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Effect of *Rhodopseudomonas palustris* as a Bioremediation on Reducing Ammonia and Nitrite Levels of Catfish Nursery Pond Water in District Kebakkramat Regency Karanganyar

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Abstract

Catfish nursery pond water generally contains ammonia and nitrites. Ammonia and nitrite in this fish farming system start with uneaten catfish feed residue, feces, and metabolic products that enter the waters. The rest will be excreted in the form of feces. Feces and food scraps that settle in this water contain ammonia and nitrites, which affect the quality of the water. Efforts to control water pollution is by using bioremediation techniques. The aim of bioremediation techniques is to restore the ecological balance in waters by utilizing microorganisms so that a process of degradation of harmful and complex compounds occurs into harmless compounds. The microorganisms used in this study were bacteria Rhodopseudomonas palustris which can to oxidize and decompose organic matter effectively reducing water quality. This research was conducted to examine the effect of bacterial efficiency R. palustris as a bioremediator for catfish pond water waste based on reducing levels of ammonia and nitrite. Testing the ammonia and nitrite levels using a spectrophotometric method with different wavelengths. Observations in this test were carried out for three days under anaerobic conditions and light at a temperature of 33°C. Observations showed that bacteria R. palustris had an effect on reducing nitrite levels 12.65% greater than the control. While the addition of bacteria to the observation of ammonia levels did not have a decreasing effect as indicated by the value F <F crit.

Keywords: *Ammonia, Bioremediation, Catfish nursery pond water, Nitrite, Rhodopseudomonas palustris*

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INTRODUCTION

Cultivation of catfish nurseries requires good water quality management of the feed consumed. The high protein content in feed can be absorbed by fish as much as 20-30% of nutrients. The rest will be excreted in the form of feces (Wahyuningsih and Gitarama 2020). Increased feces and food residue that settles in water cause poor water quality because it contains ammonia and nitrites. This poor water quality can impact the development of fish. Therefore efforts are needed to maintain water quality. The most common thing in preserving water quality is reducing and disposing of catfish pond water from streams/rivers every two days. The activity of discharging pond water into the river can be categorized as water pollution (Rasa, Rebhung, and Tallo 2018).

Bioremediation is an effective cleaning technique for removing toxic waste from polluted environments that is gaining popularity (Bala et al. 2022). Bioremediation techniques in efforts to control water pollution are very beneficial compared to methods that use chemicals. This is because there is a process of degradation of microorganisms in decomposing water pollutants. The metal pollutants in the water are absorbed by many microorganisms so that the pollutant levels in the water are reduced. On the other hand, microorganisms will die if the pollutant levels are too high when compared to the number of degrading microorganisms (Saini et al. 2020).

Water pollution control can be pursued with bioremediation techniques. The bioremediation technique aims to restore the ecological balance in the waters by utilizing microorganisms so that a process of degradation of harmful and complex compounds occurs into harmless compounds (Priadie 2012). Microorganisms that play a role in bioremediation, one of which is *Rhodopseudomonas palustris. Rhodopseudomonas palustris* is a gram-negative bacterium, purple species, capable of growing anaerobically in light and aerobically in dark conditions with an optimal growth temperature of 30^{O}C-37^{O}C (Navid et al. 2019). Bacteria *Rhodopseudomonas palustris* have the capacity *biodetosification* and *biodegradation* of livestock and industrial components waste. The content in the waste includes fat, nitrite, chloride, and aromatic compounds (Li et al. 2022). The purpose of this study was to examine the effect of bacteria *Rhodopseudomonas palustris* as a bioremediation in reducing ammonia and nitrite levels in catfish nursery pond water.

METHOD

Time and Place of Research

Sampling was carried out on November 21 2022, at the catfish nursery pond in Pulosari Hamlet, Kebakkramat District, Karanganyar Regency. Sampling was carried out in the afternoon. Sampling was carried in afternoon because the feeding was carried out in the morning, so that the metabolic results produced by fish in the form of feces became more after the morning (Pramleonita et al. 2018).

Sampling and Analysis

Samples were taken as much as 20 liters from the pool water drain to the water flow using a gallon that had previously been cleaned. Furthermore, the samples that have been taken are stored in cool a storage temperature 4^{O}C. Initial sample analysis was carried out by analyzing the total levels of bacteria, ammonia and nitrite. Furthermore, the test sample was prepared with the addition of bacteria *Rhodopseudomonas palustris* 12 mL with 3 repetitions. Meanwhile, in the control sample, bacterial medium was added a *Rhodopseudomonas palustris* 12 mL with 3 repetitions.

Samples were incubated at 33^{O}C under anaerobic conditions. Sample incubation using the help of a light that is on and monitored using a thermometer. Ammonia and nitrite levels were tested every day for three consecutive days.

Medium Creation *Rhodopseudomonas palustris*

The materials used are weighed with the following details; *malic acid* 4.02 grams, KH_2PO_4 0.6 grams, $MgSO_4$ 0.12 grams, K_2HPO_4 0.9 grams, $CaCl_2$ 0.075 grams, In EDTA 0.02 grams, $(NH_4)_2SO_4$ 1 gram. The material is dissolved in 1000 mL of distilled water and then cooked until boiling. The boiling media is then poured into bottles to be further sterilized *autoclave* at a temperature of 121^{O}C at 1 atm pressure for 20 minutes.

The autoclaved medium was allowed to cool to room temperature for further use in the control sample preparation. Furthermore, for the preparation of treatment samples using a control medium that has been inoculated with bacteria *Rhodopseudomonas palustris*.

Determination of Ammonia Content

25 mL of sample was taken, and 1 mL of phenol solution was added and homogenized. Then 1 mL sodium nitroprusside solution was added and homogenized. Also added was 2.5 mL oxidizing solution and homogenized. The next step is to cover the solution with plastic and leave it for 1 hour so that the color forms. The determination of ammonia levels was carried out using the phenate spectrophotometric method SNI 06-6989.30-2005 (Badan Standardisasi Nasional 2005), and the absorbance was read at a wavelength of 640 nm.

Determination of Nitrite Rate

The determination of nitrite content was carried out using the spectrophotometric method SNI 06-6989.9-2004 (Badan Standardisasi Nasional 2004), where the absorbance was read at a wavelength of 543 nm in an acidic atmosphere of pH 2-2.5. This is because nitrite will react with sulfanilamide and N-(1-naphthyl) ethylene diamine dihydrochloride to form azo compounds, which are purplish red in color.

Determination of Total Bacteria

Each sample weighed 10 grams and dissolved in 90 mL of 0.85% physiological salt. Homogenized using a vortex. This dilution is called 10^{-1}. Then at 10^{-1}, 1 mL was taken to be dissolved in 9 mL of 0.85% physiological salt. Homogenized using a vortex. Call this dilution 10^{-2}. Then at 10^{-2},1 mL was taken to be dissolved in 9 mL of 0.85% physiological salt. Homogenized using a vortex. Call this dilution 10^{-3}. And so on until it reaches the 10^{-6}

Each dilution was taken 1 mL to be included in a petri dish. Furthermore, each petri dish containing the inoculant is added to the media for specifications *Rhodopseudomonas palustris.* Incubation was carried out in an incubator at 33^{O}C for two days. After 2 days, the number of colonies growing in each petri dish was carried out. Determination of the number of colonies is calculated by the formula.

$$Number\ of\ colonies\ x \frac{1}{dilution\ factor} = CFU/mL$$

Data Management and Analysis Methods

This study uses descriptive and quantitative data analysis techniques. Processing data using statistical graphs comparison of the effect of bacteria *Rhodopseudomonas palustris* to each decrease in ammonia and nitrite levels. The data that was obtained was analyzed

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with Microsoft Excel using Two Way Analysis of Variance (ANOVA *Two way*).

RESULTS AND DISCUSSION

Total Bacteria *Rhodopseudomonas palustris*

Determination of total bacteria in this study using the method *for flat*. The following is the result of calculating the total bacteria *Rhodopseudomonas palustris.* The Total bacteria yield of *Rhodopseudomonas palustris* used in the treatment sample is 7,6 x 10^3 CFU/mL. The bacteria were then given 12 mL for every 1000 mL of the treatment sample. Furthermore, calculating the total bacteria in each bottle of treatment sample becomes 9,12 x 10^4 CFU/mL.

Table 1. Results of Total Bacteria *Rhodopseudomonas palustris*

No	Dilution Factor	Number of Colonies	TPC results
1	10^{-1}	TBUD	
2	10^{-2}	76	7,6 x 10^3
3	10^{-3}	3	CFU/mL
4	10^{-4}	0	

(*CFU = *Colony Forming Unit*)

Rhodopseudomonas palustris Against Ammonia

Ammonia is a chemical parameter with the initial form of N-organic in water. Based on Ernawati's 2014 research on water quality in catfish seedling cultivation, ammonia levels ranged from <0.8 mg/L (Ernawati, Prayogo, and Rahardja 2019). If the ammonia concentration in waters is > 0.8 mg/L, it is toxic to aquatic organisms. The following is a graph of ammonia observations in each sample for three days.

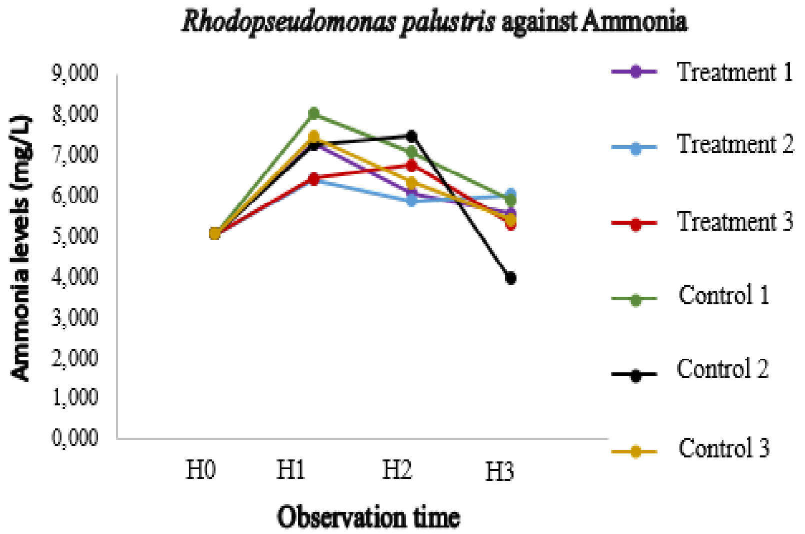

Figure 1. *Rhodopseudomonas palustris Agains Nitrite*

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The data were analyzed using the ANOVA statistical test Two way on Microsoft Excel. Table 2 shows that the effect of a significant difference is shown at the time variation. Ammonia levels decrease over time. While in the variation of the treatment of bacteria, *Rhodopseudomonas palustris* does not give a significant difference because ammonia levels tend to be unstable in waters. There are two forms of ammonia in water, namely ammonium ions ($NH4^{+}$) and non-ammonium ions (NH_3). According to research (Murti, Purwanti, and Suyati 2013), only the form of ammonium ions can be removed from the solution by ion exchange. In this study the temperature used was 33°C. This is because the optimal temperature for the growth of *Rhodopseudomonas palustris* bacteria is 30-35°C. Based on research (Barnie 2013), states that at a temperature of 10°C, the concentration of ammonia is not ionized by 60% while at a temperature of >30°C, the concentration of ammonia is not ionized by 80-90%. This means that the higher the temperature of the solution, the higher the concentration of non-ionized ammonia.

Table 2. Results of Two Way ANOVA Analysis on Ammonia

No	Variations	F	P-value	F crit
1	Treatment	1,586832	0,225845	4,493998
2	Time	22,53126	5,48E-06	3,238872
3	Interaction	2,509665	0,095681	3,238872

Administration of bacteria *Rhodopseudomonas palustris* does not have a significant effect on decreasing ammonia levels because the process of oxidation of ammonia into other forms requires oxygen with concentrations above 60% or above 5 mg/L (Wahyuningsih and Gitarama 2020).

Research results (Hidayah et al. 2018) also stated that the provision of oxygen (aeration) and a residence time of 4 days in domestic wastewater reduced ammonia levels by 97%. This is reinforced by research (Alkahf et al. 2021) which states that the ratio of ammonia levels in wastewater before aeration is 2.94 mg/L and after aeration is 1.76 mg/L. This resulted in a decrease in ammonia levels of 40.14%. This means that the bacteria *Rhodopseudomonas palustris* in anaerobic conditions (without oxygen) is less optimal in reducing ammonia levels.

Rhodopseudomonas palustris Against Nitrites

Nitrite is part of the nitrogen cycle and a transition between ammonia and nitrate. Based on Ernawati's 2014 research on the quality of catfish culture water, nitrite levels ranged from <0.05 mg/L (Ernawati et al. 2019). If the nitrite concentration in water is > 0.05 mg/L, it can be toxic to aquatic organisms. The following graph shows nitrite observations in each sample for three days.

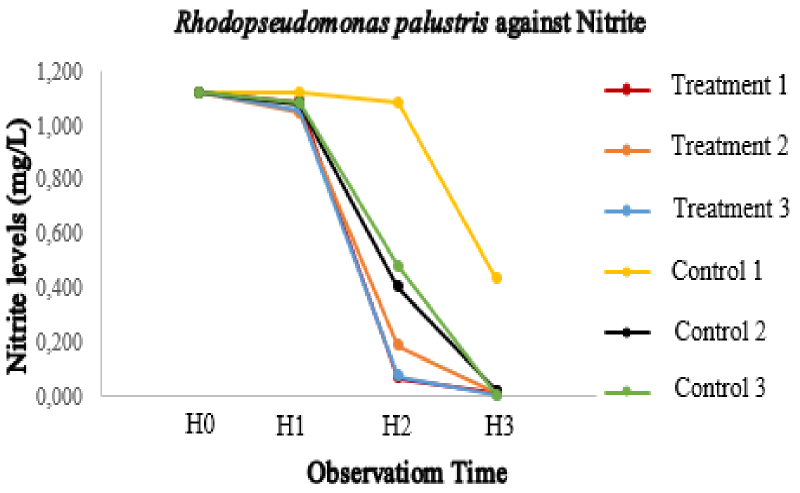

Figure 2. *Rhodopseudomonas palustris Agains Nitrite*

The data were analyzed using the ANOVA statistical test Two way on Microsoft Excel. Table 3 shows that the decrease in nitrite levels after the addition of the bacteria *Rhodopseudomonas palustris* has a significant difference in control, time variation, and interaction between the two. This is indicated by value *F* > *Fcrit* and *P-value* the resulting < 0.05, which means the confidence level of the data exceeds 95%.

Table 3. Results of Two Way ANOVA Analysis on Nitrite

No	Variations	F	P-value	F crit
1	Treatment	54,35484934	8,58715E-06	4,7472253
2	Time	1235,555602	1,27383E-14	3,8852938
3	Interaction	46,52881826	2,22086E-06	3,8852938

According to the journal (Zhai et al. 2021), the bacteria *Rhodopseudomonas palustris* included in the nonsulfur bacteria that can denitrify. Denitrification is the process of reducing nitrate to nitrogen gas through nitrites, nitrous oxide and nitric oxide.

Administration of *Rhodopseudomonas palustris* has a significantly different effect on reducing nitrite levels due to bacteria *Rhodopseudomonas palustris*'s ability to detoxify and biodegrade waste components, which include fats, nitrites, chlorides, aromatic compounds (Li et al. 2022). Anaerobic conditions also affect the stability of nitrite because there is no oxygen in it. Low dissolved oxygen levels cause the overhaul of organic matter through biological processes (Setiowati, Roto, and Wahyuni 2016).

Arashida's research, 2018 states that *Rhodopseudomonas palustris* combined with bacteria *Bacillus subtilis* capable of oxidizing iron, degrading aromatic compounds, and can have nitrogen fission under anaerobic conditions with oxygen content < 20.95% (Arashida et al. 2019).

CONCLUSION

Provision of bacteria *Rhodopseudomonas palustris* did not give a significantly different effect on decreasing ammonia levels but gave a significantly different effect on reducing nitrite levels under anaerobic conditions. The reduction in nitrite levels produced after adding these bacteria had the advantage of being 12.65% faster than without the addition of bacteria.

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