Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(1): 413 – 429 (2025)
www.ejabf.journals.ekb.eg


# Potential of Biofilm Bacteria for Ammonia Degradation in Shrimp Pond

**Fahruddin Fahruddin[1]*, Mustika Tuwo[1], Muhammad Farid Samawi[2], As'adi Abdullah[1], Ghea Farmaning Thias Putri[1], Elisurya Ibrahim[3]**

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Hasanuddin University, Makassar 90245, Indonesia
[2]Department of Marine Science, Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia
[3]Research Centre for Food Crops, National Research and Innovation Agency, Cibinong Science Center-Botanical Garden, Bogor, Indonesia

***Corresponding Author: fahruddin_science@unhas.ac.id**

## ARTICLE INFO

**Article History:**
Received: Dec. 15, 2024
Accepted: Jan. 9, 2025
Online: Jan. 17, 2025

**Keywords**:
Shrimp ponds,
Ammonia,
Biofilm bacteria,
Ammonia degradation

## ABSTRACT

Ammonia is a waste product in shrimp ponds with toxic effects on shrimp farming. It can be mitigated through the degradation of ammonia by bacteria. This study aimed to isolate and characterize biofilm bacteria from shrimp ponds and to examine their ability to degrade ammonia. Biofilm bacteria were isolated from pond materials, characterized macroscopically and microscopically, and qualitatively tested for ammonia degradation, followed by testing their ammonia degradation capability in lab-scale pond water containing 0.5ppm ammonia. Observations included bacterial growth, ammonia and nitrite concentrations, and pH measurements. The study identified 8 types of biofilm bacteria isolates, all of which showed the ability to degrade ammonia, except for isolate 8. In the ammonia degradation capability test, isolate 5 showed the highest reduction in ammonia concentration at 74%. The highest increase in nitrite concentration was observed in isolates 2, 3 and 5, recording more than 90%. The pH measurements for each treatment showed different values, with some being acidic and some being base.

## INTRODUCTION

The expansion of pond areas in Indonesia continues to increase, including shrimp farming ponds, which are one of superior commodities **(Nagel *et al.*, 2024)**. To achieve optimal results in shrimp farming, a specific environmental condition that aligns with environmental factors necessary for shrimp growth needs to be prepared. The primary factor that significantly determines the productivity of shrimp ponds is the water in the pond plots since it is the medium in which the shrimp are reared. Therefore, the most crucial effort to increase pond productivity is to provide water of good quality **(Hukom *et al.*, 2020)**.

Shrimp farming activities in ponds produce organic waste, originating from uneaten feed, shrimp faeces, and dead plankton. In the pond ecosystem, not all the feed provided is consumed by the shrimp **(Rupiwardani & Irfany, 2023; Pazmiño *et al*., 2024)**. Some of the uneaten feed remain suspended in the water, most of which settle at the bottom of the pond. The decomposition of this organic material from the feed produces ammonia **(**$NH_3$**)**, which is toxic and can inhibit shrimp growth, potentially even causing death. Furthermore, high concentrations of ammonia can increase shrimp's susceptibility to disease **(Kajornkasirat *et al*., 2021)**. The amount of ammonia will increase with rising pH; conversely, if the pH is low, the ammonia level will be lower. Thus, the pH value will affect the nitrification process in the water and will cease if the pH is low **(Kathyayani *et al*., 2019; Udume *et al*., 2022)**.

Ammonia is one of the toxic substances commonly found in fertilizer waste, residential waste, agricultural wastewater, or animal nitrogen waste disposal **(Ding *et al*., 2021)**. It is frequently used in everyday life as a bleaching agent and in the production of fertilizers, plastics, textiles, pesticides, and various other chemicals. Its widespread use results in wastewater containing high levels of ammonia. The majority of ammonia waste originates from fertilizer plants, agricultural activities, and aquaculture. Ammonia can cause eutrophication, pose a threat to aquatic life, produce foul odors, and endanger human health. Uncharged ammonia molecules, in particular, can damage gill epithelium and can reduce oxygen uptake **(Camargo & Alonso, 2006)**. Ammonia adversely affects the environment by degrading water quality when ammonia-containing waste is discharged, causing the water unsuitable for direct use as drinking water or for industrial purposes **(Anagopoulos, 2022)**. It is known that ammonia concentrations in wastewater discharges are limited to 10 and 20mg/ L. According to the Environmental Protection Agency **(**EPA**)**, the water quality criteria for acute and chronic exposure to ammonia are set at 17 and 1.9mg/ L, respectively **(USEPA, 2013)**. Additionally, in some countries, the water quality criteria for ammonia range from 4 to 12mg/ L **(Ding *et al*., 2021)**.

Ammonia concentration in pond systems is directly proportional to the amount of feed input. High ammonia concentrations will irritate shrimp gills, leading to hyperplasia, which is the swelling of gill filaments, thereby reducing the shrimp's blood capacity to bind oxygen from the water. High ammonia levels in the water can also increase the ammonia concentration in the blood. Elevated ammonia concentrations in the shrimp's blood will decrease the affinity of blood pigment (hemocyanin) for binding oxygen **(Tommerdahl *et al*., 2015; Mingming *et al*., 2020)**

In addition to affecting shrimp in ponds, ammonia can be toxic to humans if the amount that enters the body exceeds what can be detoxified. In humans, the greatest risk comes from inhaling ammonia vapours, which can result in several effects including irritation of the skin, eyes, and respiratory tract. At very high levels, inhaling ammonia vapours can be fatal. If dissolved in water, it will increase the ammonia concentration, poisoning almost all aquatic organisms **(Xiangyin *et al*., 2021)**.

Ammonia and other nitrogen compounds such as nitrite ($NO_2^-$) and nitrate ($NO_3^-$) originate from the nitrogen cycle, which is regulated by biological activity. The first stage is fixation, which is the conversion of nitrogen gas ($N_2$) into nitrogen that can be directly used by plants or, in this case, by phytoplankton in the aquatic ecosystem **(Yang *et al*., 2022)**. Phytoplankton species such as *Anabaena* sp. and *Aphanizomenon* sp. are capable of nitrogen fixation. The second stage is nitrification, which is the oxidation of ammonia ($NH_4^+$) into nitrate facilitated by the bacteria *Nitrosomonas* sp. $NH_4^+$ is converted into $NO_2^-$ and the bacteria *Nitrobacter* sp. nitrite is converted into nitrate. Nitrification also plays a role in reducing ammonia concentration and can proceed effectively under neutral pH conditions. The third stage is nitrate reduction, which is the process of converting nitrate into nitrogen gas by anaerobic bacteria, which occurs predominantly at the bottom of the pond **(Moschonas *et al*., 2017)**

The presence of ammonia in ponds causes negative impacts, thereby increasing the oxygen depletion rate in the water and the sediment oxygen demand at the pond bottom, as well as lowering the redox potential to a reduction. If this continues, it will worsen the cultivation environment, particularly the bottom water layer and the pond bottom soil, causing shrimp to experience stress, reduced appetite, and increased susceptibility to disease **(Mingming *et al*., 2020; Xiangyin *et al*., 2021)**.

Ammonia-degrading bacteria play a crucial role in improving soil quality, particularly in aquaculture. Ammonia-degrading bacteria, including nitrifying bacteria such as nitrosomonas and nitrobacter, possess the capability to convert ammonia into less harmful compounds for the aquaculture ecosystem. By reducing ammonia levels through the process of nitrification, ammonia-degrading bacteria help maintain water and soil quality in aquaculture systems, enhance the survival of aquatic organisms, and support the overall sustainability of the aquaculture ecosystem **(John *et al*., 2020; Mingming *et al*., 2020)**.

The role of ammonia-degrading bacteria in aquaculture and mining areas is crucial for maintaining ecosystem balance. These bacteria help reduce ammonia pollution, which can be toxic to living organisms, and improve soil quality by increasing nutrient availability through the process of nitrification. Thus, these bacteria support the sustainability of aquaculture ecosystems and the restoration of degraded mining land **(John *et al*., 2020)**.

This problem can be addressed by improving water quality from contamination by organic or inorganic materials using microorganisms found in the ponds, converting them into compounds that are not harmful to shrimp. This biological process is often called bioremediation, which involves the activity of microbes to reduce pollutants to safe concentration. The group of microbes widely used to remove ammonia, nitrite, and nitrate compounds from waste systems includes nitrifying autotrophic bacteria, nitrifying heterotrophic bacteria, aerobic denitrifying bacteria, and anaerobic ammonia-oxidizing bacteria **(Fahruddin *et al*., 2021; Neissi *et al*., 2022)**.

Therefore, the control of ammonia and organic materials in shrimp farming media is necessary. This can be done biologically by enhancing the decomposition activity of organic materials. One way to mitigate ammonia content in ponds is through the degradation process by bacteria, specifically by utilizing biofilm bacteria present in the ponds themselves. Biofilms can be defined as a structure of bacterial cell communities enclosed by a polymer matrix produced by the bacteria themselves and attached to a surface. These biofilm bacteria can act as natural degrading agents of ammonia in shrimp ponds **(Neissi *et al*., 2022)**.

This decomposition aims to ensure that these organic materials do not become toxic to the shrimp being cultivated in the pond. If this decomposition process runs well, the organic materials in the pond will not become toxic **(Fahruddin *et al*., 2020)**. Ammonia decomposition can be carried out biologically, one of which is through the nitrification process, which involves the conversion of ammonium nitrogen into nitrite and then into nitrate by autotrophic and heterotrophic bacteria **(Moschonas *et al*., 2017)**. The bacterial community in this biofilm will transform organic materials through the processes of ammonification and nitrification. The application of this biofilm will reduce the ammonia levels in the organic waste of shrimp ponds and will increase the nitrate levels **(Istirokhatun *et al*., 2020; Zhang *et al*., 2020)**. In the current study, an experimental growth trial of biofilm was conducted using a medium with a large surface area, allowing for denser biofilm growth and enhancing the bioconversion of organic waste in shrimp ponds. Therefore, this research offers an effective and environmentally friendly biological management of shrimp ponds.

## MATERIALS AND METHODS

### 1. Material

Materials from the pond, including water and sediment from the shrimp pond, were used as the source of biofilm bacterial inoculum. The growth media and bacterial test media included: Nutrient agar, Zobell media, nitrification media, ammonia oxidation media, biochemical test media, and physiological test media, along with several chemical reagents.

The preparation of Zobell 2216E medium was conducted as follows: 15.0g of Bacto-agar, 5.0g of Bactopeptone, 1.0g of yeast extract, and 19.5mg of ferric phosphate were placed into an Erlenmeyer flask and aquadest was added to make up a total volume of 1 liter. The mixture was then heated on a hot plate until boiling and being homogeneous. The pH was adjusted to 7.5 - 7.6, and then the medium was sterilized using an autoclave at 121°C and 1.0 atm pressure for 20 minutes **(Patrick, 1978)**.

### 2. Characterization of pond water

Water and sediment samples were taken from the shrimp pond and were then placed in sealed containers. Characterization was then performed, including the analysis of organic carbon content using the Black method, total nitrogen using the Kjeldahl method, phosphorus and potassium content using the spectrophotometer method, and pH determination using a pH meter **(Fahruddin *et al*., 2021)**.

**3. Isolation of biofilm bacteria**

Biofilm bacterial samples were collected by scraping material from the shrimp pond and were then placed in an Erlenmeyer flask containing sterile distilled water with 0.9% NaCl. The next step involved the isolation of bacteria. The scraped sample was inoculated onto nutrient agar media using the plate count method. After 24 hours of incubation, observations were made, including counting the number of colonies and identifying different colony types based on their macroscopic and microscopic morphological characteristics **(Fahrudin *et al.*, 2019)**. Different isolates were then selected for identification and ammonia degradation testing.

**4. Initial characterization of pond water**

To determine the initial chemical conditions of the pond water samples, initial characterization was performed, including the analysis of organic carbon using the TOC method, total nitrogen using the Micro Kjeldahl method, phosphorus using the Stannous Chloride method, potassium (K) using flame photometry, ammonia content measured with a spectrophotometer at 640nm wavelength, and pH measured with a pH meter.

**5. Identification of biofilm bacterial isolates**

To ensure the differentiation of bacterial isolates, characterization was performed based on morphology, physiological tests, and biochemical tests, including: Gram staining, TSIA test, motility test, indole test, MR test, VP test, Simmons citrate test, catalase test, and other tests to complete the identification of each isolate.

**6. Qualitative test for ammonia degradation**

The obtained bacterial isolates were subjected to a qualitative test for their ammonia degradation ability by inoculating them into specific ammonia oxidation media in test tubes, followed by incubation for 4x24 hours. The bacteria's ability to degrade ammonia was indicated by a positive reaction, marked by a color change in the media to brown after adding Nesler's reagent as an indicator.

**7. Testing the potential of isolates to degrade ammonia**

Each isolate was inoculated into separate 300mL Erlenmeyer flasks as a shrimp pond microcosm containing water and shrimp pond sediment with 0.5ppm of ammonia. These were incubated at room temperature. Every 24 hours, an analysis of ammonia and nitrite concentrations was performed. Subsequently, the most potential bacterial isolate for degrading ammonia was determined based on the reduction in ammonia concentration.

**8. Determination of nitrite concentration**

A 20mL sample from the isolate treatment in the Erlenmeyer flask was added with 1.0mL of sulphanilamide solution and mixed until becoming homogeneous. Subsequently, 1.0mL of NED dihydrochloride solution was added, and the nitrite concentration was measured based on absorbance using a spectrophotometer at a wavelength of 543nm.

**9. The measurement of pH**

The measurement of pH was conducted using a pH meter, which was calibrated beforehand with buffers at pH 4 and pH 7, then stabilized for 15 minutes. The pH meter electrode was then immersed in the water sample from the isolate treatment, and after a short wait, the pH value was read from the pH meter scale **(Greenberg *et al.*, 1999)**.

## RESULTS AND DISCUSSION

### 1. Pond water characterization

The analysis results of ammonia content in the shrimp pond revealed that the ammonia concentration in the pond water column was 0.520ppm and in the sediment was 0.280ppm. Results for the characterization of the shrimp pond including C, N, P, and K are exhibited in Table (1). This characterization was performed to determine the initial conditions in the pond. It was found that the ammonia content in the pond water was above the quality standards, and similarly, the sediment levels exceeded the standards. As **Yang *et al*. (2022)** stated, ammonia levels are toxic to shrimp if the concentration exceeds 0.2ppm, with normal ammonia concentrations in ponds being 0.1ppm. According to **Erfan *et al*. (2020)**, ammonia concentrations greater than 1.0mg/ l can cause shrimp mortality. Excess ammonia content in the sediment can also be toxic to nitrifying bacteria because the contained ammonia is non-ionized. The ammonia in the shrimp pond can originate from uneaten shrimp feed, which dissolves in the water or settles on the sediment. The presence of ammonia results from the decomposition of accumulated uneaten feed in either the pond water or sediment **(Tommerdahl *et al*., 2015; Mingming *et al*., 2020)**.

**Table 1.** Chemical characterization of shrimp pond water

| Type of Chemical Analysis | Pond water |
|---|---|
| Ammonia | 0.520 ppm |
| Organic carbon | 0.043 ppm |
| Nitrogen | 0,12% |
| Phosphorus | 58.90 ppm |
| Potassium | 0.87% |
| Nilai pH | 0.52 |

Based on the analysis results of C, N, P, and K, it is evident that the organic content in the pond tends to quantitatively support it as a nutrient source. The presence of appropriate organic materials will provide positive support for the pond, as they can be highly beneficial nutrients for aquatic organisms. On the other hand, the accumulation of organic materials in quantities that do not match the pond's carrying capacity will have a negative impact **(Xiangyin *et al*., 2021; Fahruddin *et al*., 2024)**. Furthermore, **Bulbul *et al*. (2022)** stated that excess organic material in pond water will disrupt the balance of dissolved oxygen because the oxygen consumption will increase more than the production rate of dissolved oxygen. This can cause the anaerobic layer to become thicker, thereby affecting the oxygen demand due to the impact of the microorganism decomposition process present in the pond.

**2. Isolation of biofilm bacteria**

From the observation of biofilm bacterial colony growth from the shrimp pond using nutrient agar media, based on macroscopic morphological characteristics including colony texture and color, as well as microscopic observations of Gram staining, 8 types of isolates were obtained, which were isolate 1, isolate 2, isolate 3, isolate 4, isolate 5, isolate 6, isolate 7, and isolate 8 (Table 2). Isolates 1, 3, 4, 6, 7, and 8 were Gram-positive, while isolates 2 and 5 were Gram-negative.

Based on the observation of colony morphology, it was found that isolates 1, 2, 3, 4, 5, 6, 7, and 8 had the same morphology with circular shape and small size, except for isolate 2. For elevation, isolate 1 had umbonate elevation; isolate 2 had convex elevation; isolates 3, 4, 7, and 8 had flat elevation; isolate 5 had pulvinate elevation, and isolate 6 had raised elevation.

**Tabel 2.** Colony and cell morphology of biofilm bacterial isolates

| Isolate | Colony Morphology | | | | Cell Morphology | |
|---|---|---|---|---|---|---|
| | Size | Shape | Elevation | Margin | Shape | Gram |
| 1 | small | circular | umbonate | undulate | rod-shaped | positive |
| 2 | medium | circular | convex | entire | rod-shaped | negative |
| 3 | small | circular | flat | undulate | rod-shaped | positive |
| 4 | small | circular | flat | undulate | rod-shaped | positive |
| 5 | medium | circular | pulvinate | entire | rod-shaped | negative |
| 6 | small | circular | raised | undulate | rod-shaped | positive |
| 7 | small | circular | flat | curled | rod-shaped | positive |
| 8 | small | circular | flat | entire | rod-shaped | positive |

Characterization of bacteria from the obtained isolates generally showed similarities with the characteristics of nitrifying bacteria groups, as observed from the shape of the bacterial colonies, which were circular and irregular. The colony colors were white, milky white, clear white, and yellowish white. Colony elevations were flat and convex, with entire and undulate colony margins. According to **Puzyr *et al*. (2001)**, nitrifying bacteria generally have round colony shapes, white, milky white, clear white, and yellowish white colony colors, with convex and flat elevations, and smooth and wavy colony margins.

Several types of primary biofilm-forming bacteria commonly found in ponds and attached to materials in shrimp ponds include *Bacillus pumilus*, and other types of bacteria such as the genus *Pseudomonas* sp., *Staphylococcus* sp., and *E. coli*. Primary biofilm-forming bacteria on material surfaces in ponds are predominantly species of *Bacillus* sp. **(Zhang *et al.*, 2020; Neissi, *et al.*, 2022)**. Furthermore, biofilm-forming bacteria on shrimp pond aeration filters are dominated by Bacteroidetes, Alphaproteobacteria, and Denitromonas, which have the potential to oxidize ammonium compounds **(Puzyr *et al*., 2001; Zhang *et al*., 2020; Neissi *et al*., 2022)**.

**3. Ammonia compound oxidation test**

The results of the ammonia degradation capability test on 8 biofilm bacterial isolates in Zobell 2216E media enriched with 100ppm ammonia nitrogen showed that 7 isolates were capable of degrading ammonia, indicated by a positive reaction with the media turning a yellowish-brown color after adding Nesler's reagent. In contrast, isolate 8 was unable to degrade ammonia, indicated by a negative reaction with the media turning yellow (Fig. 1). According to **Lehtovirta-Morley and Laura (2018)**, oxidized ammonia compounds form a colloidal dispersion, resulting in a yellowish-brown solution that follows the Lambert-Beer law. The use of selective medium for nitrifying bacteria further supports that the obtained isolates belong to the group of nitrifying bacteria capable of degrading ammonia.

Ammonia can be oxidized with bacteria from biofilms through the nitrification process under aerobic conditions. In this process, nitrification occurs in two sequential reactions: the nitridation process by ammonia-oxidizing bacteria (AOB) such as Nitrosomonas and the nitration process by nitrite-oxidizing bacteria (NOB) such as Nitrobacter, therefore nitrification plays a role in reducing ammonia concentrations **(Yang *et al*., 2021; Srivastava *et al*, 2024)**.

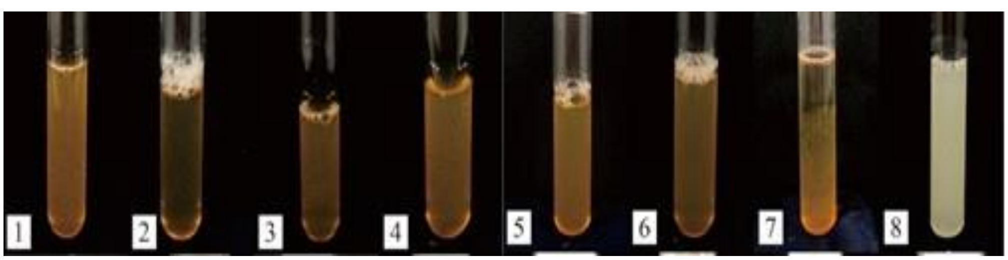


**Fig. 1.** Qualitative test of the ammonia degradation capability of isolates, with a positive reaction indicated by the media turning a yellowish-brown colour after adding Nesler's reagent

**4. Nitrite concentration**

Determination of nitrite concentration was carried out to evaluate the ability of biofilm bacterial isolates to degrade ammonia into nitrite using nitrification media. The results showed that isolate 1 and 7 yielded positive results, indicated by a pink color change. This was identified by reacting with sulfanilic acid and alpha-naphthylamine solution, forming a red complex compound. On the other hand, isolates 2, 3, 4, 5, and 6 showed negative results, as the media did not change color, indicating that these five isolates were not able to convert ammonia into nitrite (Fig. 2).

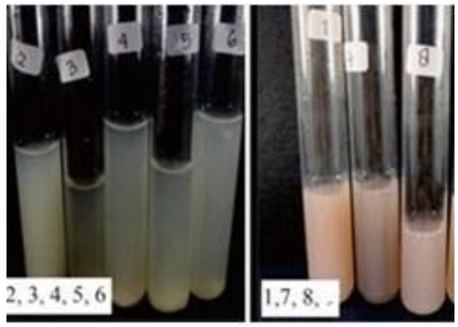


**Fig. 2.** Nitrite test in nitrification media with a positive reaction indicated by the media turning pink, and a negative reaction indicated by the media remaining yellow, showing no color change

The oxidation of ammonia compounds into nitrite is the initial step in the biological nitrogen reduction process. Bacteria isolated from waste discharge showed the ability to oxidize ammonia compounds by 46-84%. *Nitrosomonas eutropha* bacteria have the capability to perform nitrification and denitrification simultaneously. The microbial groups commonly used to remove ammonia, nitrite, and nitrate compounds from waste systems include autotrophic nitrifying bacteria, heterotrophic nitrifying bacteria, aerobic denitrifying bacteria, and anaerobic ammonia-oxidizing bacteria. One of the bacteria used for this bioremediation agent is microorganisms like *Lactobacillus* sp., enabling the oxidation of toxic ammonia into nitrate **(Fahruddin *et al*., 2021; Yang *et al*., 2021; Srivastava *et al.*, 2024)**.

## 5. Reduction of ammonia

The ammonia degradation test of isolates in shrimp pond water containing an ammonia concentration of 0.5ppm showed that each isolate was able to reduce ammonia concentration, except for Isolate 8, which did not show a relative reduction (Fig. 3). Over a duration of 96 hours, isolate 5 demonstrated the highest ability to reduce ammonia concentration, decreasing it from the initial 0.5 to 0.13ppm, which is a 74% reduction. Following this, isolate 7 reduced the concentration to 0.14ppm, a 72% reduction. Subsequently, isolate 2 showed a 58% reduction; isolate 3 indicated a 44% reduction; isolate 4 and isolate 6 both showed a 28% reduction, and finally, isolate 1 only presented a 26% reduction, as seen in Fig. (3). According to **Puzyr *et al.* (2001)**, each bacterium has different capabilities in degrading ammonia as an energy source and for cell formation.

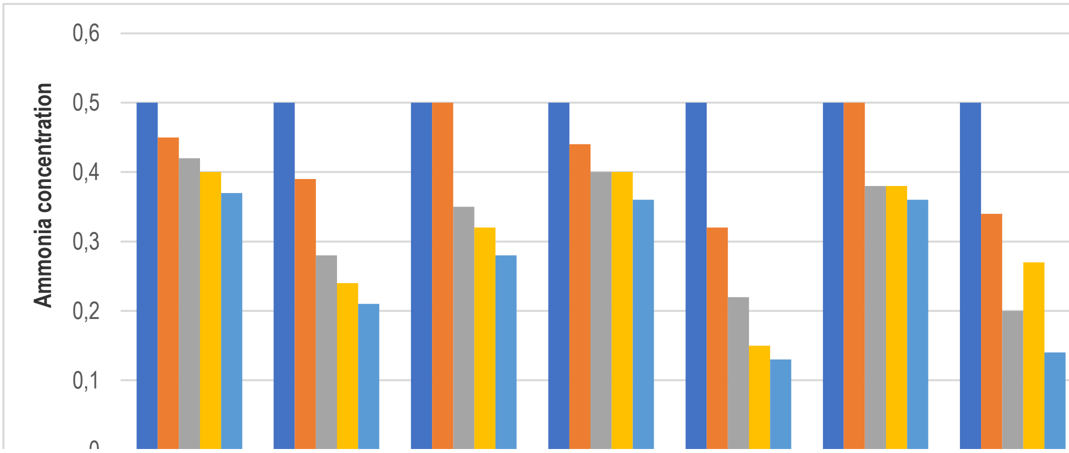


**Fig. 3.** Reduction of ammonia concentration in each isolate treatment in pond water

The presence of ammonia compounds in pond water is a result of the decomposition of organic materials originating from leftover feed. If the nitrification process does not proceed well, ammonia will accumulate in the shrimp pond. Additionally, the formation of free ammonia and ammonia ions is influenced by environmental factors such as pH and temperature **(Neissi *et al.*, 2022)**. Moreover, the composition of inorganic nitrogen is greatly influenced by the dissolved oxygen content in the water. Low oxygen concentration causes nitrogen to move toward ammonia compounds, while higher oxygen concentration causes nitrogen to move toward nitrate compounds **(Yang *et al.*, 2021)**.

### 6. Nitrite concentration

The nitrite concentration in the isolate treatments shows the results of ammonia decomposition into nitrite or nitrate. Additionally, nitrite is naturally present in the pond environment **(Kathyayani *et al.*, 2019)**. Observations showed that at the 96-hour mark, the highest nitrite concentration in pond water treated with Isolates 2, 3, and 5 more than 90% isolate 6 is 85%, isolate 1 is 64%. The lowest increase in nitrite concentration was observed in isolate 4 is 6 % and isolate 8 is 2% **(**Fig. 4**)**.

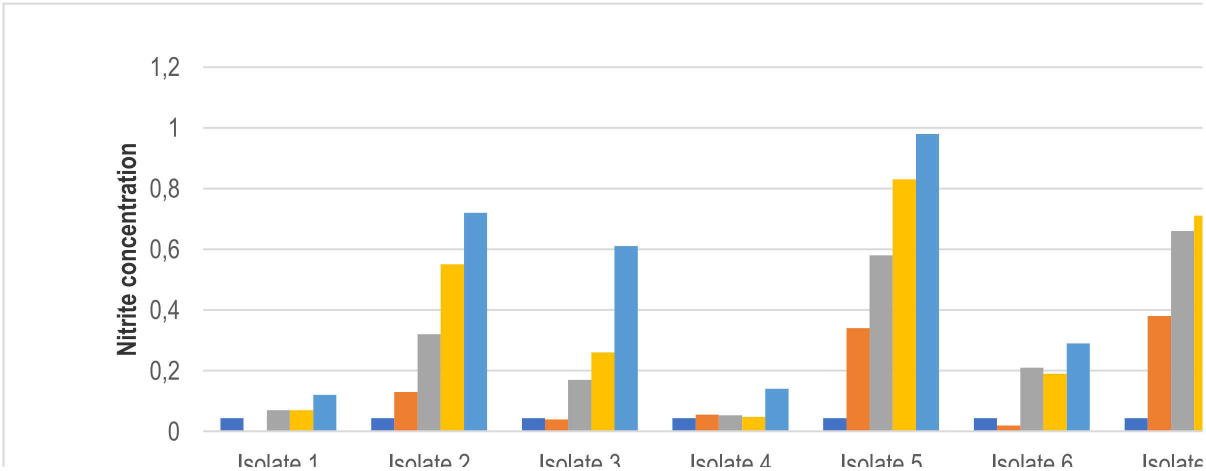


**Fig. 4.** Variation in nitrite concentration in each isolate treatment in shrimp pond water

According to **Moschonas *et al.* (2017)**, nitrite is another type of nitrogen compound originating from the nitrogen cycle, regulated by biological activity through the nitrification process, which is the oxidation of ammonia to nitrate facilitated by bacteria such as *Nitrosomonas* sp., whereby ammonia is converted into nitrite, and *Nitrobacter* sp., whereby nitrite is converted into nitrate. The nitrification process occurs not only under aerobic conditions but can also take place under anaerobic conditions with limited available oxygen, facilitated by the participation of anaerobic ammonia-oxidizing bacteria (Anammox). According to **Neissi *et al.* (2022),** Anamox bacteria only require a small amount of oxygen and no organic matter to produce dinitrogen gas.

The increase in nitrite content in the isolate treatments is influenced by many factors such as pH, salinity, and temperature. This is in line with **Jumraeni *et al*. (2020)**, who stated that if the pond has low water pH and salinity, then nitrite levels will increase. The decomposition process of organic matter such as ammonia, nitrate, and phosphate occurs more quickly if the pH range is basic. In ponds, the nitrite content naturally increases because nitrite is present in the atmosphere and then falls to the earth with rainwater, resulting in high nitrite levels in the pond. Additionally, the high nitrite levels are also caused by the decomposition of faeces or uneaten feed.

Some studies have investigated the role of biofilm bacteria in degrading ammonia and its effect on nitrite concentrations. A study conducted by **Zulkifli *et al*. (2022)** on the use of biofilm bacteria in ammonia treatment reactors revealed that biofilm bacteria effectively removed ammonia, achieving a total reduction of up to 99.7% from concentrations ranging from 16 to 900mg/ L. Similarly, research by **Rodriguez-Sanchez *et al.* (2020)** demonstrated that the biofilm microbial community in bioreactors was capable of eliminating more than 90% of organic matter and ammonia sourced from municipal waste. Furthermore, **Chaali *et al.* (2017)** and **Zulkifli *et al*. (2022)** stated that current biofilm treatments in reactors have exhibited excellent results, providing a practical approach for ammonia removal in wastewater treatment. Additionally, **Jun and**

**Wenfeng (2009)** noted that biofilm microbes play a crucial role in the two-step oxidation of ammonia: First, ammonia is oxidized to nitrite by ammonia oxidizing bacteria (AOB), such as Nitrosomonas, followed by the oxidation of nitrite to nitrate by nitrite oxidizing bacteria (NOB), such as Nitrobacter. Research by **Wu *et al*. (2021)** demonstrated that ammonia removal efficiency in reactors could reach 84% through nitrification from an influent ammonia concentration of 10.4mg N/L. In applications involving biofilm bacteria mediated by 3-hydroxybutyrate-co-3-hydroxyvalerate, results indicated a maximum ammonia removal rate of 0.45 and 0.09kg m−3 day−1. Throughout the ammonia degradation process no significant accumulation of nitrite was observed. Microbial community analysis of the biofilm in batch tests revealed that both the sludge produced and the biofilm formed play important roles in nitrogen removal **(Liu *et al.*, 2023)**.

**7. The pH values**

Results of pH measurements of water samples initially at hour 0 ranged from pH 6.2-6.6 (Fig. 5). The pH levels of the isolate treatments that experienced the lowest reduction up to 96 hours are as follows: Isolate 1 at pH 4.4; isolate 6 at pH 4.1; isolate 4 at pH 4.2. Then, isolate 3 and isolate 8 both at pH 6.3 showed relatively insignificant pH reductions, whereas isolate 2 at pH 6.9, isolate 5 at pH 7.1, and isolate 7 at pH 6.7 showed slight changes in pH.

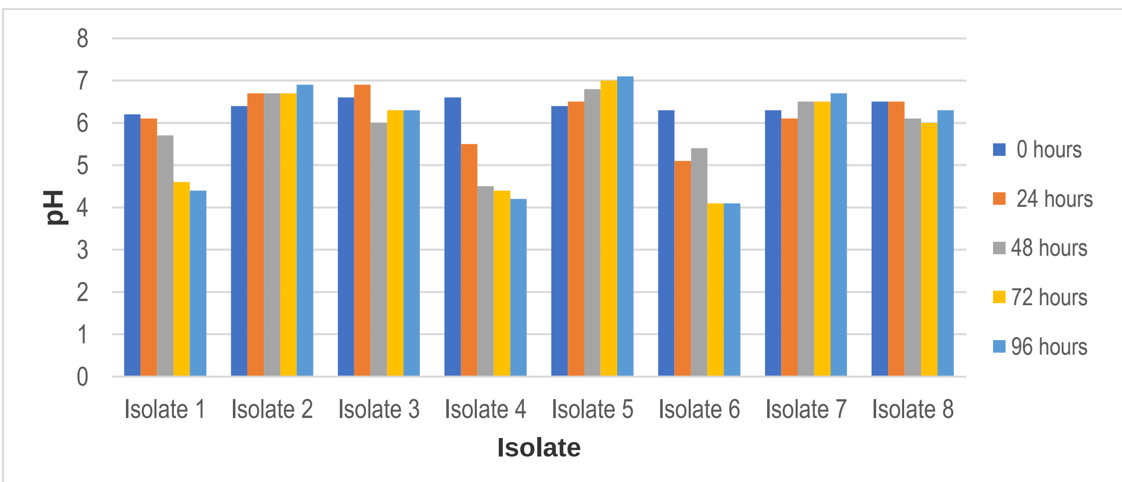


**Fig. 5.** Differences in pH values in each isolate treatment in shrimp pond water

The pH values in pond water are closely related to physical, chemical, and biological water factors. The decrease in pond water pH occurs due to the decomposition of organic matter by microorganisms that release carbon dioxide ($CO_2$) during the process, and the reduction of oxygen levels, which leads to a decrease in pH. This also results in an increase in the total organic material content. The increase in carbon dioxide, which lowers the pH, implicates a reduction in free ammonia levels. Additionally, the pH reduction is a result of respiration and the decomposition of organic substances present in the pond **(Supriatna *et al*., 2023)**. Sediments in the pond can release chemicals into the water column sourced from decaying organic material, forming organic acids that can seep into the water and lower the pH levels. Moreover, the anaerobic decomposition of total organic matter **(**TOM**)** by bacteria will lead to an increase in pH **(Matthew *et al*., 2022)**.

According to **Jumraeni *et al.* (2020)**, in aquatic environments, the amount of free ammonia will increase with rising the pH. At high pH levels, there is more ammonia present. Conversely, if the pH is low, the amount of ammonia will be less. At high pH, the formed ammonia is un-ionized. The pH value will affect the nitrification process in water and will cease if the pH is low. Nevertheless, brackish water is well-buffered. Hence, its pH rarely drops below 6.5 or rises above 9 **(Supriatna *et al*., 2023)**.

## CONCLUSION

Eight types of bacteria were isolated from the biofilm in shrimp ponds, named isolate 1 through Isolate 8. The results of the qualitative ammonia degradation test indicated that only isolate 8 was unable to degrade ammonia. In the ammonia degradation test conducted with pond water treatments, each isolate demonstrated the ability to degrade ammonia. Similarly, the nitrite concentration test in pond water revealed differences in ammonia concentration increases across the treatments. Additionally, the pH measurements showed variation, with some treatments exhibiting relatively acidic properties (pH values ranging from 4 to 4.4), while other isolates resulted in more basic conditions (pH values ranging from 6.3 to 7.1).

## ACKNOWLEDGEMENT

The authors appreciate the efforts the Hasanuddin University for the financial assistance to support this research under grant research PFK 2024 of contract number 00309/UN 4.221PT.01.A3/2A24.

## REFERENCES

**Anagopoulos, A.** (2022). Brine management **(**saline water & wastewater effluents**)**: Sustainable utilization and resource recovery strategy through Minimal and Zero Liquid Discharge **(**MLD & ZLD**)** desalination systems. Chemical Engineering and Processing - Process Intensification 176:108944. https://doi.org/10.1016/j.cep.2022.108944

**Bulbul, A.**; **Anushka and Abha, M.** (2022). Effects of dissolved oxygen concentration on freshwater fish: a review. International Journal of Fisheries and Aquatic Studies, 10 **(**4**)**:113-127. https://DOI:10.22271/fish.2022.v10.i4b.2693

**Camargo, J.A. and Alonso, Á.** (2006). Ecological and toxicological effects of inorganic nitrogen pollution in aquatic ecosystems: A global assessment. Environment international, 32 **(**6**)**: 831-49.

**Chaali, M.**; **Naghdi, M.; Satinder, K. B. and Avalos-Ramirez, A.** (2017). A review on the advances of nitrifying biofilm reactors and their removal rates in wastewater treatment. Journal of Chemical Technology & Biotechnology, 93. https://doi.org/10.1002/jctb.5692

**Ding, T.; Shi-Lin, D.; Zi-Yan, H.**; **Ze-Jun, W. ; Jin, Z.; Ya-Hui, Z.; Shu-Shen, L. and Lian-Sheng, H.** (2021). Water quality criteria and ecological risk assessment for ammonia in the Shaying River Basin, China. Ecotoxicology and Environmental Safety, 215:112141, https://doi.org/10.1016/j.ecoenv.2021.112141

**Erfan; Hendrajat; Sahabuddin and Nafisah.** (2020). Tiger Shrimp farming in rice-fish farming system using salinity-tolerant rice lines. AACL Bioflux, 13 **(**6**)**:694-3705.

**Fahruddin, F.; La Nafie, N.; Abdullah, A.; Tuwo, M. and Awaluddin** (2021). Treatment of compost as a source of organic material for bacterial consortium in the removal of sulfate and heavy metal lead **(**Pb**)** from acid mine drainage. Journal of Degraded and Mining Lands Managemen, 9**(**1**)**: 3083-3091.https://doi:10.15243/jdmlm.2021.091.3083

**Fahruddin, F.; Abdullah, A.; Haedar, N. and La Nafie, N.** (2020). Estuary sediment treatment for reducing sulfate in acid mine water. Environment and Natural Resources Journal, 18**(**2**)**:191–199. https://DOI:10.32526/ennrj.18.2.2020.18

**Fahruddin, F.; Johannes, E. and Zaraswati, D.** (2019). Antifouling potential of *Thalassia hemprichii* extract against growth of biofilm-forming bacteria. ScienceAsia, 45:21-27. https://10.2306/scienceasia1513-1874.2019.45.021.

**Fahruddin, F.; Samawi, M. F.; Tuwo, M. and Ramlan, T**. (2021). The effect of heavy metal lead **(**Pb**)** on the growth of ammonia-degrading bacteria and physical changes of eichhornia crassipes in groundwater phytoremediation. International Journal on Advanced Science, Engineering and Information Technology, 11**(**3**)**:994-1000. https://doi:10.18517/ijaseit.11.3.12588.

**Fahruddin, F., Syahri, Y.F., Fauziah, S., Samawi, M.F., Johannes, E., Tambaru, E., Tuwo, M. and Abdullah, A.** (2024). Combining biochar with sediment in the treatment for the effectiveness of sulfate and heavy metal Pb reduction of acid mine drainage. Journal of Degraded and Mining Lands Management, 11**(**4**)**:6329-6335. https://doi:10.15243/jdmlm.2024.114.6329.

**Greenberg, A.E.; Clesceri, L.S. and Eaton, A.D**. (1992). Standard methods for the examination of water and waste water, Public Health Association, Washington, DC, American. https://DOI:10.1093/femsle/fny058

**Hukom, V.; Nielsen, R.; Asmild, M. and Nielsen, M**. (2020). Do aquaculture farmers have an incentive to maintain good water quality? the case of small-scale shrimp farming in Indonesia, Ecological Economics, 176:106717. https://doi.org/10.1016/j.ecolecon.2020.106717.

**Istirokhatun, S. N.; Aufar; Munasik and Sudarno**. (2020). Effects of biofilms on ammonium removal efficiency in fish pond effluents. IOP Conf. Series: Earth and Environmental Science 448:012053. https://Doi:10.1088/1755-1315/448/1/012053

**John, E.M.; Krishnapriya, K. and Sankar, T.V.** (2020). Treatment of ammonia and nitrite in aquaculture wastewater by an assembled bacterial consortium. Aquaculture, 256:735390. https://doi.org/10.1016/j.aquaculture.2020.735390

**Jumraeni, A.; Khaeriyah; Burhanuddin, and Anwar, A.** (2020). The effect of disposal model on accumulation of organic material in intensive pond of vaname shrimp **(***Litopenaeus vannamei***)**". Octopus: Jurnal Ilmu Perikanan, 9**(**1**)**:11-18.

**Jun, Y. and Wenfeng, X.** (2009). Ammonia biofiltration and community analysis of ammonia-oxidizing bacteria in biofilters. Bioresource Technology, 100 **(**17**)**:3869-76. https://doi.org/10.1016/j.biortech.2009.03.021

**Kajornkasirat, S.; Ruangsri, J.; Sumat, C. and Intaramontri, P.** (2021). Online analytics for shrimp farm management to control water quality parameters and growth performance. Sustainability, 13 **(**11**)**: 5839. https://doi.org/10.3390/su13115839.

**Kathyayani, Z.A.; Poornima, M.; Sukumaran, S.; Nagavel, A. and Muralidhar, M.** (2019). Effect of ammonia stress on immune variables of pacific white shrimp *Penaeus Vannamei* under varying levels of pH and susceptibility to white spot syndrome virus. Ecotoxicology and Environmental Safety, 30**(**184**)**:109626. https://doi.org/10.1016/j.ecoenv.2019.109626

**Lehtovirta-Morley and Laura E.** (2018). Ammonia oxidation: ecology, physiology, biochemistry and why they must all come together. FEMS Microbiology Letters, 365:1–9.

**Matthew, H.; Thornhill, I.; Castro-Castellon, A.; Hernández-Avilés, J.; Arantza, D.; Victor, S. and Hobbs, S.** (2022). Organic-matter decomposition in urban stream and pond habitats. Ecological Indicators, 142:109232. https://doi.org/10.1016/j.ecolind.2022.109232.

**Mingming, Z.; Defu, Y.; Shengkang, L.; Yueling, Z. and Jude, J.A.** (2020). Effects of ammonia on shrimp physiology and immunity: a review. Reviews in Aquaculture, 12 **(**4**)**:2194-2211 https://doi.org/10.1111/raq.12429

**Moschonas, G.; Gowen, R. J. and Paterson, R.F.** (2017). Nitrogen dynamics and phytoplankton community structure: the role of organic nutrient., Biogeochemistry, 134:125 – 145. https://DOI:10.1007/s10533-017-0351-8

**Nagel, B., Nurliah and Stefan, P., Artelow, P**. (2024). Archetypes of community-based pond aquaculture in Indonesia: applying the social-ecological systems framework to examine sustainability tradeoffs. Environmental Research Letters, 19 **(**4**)**: 04402. https://DOI:10.1088/1748-9326/ad2e71.

**Neissi, A.; Rafiee, G.; Rahimi, S.; Farahmand, H.; Pandit, S. and Mijakovic, I.** (2022). Enriched microbial communities for ammonium and nitrite removal from recirculating aquaculture systems. Chemosphere, 295:133811 .https://doi.org/10.1016/j.chemosphere.2022.133811.

**Patrick, F.M.** (1978). The use of membrane filtration and marine agar 2216E to enumerate marine heterotrophic bacteria. Aquaculture, 13**(4)**:369-372. https://doi.org/10.1016/0044-8486(78)90186-2

**Pazmiño, M.L.; Chico-Santamarta, L.; Boero, A. and Ramirez, A.D.** (2024). Environmental life cycle assessment and potential improvement measures in the shrimp and prawn aquaculture sector: a literature review. Aquaculture and Fisheries, https://doi.org/10.1016/j.aaf.2024.06.003.

**Puzyr, A.P.; Mogil'naya, O.A.; Gurevich, Y.L. and Babkina, E. A.** (2001). Colony structure of a consortium of nitrifying bacteria. Microbiology, 70:84–90. https://doi.org/10.1023/A:1004853123326

**Rodriguez-Sanchez, A.; Leyva-Diaz, J.C.; Muñoz-Palazon, B.; Gonzalez-Lopez, J. and Poyatos, J.M.** (2019). Effect of variable salinity wastewater on performance and kinetics of membrane-based bioreactors. Journal of Chemical Technology & Biotechnology, 94: 3236–3250.

**Rupiwardani, S., and Irfany, F.** (2023). Unveiling vannamei shrimp farming's impact on water pollution in Wonocoyo Village. Jurnal Pembangunan dan Alam Lestari, 2 **(**14**)**:70-75.

**Srivastava, P.; Marquez, G. P.; Gupta, S.; Mittal, Y.; Soda, S.; Dwivedi, S.; Ajibade, F. O. and Freguia, S.** (2024). Diversity of anaerobic ammonium oxidation processes in nature. Chemical Engineering Journal, 483:149257. https://doi.org/10.1016/j.cej.2024.149257

**Supriatna, A.; Darmawan and Maizar, A**. (2023). Pathway analysis of pH in whiteleg shrimp, *Litopenaeus vannamei* concrete pond intensifies in Banyuwangi East Java.

IOP Conf. Series: Earth and Environmental Science, 1191: 012015. https://doi:10.1088/1755-1315/1191/1/012015.

**Tommerdahl, A.P.; Burnett, K.G. and Burnett, L.E.** (2015). Respiratory Properties of Hemocyanin From Wild and Aquacultured Penaeid Shrimp and the Effects of Chronic Exposure to Hypoxia. Biological Bulletin, 228 **(**3**)**: 242–252. http://www.jstor.org/stable/24588179

**Udume, B.U.; Ota, H.O.; Zhifeng, Y. and Onyewuchi, C.C.** (2022). Evaluating the effect of acute ammonia toxicity in a freshwater ecological environment on the growth and hematological functioning of *Clarias Gariepinus.* Journal of Water Supply: Research and Technology-Aqua, 71**(**3**)**: 464-477. https://DOI:10.2166/aqua.2022.138.

**Xiangyin, Z.; Liu, R.; Li, Y.; Li, J.; Zhao, Q.; Li, X. and Bao, J.** (2021). Excessive ammonia inhalation causes liver damage and dysfunction by altering gene networks associated with oxidative stress and immune function. Ecotoxicology and Environmental Safety, 217:112203. https://doi.org/10.1016/j.ecoenv.2021.112203.

**Yang, J.; Pei, H. and Lü, J.** (2022). Effects of phytoplankton community and interaction between environmental variables on nitrogen uptake and transformations in an urban river. Journal of Oceanology and Limnology, 40: 026.

**Yang, X.; Li, R.; Su, J. and G. Zhou.** (2021). Anammox bacteria are potentially involved in anaerobic ammonium oxidation coupled to iron **(**III**)** reduction in the wastewater treatment system. Frontiers Microbiology, 12: 717249. https://doi.org/10.3389/fmicb.2021.717249

**Zhang, Q.; Futian, R.; Fadong, Li.; Guoliang, C.; Guang, Y.; Jianqi, W.; Kun, D.; Shanbao, L. and Zhao, L.** (2020). Ammonia nitrogen sources and pollution along soil profiles in an in-situ leaching rare earth ore. Environmental Pollution, 267:15449. https://doi.org/10.1016/j.envpol.2020.115449.

**Zhang, Y., Zhao, L., Song, T., Cheng, Y., Bao, M. and Li, M.Y.** (2020). Simultaneous nitrification and denitrification in an aerobic biofilm biosystem with loofah sponges as carriers for biodegrading hydrolyzed polyacrylamide-containing wastewater. Bioprocess and Biosystems Engineering, 43:529-540. https://doi.org/10.1007/s00449-019-02247-x

**Zulkifli, M.; Hassimi, A.H.; Siti, R.; Sheikh, A. and Mohd, H. M.** (2022). A review of ammonia removal using a biofilm-based reactor and its challenges. Journal of Environmental Management, 315:115162.