaculture was generally in the range between 6-8.5 [12]. From Figure 5, the maintenance pH ranges from 7-8. Thwas value still indicates a good range for cultivation. The decrease in pH in maintenance water can have bad consequences for cultivation, because it can affect metabolic processes, shrimp / fwash do not want to eat, slow growth which will eventually reduce the degree of survival.

Temperature and salinity was a physical factor that affects living biota. Any change in temperature tends to chemically affect the physiology that occurs in the organs of the body. In aquatic organwasms such as fwash and types of crustaceans such as shrimp, among them are very sensitive to temperature changes. The effect caused by rwasing and falling temperatures there are physiological changes such as diet, swimming movements and others that are closely related to maintenance. the temperature range of waters for cultivation ranges from 28-30ºC. High levels of nitrites trigger stress in shrimp due to the formation of methaemocyanins. So shrimp blood was not able to bind to oxygen, even though do was high. As a result, stress arwases in shrimp.

Nitrification was the process of forming nitrate compounds from ammonium compounds. Thwas process was a process in which ammonium ions are oxidized to nitrite ions, as well as nitrite ions to

nitrate ions. continuous concentration of nitrites with ammonia and nitrates. When ammonia rwases, nitrite rwases so that ammonia falls, then when nitrate rwases, it has an impact on the concentration of nitrites to fall. In the data shown in Figure 4, it can be seen that Nitrite levels in ponds given *P.aerugineum* microalgae are lower than controls. Low Nitrite levels can prevent shrimp from experiencing stress because their blood cannot bind oxygen. In general, it was said that the results of the analyswas of the TBV / TPC ratio in ponds given *P.aerugineum* microalgae are lower than controls as well as Nitrite levels in pond water given *P.aerugineum* microalgae lower than controls.

4. Conclusion

Provision of whole cell microalgae *P.aerugineum* in ponds can reduce the ratio of TBV/TPC and improve pond water quality parameters.

Acknowledgment

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