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Effect of Detergent Concentration and Exposure Duration on the Growth of Heterotrophic Bacteria in Seawater

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ABSTRACT

This research was conducted from June to July 2024 at the estuary of the Selat Morong River, Rupat District, Bengkalis Regency, Riau Province. This study aimed to examine the effect of different detergent concentrations on the growth of heterotrophic bacteria, as well as to investigate the influence of varying detergent exposure durations on their growth. The experimental method employed a two-factor completely randomized design (CRD) consisting of different detergent concentrations (Factor A) with five levels: A1 (0 ppm) as the control, A2 (3000 ppm), A3 (6000 ppm), A4 (9000 ppm), and A5 (12,000 ppm), and varying exposure durations (Factor B): 1 day, 6 days, 11 days, 16 days, and 21 days. The results showed that the logarithmic values of heterotrophic bacterial growth over the 21 days indicated the highest growth occurred on day 1 at a concentration of 0 ppm, with a value of 7.09 CFU/mL. However, bacterial growth declined over time, reaching its lowest point on day 11 at a concentration of 3000 ppm, with a value of 6.95 CFU/mL. Statistical analysis revealed that different concentrations and exposure durations had no statistically significant effect on the growth of heterotrophic bacteria ($P>0.05$), meaning that variations in detergent concentration and exposure time did not cause meaningful differences in bacterial growth under the experimental conditions. This study highlights the potential resistance of heterotrophic bacteria to detergent exposure. It provides a basis for further research into the resilience of microbial communities in marine environments affected by pollution.

Keywords: Detergent, Heterotrophic Bacteria, Seawater

1. INTRODUCTION

Water is a fundamental requirement for all life processes on Earth; life would not exist without it. However, water can also become a source of disaster if it is not available in the proper conditions in terms of quantity and quality. Relatively clean water is essential for human daily needs, industrial processes, urban sanitation, agriculture, and other purposes. Although water is a renewable natural resource, it can be easily contaminated by various human activities, making it highly susceptible to pollution (Kristanto, 2004).

Among the many causes of water pollution, detergents are considered one of the most potentially hazardous pollutants due to their close association with daily human activities. Detergents are cleaning products that are an advancement over traditional soap. One of the main advantages of detergents over soap is their effectiveness in hard water and acidic solutions. The growth of the detergent industry parallels the increase in population. However, as detergent use increases, so does the volume of detergent waste. The resulting wastewater, containing detergent residues, is often discharged directly into aquatic environments without adequate treatment, contributing to the deterioration of water quality.

Aquatic environments contaminated by high concentrations of hard detergent waste pose a serious threat to the survival of marine organisms and to humans who consume these organisms (Lichtenberg et al., 2013). Some environmental impacts of detergent waste include eutrophication, a decrease in dissolved oxygen levels in the water, alterations in the physical and chemical properties, and aesthetic disturbances caused by white foam on the water surface. Despite the known impacts, studies examining the response of native microbial communities, such as heterotrophic bacteria, to detergent pollution in estuarine environments remain limited.

Bacteria capable of degrading detergent compounds utilize these substances as a source

of nutrients. As a result, detergents broken down by bacteria no longer act as pollutants that produce toxic effects harmful to marine organisms. Heterotrophic bacteria belong to a group of bacteria that can utilize and degrade organic compounds. According to Gunawati (2002), the role of heterotrophic bacteria in aquatic environments is to decompose organic matter in the water. Organic materials originating from terrestrial sources are the dominant inputs into marine ecosystems.

Unstable changes in aquatic environments, such as high concentrations of detergents, can affect the growth of heterotrophic bacteria. The chemical compounds in detergents, once present in seawater at toxic levels, can drastically decline these bacteria' growth (Pakpahan et al., 2017). With fewer heterotrophic bacteria in the marine environment, the decomposition of waste entering the water will take longer. Therefore, bacteria play an essential role in maintaining the health and balance of marine ecosystems.

Given the increasing discharge of detergent waste into aquatic systems and the critical role of heterotrophic bacteria in maintaining ecosystem balance, it becomes urgent to investigate how detergent exposure affects these bacterial populations, particularly in estuarine areas like the Selat Morong River estuary in Rupat District, Riau Province. This area has a significant potential risk of domestic waste pollution, yet research on the impacts of pollution on the aquatic ecosystem in this region remains limited. Therefore, this study is vital to expand understanding of the effects of detergents on heterotrophic bacterial growth and water quality.

This study aims to examine the effect of varying detergent concentrations on the growth of heterotrophic bacteria and assess the impact of different detergent exposure durations on their growth.

2. RESEARCH METHOD

Time and Place

This research was conducted from June to July 2024 in the estuarine area of the Selat Morong River, Rupat District, Bengkalis Regency, Riau Province.

Method

This research was conducted using an experimental method. It employed a two-factor, completely randomized design consisting of different detergent concentrations (Factor A) and varying exposure durations (Factor B). Factor A included five levels: A1 (0 ppm) as the control, A2 (3000 ppm), A3 (6000 ppm), A4 (9000 ppm), and A5 (12,000 ppm). Factor B consisted of different exposure durations: 1 day, 6 days, 11 days, 16 days, and 21 days. The bacterial count was determined using the spread plate method.

Procedures

Seawater Sampling

Seawater was collected in a 5-liter jerrycan. The seawater samples were then stored in an icebox and transported to the Marine Microbiology Laboratory, Department of Marine Science, Faculty of Fisheries and Marine Science, Universitas Riau. In this study, the test containers used were microcosm bottles.

Test Container Preparation

The microcosm bottles were filled with 500 ml of seawater. Each microcosm was treated with different concentrations of Soklin detergent: 0 ppm (control), 3000 ppm, 6000 ppm, 9000 ppm, and 12,000 ppm. The microcosms were wrapped with aluminum foil to minimize the effects of light on microbial growth. All microcosms were placed at room temperature, with a range of approximately ±20°C (Adithiya et al., 2017).

Preparation of Saline Solution

A 0.9% saline solution is prepared by dissolving 9 g of NaCl in 1000 mL of distilled water. The saline solution is then transferred into test tubes, with 9 mL of the solution in each tube, followed by serial dilutions of 10^{-1}, 10^{-2}, and up to 10^{-5}. A 1 mL seawater sample is taken using a micropipette, added to the 10^{-1} saline solution test tube, and mixed using a shaker. After mixing, 1 ml of the 10^{-1} saline solution is transferred to the 10^{-2} saline solution and repeated using the shaker. This procedure is continued until the 10^{-5} dilution is achieved. The 10^{-3}, 10^{-4}, and 10^{-5} dilutions are used for bacterial plating with three replicates (Pakpahan et al., 2017).

Preparation of Media

The media used is plate count agar (PCA). To prepare 1 L of PCA media, 22.5 g of PCA powder is placed into an Erlenmeyer flask, dissolved in 1000 mL of distilled water, and

stirred until homogeneous. The Erlenmeyer flask containing the media is then covered with aluminum foil and heated until boiling using a magnetic stirrer. Sterilization is then performed in an autoclave for 15 minutes. After sterilization, 15-20 ml of the media is poured into a sterile Petri dish and cooled (Adithiya et al., 2017).

Bacterial Counting

Bacterial population growth is counted using the spread plate method. 0.1 mL is taken from each dilution and placed into a Petri dish containing PCA media. The sample liquid is then spread evenly using a spreader stick. The Petri dish is then incubated at 37°C for 48 hours. The formula for calculating the number of bacteria in the Petri dish is as follows:

$$\text{Number of bacteria} = \text{Number of colonies} \times \frac{1}{\text{Dilution factor}}$$

Colony counting samples are taken from Petri dishes with a colony count range of 30-300. Observation of bacterial population is conducted at intervals of growth pattern by taking samples on days 1, 6, 11, 16, and 21 (Pakpahan et al., 2017).

Data Analysis

The analysis includes a parametric test, ANOVA, if the data meet the requirements. If not, a non-parametric Friedman test is used. The normality test determines whether the heterotrophic bacterial growth data obtained from different detergent concentrations and exposure times are typically distributed. This is one of the assumptions used for performing parametric statistical tests (Nasrum, 2018). This test is performed using the Shapiro-Wilk method with a significance level 0.05. The homogeneity test determines whether the heterotrophic bacterial growth data between groups with different detergent concentrations and exposure times have the same variance (Flynn, 2003). In this study, the homogeneity test uses the Levene test with a significance level 0.05.

3. RESULT AND DISCUSSION

Water Quality

Measuring water quality parameters can provide an overview of the water conditions, including physical, chemical, and biological conditions. Good water conditions will support the growth of heterotrophic bacteria. Water temperature is one of the critical water quality parameters. Water temperature affects the metabolic activity of heterotrophic bacteria. The ideal water temperature for the growth of heterotrophic bacteria is between 20-30°C.

Water salinity is a measure of the dissolved salt content in the water. Water salinity affects the metabolic activity of heterotrophic bacteria. Heterotrophic bacteria can grow at varying salinity levels, ranging from low to high salinity. Water pH measures the concentration of hydrogen ions in the water. The ideal pH for the growth of heterotrophic bacteria is between 6.5 and 8.5. Heterotrophic bacteria require dissolved oxygen for respiration. Low dissolved oxygen levels can inhibit the growth of heterotrophic bacteria.

Table 1. Water quality of the Selat Morong River estuary

No	Parameter	Unit	Observation Value
1	Temperature	°C	28,5
2	pH	-	7,8
3	Salinity	‰	30,5
4	Dissolved oxygen	mg/L	4,8

Based on Table 1, it is known that the water in the Selat Morong River estuary has a temperature of 28.5°C, a pH of 7.8, a salinity of 30.5‰, and dissolved oxygen of 4.8 mg/L.

The Effect of Detergent Concentration on Bacterial Growth

The growth of heterotrophic bacteria at different detergent concentrations and exposure times fluctuated in each treatment with detergent concentrations in the medium. More details can be seen in Table 2, and it can be observed that on day 1 of exposure, the highest growth of heterotrophic bacteria occurred at concentration A1, which was 1.23 x 10^7 (CFU/mL), while the lowest bacterial growth occurred at concentration A5, which was 9.0x10^6 (CFU/mL).

On day 6 of exposure, the growth of heterotrophic bacteria decreased in treatments A1 through A3, but an increase in heterotrophic bacterial growth occurred in treatments A4 and

A5 within the media. On day 6 of exposure, the highest growth of heterotrophic bacteria occurred at concentration A1, 1.13 x 10^7 (CFU/mL), while the lowest bacterial growth occurred at concentration A3, which was 9.0 x 10^6 (CFU/mL).

On day 11 of exposure, the growth of heterotrophic bacteria decreased in treatments A1 through A3, while an increase in heterotrophic bacterial growth occurred in treatments A4 and A5 within the media. On day 11 of exposure, the highest growth of heterotrophic bacteria occurred at concentration A1, 1.2 x 10^7 (CFU/ml), while the lowest bacterial growth occurred at concentration A3, which was 1.1 x 10^7 (CFU/mL).

On day 16 of exposure, the growth of heterotrophic bacteria tended to decrease in each treatment as the detergent concentration in the media increased, with bacterial growth observed in treatment A4. On day 16 of exposure, the highest bacterial growth occurred at concentration A1, 1.17 x 10^7 (CFU/mL), while the lowest bacterial growth occurred at concentration A3, which was 1.03x10^7 (CFU/mL).

Table 2. The growth of heterotrophic bacteria in each treatment

Incubation Time (Days)	Treatment of detergent concentration				
	0 ppm	3000 ppm	6000 ppm	9000 ppm	12.000 ppm
1	1,23 x 10^7	1,2 x 10^7	1,2 x 10^7	1,17 x 10^7	9,0 x 10^6
6	1,13 x 10^7	1,1 x 10^7	9,0 x 10^6	1,13 x 10^7	1,13 x 10^7
11	1,2 x 10^7	1,17 x 10^7	1,1 x 10^7	1,17 x 10^7	1,17 x 10^7
16	1,17 x 10^7	1,13 x 10^7	1,03 x 10^7	1,13 x 10^7	1,1 x 10^7
21	1,2 x 10^7	9,6 x 10^6	1,17 x 10^7	1,1 x 10^7	1,1 x 10^7

On day 21 of exposure, the growth of heterotrophic bacteria fluctuated in each treatment as the detergent concentration in the media increased. On day 21, the highest bacterial growth occurred at concentration A1, 1.2 x 10^7 (CFU/mL), while the lowest bacterial growth occurred at concentration A2, 9.6 x 10^6 (CFU/mL).

The results of this study indicate that variations in detergent concentration do not significantly affect heterotrophic bacterial growth, with a p-value > 0.05 in the Friedman test. This finding contrasts with the results of Pakpahan et al. (2017), which showed significant findings where higher detergent concentrations significantly affected the reduction in heterotrophic bacterial growth in detergent-polluted aquatic environments. Detergents can inhibit bacterial growth by interacting with bacterial cellular components, particularly at higher detergent concentrations.

The discrepancy between the results of this study and Pakpahan et al. (2017) may be due to several factors, one of which is the difference in detergent concentrations used and the volume of the test container. Higher detergent concentrations and smaller test container volumes allowed for stronger interactions between the detergent and the bacteria, which could cause more significant effects on bacterial growth. In contrast, in this study, with lower detergent concentrations and larger test container volumes, the interaction between the detergent and the bacteria may not have been strong enough to affect bacterial growth significantly.

The Effect of Exposure Time on Bacterial Growth

The log of heterotrophic bacterial growth at different exposure times over 21 days fluctuated, but not significantly, as the detergent concentration increased. The log of heterotrophic bacterial growth over 21 days showed that the highest bacterial growth occurred on day 1 at a concentration of 0 ppm, with a value of 7.09 CFU/mL. However, bacterial growth declined over time, and the lowest growth occurred on day 11 at a concentration of 3000 ppm, reaching a value of 6.95 CFU/mL.

This decline may indicate the presence of factors that inhibit bacterial growth, such as the negative effects of detergent concentration or other environmental factors that affect bacterial metabolism. Although bacterial growth shows potential for recovery and growth on days 11 and 16, the graph on day 21 shows a further decline. This suggests that even though bacteria can recover and grow again after a decline, there is an optimal limit to the exposure time that needs to be considered, where emerging inhibitory

factors can influence bacterial growth. For a clearer understanding of bacterial growth, it can be seen in Figure 1.

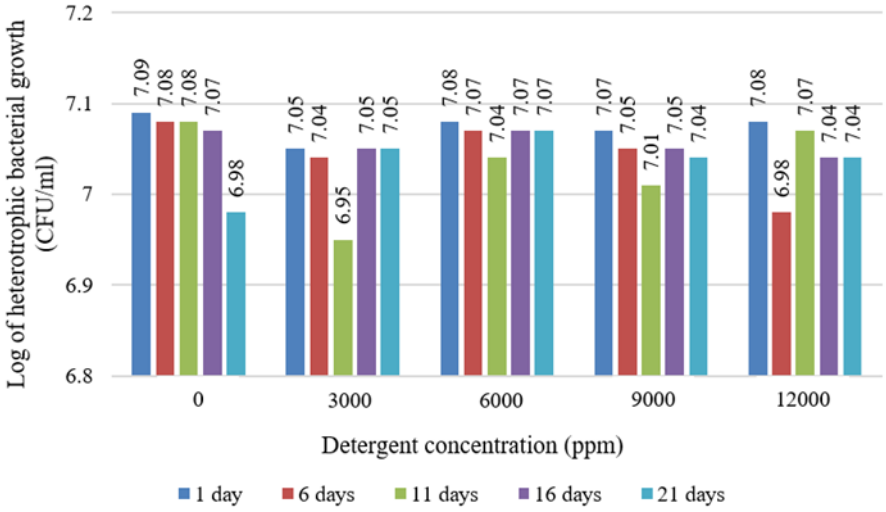

Figure 1. Effect of exposure time on the growth of heterotrophic bacteria

Environmental factors in the test containers, such as nutrient availability and interactions between microorganisms, can influence bacterial growth over the 21 days. This indicates that the detergent concentration may still be within the bacterial tolerance threshold, meaning its effect on growth has not been significant enough to produce noticeable differences.

The statistical test results showed a value of 0.733, indicating no significant difference in heterotrophic bacterial growth based on exposure time, with a p-value > 0.05 in the Friedman test. This suggests that variations in exposure duration did not significantly affect bacterial growth under these experimental conditions. The different exposure times in this study may have caused the accumulation of bacterial excretions, affecting bacterial growth. Waluyo (2004) stated that the presence of metabolic waste products, which may be toxic, can inhibit bacterial growth.

Analysis of the Effect of Detergent Concentration on Bacterial Growth

One of the factors influencing bacterial growth during the study is the change in environmental conditions from their natural state. Bacteria can adapt to their environment as a form of self-defense against extreme conditions such as changes in pressure, temperature, pH, and salinity. Bacteria can produce bioactive compounds and secondary metabolites with specific and unique structures. These compounds serve as a means of self-protection against environmental changes and disturbances from other organisms (Carvalho & Fernandes, 2010). This demonstrates the bacteria's ability to survive and reproduce even in the presence of antimicrobial agents or chemical substances that can inhibit their growth.

The findings of this study show that several factors contribute to the fluctuating average growth of heterotrophic bacteria at different exposure times over 21 days, along with the increasing concentrations of other detergents. Detergents can inhibit the processes of nutrient dissolution and absorption by bacteria. Detergents reduce the surface tension of water by surrounding water molecules, which causes the water molecules to separate more easily and mix with other molecules. In addition, detergents can also inhibit the activity of bacterial enzymes involved in nutrient metabolism. Enzymes are proteins that function to accelerate chemical reactions within living organisms. Detergents can inhibit enzyme activity by binding to the enzymes.

Kusrini (2022) explained that detergents can disrupt the cell membranes of bacteria and aquatic organisms. The cell membrane is a crucial structure for cellular life, as it protects the cell from the external environment, regulates the transport of substances in and out of the cell, and serves as a site for various metabolic reactions. Yudo (2018) stated that detergents are surfactant compounds with two ends, a hydrophilic and a hydrophobic end. Furthermore, Yudo (2018) also explained that

detergents can bind to nutrients, making them unavailable for bacterial absorption. This occurs because detergents exhibit amphoteric properties, meaning they can behave as both acids and bases. The nutrients that detergents can bind include proteins, carbohydrates, and lipids. Proteins are essential nutrients for bacterial growth and development, carbohydrates serve as an energy source, and lipids function as both an energy reserve and a structural component for bacteria. Acidic detergents can bind covalently to proteins, rendering them unusable for bacteria, while basic detergents can bind non-covalently to carbohydrates and lipids, preventing their utilization by bacteria.

Detergents can also interfere with enzyme function. Enzymes are proteins that act as catalysts in metabolic reactions. Detergents can disrupt enzyme function by binding to the enzymes. The bond formed between detergents and enzymes can cause structural changes in the enzymes, preventing them from functioning optimally. This disruption may hinder various metabolic reactions within the cell, potentially leading to cell death (Kusrini, 2022). Heterotrophic bacteria play an important role in the decomposition process within aquatic environments; therefore, their growth directly affects the fertility of the water. This aligns with the statement by Kurnia et al. (2016), who noted that heterotrophic bacteria possess metabolic capabilities crucial to aquatic systems. These bacteria can alter their environment's physical and chemical conditions, influencing bacterial activity, growth, and survival.

Analysis of the Effect of Exposure Duration on Bacterial Growth

Detergents can cause changes in water quality, which in turn can disrupt the life of aquatic organisms. Achmad (2004) stated that shifts in water pH, whether toward more acidic or more alkaline conditions, can drastically affect the survival of fish and other aquatic organisms. A pH level that is too low can lead to the death of aquatic organisms that are sensitive to pH changes. Additionally, detergents can reduce the concentration of dissolved oxygen in water. Dissolved oxygen is essential for the respiration of aquatic organisms. Detergents can also promote the growth of algae and pathogenic bacteria. Both algae and pathogenic bacteria can produce toxins that pose a threat to aquatic life (Pakpahan et al., 2017). Furthermore, detergents can lead to a decline in biodiversity. Biodiversity is essential in maintaining the balance of aquatic ecosystems (Megawati, 2015).

In addition, the duration of seawater detergent exposure also affects the inhibition level of heterotrophic bacterial growth. The longer the detergent remains in seawater, the more time it has to interact with the bacteria, which leads to an increasingly higher level of growth inhibition. Some components of detergents, such as surfactants, can be gradually degraded by bacteria; however, this process often requires considerable time. During the degradation period, surfactants continue interacting with the bacteria, resulting in a progressively more potent inhibition of bacterial growth.

The rate of detergent biodegradation is influenced by several factors, including detergent concentration, the number of microorganisms present, and pH conditions. The lower the detergent concentration or the higher the microbial population, the faster the degradation rate. The degradation occurs more rapidly under neutral or near-neutral pH conditions than in acidic or alkaline environments (Said, 2006). Resistance refers to the ability of a microorganism to withstand specific antimicrobial or antibiotic agents (Artati et al., 2018). Microbial resistance tends to increase in the environment due to the continuous discharge of waste containing antimicrobial substances, including antifungal agents in laundry detergents, household cleaning products, cosmetics, and pharmaceuticals (Tarigan, 2019).

4. CONCLUSION

This study aimed to analyze the effect of detergent concentration and exposure duration on the growth of heterotrophic bacteria in seawater. Based on the results of the tests conducted, it can be concluded that variations in detergent concentration at five different levels, 0 ppm, 3000 ppm, 6000 ppm, 9000 ppm, and 12,000 ppm, did not have a significant effect on the growth of heterotrophic bacteria. Variations in detergent exposure duration 1 day, 6 days, 11 days, 16 days, and 21 days did not significantly affect the growth of heterotrophic bacteria.

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