Bioscience
Volume 3 Number 2, 2019, pp. 121-126
ISSN: Print 1412-9760 – Online 2541-5948
DOI: 10.24036/0201932105264-0-00

BIOSCIENCE
http://ejournal.unp.ac.id/index.php/bioscience

Growth Response Test on Potential Indigenous Bacteria to Degrade Naphthalene and Phenanthrene on various pH

Alfi Rizca Hardianti *, Helga Lusiana , Dita Widiyanti Sawitri

Biology Departement, Faculty of Sains and Technology, Airlangga University, Surabaya

*Alfirizcahardianti1990@gmail.com

Abstract. This study aims to understand the growth response of indigenous bacteria originating from oil sludge in Dumai which has the potential as Naphthalene and Fenantren degrading agents based on variations in substrate pH. The isolates used in this study were Isolate A, Isolate E, and Isolate F as a result of isolation from oil sludge in Dumai. Based on the results of the growth response test on various concentration in previous studies, we chose E isolates to be tested for its degradation ability. The bacterial growth response data was obtained based on OD measurements every 24 hours using a spectrophotometer and TPC at 72 hours using Nutrient Agar media. Based on these data, we determined the optimum pH for indigenous bacterial growth potential to degrade naphthalene or phenanthrene. In all variations of pH, bacterial isolate E experienced growth. This shows that bacterium E can use naphthalene and fenantren at normal pH range (pH 5-9).

Key words: growth response, indigenous bacteria,naphthalene,phenanthrene,pH

1. INTRODUCTION

Dumai is one of the largest oil port and producing cities in Indonesia. However, oil also has an impact on environmental sustainability because oil sludge deposits as waste from refineries. Oil sludge can damage oil tanks because they trigger the rust process (Sugiarto, 2004; Banat and Rancich, 2009).

Oil sludge also contains aliphatic, aromatic and poliaromatic hydrocarbons (PAHs) that are carcinogenic, mutagenic, and immuno-toxic, and also have the potential to pollute the environment. The Environmental Protection Agency in the United States categorizes 16 types of PAHs as the main environmental polluters (Skupinska *et al.*, 2004; Seo *et al*., 2012; Masakorala *et al*., 2013). Naphthalene and phenanthrene are types of PAHs that have two or three rings. Both can be degraded more easily than other PAHs (Kafilzadeh and Fatemeh, 2012; Stingley *et al.*, 2004; Mallick and Dutta, 2008; Seo *et al*., 2012; Pawar *et al.*, 2013).

pH is one of the environmental factors that influence microbial growth. During the biodegradation process, pH changes occur due to the formation of organic acids and produce biosurfactants as secondary metabolites (Walker *et al.*, 1975; Kokub *et al.*, 1990, Rosenberg *et al.*, 1980; Nghia, 2007). Biodegradation will not be successful if the pH reaches the optimal range of microbial growth. This study aims to understand the growth

response of indigenous bacteria originating from oil sludge in Dumai which has the potential as Naphthalene and Fenantren degrading agents based on variations in substrate pH. In the future, this research is very important to understand the most ideal pH range for hydrocarbon degradation processes.

2.METHOD

This research was conducted in November 2018 - July 2019 in Laboratorium Terpadu, Microbiology Laboratory, and Molecular Genetic Laboratory, Department of Biology, Faculty of Science and Technology, Airlangga University. The isolates used in this study were Isolate A, Isolate E, and Isolate F as a result of isolation from oil sludge in Dumai. Some of the media used are Nutrient Broth, Nutrient Agar, and Synthetic Mineral Water (AMS). The compounds used as substrate in this study are naphthalene (MERCK) and phenanthrene (MERCK).

Isolates of bacteria A, E, and F are rejuvenated in Nutrient Broth media. Culture was incubated at room temperature for 24 hours. Bacterial cultures are regulated to have cell turbidity (Optical Density / OD) 0.5 at wavelength (□) 660 nm using a spectrophotometer. The growth response test was carried out by growing 20 mL of bacteria in AMS media which added PAH substrate at a concentration of 200 ppm with varying pHs namely 5, 6, 7, 8, and 9. Bacterial cultures that had previously been measured OD660nm = 0.5 were added in media as much as 5% (v / v). Furthermore, the culture was incubated for 72 hours with a shaker speed of 120 rpm at room temperature. The bacterial growth response data was obtained based on OD measurements every 24 hours using a spectrophotometer and TPC at 72 hours using Nutrient Agar media. Based on these data, we determined the optimum pH for indigenous bacterial growth potential to degrade naphthalene or phenanthrene.

3. RESULT AND DISCUSSION

Based on the results of the growth response test on various concentration in previous studies, we chose E isolates to be tested for their degradation ability. To determine the optimum condition of bacterial growth in degrading hydrocarbons, a bacterial growth response test was carried out at various hydrocarbon pHs. This test is carried out at variations in pH 5, 6, 7, 8, and 9. The results can be seen in Figure 1.

It can be seen that in both the naphthalene and phenanthrene substrates at 24-hour incubation, the highest OD values were found in the pH 7 treatment which continued to increase (see Figure 1). At 72 hours incubation the values of 0.2 ± 0.1e were obtained on the naphthalene substrate, which was significantly different from the other treatments. On the other hand, on the phenanthrene substrate, the pH 7 treatment obtained the best value of 0.19 ± 0.1e but was not significantly different from the treatment at pH 6 which was 0.18 ± 0.1e.

Comparison of OD values and TPC results at 72 hours incubation visualized in Figure 2. This comparison shows that there is a correlation between OD and TPC log values. The trend of increasing OD values from 0 to 72 hours is parallel with the TPC log increase trend. It can be concluded that the increasing OD value is due to increased bacterial growth.

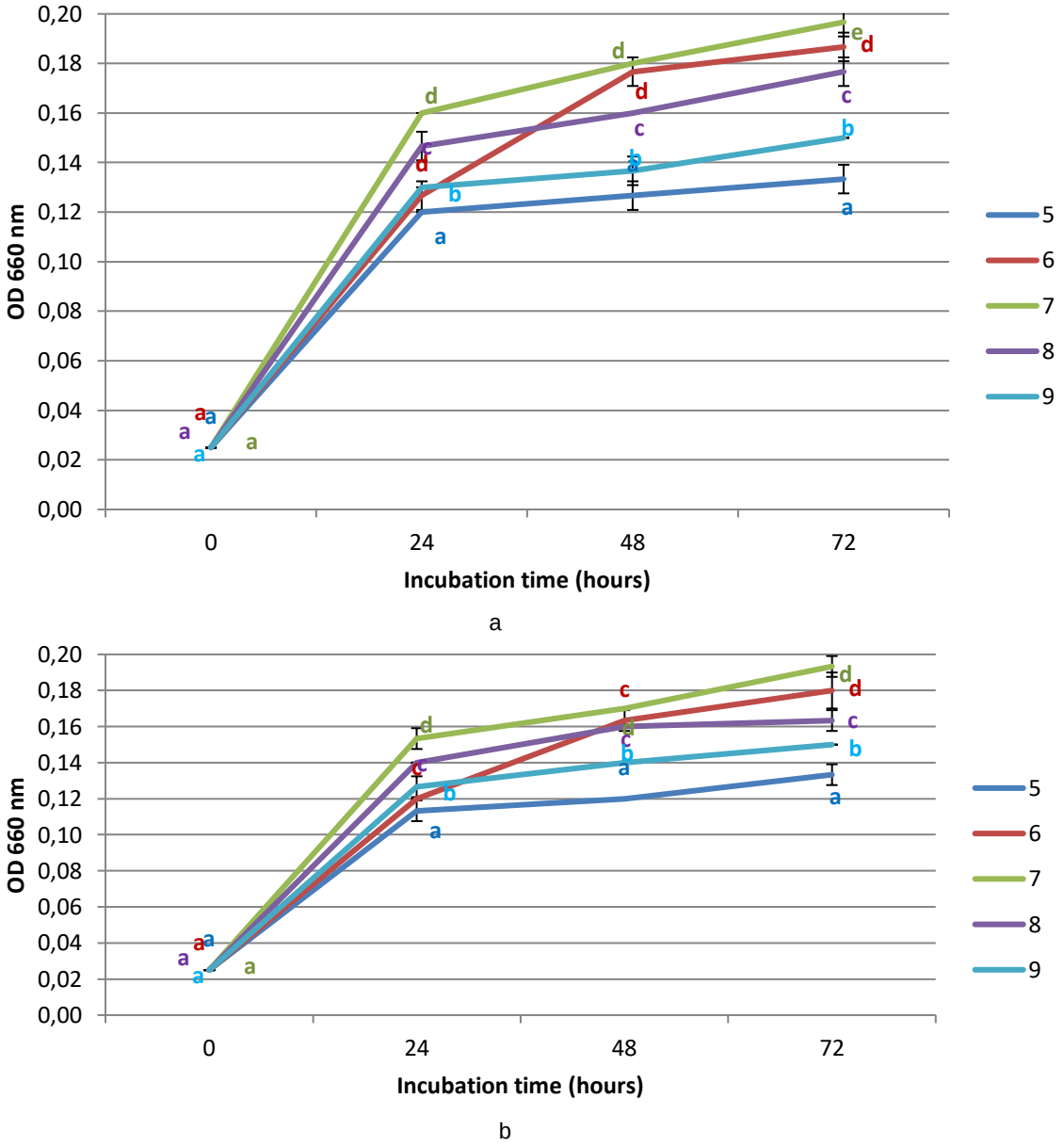

Figure 1. Growth response of isolate E on substrate a) Naphthalene, and b) Phenanthrene in various pH

In all variations of pH, bacterial isolate E experienced growth. This shows that bacterium E can use naphthalene and fenantren at pH 5-9. This shows that bacterium E is neutrophilic which can live in the normal pH range. pH is one of the environmental factors that affect bacterial cell growth. The biodegradation process cannot succeed if the pH is not in the optimal range of microbial growth. The microbes used in the crude oil bioremediation process can live well at pH 6-8. This range becomes a standard for the application of bacteria in the process of degradation of naphthalene and phenatrene (Nghia, 2007; Pratiwi 2014). The degradation process is characterized by the metabolism that produce fatty acids as the final product. This product is further oxidised to acetic acid and propionic acid (Holifah et al., 2018).

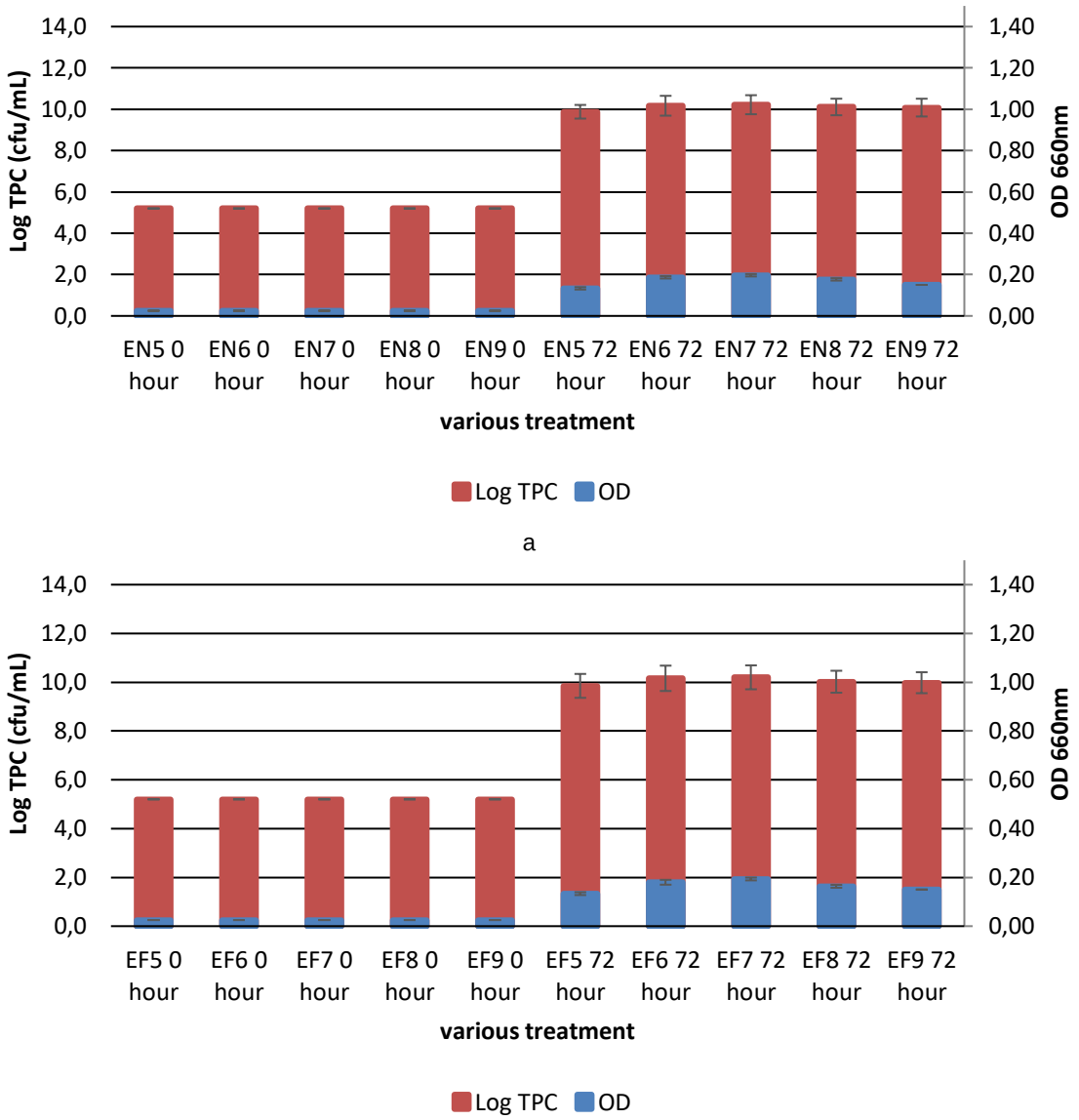

Figure 2. Histogram comparison of OD and TPC of Isolate E on substrate a) Naphthalene and b) Phenanthrene with various pH (Incubation for 0 and 72 hours)

Hydrocarbon compounds are used by microbes as an energy source for metabolism and propagation. The process of degradation occurs in carbonclastic (carbon degrades) microbial groups by hydrocarbon oxidizing enzyme. This allows microbes to cut the hydrocarbon chain. In addition, carbon-plastic microbes have the ability to free themselves (desorption) from hydrocarbons. The synthesis of hydrocarbon oxidizing enzymes is encoded by chromosomes and plasmids that are mutated due to their habitats that are in rich hydocarbons environment (Cookson, 1995).

4. CONCLUSION

Isolate E can grow well on naphthalene and phenanthrene substrates with normal pH tolerance (5-9). This is the basis of knowledge about the ideal pH conditions for the application of Naphthalene and Fenantren degrading bacteria in oil sludge.

5. ACKNOWLEDGEMENT

Authors thank to Dr. Ni'matuzahroh and Dr. Fatimah who accompanied and provided suggestions regarding this manuscript. Authors also thank to members of the Microbiology Laboratory who have provided technical assistance to the research.

REFERENCES

Banat I., and Rancich I.. 2009. No Man Entry Tank Cleaning and Voc Control Using Biotechnological Intervention. *Enviromnemtal Technologies for Refineries*. University of Ulster. Europe

Cookson, JT. 1995. *Bioremidiation engineering design and application.* Mc Graw-hill. USA

Holifah S, Supartono, dan Harjono. 2018. Analisis Penambahan Kotoran Kambing dan Kuda pada proses Bioremidiasi *Oil Sludge* di Pertambangan desa Wonocolo. *Indonesian Journal of Chemical Science.* 7(1):35-42

Kafilzadeh F., and Fatemeh H.P. 2012. Degradation og Naphthalene, Phenanthrenem and Pyrene by *Pseudomonas* sp. and *Corynebacterium* sp. in the Landfill. *International Journal of Bioscience.* 2(9):77-84.

Kokub, D., Shafeeq, Khalid M., Hussain A., and Malik K.A. 1990. Comparative Studies on Emulsification and Biodegradation of *Indigenous* Crude Oils by Enriched Bacteria Cultures. *Biorecovery.* 2:55-68.

Mallick S., and Dutta, T.K.. 2008. Kinetics of Phenanthrene Degradation by *Staphylococcus* sp. strain PN/Y involving 2-hydroxy-1-naphtoic acid in a novel metabolic pathways. *Journal Process Biochemistry* 42: 1004-1008.

Masakorala K., Yao J, Cai M, Chandankere R, Yuan H, and Chen H. 2013. Isolation and Characterization of A Novel Phenanthrene (PHE) Degrading Strain *Pseudomonas* sp. USTB-RU from Petroleum Contaminated Soil. *Journal of Hazardouz Materials.* 263: 493-500.

Nghia NK. 2007. Degradation of Aged Creosete and Diesel Contaminated Soils by Phytoremediation or Biostimulation (nutrients). Unpublished Thesis. Swedish: Sveriges Lantbruks Universitet.

Pawar AN, Ugale SS, More MG, Kokani NF, Khandelwal SR. 2013. Biological Degradation of Naphhalene: A New Era. *J. Bioremediation Biodegradation.* 4:7.

Pratiwi IA. 2014. Biodegradasi Komponen Hidrokarbon *Oily Sludge* oleh *Micrococcus* sp. L II 61. Tesis. Universitas Airlangga Seo, Jong-Su, Keum YS, Li Q.X. 2009. Bacterial Degradation of Aromatic Compound. *International Journal Environmental Res*earch and *Public Health.* 6:278-309.

Rosenberg M, Gutnick D, and Rosenberg E. 1980, Adherence of Bacteria to Hydrocarbons: A Simple Method for Measuring Cell-Surface Hydrofobicity. *FEMS Microbiol Lett.* 9: 29–33.

Seo J, Keum YS, and Li QX. 2012. Mycobacterium aromativorans JS19b1^{T} Degrades Phenanthrene through C-1,2, C-3,4 and C-9-10 dioxygenation pathways. *International Biodeterioration & Biodegradation.* 70: 96-103.

Skupinska K, Misiewicz I, and Guttman T. 2004. Polycyclic Aromatic Hydrocarbon Physiochemical Properties, Environmental Appearance and Impact on Living Organism. *Acta Poloniae Pharmaccutica-Drug Research.* 3(3):233-240.

Sugiarto AT. 2004. Teknologi Plasma Untuk Daur Ulang Limbah Oil Sludge. http://www.beritaiptek.com. Acessed on July 27, 2019

Walker JD, Austin HF, and Colwell RR. 1975. Utilization of Mixed Hydrocarbon Substrates by Petroleum-degrading Microorganism. *Journal of General Appication Microbiol.* 21:27-39.