JPPIPA 11(2) (2025)

Jurnal Penelitian Pendidikan IPA

Journal of Research in Science Education

http://jppipa.unram.ac.id/index.php/jppipa/index

Evaluation of the Effectiveness of Organic Matter Biodegradation by a Bacterial Consortium in Vannamei Shrimp Farming Wastewater

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Received: July 08, 2024
Revised: December 11, 2024
Accepted: February 25, 2025
Published: February 28, 2025

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DOI: 10.29303/jppipa.v11i2.9983

Abstract: This study evaluated the effectiveness of a bacterial consortium for biodegrading organic waste in vaname shrimp (*Litopenaeus vannamei*) farming wastewater. A completely randomized design with four treatments (1, 2, and 3 ppm, plus a control) and three replicates per treatment was used. The consortium, consisting of *Lactobacillus casei*, *Bacillus subtilis*, *Bifidobacterium sp.*, *Nitrosomonas sp.*, and *Nitrobacter sp.*, was cultured in a medium containing molasses, rice bran, brown sugar, and whole milk. Over a 5-day incubation period, key parameters (TOM, BOD, ammonia, nitrite, nitrate, and bacterial viability) were measured. The 3 ppm treatment achieved the highest reductions TOM by 61.4%, BOD by 69.57%, ammonia by 68.92%, nitrite by 67.59%, and nitrate by 62.02% with bacterial viability increasing by 165% compared to the control. These results demonstrate that the optimal dose significantly enhances biodegradation under conditions of 24.3–28.9°C, pH 7.8–8.5, and DO 3.5–6.8 mg/L. In conclusion, applying the bacterial consortium at 3 ppm offers an eco-friendly and effective strategy to improve water quality in intensive shrimp aquaculture, supporting national food security and Blue Economy initiatives.

Keywords: Bacterial consortium; Biodegradation; Organic waste; Shrimp farming wastewater; Water quality

Introduction

Farmers increasingly intensify *L. vannamei* farming to meet the increasing market demand yearly. However, this practice faces significant challenges, mainly related to the decline in water quality due to the accumulation of organic waste (Khoa et al., 2020). This waste comes from feed residues, shrimp excretions, and other organic compounds formed during farming (Nguyen et al., 2019). If not appropriately managed, organic waste can lead to decreased dissolved oxygen levels, increased Total Organic Matter (TOM), and accumulation of toxic compounds such as ammonia and nitrite, negatively affecting shrimp health and survival rates (Anwar et al., 2024).

The impact of water quality degradation due to organic waste is detrimental to farmers through reduced productivity and increased shrimp mortality (Pratiwi et al., 2023). It contributes to the degradation of the surrounding aquatic environment. Untreated effluent discharge can trigger eutrophication and cause imbalance in aquatic ecosystems (Ayilara & Babalola, 2023). Therefore, environmentally friendly technology-based aquaculture effluent management is urgently

How to Cite:
Nada, A. F., Susilowati, S. R., Hertika, A. M. S., & Maftuch. (2025). Evaluation of the Effectiveness of Organic Matter Biodegradation by a Bacterial Consortium in Vannamei Shrimp Farming Wastewater. *Jurnal Penelitian Pendidikan IPA*, *11*(2), 172–183. https://doi.org/10.29303/jppipa.v11i2.9983

needed to ensure the aquaculture industry's sustainability (Tangguda & Prasetia, 2019).

One solution has been developed using bacterial consortium-based biotechnology to accelerate the biodegradation process of organic waste, thereby effectively maintaining water quality (Ulfah et al., 2024). This process involves microorganisms breaking down complex organic compounds into more straightforward and non-toxic forms. Several studies have shown that lactic acid and nitrifying bacteria can accelerate the decomposition process and reduce the concentration of pollutants in cultured water (Muthu et al., 2024).

The efficiency of the biodegradation process is highly dependent on the type and composition of bacteria used (Kumari & Kumar, 2023). Therefore, this study aims to evaluate the effectiveness of a self-cultured bacterial consortium with a bacterial composition of *Lactobacillus casei*, *Bacillus subtilis*, *Bifidobacterium sp*., *Nitrosomonas sp*., and *Nitrobacter sp*. in degrading organic waste in *L. vannamei* farming wastewater. By using biodegradation methods, this research is expected to provide practical solutions in pond waste management and contribute to the sustainability of the aquaculture sector.

Method

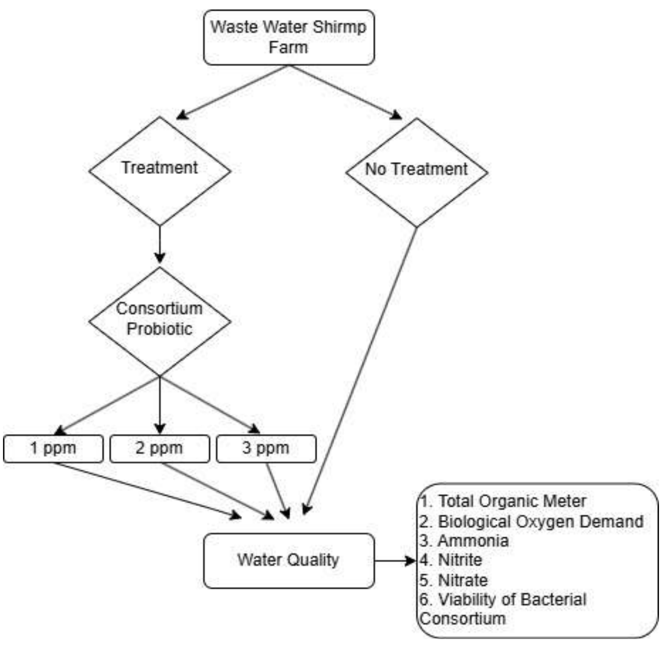

Figure 1. Flowchart of research flow

This study evaluated the impact of using bacterial consortium as a biodegradation agent to handle organic matter waste in *L. vannamei* farming. The bacterial consortium was obtained from the results of a pure isolate culture. The sample water was obtained from shrimp farming water that had been reared for 54 days. The research method experimentally used a complete randomized design with 3 treatments and 1 control with 3 replicates each. Samples of residual water from *L. vannamei* farming were put into 12 treatment containers of 3 liters. Then, every 3 treatment containers were added with bacterial consortium by the predetermined treatment dose, namely treatment A: 1 ppm, treatment B: 2 ppm, treatment C: 3 ppm, and treatment K without the addition of bacterial consortium as control. This study was conducted for 5 days or 120 hours by measuring the concentration of total organic matter (TOM), *biological oxygen demand* (BOD), ammonia (NH3), nitrite (NO2), and nitrate (NO3). In addition, bacterial viability was also measured to determine the density of bacteria in each treatment. Furthermore, water quality parameters, including temperature, pH, and dissolved oxygen, were also observed to determine more specific characteristics of the sample water. The flowchart is presented in Figure 1. to clarify the research flow.

Bacterial Consortium Culture

The bacterial consortium in this study comprised *Lactobacillus casei*, *Bacillus subtilis*, *Bifidobacterium sp*., *Nitrosomonas sp*., and *Nitrobacter sp*. These isolates were obtained from the Faculty of Medicine, Universitas Brawijaya, Malang. The consortium was cultured in a mass medium containing 1 liter of molasses, 600 grams of rice bran, 1 kg of brown sugar, and 1 liter of whole milk, all dissolved in water to 20 liters. The medium was boiled, cooled to room temperature, and transferred to an airtight container. The bacterial species were mixed into the medium, sealed, wrapped in black plastic to prevent contamination and sunlight exposure, and allowed to ferment for 36 hours.

Total Organic Matter (TOM) Analysis

The total organic matter (TOM) was measured using the $KMnO_4$ titrimetric method. In summary, 25 mL of the sample water was placed in an Erlenmeyer flask, then 4.75 mL of 0.01N $KMnO_4$ and 5 mL of H_2SO_4 (1:4) were added. The mixture was heated on a hotplate to 75 °C and then maintained at 60°C. Next, 0.01N oxalic acid was added slowly until the solution became colorless, followed by titration with 0.01N $KMnO_4$ until a faint pink color appeared; the volume used was recorded as x mL. A blank titration with distilled water (recorded as y mL) was also performed, and the TOM was calculated using the formula provided.

$$\text{TOM (mg/L)} = \frac{(x - y) \times 31.6 \times 0.01 \times 1000}{\text{mL water sample}} \quad (1)$$

Biological Oxygen Demand (BOD) Analysis

BOD analysis is performed to determine the oxygen demand of a water sample by incubating it for 5 days at 20 °C under dark conditions to prevent any photosynthetic oxygen production. First, 250 mL of sample water is collected, and the initial dissolved oxygen (DO) level (DO_0) is measured using a DO meter. The sample is then transferred to a Winkler bottle, which is tightly sealed to prevent any oxygen exchange with the environment. The sealed bottle is placed in a BOD incubator cabinet maintained at 20 °C for 5 days. After the incubation period, the DO is measured again and recorded as DO_5. The BOD value is calculated by taking the difference between the initial and final DO concentrations, indicating the amount of oxygen consumed by microbial activity during the incubation period.

$$\mathrm{BOD} = \mathrm{D_0} - \mathrm{D_5} \tag{2}$$

Ammonia (NH3) Analysis

Analysis of ammonia concentration is carried out according to the standard method of APHA (2017), using a spectrophotometer with the help of Nessler solution, which reacts with ammonia to form a yellow-brown complex compound. The ammonia measurement procedure are: enter 10 mL of sample water into Erlenmeyer; add 1 mL of Nessler solution, then stir until homogeneous and let stand for 10 minutes at room temperature to complete the reaction; and measure using a UV-Vis spectrophotometer at a wavelength of 425 nm, with deionized water used as a blank. The concentration of ammonia in the samples was calculated based on the calibration curves obtained from ammonia standard solutions of various concentrations.

Nitrite (NO2) Analysis

Analysis of nitrite concentration was performed using a spectrophotometric azo staining method based on APHA (2017). In brief, 10 mL of sample water was filtered using 42 µm Whatman filter paper, then 0.5 mL of sulfanilamide solution was added and allowed to stand for 2–3 minutes. Next, 0.5 mL of Naphthyl ethylenediamine dihydrochloride (NED) solution was added, and the mixture was left for 10 minutes at room temperature until the color stabilized. The absorbance was measured at 543 nm using a UV-Vis spectrophotometer with deionized water as the blank, and nitrite concentration was calculated using calibration curves from standard nitrite solutions.

Nitrate (NO3) Analysis

Nitrate concentration analysis uses a spectrophotometric azo staining method (APHA, 2017). Briefly, 10 mL of sample water is filtered through 42 µm Whatman filter paper. Then, 0.5 mL of sulfanilamide solution is added and allowed to stand for 2–3 minutes, followed by adding 0.5 mL of Naphthyl ethylenediamine dihydrochloride (NED) solution. The mixture is left at room temperature for 10 minutes until the color stabilizes. Absorbance is measured at 543 nm using a UV-Vis spectrophotometer with deionized water as the blank, and nitrite concentration is determined from calibration curves using standard nitrite solutions.

Analysis of Bacterial Viability

Bacterial viability was measured using the Total Plate Count (TPC) testing method. Calculation of bacterial viability was carried out with the following procedure: 1 ml of chili water was suspended in 9 ml of sterile 0.85% NaCl solution and vortexed until homogeneous to produce multilevel dilutions; 100 µL of the dilution results to be planted on agar media using the spread plate method on a Petri dish; then incubate at 30-37 °C for 48 hours. After the incubation period, count the number of bacterial colonies that grow on the dish using a *colony counter*, and bacterial viability (cfu/ml) is calculated using the formula.

$$\text{Viability (cfu/mL)} = \frac{\text{Colony count} \times \text{Total dilution factor}}{\text{Volune of culture plated}} \tag{3}$$

Data Analysis

The results were then analyzed using OneWay Variance Analysis with the IBM SPSS 27 Application to determine the effect of giving 3 doses of bacterial consortium on the effectiveness of organic waste biodegradation for 5 days of observation. The results, with a significant impact with a confidence level of < 0.05%, were then continued with the Duncan test to determine the correlation between treatments.

Result and Discussion

Organic waste management in *L. vannamei* farming is a significant challenge that affects water quality and production sustainability. This study evaluated the effectiveness of bacterial consortium as a biodegradation agent in overcoming organic waste through the analysis of several water quality parameters. The parameters analyzed included Total Organic Matter (TOM), Biological Oxygen Demand (BOD), ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), and viability of the bacterial consortium, as well as supporting parameters such as pH, temperature, and dissolved oxygen observed during the 5-day study. The observation results of each parameter are presented in Figures 1, 2, 3, 4, 5, and 6.

Total Organic Matter (TOM)

The measurement results of Total Organic Matter (TOM) levels showed a decrease in all treatments for five days of observation. The initial TOM value before treatment was 92.5 mg/L. In the control treatment (K), without the addition of bacterial consortium, the TOM level decreased to 69.4 mg/L or about 44.4%. In treatment A (1 ppm), the TOM level decreased to 51.6 mg/L, showing a decrease of 52.9%. Treatment B (2 ppm) recorded an even more significant reduction of 57.5%, with final TOM levels of 32.4 mg/L. Meanwhile, treatment C (3 ppm) gave the best results with a 61.4% reduction in TOM, from 92.5 to 19.3 mg/L. The results of the measurement of total organic matter levels can be seen in Figure 2.

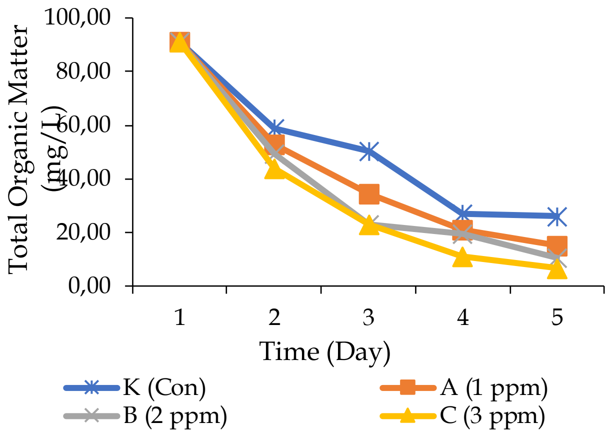

Figure 2. Observation of the rate of TOM reduction with different doses of bacterial consortium treatment

The more significant decrease in TOM in the treatment with bacterial consortium indicates the effectiveness of bacteria in accelerating the decomposition of organic matter. This result is in line with the research of Zheng et al. (2024), which showed that the use of bacterial consortium in aquaculture systems can increase the efficiency of organic waste decomposition by up to 60%, especially in the presence of bacterial species such as *Bacillus subtilis* and *Lactobacillus casei*, which have high enzymatic ability in breaking down complex organic compounds into simpler compounds. In addition, research by Widowati et al. (2021), found that *Bifidobacterium sp*. and *Nitrosomonas sp*. accelerate the oxidation of organic compounds into more stable forms. The bacterial consortium in this study also showed a similar pattern, where treatments with higher bacterial doses resulted in a more significant reduction in TOM.

The results of one-way analysis of variance (ANOVA) showed that the treatment with the addition of bacterial consortium significantly reduced TOM levels. The F-calculated value of 24.95 is much greater than the F-table at the 5% (4.066) and 1% (7.59) significance levels, with a p-value of 0.001, which indicates that the difference between treatments is very significant. Duncan's further test results also showed that treatment C (3 ppm) had the lowest average TOM levels (43.77 mg/L) and was significantly different compared to treatment B (49.23 mg/L), treatment A (52.62 mg/L), and control (58.57 mg/L). These results are consistent with the research of Paena et al. (2020), which states that increasing the concentration of bacteria in aquaculture systems contributes to improving the effectiveness of organic compound degradation through higher enzymatic activity. This is also supported by the research of Sutanto et al. (2024), who found that the application of bacterial consortium in intensive shrimp ponds was able to reduce TOM levels by more than 55% within five days, with the dominance of Bacillus and Pseudomonas which have high protease and lipase activities to degrade protein- and fat-based waste from shrimp feed.

Research by Kwoji et al. (2021), emphasized that the success of TOM reduction depends not only on the concentration of bacteria but also on environmental conditions such as pH, temperature, and dissolved oxygen levels. In this study, dissolved oxygen levels during treatment were still in the optimal range (3.5-6.8 mg/L), which allowed bacteria to work optimally in degrading organic waste. Overall, this study proved that using bacterial consortium could effectively reduce the organic load in *L. vannamei* farming wastewater.

Biological Oxygen Demand (BOD)

Biochemical Oxygen Demand (BOD) measurement results show that the higher the concentration of bacterial consortium given, the more significant the decrease in BOD value occurs. The control's BOD value only decreased to 3.53 mg/L. Treatment A (1 ppm) decreased BOD to 3.30 mg/L (51.97% decrease compared to the control), treatment B (2 ppm) decreased BOD to 2.90 mg/L (59.72%), and treatment C (3 ppm) gave the best result with BOD of 2.10 mg/L (69.57% decrease). The more significant reduction in BOD value in the treatment with the addition of bacterial consortium compared to the control shows that bacteria have an essential role in accelerating the decomposition process of organic matter in aquaculture waste. This result aligns with the research of Yanqoritha et al. (2022), who reported that using probiotic microorganisms in bio-remediation systems can reduce BOD values by more than 65% in five days. These microorganisms work through enzymatic activities that break down complex organic compounds into more straightforward and biodegradable forms, thereby reducing oxygen demand in the water. The results of the BOD measurement are presented in Figure 3.

Combining *Bacillus subtilis* and *Lactobacillus casei* in aquaculture wastewater can reduce BOD by up to 60% in the same period. This is because these bacteria can produce enzymes such as proteases and amylases that play a role in the breakdown of organic compounds from feed residues and shrimp excretions, which are the leading causes of high BOD (Jannah et al., 2024). The results of this study show the same pattern, where treatments with higher doses of bacteria provide a more significant reduction in BOD than treatments with lower doses or no bacteria.

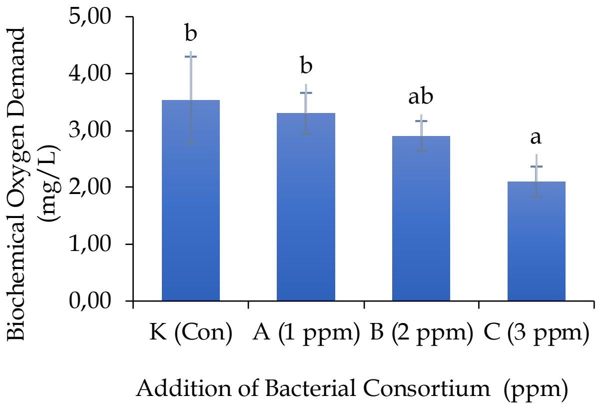

Figure 3. Observation of BOD reduction rate with different doses of bacterial consortium treatment

The results of one-way analysis of variance (ANOVA) showed that the treatment with the addition of bacterial consortium significantly reduced BOD values ($F = 5.57$; $p = 0.023$). The F-count value, more significant than the F-table at the 5% significance level (4.066), indicates a substantial difference between the treatments. Duncan's further test results showed that treatment C (3 ppm) had the lowest average BOD and significantly differed from the other treatments. Success in reducing BOD depends on bacterial activity and environmental factors such as pH, temperature, and dissolved oxygen levels. According to research by Elumalai et al. (2021), microorganisms that decompose organic matter work optimally in the pH range of 7-8.5 and require sufficient oxygen to support the metabolic process. In this study, dissolved oxygen levels were 3.5-6.8 mg/L, which is still adequate to support bacterial activity in biodegradation. Overall, the results of this study prove that the use of bacterial consortium is an effective solution to reduce BOD values in *L. vannamei* farming wastewater.

Ammonia

The measurement of ammonia levels significantly decreased during the five days of observation in all treatments. The initial ammonia level was recorded at 0.06 mg/L on the first day. The control treatment (without addition) only reduced ammonia to 0.02 mg/L (46.6%). Meanwhile, treatment A (1 ppm) reduced ammonia levels to 0.015 mg/L (56.77%), treatment B (2 ppm) to 0.012 mg/L (62.84%), and treatment C (3 ppm) gave the best results with final ammonia levels of 0.01 mg/L (68.92% reduction). The more significant ammonia reduction in the treatment with bacterial consortium compared to the control showed that microorganisms in the consortium play an active role in nitrification and denitrification. This is supported by the research of John et al. (2020), which showed that the combination of *Nitrosomonas sp.* and *Nitrobacter sp.* bacteria can oxidize ammonia to nitrite and then to nitrate, thus significantly reducing ammonia levels in aquaculture wastewater. The results of the ammonia measurement are presented in Figure 4.

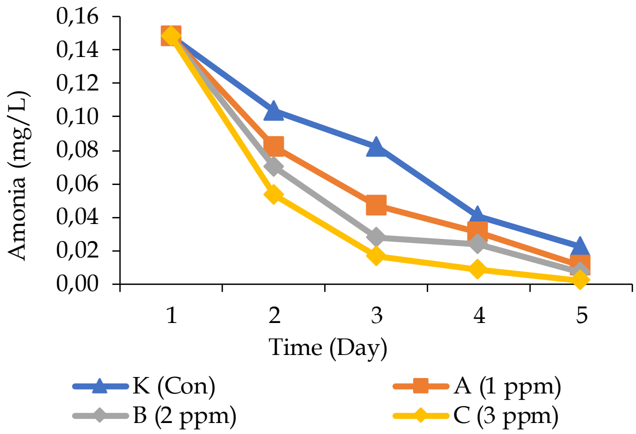

Figure 4. Observation of Ammonia Reduction rate with different doses of bacterial consortium treatment

Research conducted by Shahzad et al. (2025) explained that the bio-remediation system based on nitrifying bacteria reduced ammonia levels by 70% within five days. These results align with this study, where treatments with higher concentrations of bacteria showed greater effectiveness in reducing ammonia levels. Another survey by Supardiono et al. (2023) found that nitrifying bacteria work optimally at pH conditions of 7-8.5 and require sufficient oxygen to support microbial metabolism. In this study, dissolved oxygen levels were 3.5–6.8 mg/L, which still promotes effective nitrification activity.

The results of ANOVA analysis showed that the treatment with the addition of bacterial consortium had a significant effect on ammonia levels ($F = 15.19$; $p = 0.001$). The F-count value, more important than the F-table at the 5% significance level (4.066), indicates a significant difference between treatments. Duncan's further test also showed that treatment C (3 ppm) had the lowest ammonia levels and significantly differed from the other treatments. Research by Kunjiraman et al. (2024) found that applying a consortium of nitrifying bacteria in an intensive cultivation system reduced ammonia levels by 65% within four days. This is due to the synergy between heterotroph and autotroph bacteria that accelerate the conversion of ammonia into more stable nitrogen compounds (Fakhriyah et al., 2022). Bacteria such as *Bacillus subtilis* and *Lactobacillus casei* can help improve the performance of nitrifying bacteria by providing organic compounds as additional energy sources (Rahmi et al., 2023). So, this study shows that adding bacterial consortium significantly accelerates the reduction of ammonia levels in *L. vannamei* farming wastewater. Results show significant ammonia reduction, especially in treatments with higher bacterial doses, and this approach can be an effective and environmentally friendly bio-remediation solution in shrimp rearing water quality management.

Nitrite

The measurement of nitrite levels showed a significant decrease in all treatments during the five days of observation. Before treatment, the initial nitrite level was recorded at 1.25 mg/L. In the control treatment (without addition), nitrite levels decreased to 1.05 mg/L (43.59%). Treatment A (1 ppm) reduced nitrite to 0.87 mg/L (53.85%), treatment B (2 ppm) reduced nitrite to 0.68 mg/L (59.00%), while treatment C (3 ppm) gave the best results with final nitrite levels of 0.50 mg/L (67.59% reduction). The measurement results of the observation of the Nitrite parameter can be seen in Figure 5.

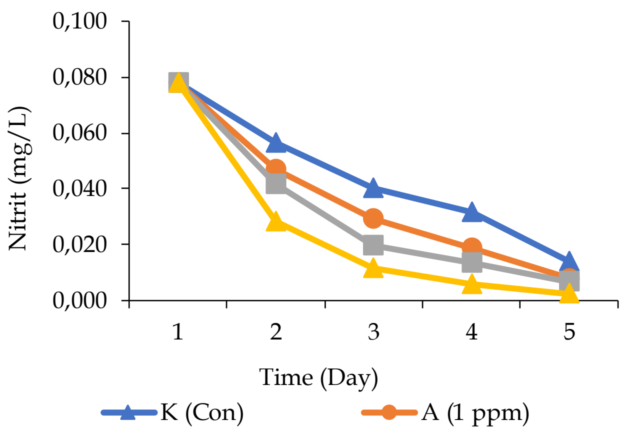

Figure 5. Observation of nitrite reduction rate with different doses of bacterial consortium treatment

The more significant decrease in nitrite levels in the treatment with the addition of bacterial consortium compared to the control indicates that the microorganisms in the consortium play an essential role in the nitrification and denitrification processes. According to Octovianus et al. (2023), nitrifying bacteria such as *Nitrosomonas sp*. convert ammonia into nitrite, while denitrifying bacteria such as *Pseudomonas sp*. and *Paracoccus sp*. convert nitrite into nitrogen gas, which is safer for the environment. This finding is also reinforced by the research of Ghaly & Ramakrishnan (2015), who found that a bio-remediation system based on nitrifying bacteria reduced nitrite levels by 65% in five days through an optimal biological conversion process. The results of this study showed a similar pattern, where a higher dose of bacterial consortium (3 ppm) gave the best results in reducing nitrate. In this study, dissolved oxygen levels ranged from 3.5-6.8 mg/L, which still supported bacterial activity in effectively decomposing nitrite.

The results of ANOVA analysis showed that the treatment with the addition of bacterial consortium had a significant effect on nitrite levels ($F = 23.75$; $p = 0.001$). The F-count value, more important than the F-table at the 5% significance level (3.49), indicates a significant difference between treatments. Duncan's further test results showed that treatment C (3 ppm) had the lowest nitrite levels and significantly differed from the other treatments. Research by Iber & Kasan (2021), also stated that applying nitrifying and denitrifying bacteria in intensive pond systems can reduce nitrite levels by 60% in four days. This is due to the synergistic work between nitrifying bacteria that convert ammonia into nitrite and denitrifying bacteria that convert nitrite into nitrogen gas. In addition, research by Merino et al. (2019), showed that increasing the dose of bacteria in the bio-remediation system accelerated the conversion of nitrite significantly due to an increase in the microbial population active in the nitrogen cycle.

This study's significant reduction in nitrite levels indicates that the bacterial consortium has potential as a bio-remediation solution in *L. vannamei* farming systems. Nitrite is a highly toxic compound for aquatic organisms because it can disrupt the shrimp respiration system by inhibiting oxygen transport in the hemolymph (Inayah et al., 2023). According to Maysabila et al. (2023), high nitrite levels in ponds can cause stress in shrimp and significantly reduce their survival rate. Therefore, the application of bacterial consortium in this study can be an environmentally friendly solution to reduce the negative impact of nitrite in intensive aquaculture systems.

Nitrate

The measurement of nitrate levels showed a significant decrease during the five days of observation in all treatments. Before treatment, the initial nitrate level was recorded at 12.8 mg/L. In the control treatment (no treatment), nitrate levels only decreased to 10.2 mg/L (34.88%). Treatment A (1 ppm) reduced nitrate levels to 8.5 mg/L (47.30%), treatment B (2 ppm) reduced nitrate levels to 6.4 mg/L (54.30%), while treatment C (3 ppm) gave the best results with final nitrate levels of 4.5 mg/L (62.02% reduction). Nitrate observation results are presented in Figure 6. The more significant decrease in nitrate levels in treatments with bacterial consortium indicates the effectiveness of microorganisms in the denitrification process (Chan et al., 2022). The application of denitrifying bacteria in a bio-remediation system can reduce nitrate levels by 60% in five days, which aligns with this study. Another study by Garrido-Amador et al. (2021) explained that the effectiveness of the denitrification process is highly dependent on environmental conditions, such as pH 7-8.5 and oxygen concentrations that are low enough to allow the denitrification process to take place optimally. In this study, dissolved oxygen levels were 3.5-6.8 mg/L, which still supported bacterial activity in nitrate reduction.

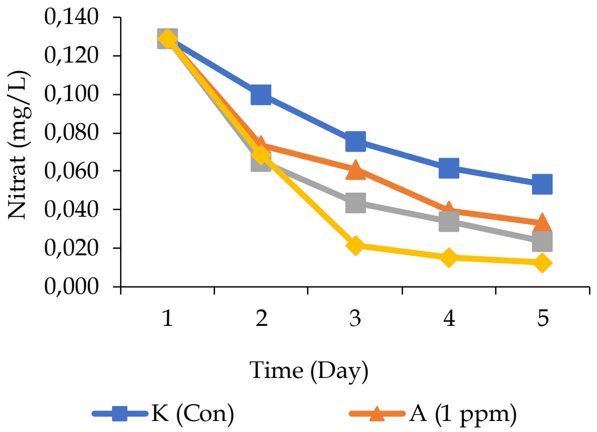

Figure 6. Observation of nitrate reduction rate with different doses of bacterial consortium treatment

The results of ANOVA analysis showed that treatment with bacterial consortium significantly affected nitrate levels ($F = 28.43$; $p = 0.001$). The F-count value is more significant than the F-table at the 5% significance level (3.49), indicating an essential difference between treatments. Duncan's further test results showed that treatment C (3 ppm) had the lowest nitrate levels and significantly differed from the other treatments. This finding is also supported by the research of Turista (2017), who found that applying denitrifying bacteria in intensive ponds can reduce nitrate levels by 65% within five days. This is due to an increase in the population of denitrifying bacteria that convert nitrate to nitrogen gas through anaerobic metabolic pathways. In addition, research by Gupta (2021), showed that increasing the dose of bacteria in the bio-remediation system accelerated the denitrification process, thus accelerating the rate of nitrate reduction in the culture system. The results of this study showed that the addition of bacterial consortium significantly accelerated the decrease in nitrate levels in *L. vannamei* farming wastewater. Results show significant nitrate reduction, especially in treatments with higher bacterial doses, and this approach could be an effective bio-remediation solution to improve water quality in sustainable aquaculture systems.

Viability of Bacterial Consortium

The results of measuring the viability of the bacterial consortium through the Total Plate Count (TPC) method showed an increase in the number of bacterial colonies along with the increase in the dose of bacteria given. In the control treatment (no treatment), the TPC value was 2.3×10^2 CFU/mL. In treatment A (1 ppm), the number of bacteria increased to 3.5×10^2 CFU/mL (52% increase compared to the control), treatment B (2 ppm) increased to 4.8×10^2 CFU/mL (108%), and treatment C (3 ppm) showed the highest bacterial growth, reaching 6.1×10^2 CFU/mL (165% increase). The increase in bacterial viability in the treatment with the addition of bacterial consortium indicates that the available environment supports microbial growth and activity (Irdawati et al., 2023). This result is in line with the research of Arifan et al. (2019), which explained that bacteria given in the form of a consortium can adapt quickly to water conditions and develop better than natural populations. The results of the bacterial viability measurement are presented in Figure 7.

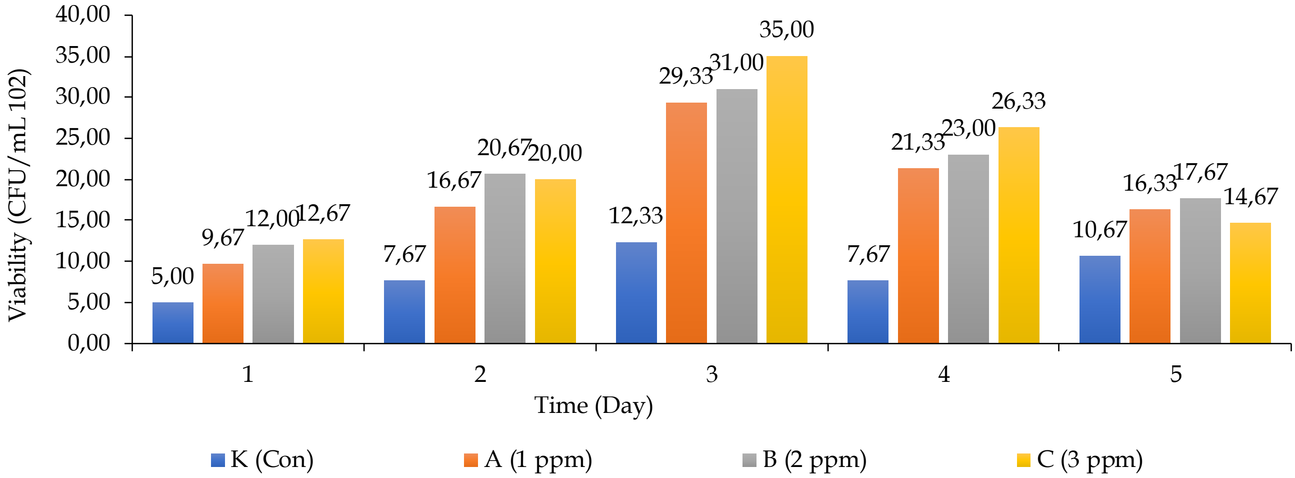

Figure 7. Observation of the rate of TPC decrease with different doses of bacterial consortium treatment

An increase in the number of microorganisms in a bio-remediation system is directly related to the rise in the effectiveness of organic matter decomposition and wastewater treatment (Yuka et al., 2021). In this study, the increase in the number of bacteria was higher at the higher dose (3 ppm), which indicates that the bacteria have a source of nutrients and environmental conditions that support their optimal growth (Çelebi et al., 2023). According to Suwartha & Pujiastuti (2017), the main factors that affect bacterial viability in aquaculture systems include pH, temperature, nutrient availability, and competition between microorganisms. This study's environmental conditions remained within the optimal range (pH 7.8-8.5, temperature 24.5-28.9°C, and dissolved oxygen 3.5-6.8 mg/L), allowing microbial growth to occur correctly.

The results of ANOVA analysis showed that the treatment with the addition of bacterial consortium had a significant effect on bacterial viability ($F = 21.68$; $p = 0.001$). The F-count value, which is greater than the F-table at the 5% significance level (3.49), indicates a significant difference between treatments. Duncan's further test confirmed that treatment C (3 ppm) produced the highest number of bacteria and significantly differed from other treatments. This is supported by the research of Khastini et al. (2022), which found that increasing bacterial populations in aquatic systems can improve the effectiveness of the bio-remediation process because more microorganisms are available to degrade organic matter and toxic compounds. This was also confirmed by Latupeirissa & Latupeirissa (2022), who reported that bacteria in consortia work synergistically, where one species can produce compounds that support the growth of other species, thereby increasing microbial resilience and competitiveness in aquatic environments.

The high viability of bacteria in this study indicates that the application of microbial consortium has great potential in improving the effectiveness of aquaculture wastewater management. The higher the bacterial population, the faster the degradation of organic compounds, ammonia, nitrite, and nitrate. According to Ngabito et al. (2024), bacteria such as *Bacillus subtilis* and *Lactobacillus casei* play an essential role in improving the health of pond ecosystems by suppressing the growth of pathogenic bacteria and improving water quality. Overall, this study proved that the bacterial consortium was effective in reducing pollutant levels in *L. vannamei* farming wastewater and had high durability and viability. This suggests that using microbes as bio-remediation agents can be an environmentally friendly solution to improve the sustainability of intensive aquaculture systems.

Water Quality Parameter

Water quality is a significant factor in supporting the survival and growth of *L. vannamei* in aquaculture systems (Mohanty et al., 2018). One of the factors of water quality conditions is very important to note is to create environmental conditions of shrimp rearing waters that are comfortable and by the needs of shrimp, furthermore to produce wastewater with a quality that is still safe for coastal aquatic ecosystems or still within the limits allowed based on seawater quality standards for aquaculture activities (Akinnawo, 2023). Therefore, in *L. vannamei* farming activities, water quality measurements are necessary to determine the actual conditions, while water quality parameters include physical and chemical parameters. The main water quality parameters include dissolved oxygen (DO), pH, and temperature.

The results of temperature measurements during the study showed a temperature range between 24.5-

28.9 °C. This temperature is within the range that corresponds to the optimal conditions for most decomposing bacteria involved in the biodegradation of organic matter. According to research conducted by Yusoff et al. (2024), the optimal temperature for decomposing bacteria activity usually ranges from 25 - 30°C, which supports efficient bacterial metabolic processes.

The pH measurement results in the study ranged from 7.8 to 8.5. This pH value is still in a good range to support the life of decomposing bacteria because the optimal pH for microbes is 5-9 (Djumanto et al., 2018). A higher pH indicates a more alkaline environment, which can support bacterial activity in decomposing organic matter. Process of decomposing organic matter by bacteria can affect changes in pH, with more intensive decomposition tending to reduce pH due to increased acid production and CO_2 produced during the metabolic process (Naz et al., 2022).

The results of dissolved oxygen (DO) measurements in this study ranged from 3.5 to 6.8 mg/L. Oxygen levels in the water, essential for metabolizing decomposing bacteria. DO levels also affect the rate of decomposition of organic matter. The higher the DO, the slower the decomposition of organic matter, as facultative anaerobic decomposing bacteria are more active in low oxygen conditions (Visser et al., 2016; Prasetyawan et al., 2017).

Conclusion

Of all the parameters measured, the dose of 3 ppm (treatment C) was the best among other treatments. This treatment decreased Total Organic Matter (TOM) by 61.4%, BOD by 69.57%, ammonia by 68.92%, nitrite by 67.59%, and nitrate by 62.02%. In addition, bacterial viability increased by 165% compared to the control. The success of the 3 ppm dose was due to the synergistic work of the bacterial consortium consisting of *Lactobacillus casei*, *Bacillus subtilis*, *Bifidobacterium sp*., *Nitrosomonas sp*., and *Nitrobacter sp*. in decomposing organic and nitrogen contaminants in culture water. Optimal environmental conditions (temperature 24.3-28.9°C, pH 7.8-8.5, and DO 3.5-6.8 mg/L) also support enzymatic activity and bacterial metabolism so that a dose of 3 ppm can be used as an applicable and environmentally friendly solution to improve water quality in vannamei shrimp cultivation.

Acknowledgments
This work was supported by Universitas Brawijaya, Indonesia.

Author Contributions
Conceptualization, M., A.M.S.H., and A.F.N.; methodology, writing—review and editing, A.F.N. and S.R.S.; software, writing—original draft preparation, visualization, A.F.N.; validation, M. and A.M.S.H.; supervision, funding acquisition, M.; project administration, S.R.S. The authors listed in this article have read and agree to the published version of the manuscript.

Funding
Professor Grant Research Scheme, Universitas Brawijaya, with contract number: 3709/UN10.F06/KS/2024.

Conflicts of Interest
The authors declare no conflict of interest.

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