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Bioremediation of shrimp pond wastewater using effective microorganism-4 (EM4)

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ABSTRACT

The problems of shrimp farming with semi-intensive systems are leftover feed, organic matter, and toxic compounds that can reduce water quality. Shrimp pond wastewater treatment can be done through several technologies, such as chemical, physical, and biological treatment. One of biological treatment that widely used for wastewater treatment is bioremediation. Bioremediation is a biological process that utilizes microorganisms to decompose waste in aquaculture ponds into compounds that are not harmful to aquatic biota. Therefore, this research aims to determine the effect of effective microorganism-4 (EM4) in reducing ammonia compounds and BOD levels in shrimp pond wastewater. The research method used is a laboratory-scale experimental method. A total of 6 aquariums were used for 3 different treatments with two replications, control, adding EM4 2.5% and EM4 3% into 5 L of wastewater. The water quality parameters observed were dissolved oxygen, temperature, pH, ammonia, and BOD. The *one-way* ANOVA test was used in this study. The results showed that there was a decrease in ammonia and BOD levels. The highest reduction of ammonia was found in the treatment with the addition of EM4 2.5%. The decrease in BOD with the highest effectiveness was found in the control treatment (37.73%) with the initial BOD value 5.3 to 3.3. mg/l, which fulfilled the allowed maximum level of standard of Ministerial Decree No. KKP. KEP.28/MEN/2004 concerning General Guidelines for Shrimp Cultivation in Ponds. In conclusion, the small dosage addition of EM4 did not have a significant effect on decreasing levels of ammonia and BOD.

Introduction

Aquaculture is a fishery activity that continues to advance today. According to FAO, aquaculture is the fastest growing food supplier, providing nearly 50% of the fish, shrimp and shellfish needed for human consumption and by 2030, aquaculture is projected to increase to 62% with total production of more than 100 million ton/year. Whiteleg shrimp (*Litopenaeus vannamei*) is one of the superior aquaculture commodities that are widely cultivated in Indonesia. Whiteleg shrimp was first introduced and cultivated in the early 2000s and showed satisfactory production results. Therefore, whiteleg shrimp is also known as powerhouse of shrimp farming business in Indonesia. Whiteleg shrimp has several advantages than other aquaculture commodities, their productivity value reaches more than 13,600 kg/ha, whiteleg shrimp has a high survival rate of 80-100%, quality available fry, disease resistance, high stocking density, and low feed conversion (Sambu *et al.*, 2021).

Semi intensive aquaculture system is one of the aquaculture systems that are widely used, especially for cultivating shrimp, but the problem with shrimp farming with in a semi-intensive system is that residual feed, organic matter, and toxic compounds accumulate quickly (Elfidiah, 2016). The high organic matter, suspended solids, carbon, nitrogen, and phosphorus from feed residues or shrimp metabolism residues, semi-intensive cultivation systems have great potential in reducing pond water quality (Herbeck *et al.*, 2021). Shrimp pond wastewater can generate environmental impacts as

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effluent discharged from shrimp pond, which will potentially be the major source of pollution in several water bodies such as estuarine and marine ecosystem (Bui *et al*., 2012).

Problems regarding the wastewater can be solved by improving water quality management. Improving water quality from contaminants is a process that breaks down harmful organic and in-organic materials into harmless compounds. There are several methods that can be used to improve wastewater quality including chemical, physical, and biological treatments. Although chemical treatment removes nutrient from wastewater, their residual effect due to precipitation can linger in the discharged water for a long time causing further harm for the environment (Iber *et al*., 2021). Current approach to improving aquaculture wastewater is bioremediation (Barman, 2020). Bioremediation refers to optimizing naturally occurring remediation processes carried out by living organisms that degrade, change, or remove toxic organic compounds (Mora-Ravelo *et al*., 2017). Bioremediation is an environmentally safe and cost-effective method to decontaminate pollutant from soil and water, and is emerging as an alternative to costly physicochemical remediation technologies. Bioremediation method has been widely used in various types of aquaculture ponds, such as natural ponds, biofloc system, tidal flat ponds, and etc. (Pujiastuti *et al*., 2017; Sharma *et al*., 2018; Putra *et al*., 2020; Ni *et al*., 2021).

EM4 is a solution containing decomposer microorganisms, *Lactobacillus* sp., *Streptomyces,* cellulose-degrading fungi, lactic acid bacteria, phosphorus solubilizing bacteria, and photosynthetic bacteria (Walandow *et al*., 2020). EM4 is one of probiotics trademark that widely used as feed additive (Fadri *et al*., 2016; Sitorus *et al*., 2018; Dewi *et al*., 2023) as well as improving pond water quality (Khartiono, 2020). The use of EM4 in this research aims to decompose and ferment organic materials deposited on the bottom of the pond, increasing dissolved oxygen levels in the water so that the water quality of the aquaculture ponds remains in good condition for the survival of shrimp. The use of EM4 in reducing organic matter in shrimp pond waste has been carried in a previous research with a percentage decrease in organic matter of 91% from the initial level of 87.74 mg/L (Arfiati *et al*., 2021). The combination of bio-floc system and the addition of EM4 for shrimp farm wastewater in the previous research by Pujiastuti *et al*. (2017) has shown a significant ammonia removal rate up to 88.9%. Therefore, this study aims to determine the effectiveness of the use of Effective Microorganisms (EM4) in reducing ammonia and BOD compounds in shrimp pond wastewater.

Materials and Methods

Site and Time

This research was conducted in May – June 2022 at the Tropical Fisheries and Marine Laboratory, Perikanan Pangandaran, Universitas Padjadjaran. Observations were made in the laboratory to minimize the possibility of environmental contamination. Wastewater samples were collected from Teaching Factory of Shrimp Cultivation, Pangandaran Marine and Fishery Polytechnic. Sampling was carried out from the first discharge of culture pond. As much as 30 L shrimp pond composite wastewater was accommodated in two 15 L gallons of water to the laboratory. The water quality parameters measured were DO (*Dissolved Oxygen*), temperature, pH, ammonia, and BOD. The method used in this research is a laboratory-scale experimental method with three treatments, namely control (K), addition of 2.5% EM4, and addition of 3% EM4 for 5 L of wastewater in each treatment. The percentage of EM4 addition used aims to see its effectiveness in low dosage. All treatment was conducted in two repetitions. Each treatment was tested in duplicate, namely two repetitions.

Seedling

Seeding process is the initial stage that aims to activate microorganisms contained in Effective Microorganism-4 through fermentation. The aims of activation are to ensure the total of bacteria is sufficient enough to carry out oxidation during observation. The materials used in the fermentation comprised of aquarium, aerator, EM4 and pure water. Activation of EM4 was done by mixing as much as 4750 ml of distilled water and 250 ml of EM4, the solution was homogenized in the aquarium, then aeration was carried out for 8 days. Bacterial density was measured every two days using a spectrophotometer to measure the level of turbidity (*Optical Density*) based on the absorbance value. The wavelengths used in measuring absorbance in this research are 600 nm, the wavelength scale used aims to damage and inhibit the growth of bacteria in it. Bacterial density is estimated from the turbidity of the solution and expressed as optical density that typically used at a wavelength of 600 nm (Mira *et al*., 2022).

Water quality measurements

Water quality parameters were measured every five days. Water quality parameters in this research consisted of physical and chemical characteristic. Dissolved oxygen (DO) and temperature were measure using DO meter equipped with probe, while pH was measured using litmus paper. Ammonia value was obtained from testing using an ammonia test kit. The BOD value was obtained from the dissolved oxygen measurement on the first day minus the dissolved oxygen value on day 5, where DO*i* was the initial dissolved oxygen content and DO5 was the dissolved oxygen content in the sample after being incubated for 5 days in the dark bottle and at a constant temperature. The BOD value is the difference between DO*i* and DO5 expressed in milligrams of oxygen per liter (Atima, 2015).

Data Analysis

The analysis used in this research is quantitative descriptive analysis. Decreased in in ammonia and BOD concentration were calculated to determine the effectiveness of the addition of EM4 in shrimp pond waste at the beginning of the observation and at the end of the observation. Decreased concentrations of BOD and ammonia were analysed using statistical analysis one-way ANOVA with F test ($p > 0.05$). The effectiveness formula is calculated according to Saputra *et al.* (2016) as follow:

$$\text{Effectiveness}(\%) = \frac{a - b}{a} x100$$

Where, a = initial value of parameter, b = final value of parameter

Results

The results of water quality measurements obtained are physical and chemical parameters, namely DO, temperature, pH, ammonia, and BOD. The values resulting from the measurement of pH, ammonia, and BOD were compared with the effluent quality standard of shrimp pond waste Ministerial Decree No. KKP. KEP.28/MEN/2004 concerning General Guidelines for Shrimp Cultivation in Ponds.

Bacterial growth

Optical density (OD) expressing the turbidity of the microorganism solution which used to estimate population density. The absorbance value data obtained at 600 nm are presented in the Figure 1. Based on the observation of bacterial growth, the highest absorbance value reached 0.50 in the day 8, while the lowest absorbance value was 0.30 in the second day of fermentation. The absorbance value continuously increased every day during the fermentation process which indicates an increase in bacterial growth.

Characterization of shrimp pond wastewater

Observations shows the temperature value tend to be stable and not volatile. The temperature value of whiteleg shrimp pond wastewater is around 26°C. pH level maintained in stable value during observations for all treatment, namely 8, as can be seen in the Table 1.

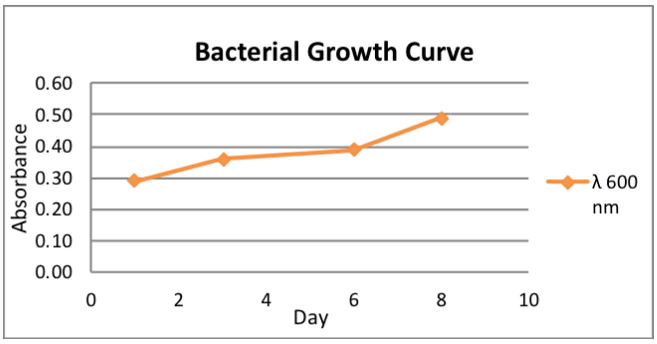

Figure 1. Bacterial growth curve shows an increase in the absorbance value

Table 1. Wastewater characteristics results comparing with quality standard of shrimp pond waste Ministerial Decree No. KKP. KEP.28/MEN/2004 concerning General Guidelines for Shrimp Cultivation in Ponds

Treatments	Parameters							
	pH		Temperature (°C)		Ammonia (mg/l)		BOD (mg/l)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final
K	8	8	26.2	25.0	2	0.5	5.3	3.3
$EM4_{2,5}$	8	8	26.3	24.9	2	0	4.4	3.7
$EM4_3$	8	8	26.3	25.1	0.8	0	4.9	4.3
Standards	6.0-9.0		-		<0.1		<45	

Ammonia reduction

Based on the final ammonia test, the treatment with the addition of EM4 2.5% and 3% resulted in an ammonia value of 0 mg/l in each treatment. This shows that there is a decrease in ammonia levels compared to the first day of observation. The levels of ammonia produced have met the quality standard for effluent from shrimp pond waste Ministerial Decree No. KKP. KEP.28/MEN/2004 concerning General Guidelines for Shrimp Cultivation in Ponds, namely <0.1 mg/l.

BOD reduction

Observations showed that there was a decrease in BOD levels on the 10th day of treatment, although the values obtained were not significant. Control treatment (K) showed the highest BOD reduction percentage which is up to 37.73%. The addition of 2.5% and 3% of EM4 treatment resulted in the lower BOD reduction percentage, namely 15.90% and 12.24%, respectively. Changes in the

BOD value during observations can be seen in the Figure 1.

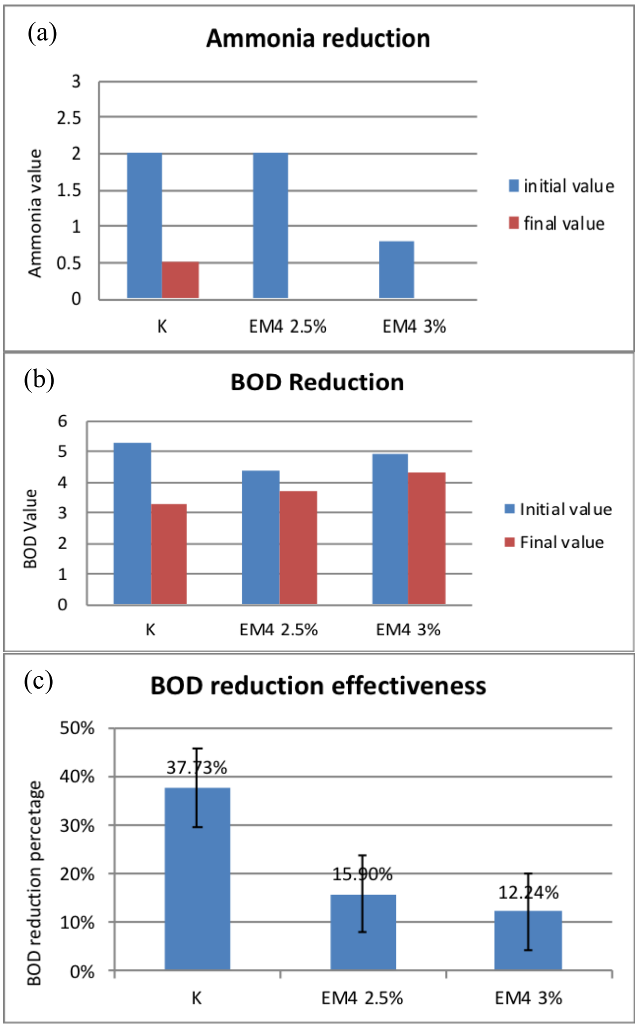

Figure 2. Reduction of ammonia and BOD concentrations (a) Decreased in ammonia concentration was higher in the treatment of EM4 addition (b) Decreased in BOD concentrations showed lowest reduction in the addition of 3% EM4 treatment (c) Percentage of reduction effectiveness showed that control treatment has the highest value

Discussion

Microbial growth is divided into growth phase, namely the lag phase, the exponential phase, the stationary phase, and the death phase. The lag phase is also known as the adaptation phase, which is the initial phase where microbial activity is adjusted to its new environment. The exponential or logarithmic phase is a phase where there is an increase in activity and the number of bacteria that reaches the maximum speed. The stationary phase is a phase of activity balance or a balance occurs between individuals who die and the addition of individuals. The stationary phase can occur due to availability of nutrient sources are decreasing, inhibitory compounds begin to form, and environmental factors are unfavorable. The death phase is the phase where the activity or colony growth begins to stop. In this phase, individual mortality begins to exceed its addition (Nurhajati *et al.*, 2016).

The application of microorganisms-4 (EM4) did not show a significant effect on the growth performance. The lag phase occurs on day 3 where the bacteria adjust to their new environment. The exponential phase is clearly visible at a wavelength of 600 nm where there is an increase in bacterial activity. On day 8, there was an increase in bacterial growth, where in that phase the fermented bacteria were directly added to the shrimp pond wastewater. The increase of bacterial growth are influenced by the availability of adequate nutrients, optimum pH, and favorable temperature (Lizayana *et al.*, 2016). The absorbance value of the light passed is proportional to the concentration of the OD solution in the cuvette (Seniati *et al.*, 2019).

Wastewater temperature showed a stable value in 15 days observations. The stability of the wastewater temperature is caused by the small concentration of EM4 in the treatment so that there is not much increase in the density of microorganisms in the media and the gas exchange in the media is relatively small. If the higher concentration of EM4 was used, it is estimated that there will be an increase in the density of microorganisms so that the population in the media increases and the gas exchange in the media increases. With such conditions, there is a possibility of an increase in the temperature of the media (Maharsyah *et al.*, 2013). In addition, other factors that cause temperature fluctuations are environmental factors. Waste temperatures are generally in the value of 25-29°C because this temperature range is the optimal temperature for the growth of microorganisms (Widiatmono *et al.*, 2018).

Based on the observations made, a stable pH value was obtained. The pH level obtained was 8, which is belongs to the alkaline. The high pH value is caused by the large amount of organic matter contained in the waters. pH is also one of the environmental factors that affect the growth and activity of ammonia-oxidizing bacteria. Acidic bacteria activity allows increase in pH due to NH_4^+ that bond with OH^- which enabled the NH_4OH (alkaline) formation (Arfiati *et al.*, 2021). The optimum pH value for autotroph bacteria in order to oxidize ammonia is between 7.5-8.5 (Hastuti, 2011). The pH value in waters is influenced by DO concentration, the higher the DO concentration, the smaller CO_2 concentration value so that the pH value decreases and is in normal condition (Romadhona *et* *al.*, 2016). This statement is in line with the treatment given during the observations and the results of the observations that have been made. Based on the quality standard of shrimp pond waste effluent, Decree of the Minister of Marine Affairs and Fisheries No. KEP.28/MEN/2004, the pH value of 8 meets the pond wastewater quality standard.

All treatments showed that there was a decrease in ammonia levels. However, the highest reduction in ammonia levels occurred in the treatment with the addition of 2.5% EM4. The decrease in ammonia can occur due to the heterotrophic process of bacterial biosynthesis that utilizes ammonia and the chemoautotrophic nitrification process that converts nitrite compounds into nitrates (Saputra *et al.*, 2016). The addition of EM4 aims to decompose organic compounds such as carbon, hydrogen, oxygen, and nitrogen. Microorganisms will then decompose these compounds into simpler organic compounds such as carbon dioxide and ammonia (Badrah *et al.*, 2021).

The results of BOD removal effectiveness in this research showed that the control treatment had an effectiveness level of 37.73%. The addition of 2.5% EM4 treatment gave an effectiveness percentage of 15.90%, while the treatment with the addition of 3% EM4 has an effectiveness level of 12.24%. Pitriani *et al.* (2015) reported that the addition of EM4 (5%) is able to accelerate the efficiency of pollutant removal in hospital wastewater using biofilter. The decrease in BOD levels indicated the activity of lactic acid bacteria (*Lactobacillus* sp.) contained in EM4. These bacteria ferment organic matter in shrimp pond waste into lactic acid compounds that can accelerate the decomposition of organic matter in water compared to natural decomposition (Sari *et al.*, 2017). The less significant effect on the observation results is influenced by several factors such as the low microorganisms concentrations ratio with wastewater used. This is in accordance with the previous research (Saputra *et al.*, 2016), where the best microorganism concentration range is between 1 mL – 10 mL, the lower the concentration of fermented microorganisms, the lower the density level will be.

The results of the *one-way* ANOVA test showed that the effect of adding EM4 to wastewater where F-count<F-table then the treatment has no significant effect. In addition, p-value>0.05, which means the addition of EM4 does not have a significant effect on reducing ammonia and BOD levels. This is in accordance with the research of (Badrah *et al.*, 2021), where the addition of EM4 did not produce a difference in the reduction of ammonia levels in wastewater. The insignificant effect can be caused by several factors such as the ratio of wastewater to the concentration of bacteria used.

Further research is highly recommended to determine the effective concentration of EM4 for reducing ammonia and BOD levels in shrimp pond wastewater. The findings of this study indicate that higher concentration of EM4 might be resulting in significant effect of nutrient removal. As for the bacterial fermentation process, the addition of molasses can be done as carbon source in bacterial growth.

Conclusions

Based on our observation, it can be concluded that the addition of EM4 at a dose of 2.5% and 3% did not significantly affect the improvement of wastewater quality during the bioremediation process. Temperature and pH values at the time of observation tended to be stable, there was an increase and decrease in several treatments, as well as an insignificant decrease in BOD values. Thus, the addition of EM4 with the concentration 2.5% and 3% did not show any significant effect on improving the quality of shrimp pond wastewater.

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