Lubricant Oil Bioremediation by *Rhodococcus erythropolis* Bacteria and Indigenous Bacteria Isolated from Water Contaminated with Lubricant Oil

Frentina Murti Sujadi 1), Yahya 2) and Andi Kurniawan 3), Abd. Aziz Amin 2)

1) Fisheries and Marine Science Faculty, University of Brawijaya, Indonesia
2) Coastal and Marine Research Center Brawijaya, University of Brawijaya, Indonesia

Email Address: frentinamurtis@gmail.com

**Abstract** The bioremediation system can be improved by using specific bacterial potential as oil-degrading bacteria which microorganisms can use hydrocarbons as a carbon source for their metabolic processes. The aim of this study is to identify *R. erythropolis* on degradation oil contamination and to obtain indigenous bacteria as new agent bacteria on bioremediation of oil contamination. The polluted water samples from used oil were taken from PPN Prigi, Trenggalek, East Java, Indonesia. The density of bacteria was analyzed in the interval time of days 0, 2, 4, 6 and 7 and TPH was analyzed at final incubation. The results of this study indicate that the effectiveness of reducing oil concentration was used in testing the potential of bacteria from the highest was *Pseudomonas aeruginosa* which as indigenous bacteria isolated from water contaminated sites with application cell rate $1 \times 10^8$ cells/ml. It reduced of oil concentration up to 53%, and $1 \times 10^6$ cells/ml reduced oil concentration up to 47%. While, *R. erythropolis* with application cell rate $1 \times 10^8$ cells/ml reduced 47%. This result was found that *Pseudomonas aeruginosa* was effectively removed of oil concentration.

**Introduction** Water contamination in the sea due to careless disposal of used oil is increasing in fishing ports. Activities related to ship activities such as mooring of ships, landing and marketing of catches, loading needs of the sea, construction and repair of ships and repairs to fishing equipment have the potential to be a source of environmental pollution. The negative impact of the existence of fishing port activities in the form of solid and liquid waste is the cause of environmental pollution. Puspito (2013) stated that one of the sources of pollution in the coastal and port environment is derived from the activities of ships in the port. Obi et al. (2014) also stated that the dominant waste produced from ships is plastic, scattered used oil and liquid waste.

One of the problems of sea pollution is hydrocarbon, particularly, oil and oil products. The development of the fishing port industry is directly proportional to the increase in ship activities. According to several studies it was reported that the activities of ships are a source of pollution in the aquatic environment of fishing ports. Waste that dominates in the fishing port is plastic, liquid waste and scattered used oil. The bad impact of the spillage of used oil vessels which affects the development of marine biota can even cause death (Bhattacharyya et al., 2003). Hydrocarbons in used oil waste can have different effects on organism, depending on...
their physiological state, the chemical composition of the oil, its concentration, temperature, water, etc. Thus, at a concentration of 0.001 mg·dm⁻³ oil and petroleum accelerate the death of aquatic organism. The zooplankton mortality rate increased to 1-56 times. The copepodic mortality rate has risen to 70%. (Obi et al., 2014).

Conventional pollution prevention efforts that are based on mechanical, physical and chemical processes, have often been unsatisfactory and inadequate. Weaknesses use physical methods which cannot be done completely if the oil has spread everywhere. Chemical countermeasures are carried out by looking for chemicals that could disperse hydrocarbon compounds (Das and Chandran, 2010). However, the use of chemical compounds is only a matter of moving, on the one hand the treatment of dispersants can disperse hydrocarbon compounds thereby reducing the level of pollution, but on the other hand the use of dispersants has been reported to be highly toxic to marine biota (Bhattacharyya et al., 2003).

Therefore, one alternative method that is more effective, efficient and environmentally friendly is bioremediation, namely the technology of using living things especially bacteria as biological agents capable of utilizing petroleum hydrocarbons as a carbon source for their metabolic processes which is then converted to CO₂, H₂O and biomass (Dhar et al., 2014). Microorganisms may be able to removal hydrocarbon so it can be bioremediation agent in the detoxification of pollution in environment. The process of using microorganisms can be used to biodegrade hydrocarbons in used oil waste. Microorganisms can remodel pollutants released in the environment as a source of energy and produce biomass in the metabolic process. Bacteria that can degrade compounds found in petroleum hydrocarbons are known as hydrocarbon clastic bacteria. These bacteria have characteristics that are not possessed by other microbes, namely their ability to express alkane hydroxylase enzymes, namely hydrocarbon oxidizing enzymes, so that these bacteria can degrade petroleum hydrocarbons by cutting the hydrocarbon chains shorter (Hozumi, 2013). Biodegradation of hydrocarbons is influenced by oxygen, pH, temperature, microbial population (bioaugmentation) and nutrition (biostimulation) (Suja et al., 2014). Bioremediation technology can be used in the process of reducing hydrocarbons in used oil waste with the use of karene bacteria more easily and quickly multiply. Bioremediation of used oil waste provides a lot of money which is one of the best environmentally friendly and does not require a large amount of money in comparison with the physical methods of chemical.

Isolation technique Isolation techniques are carried out to count pure bacteria that are not contaminated with other microorganisms. The research of Yetti (2016), obtained bacterial isolates from consortiums from marine waters that have the potential to degrade PAH compounds. Some experts have reported that bacteria from the genus *Rhodococcus* have the ability to reduce hydrocarbons including long chain hydrocarbons (Bourguignon et al., 2014). *Rhodococcus* also supports the ability to overhaul dangerous competencies such as phenathene. *Rhodococcus* consumed near 70% of coil in the contamination sites and consider that *Rhodococcus* was to be capable of use for developing a purification technology for being bioremediation agent bacteria in the ecosystem of pollution by petroleum hydrocarbon. Here, development of an efficient bioremediation system in water contamination sites for oil hydrocarbon is described. Thus, the aim of this study to
identified *R. erythropolis* on degradation oil contamination and obtaining indigenous bacteria as new agent bacteria on bioremediation of lubricant oil contamination.

**Materials and methods**

**Description of The Site and Sampling**

This research was conducted in December 2018 - January 2019. The sampling site was located in Prigi, Trenggalek, East Java, Indonesia. This location in-situ and ex-situ parameter of water quality also be measured that consisted of temperature, pH, DO, salinity, and total petroleum hydrocarbon. Water Sample for isolation bacteria was collected from most contamination sites and Laboratory analysis was in the Laboratory of Fishery Products Safety and Handling of Fisheries Products (Faculty of Fisheries and Marine Sciences, Brawijaya University, Indonesia). Analyze of Hydrocarbon was analyzed in Microbiology laboratory. Faculty of Science, Islamic of Malang University, Indonesia.

**Isolation of Indigenous Bacteria**

Bacterial species were isolated from the collected water sample by serial dilution $10^1$ to $10^5$ dilutions, and the diluted samples were spread on selective Bushnell-Hass media with 1 ml of oil as carbon sources. The inoculated plates were incubated at 30°C for 1 week. The isolates were grown on the media until a single colony was obtained. Microbial colony was transferred with an inoculation loop onto solid Luria Bertani broth medium for separation and purification.

**Biochemical Test of Indigenous Bacteria**

Biochemical tests for bacterial identification are used API 20E. Pure bacterial isolates grown on slant NA media were then incubated at 37°C for 1 day. The isolates which had been bred for 24 hours were then taken 1 colony and bred first in 5 ml 0.85 % NaCl. The bacterial suspension is then put into an API paper strip consisting of 20 microtavings which have been filled with dry reagents. After 24 hours color changes were observed in the microtube. Data from the biochemical test results are then entered into the API KIT 20E software.

**Test The Potential of Bacteria Against Lubricant Oil**

The exogenous bacteria (*R. erythropolis*) and indigenous isolated bacteria used were prepared in the LB broth medium. Then, the following treatment consisted; (A): Indigenous isolation bacteria ($1 \times 10^6$ cells/ml); (B): Indigenous isolation bacteria ($1 \times 10^8$ cells/ml); (C): *R. erythropolis* ($1 \times 10^8$ cells/ml) and control without bacteria. Thus, all of treatments were added by oil 30 ppm and incubated in water bath shaker for 7 days at 32°C, 170 rpm speed shaker.

**Estimated Density of Bacterial Cells**

The calculation of bacterial density was carried out under a 400× zoom microscope using the hemocytometer method. Counted cells are usually colored to facilitate visualization and calculation. The calculated cell coloring can use trypan blue (0.4% (w/v) in PBS). Trypan blue is a diazo dye which is very important in its use in staining dead cells. Living cells will look to have clear cytoplasm, while dead cells will appear blue.

$$\Sigma \text{cells/ml} = \Sigma \text{cell count} \times 250,000 \times n \times a$$

**Information:**

- $\Sigma \text{cells/ml}$: Total bacteria (cells/ml)
- $n$: dilution factor
- $a$: trypan blue volume

---

Copyright © 2020 Universitas Brawijaya

E-ISSN 2355-9926
Analysis of Removal Lubricant Oil Levels

Analysis of removal oil was done at day 0 and final incubation. Added 5 ml of 3 N HCl and 60 ml of n-hexane from purified distillation at 60°C, then shaken for ±15 minutes, let stand until n-hexane is separated. There are 3 layers, namely n-hexane, used oil and water. The water is removed, the layer of used oil and n-hexane is filtered with filter paper which has been smeared ±0.5 g of Na₂SO₄ into a 100 ml beaker that has been weighed. The chemical glass is heated at 60°C until n-hexane runs out. The beaker is removed and left to cool until it is weighed and weighed. The levels of used oil are calculated by:

\[
\text{Level of used oil (g)} = W2 - W1
\]

Information:
W1: dry beaker (g)
W2: beaker with the amount of used oil obtained (g)

Results and Discussion

Water Quality Analysis

The results of water quality parameters. The purpose of measuring water is to find out whether the quality of water in Prigi Trenggalek PPN, East Java is in accordance with water quality standards. This measurement has been carried out at the Hydrology Laboratory of the Division of Aquatic Biotechnology and Environment, Faculty of Fisheries and Marine Sciences, University of Brawijaya, Malang. The following results of water quality (Table 1).

| Parameter | Unit | Observation Value | Optimum Value | Reference |
|-----------|------|-------------------|---------------|-----------|
| Temperature | °C | 27 | 20-30 | Perda Jatim No.2 Th.2008 |
| pH | - | 8.16 | 6-9 | PP No. 82 tahun 2001 |
| DO | (mg/l) | 8.2 | <10 | PP No. 82 tahun 2001 |
| Salinity | (ppm) | 27 | 33 – 34 | Kepmen LH No.51 th 2004 |
| TPH | (ppm) | 30 | <20 | Kepmen LH No. 19 th 2010 |

1. Temperature

The results of temperature show a water temperature of 27°C. The temperature condition is still in accordance with class II water quality criteria according to East Java Provincial Regulation No. 2 of 2008 which is at 3°C deviation from its natural temperature, so the condition of water quality in terms of temperature parameters is still in water quality criteria according to its designation. According to (Yan et al., 2018), there are several factors governing the balance of temperature, namely rainfall, evaporation, humidity, air temperature, wind speed and solar radiation.

Increasing the water temperature by 10°C causes an increase in oxygen consumption by aquatic organisms around 2-3 times which is followed by a decrease in dissolved oxygen levels (El-khawaga et al., 2015). According to Septriady (2017), the temperature will affect the level of availability of oxygen and nutrients in water. Changes in temperature will also affect the life of aquatic organisms and can even cause the extinction of aquatic organism that are sensitive to high temperatures (Delille et al., 2004).

2. Acidity (pH)

The results of pH show that the pH value is alkaline, the value is still within the range of the port area's environmental quality standards. As suggested by Ajoku & Oduola (2013), the acidity (pH) of water shows the levels of hydrogen ions or protons contained in water.
pH is a limiting factor for organisms living in waters. Waters with a pH that is too high or low will affect the survival of living organisms in it (Obahiagbon et al., 2014), but according to Zhang et al. (2018), a high pH also affects heavy metal content.

The degree of acidity (pH) in a waters is one of the important chemical parameters in monitoring water stability (El-Rahim et al., 2009). Changes in the pH value of waters against aquatic organisms have certain limits with varying pH values. The pH value of the environment affects the cell membrane transport process and balances the catalytic reaction of enzyme activity (Pawar, 2015).

3. Dissolved Oxygen (DO)

The results of DO show DO values of 8.2 mg/l, still below the threshold and indicating good water conditions. DO shows the level of oxygen dissolved in water. DO is influenced by temperature and mineral water. The lower the temperature, the higher the DO and the better the condition of the waters. DO is needed in the process of respiration, photosynthesis and organism metabolism (Yu et al., 2012).

Oxygen plays an important role as an indicator of water quality because dissolved oxygen plays a role in the oxidation process and reduction of organic and inorganic materials. The speed of diffusion of oxygen from the air depends on several extracts factors, such as turbidity of water, movement of water and air masses such as currents, waves and tides recede. Dissolved oxygen levels in waters are also affected by high temperatures and more salinity height. If the temperature of a temperature increases, the DO value will decrease and if the salinity a waters increase, the DO value will also decrease (Viyakarn et al., 2015).

4. Salinity

Salinity is defined as the amount of weight all salt (in grams) dissolved in one liter of water, usually expressed in units of grams per liter. Salinity distribution in the sea is affected by various factors such as water circulation, evaporation, bulk rain and river flow. Salinity shows the level of salt in a certain volume of water which is directly proportional to TDS. Salinity describes the total ion concentration of water with the main constituent ions namely sodium, potassium, magnesium, chloride, sulfate and bicarbonate. The salinity gradient pattern varies depending on the season, estuary topography, tide ebb and amount and freshwater (Thavasi et al., 2007).

Water salinity describes the womb salt in waters and its size expressed in per mil. Fluctuations salinity is a common condition in estuary area. Qin et al., (2012), states the mix of seawater mass with freshwater makes the estuary region have uniqueness, namely with the formation of water brackish with fluctuating salinity. Salinity affects biological processes and directly will affect the life of the organism between another aspect of the growth rate, the amount of food that is consumed, food conversion value, and power survival (Al-Hawash et al., 2018).

5. TPH (Total Petroleum Hydrocarbon)

The results of TPH shows TPH value of 30 ppm so that the value exceeds the standard threshold of port waters, the condition of water quality in terms of TPH parameters is not in the water quality criteria according to its designation so efforts should be made to reduce hydrocarbon contaminants in these waters.

According to Schunck et al. (2004), decrease in TPH concentration in the reactor without addition of inoculum caused by influence physical factors, such as the effect of shuffling on shaker
media, room temperature, and photosun oxidation. This process is called weathering. This process produces compounds with low molecular weight (volatile hydrocarbons) and volatile.

**Bacterial Density during Oil Bioremediation Process**

The total bacterial density cells were counted for 7 days (Figure 1). The results of bacterial density show that bacterial density increased their activity on a response oil degradation process. The results of the study found an abundance of the highest population of bacterial cells in treatment C (*Rhodococcus erythropolis* 1 ×10⁸ cells/ml) increased of total bacterial up to 1.95 ×10⁹ cells/ml. While, *Pseudomonas aeruginosa* in treatment A increased up to 1.28 ×10⁹ cells/ml and treatment B up to 1.33 ×10⁹ cells/ml. According to Subramaniam *et al.* (2014), in bioremediation the use of indigenous microorganisms (indigen) is still not maximum so that inoculation of exogenous (exogenous) microorganisms is needed which is a mixture of several types of bacteria or fungi that have the potential to degrade these pollutants. The difference in growth in each treatment was due to the different adaptation processes. Bacteria will show different growth patterns, the periode time needed to grow or adapt, and the metabolites produced (Bourguignon *et al.*, 2014).

![Figure 1. Bacterial Cells Growth (n=2)](image)

Figure 1 shows that at 7th days observation is the stationary bacteria on oil degradation. Maximum stationary phase during which is there is practically no increase in the number of bacteria. There are several reasons why a batch culture may reach stationary phase. One common reason is that the carbon and energy source or an essential nutrient becomes completely used up. When a carbon source is used up it does not necessarily mean that all growth stops. This is because dying cells can lyse and provide a source of nutrients (Fu *et al.*, 2015).

**Lubricant Oil Removal**

The percentage of degradation lubricant oil by hydrocarbonoclastic bacteria can be seen in Figure 2.
The percentage of degradation is the total amount of hydrocarbons that have already been degraded by hydrocarbonoclastic bacteria. The results of the measurement of the concentration of used oil on treatments A, B and C shows the most significant decrease occurred in treatment B of 53% with the value of the used oil final concentration of 14 ppm, then in treatments A and C there was a similar decrease 46% with the value of used oil final concentration of 16 ppm (Figure 2). If referring to the Regulation of the Minister of Environment No. 19 of 2010 concerning waste water quality standards for businesses and / or petroleum processing activities the maximum level of TPH is 20 ppm.

Bioremediation of used oil waste using indigenous bacteria, used oil can be reduced with the help of indigenous bacteria and exogenous bacteria through metabolic processes. The bioremediation process of hydrocarbons occurs most rapidly in aerobic conditions. The first step in the mechanism of alkane degradation by bacteria in aerobic conditions is the oxidation of alkanes by the class of oxygenase enzymes (enzymes that catalyze the incorporation of oxygen into the substrate) namely alkane hydroxylase enzymes that catalyze the addition of hydroxyl groups by attacking the O atom through oxidation during the alkane hydroxylation reaction. Alkanes are oxidized to alcohol and subsequently become fatty acids (Schunck et al., 2004). The next pathway of fatty acid metabolism can be through cellular lipid pathways, β-oxidation, and α-oxidation.

Through the β-oxidation pathway fatty acids will be converted into acetyl co-A and enter into the TCA cycle, converted into CO₂ and energy. If through the fatty acid α-oxidation pathway it will be converted directly into CO₂ and fat derivatives (Al-Hawash et al., 2018).

The degradation process of hydrocarbons (used oil) can be mediated by the enzymatic system. The mechanism of biodegradation of hydrocarbons is first attaching microbial cells to the substrate and both biosurfactant production (Yetti, 2016). Biosurfactants are complex compounds produced by various microorganisms (Dhar et al., 2014; Hozumi, 2013). Biodegradation is enhanced by surfactants due to an increase in solubilization and bioavailability of pollutants (Dasari et al., 2014; Bourguignon et al., 2014).

Characterization and Identification of Indigenous Bacteria

The isolates obtained generally have similarities with the characteristics of the group of hydrocarbon clastic bacteria, when viewed from the form of bacterial colonies obtained, that is round. Color of yellowish white colonies. Flat elevation of colonies. Edge of jagged colonies. The results of microscopic observations and gram staining tests showed that indigenous bacterial
isolates were rod-shaped bacteria and included gram-negative bacteria. According to Romo et al. (2013) that hydrocarbonoclastic bacteria generally have the form of round colonies, white colony, milky white, clear white and yellowish white, with convex and flat elevation, the edge of the clam colony, flat and jagged. Based on the results of the similarity of characterization, the isolates obtained were thought to also have the ability to reduce the hydrocarbon concentration of used oil waste. This is supported by the use of a selective medium for hydrocarbonoclastic bacteria that the isolates obtained are a group of hydrocarbonoclastic bacteria that can degrade hydrocarbons.

Figure 3. The positive results of Pseudomonas aeruginosa use the API 20E kit

Figure 3 shows the biochemical reactions that gave positive identification results of Pseudomonas aeruginosa from the sample of Prigi, Trenggalek PPN waters contaminated with used oil waste using the API 20E kit. According to Nucera et al. (2006) API 20E kit contains dry substrate which will show the results of enzymatic activity of several types of amino acids and carbohydrate fermentation. The identification results are obtained based on numerical profiles which are divided into 3 numerical categories, namely 1, 2 and 4. The positive numbers generated are then added up and entered into the API 20E software. Samples that give positive results of Pseudomonas with a probability of ≥90% can be confirmed as Pseudomonas aeruginosa.

Identification using the API 20E kit method is a standard method that is often carried out to identify bacteria belonging to the family Enterobacteriaceae, gram negative, and non-fastidious bacteria (Nucera et al., 2006). Therefore, these indigenous bacterial isolates can be identified in the API 20E kit because the API 20E kit can identify bacteria from the Enterobacteriaceae family and gram-negative bacteria. The advantage of identification using the API 20E kit is that it does not require a long time and is easy to do, but costs are not small to identify with this kit when compared to other biochemical identification standards (Romo et al., 2013).

The discovery of Pseudomonas aeruginosa bacteria in the Prigi, Trenggalek PPN waters contaminated with used oil waste indicates that Pseudomonas aeruginosa has the potential to degrade used oil waste in the waters. This is supported by the study of El-khawaga et al. (2015) who found the Pseudomonas sp. from soil contaminated with used oil waste. Microbes that are capable of making biodegradation into a better environment. Pseudomonas sp. including the group of obligate aerobes that have stem cell forms with a size of 0.5-1.0 µm. These bacteria are widespread in nature and predominant. According to Shah et al. (2013), Pseudomonas sp. is an important key in the carbon cycle so that these bacteria are often used as bioremediation agents. P. putida, P. fluorescent, P. aeruginosa is a type of Pseudomonas bacteria that is often used as a bioremediation agent for hydrocarbon and heavy metal wastes.
According to Yetti (2016), most microbes that live and play a role in the petroleum hydrocarbon environment are mostly bacteria. Bacteria have physiological and metabolic abilities to degrade pollutants (Septriady, 2017). Bacteria that can degrade compounds found in petroleum hydrocarbons are known as hydrocarbonlastic bacteria. These bacteria have characteristics that are not possessed by other microbes, namely their ability to express hydroxylase enzymes namely hydrocarbon oxidizing enzymes, so that these bacteria are able to degrade petroleum hydrocarbons by cutting the hydrocarbon chains shorter (Peixoto et al., 2011).

**Statistical Analysis**

Relationship between pace decrease in TPH concentration and increase in number of bacteria can be seen in Table 2.

**Table 2. Results of Variant Analysis (ANOVA) using SPSS Software**

| Source          | Type III Sum of Squares | df | Mean Square | F      | Sig   |
|-----------------|-------------------------|----|-------------|--------|-------|
| Corrected Model | 374.66*                 | 3  | 124.889     | 187.333| .005  |
| Intercept       | 2400.000                | 1  | 2400.000    | 3.60E3 | .000  |
| Perlakuan       | 109.000                 | 2  | 54.000      | 91.000 | .012  |
| Kepadatan       | 266.667                 | 1  | 266.667     | 400.000| .002  |
| Error           | 1.333                   | 2  | .667        |        |       |
| Total           | 2776.000                | 6  |             |        |       |
| Corrected Total | 376.000                 | 5  |             |        |       |

The substrate decrease rate is in the form of TPH concentration is followed by an increase in number microorganism population and involving substrate as a source of energy and carbon. The number of *Pseudomonas aeruginosa* and *Rhodococcus erythropolis* bacterial isolates was calculated using the hemocytometer method through the duration of measurement every two days for one week. Based on Variant analysis (ANOVA) at the level of error δ = 5% regarding the conclusion of the sig value. if the value is sig. <0.05 then there is a significant relationship and vice versa table so there is no significant relationship. Based on the results of the analysis it was found that the value of sig. at 0.012 <0.05 for a 5% error level, then the treatment of bacterial starter density has a fairly good influence on decreasing the concentration of used oil waste.

The number of bacterial populations after the addition of bacterial isolates in the bioremediation process had a significant effect on the rate of decline in TPH. Bacteria will form a preference for choosing a substrate so that pathway reaction pathways are created from hydrocarbon pollutant contaminants (Dianou et al., 2016). Bacterial population after addition of bacterial isolates in the process bioremediation has a significant influence the rate of decline in TPH. Bacteria will form a preference for choosing a substrate so that the pathway reaction path is created from hydrocarbon pollutant contaminants. In this control reactor, natural activity indigenous bacteria in actual water has been detected and has the ability to utilize hydrocarbon pollutants as food source. However, the ability of bacteria it is still latent or not expressed out (Hozumi, 2013).
Conclusions and Suggestions

Based on the characteristic test bacteria that play a role in the treatment of wastewater containing identified used oil as *Pseudomonas aeruginosa*. The highest decreasing effectiveness of used oil concentration on bacterial potential test was *Pseudomonas aeruginosa* with a density of $1 \times 10^8$ cells/ml (53%), *Rhodococcus erythropolis* with a density of $1 \times 10^6$ cells/ml (47%) and *Pseudomonas aeruginosa* with a density of $1 \times 10^6$ cells/ml (46%). The ability of *Pseudomonas aeruginosa* and *Rhodococcus erythropolis* isolates showed significant differences ($P<0.05$) compared to controls.

From the results of this study, further research is needed to find out various other types of bacteria that have the ability to degrade hydrocarbons from used oil waste. Further research needs to be done on gram staining to see the shape of the cell (bacillus / coccus) of bacteria and need to examine the type of bacteria with more accurate methods such as analysis of RDNA 16s.

Acknowledgements

The research is supported by the LPDP scholarship, the Ministry of Finance of the Republic of Indonesia. Dr. Ir. Yahya, M.P. and Andi Kurniawan, S.Pi., M.Eng., D.Sc as a great supervisor and advisor.

References

Ajoku, G. A. O. & Oduola, M. K. (2013). Kinetic Model of pH effect on Bioremediation of Crude Petroleum Contaminated Soil. 1. Model Development, *American Journal of Chemical Engineering*, 1(1), 6-10. doi: 10.11648/j.ajche.20130101.12.

Al-Hawash, A. B., Dragh, M. A., Li, S., Alhujaily, A., Abbood, H., A., Zhang, X., Ma, F. (2018). Principles of microbial degradation of petroleum hydrocarbons in the environment. The *Egyptian Journal of Aquatic Research*, 44(2), 71–76. doi: 10.1016/j.ejar.2018.06.001.

Bhattacharyya, S., Klerks, P. L. and Nyman, J. A. (2003). Toxicity to freshwater organisms from oils and oil spill chemical treatments in laboratory microcosms. *Environmental Pollution*, 122(2), 205–215. doi: 10.1016/S0269-7491(02)00294-4.

Bourguignon, N., Isaac, P., Alvarez, H., Amoroso, M. J., Ferrero, M. A. (2014). Enhanced polyaromatic hydrocarbon degradation by adapted cultures of actinomycete strains. *Journal of Basic Microbiology*, 54(12), 1288–1294. doi: 10.1002/jobm.201400262.

Das, N. and Chandran, P. (2010). Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Biotechnology Research International*, 2011, 1–13. doi: 10.4061/2011/941810.

Dasari, S., Subbaiah, K. C. V., Wudayagiri, R., Valluru, L. (2014). Biosurfactant-mediated biodegradation of polycyclic aromatic hydrocarbons-naphthalene. *Bioremediation Journal*, 18(3), 258–265. doi: 10.1080/10889868.2014.933169.

Delille, D., Coulon, F. and Pelletier, E. (2004). Effects of temperature warming during a bioremediation study of natural and nutrient-amended hydrocarbon-contaminated sub-
Antarctic soils. *Cold Regions Science and Technology*, 40(1–2), 61–70. doi: 10.1016/j.coldregions.2004.05.005.

Dhar, K., Dutta, S. and Anwar, M. N. (2014). Biodegradation of Petroleum Hydrocarbon by indigenous Fungi isolated from Ship breaking yards of Bangladesh, *International Research Journal of Biological Sciences*, 3(9), 22–30. http://www.isca.me/IJBS/Archive/v3/i9/4.ISCA-IRJBS-2014-62.pdf

Dianou, D., Swadogo, A., Otidobiga, H. C., Nitiema, L. W., Traoré, A. S. (2016). Optimization of Hydrocarbons Biodegradation by Bacterial Strains Isolated from Wastewaters in Ouagadougou, Burkina Faso: Case Study of SAE 40/50 Used Oils and Diesel. *Journal of Agricultural Chemistry and Environment*, 5(1), 1–11. doi: 10.4236/jacen.2016.51001.

El-khawaga, M. A., El-Din, R. A. S., Ghonem, R. A., Moussa, L. A., and (2015). Bioremediation of Petroleum Oil By Pseudomonas Aeruginosa and Pseudomonas Fluorescens ( Biotype a) Isolated From Petroleum Oil Contaminated Soil. *Egypt J. Biotechnol.*, 50, 65–81.

El-Rahim, W. M. A., El-Ardy, O. A. M. and Mohammad, F. H. A. (2009). The effect of pH on bioremediation potential for the removal of direct violet textile dye by Aspergillus niger, *Desalination*. Desalination, 249(3), 1206–1211. doi: 10.1016/j.desal.2009.06.037.

Fu, W. J., Chi, Z., Ma, Z. -C., Zhou, H. -X., Liu, G. -L., Lee, C. -F., Chi, Z., -M. (2015). Hydrocarbons, the advanced biofuels produced by different organisms, the evidence that alkanes in petroleum can be renewable. *Applied Microbiology and Biotechnology*, 99(18), 7481–7494. doi: 10.1007/s00253-015-6840-6.

Hozumi (2013). Bioremediasi Lahan Tercemar Limbah Lumpur Minyak Menggunakan Campuran Bulking Agents yang Diperkaya Konsorsia Mikroba Berbasis Kompos Irradiasi. *Jurnal Ilmiah Aplikasi Isotop dan Radiasi*, 9(2), 139–150.

Nucera, D. M., Maddox, C. W., Hoien-Dalen, P., Weigel, R. M. (2006). Comparison of API 20E and invA PCR for identification of Salmonella enterica isolates from swine production units. *Journal of Clinical Microbiology*, 44(9), 3388–3390. doi: 10.1128/JCM.00972-06.

Obahiagbon, K. O., Amenaghawon, A. N., Agbonghae, E. (2014). Effect of Initial pH on the Bioremediation of Crude Oil Polluted Water Using a Consortium of Microbes. The Pasific Journal of Science and Technology, 15(1), 452–457.

Obi, E. I., Kamgba, F., Obi, D. (2014). Techniques of Oil Spill Response in the sea, *IOSR Journal of Applied Physics*, 6(1), 36–41. doi: 10.9790/4861-06113641.

Pawar, R. M. (2015). The Effect of Soil pH on Bioremediation of Polycyclic Aromatic Hydrocarbons (PAHs). *Journal of Bioremediation & Biodegradation*, 6(3). doi: 10.4172/2155-6199.1000291.

Peixoto, R. S., Vermelho, A. B. and Rosado, A. S. (2011). Petroleum-Degrading Enzymes: Bioremediation and New Prospects. *Enzyme Research*, 2011(3), 1–7. doi:
Puspito, G. (2013). Jenis Umpan dan Bentuk Perangkap Plastik yang Efektif untuk Menangkap Rajungan. Marine Fisheries Journal of Marine Fisheries Technomogu and Management, 2(2), 11. doi: 10.29244/jmf.2.2.111-119.

Qin, X., Tang, J. C., Li, D.S., Zhang, Q. M. (2012). Effect of salinity on the bioremediation of petroleum hydrocarbons in a saline-alkaline soil. Letters in Applied Microbiology, 55(3), 210–217. doi: 10.1472-765X.2012.03280.x.

Romo, D. M. R., Obando, M. C., Perdomo, M. I. G., Bravo, D., Izquierdo, P., F. (2013). Characterization of hydrocarbonoclastic marine bacteria using the 16S rRNA gene: a microcosm case study. Dyna, 80(180), 122–129. http://digital.unal.edu.co/index.php/dyna/article/view/26270.

Schunck, W.-H., Blasig, R., Mauersberger, S., Riege, P. (2004). Degradation of long-chain n-alkanes by the yeast Candida maltosa. Applied Microbiology and Biotechnology, 28(6), 589-597. doi: 10.1007/bf00250418.

Septriady, D. A. (2017). Peningkatan Kualitas Supply Chain Material Melalui Teknologi Material Traceability System di PT. Pertamina Gas Eastern Java Area Operation. Journal Earth Energy Engineering, 6(1), 11–29.

Shah, M. P., Patel, K. A. and Darji, A. M. (2013). Microbial Degradation and Decolorization of Methyl Orange Dye by an Application of Pseudomonas Spp. ETL-1982. International Journal of Environmental Bioremediation & Biodegradation, 1(1), 26–36. doi: 10.12691/ijebb-1-1-5.

Subramaniam, S., Kansrajh, C., Murugan, D., Satyan, R. S. Kiruthika, S., and Tharanipriya, A. (2014). Application of indigenous microbial consortia in bioremediation of oil-contaminated soils. International Journal of Environmental Science and Technology, 11(2), 367–376. doi: 10.1007/s13762-013-0366-1.

Suja, F., Rahim, F., Taha, M. R., Hambali, N., Razali, M. R., Khalid, A., Hamzah, A. (2014). Effects of local microbial bioaugmentation and biostimulation on the bioremediation of total petroleum hydrocarbons (TPH) in crude oil contaminated soil based on laboratory and field observations. International Biodeterioration and Biodegradation, 90(May 2014), 115–122. doi: 10.1016/j.ibiod.2014.03.006.

Thavasi, R., Jayalakshmi, S., Balasubramanian, T., and Banat, I. M. (2007). Effect of Salinity, Temperature, pH and Crude Oil Concentration on Biodegradation of Crude Oil by Pseudomonas aeruginosa. J. Biol. Environ., Sci, 1(2), 51–57.

Viyakarn, V., Kuanui, P., Chavanich, S. A., Omori, M., Lin, C. (2015). Effects of temperature and salinity on survival rate of cultured corals and photosynthetic efficiency of zooxanthellae in coral tissues. Ocean Science Journal, 50(2), 263–268. doi: 10.1007/s12601-015-0023-3.
Yan, K., Yang, M.-Z., Gao, S., Li, J.-Y. (2018) Variation characteristics and influencing factors of free water evaporation in recent 58 years in Tai’an area. *MATEC Web of Conferences*, 246(9), 02003. doi: 10.1051/matecconf/201824602003.

Yetti, E. (2016). Polycyclic aromatic hydrocarbon degrading bacteria from the Indonesian Marine Environment. *Biodiversitas Journal of Biological Diversity*, 17(2), 857–864. doi: 10.13057/biodiv/d170263.

Yu, Y. T., Chen, T. Y., Kao, C. M., Chiou, H. Y. (2012). Application of Oxygen-Releasing Material to Enhance In Situ Aerobic Bioremediation. *Advanced Materials Research*, 430–432(January), 1401–1404. doi: 10.4028/www.scientific.net/amr.430-432.1401.

Zhang, Y., Zhang, H., Zhang, Z., Liu, C., Sun, C., Zhang, W., and Marhaba, T. (2018). PH Effect on Heavy Metal Release from a Polluted Sediment. *Journal of Chemistry*, 2018, 1-7. doi: 10.1155/2018/7597640.