The Potential Use of Microorganisms as Degradation Agent on Naphthalene and Phenanthrene

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Abstract

This study aims to find the most appropriate indigenous bacteria as Naphthalene and Phenanthrene degrading agents carried out in the growth response test. This research is very important to find the best bacterial agent, which in the future can reduce the impacts of oil pollution, as well as from the presence of oil sludge Bacterial cultures added in the media as much as 5% (v/v), which had previously been measured OD660nm = 0.5. The growth response test was carried out by growing 20 mL of bacteria in AMS media which was added with PAH substrate, naphthalene or Phenanthrene in various concentrations: 200 ppm, 500 ppm, 800 ppm, and 1000 ppm. In the naphthalene substrate with 24-hour incubation, the best treatment was in isolates A with a concentration of 200 ppm. In the phenanthrene substrate 24 hours incubation, the best treatment was in isolate A at 200 ppm, and at 48 hours incubation. Bacterial isolates F in 24-hour incubation increased growth in all treatments and controls. The bacterial E isolate on the phenanthrene substrate at 24-hour incubation has increased growth, which occurs until the incubation time is 48 hours, both in the control and phenanthrene substrate. E Isolate is tolerant of increasing substrate concentrations up to 1000 ppm, both naphthalene and phenanthrene and has the best growth ability. It can be concluded that isolate E has the best growth ability and is tolerant of increasing substrate concentrations up to 1000 ppm. E isolates were designated as the best isolates of naphthalene and phenanthrene degradation.

Keywords: Dumai, Growth Response, Naphthalene, Phenanthrene

INTRODUCTION

Pollution is one of the important problems both pollution of organic matter and inorganic materials. Pollution of organic matter, which gets the most attention, is hydrocarbon pollution because of its abundance. Oil sludge is a precipitate formed at the bottom of the storage tank due to contact between oil, air, and water. Increasing the accumulation of oil sludge can cause a reduction in oil storage capacity and accelerate the occurrence of the rusting process [1,2]. In addition, there are aliphatic, aromatic, and polyaromatic hydrocarbons (PAHs) in oil sludge. PAH content in oil sludge reached 13.24% [3]. These compounds are one of the concerned pollutant sources because they are carcinogenic, mutagenic, and immuno-toxic. The Environmental Protection Agency in the United States categorizes 16 types of PAHs as the main environmental pollutants [4,5,6]. Types of PAHs that have two or three rings (naphthalene and phenanthrene) are compounds that easily degraded compared to other types of PAH [7].

As one of the largest petroleum refining locations in Indonesia, Dumai City, Riau Province, is inseparable from the impacts of pollution and the presence of oil sludge and harmful compounds inside. This study aims to find the most appropriate indigenous bacteria as Naphthalene and Phenanthrene degrading agents carried out in the growth response test. This research is very important to find the best bacterial agent, which in the future can reduce the effects of oil pollution as well as from the presence of oil sludge.

MATERIAL AND METHOD

Research was carried out in Laboratorium Terpadu, Biology department, Faculty of Science and Technology, Airlangga University in November 2018-July 2019. The bacteria used in this study were seven isolates of pure indigenous bacteria from Dumai oil sludge, which obtained from previous research and coded A (4), A (4) 2, Ao (5) 2, A (5) 3, A (5) 5, A (6) 1, A (6) 2, which are then sequentially called isolates A, B, C, D, E, F, G. The media used in this study are Nutrient Broth, Nutrient Agar, and Synthetic Mineral Water (AMS), which was a modified composition [8].

Bacterial cultures added in the media as much as 5% (v/v), which previously had been measured OD660nm=0.5. The culture incubated for three days (72 hours) with a shaker speed of 120 rpm at room temperature. The data obtained in the form of bacterial growth response through measurement of OD every 24 hours using a spectrophotometer and TPC at 72 hours. OD measurement intended to determine the growth

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ISSN. 2087-2852
E-ISSN. 2338-1655

of total bacterial biomass as indicated by increasing substrate turbidity. The TPC method carried out to confirm and count the number of living bacterial colonies.

The growth response test carried out by growing 20 mL of bacteria in AMS media, which added with PAH substrate, naphthalene, or phenanthrene, with various concentrations of 200 ppm, 500 ppm, 800 ppm, and 1,000 ppm. Bacterial cultures added in the media as much as 5% (v/v), which previously had been measured OD660nm=0.5. Furthermore, the culture incubated for 72 hours with a shaker speed of 120 rpm at room temperature. The data obtained were in the form of bacterial growth response through measurement of OD every 24 hours using a spectrophotometer and carried out TPC at 72 hours using Nutrient Agar media. We also conducted a one-way ANOVA and Duncan statistics test to find out the difference between the treatment of the substrate treatment concentration and the incubation time.

RESULT AND DISCUSSION

We use bacterial isolates A, E, and F based on previous research. The bacterial growth response test carried out on naphthalene and phenanthrene substrates in different cultures with different concentrations: 200, 500, 800, 1000 ppm [9]. There is a growth response of bacteria A, E, and F on naphthalene substrates with different concentrations observed for 72 hours (Fig. 1), and the results on phenanthrene substrates (Fig. 2).

Bacteria Growth on Naphthalene Substrates

Bacteria grown on naphthalene substrates show a varied growth response (Fig. 1). The bacterial isolate A at the 24-hour incubation period shows the log (exponential) phase. It proved by the value of OD, which becomes very high at 24 hours incubation time. At 48 hours incubation, bacterial A OD decreased in control, 800 ppm and 1,000 ppm naphthalene concentration treatment. It continued to decrease until 72 hours incubation, while 200 ppm and 500 ppm concentration treatment increased up to 72 hours, and continued to increase. It happens because, at 800 ppm and 1,000 ppm naphthalene concentration, it becomes an inhibitor concentration that can inhibit cell growth because it is toxic [10].

In the 24 hours incubation of bacterial isolates, there was an increase in growth, both in the control and treatment of concentration. This increase in growth occurred until 48 hours incubation for the treatment of concentrations of 200 ppm to 1,000 ppm, while in the control of bacterial growth began to decline. In the control, the OD value dropped because there was no carbon source for bacterial growth. The same thing also happened at 72 hours incubation, where growth continued to increase in the concentration treatment, while in the control there was a continuous decline.

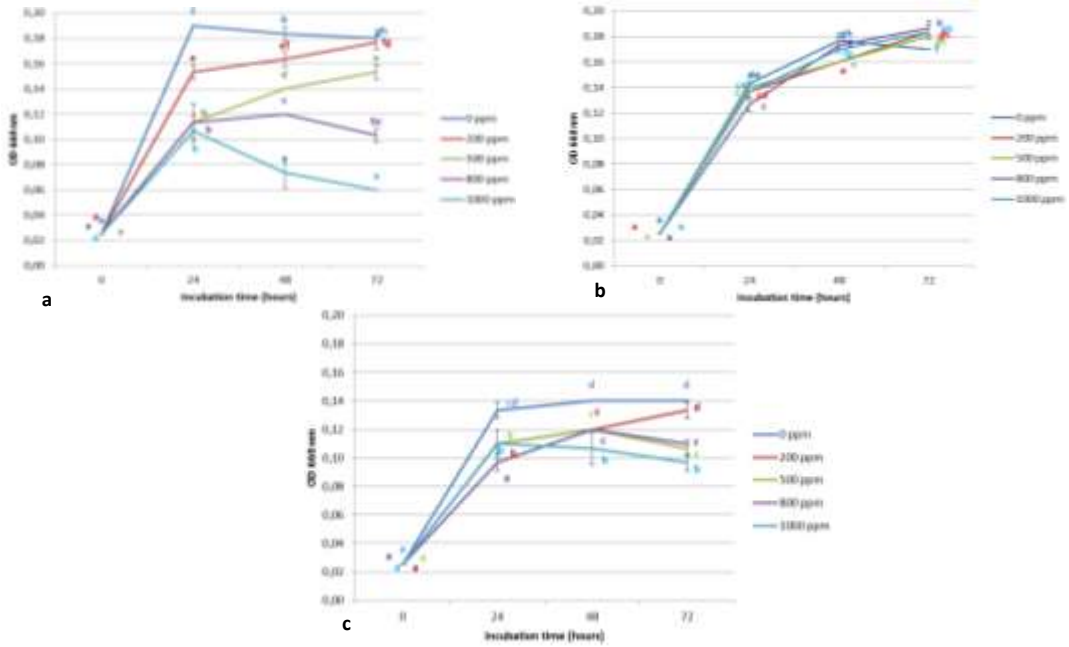

Figure 1. Response to Growth of Bacterial Isolates on the Naphtalene substrate with different concentrations
Description: a) Isolates A, b) Isolates E, and c) Isolates F

ISSN. 2087-2852
E-ISSN. 2338-1655

Bacterial isolates F in 24-hour incubation increased growth in all treatments and controls. During the 48-hour incubation period, there was a varied growth: in the treatment concentration of 200 ppm and control, there was an increase in growth, whereas in naphthalene 500 ppm, 800 ppm and 1,000 ppm there was a decrease in OD values up to 72 hours incubation. At the treatment of 200 ppm at the incubation time of 72 hours, bacterial growth continues to increase, while in the control, growth has entered the stationary phase.

Bacterial Growth on Phenanthrene Substrates

The bacterial growth response on phenanthrene substrates with different concentrations showed in Figure 2. The bacterial isolate A at 24 incubation time reached the log phase. At 48 hours incubation time in phenanthrene 200 ppm and 500 ppm, there was still an increase in growth, whereas in concentrations of 800 ppm and 1,000 ppm there was a decrease, and in the control entering the stationary phase.

This happened until 72 hours incubation. At phenanthrene concentrations of 800 ppm and 1,000 ppm, recorded a decrease in growth. It happened because, at this level, it reached an inhibitor concentration that inhibits bacterial growth. While a decrease in control has occurred because the media has no carbon source for bacterial growth.

The classic problem with microbiology is that bacteria exhibit two types of growth when they are in a culture of mixing two carbon sources. In the case of co-utilization, there is a model that can predict each carbon source in the supply of amino acids [11].

The bacterial E isolate on the phenanthrene substrate at 24-hour incubation has increased growth. It occurred until the incubation time of 48 hours, both in the control and phenanthrene substrate. At 72 hours of incubation, in the treatment of variations in concentration, bacterial growth still increased while in the control decreased. It happened because, in the control, there is no carbon source that can be used by bacteria for growth whereas in the treatment of phenanthrene concentration variations there are still carbon sources to grow [12].

There was a growth of bacterial isolates F at 24 hours incubation time in various treatments and controls. At 48 hours incubation, the addition of phenanthrene substrate with a concentration of 200 ppm increased while the addition of 500 ppm, 800 ppm, 1,000 ppm, and control decreased. At 72 hours of incubation, all treatments experienced a decrease in growth, both in the control and various concentration of the phenanthrene substrate. It happened because the age of the bacteria reaches the Lag phase. This phase shows that bacteria cannot duplicate or modify their physiological performance to cope with their environmental influences [13].

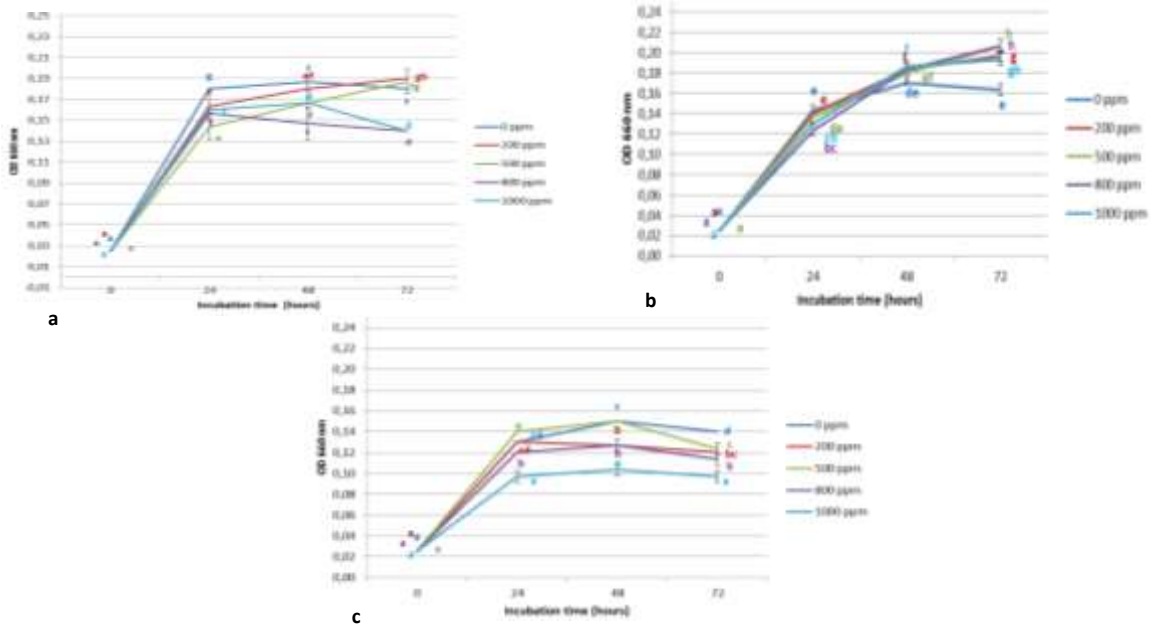

Figure 2. Response to Growth of Bacterial Isolates on the Phenanthrene Substrate with Different Concentrations
Description: a) Isolates A, b) Isolates E, c) Isolates F

OD and TPC values

To strengthen OD measurement data, we measured TPC in log CFU.mL^{-1} at 72 hours incubation time, then compared OD values and TPC values at 72 hours incubation time presented in Figure 3. Based on the one-way ANOVA statistical test followed by the Duncan test, it produces distinguishing notations between the treatment of substrate concentration and incubation time (Fig. 1 and Fig. 2).

In the naphthalene substrate with 24-hour incubation, the best treatment was in isolates A with a concentration of 200 ppm (there were significant differences). However, at 48 and 72 hours incubation, the best treatment was E isolates at a concentration of 800 ppm. In the phenanthrene substrate 24 hours incubation, the best treatment was in isolates A at 200 ppm, and at 48 hours incubation, isolates E at 1000 ppm was the best treatment, but not significantly different from treatment E at 800 ppm. At the incubation time of 72 hours, the treatment of isolates E at 800 ppm was the best, but not significantly different from E treatment at 500 ppm.

Based on its growth response, bacterial isolates E tend to be resistant to variations in substrate concentration, both naphthalene, and phenanthrene. The physical-chemical properties are one of the determinants of the ease of accessibility of hydrocarbons by microbes, such as type, form, solubility, and toxicity [9,14].

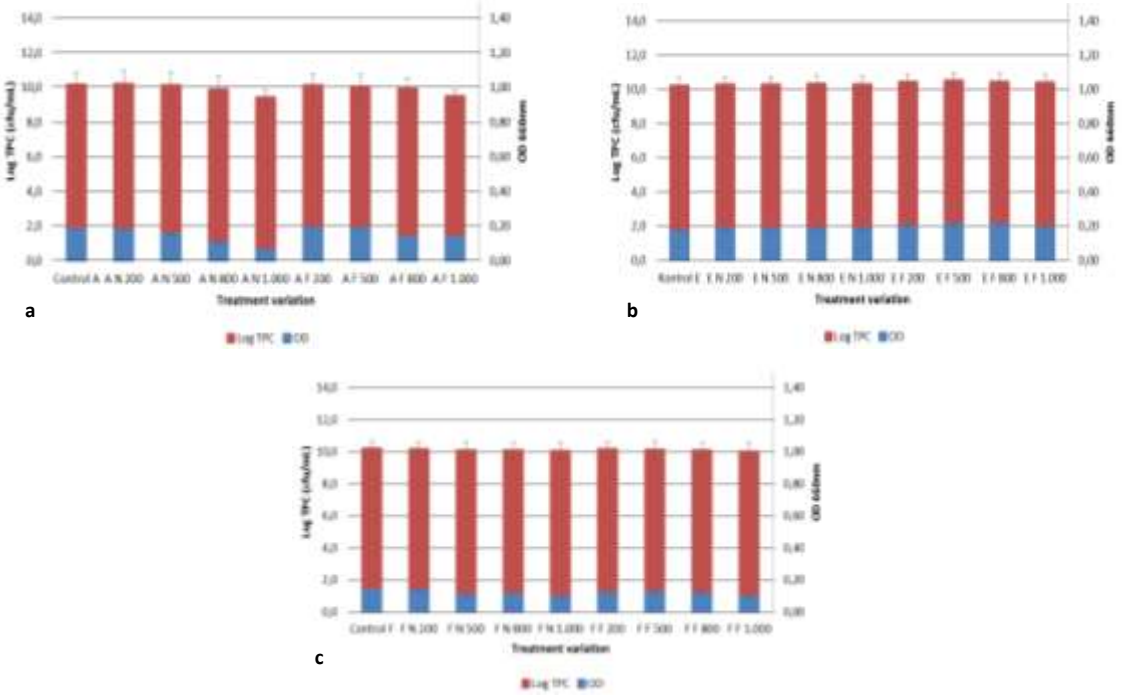

Figure 3. Histogram Comparison of OD and TPC values for N Naphthalene and Phenanthrene at 72 hours incubation period
Description: a) Isolates A, b) Isolates E, c) Isolates F

CONCLUSION

From the results of the growth response test, it concluded that isolate E has the best growth ability and is tolerant of increasing substrate concentrations up to 1000 ppm. Therefore, E isolates designated as the best isolates for naphthalene and phenanthrene degradation.

ACKNOWLEDGEMENTS

We thank Dr. Ni'matuzahroh who accompanied this research. We also thank the managers of the Integrated Laboratory, the Biology department, FST, for providing their permission and assistance in this study.

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