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

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KEYWORDS

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Pseudomonas aeruginosa
R. erythropolis
Lubricant

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 parameters considered were the detection and characterization of indigenous bacteria that degraded used oil. 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 1×10^8 cells/ml. It reduced of oil concentration up to 53%, and 1×10^6 cells/ml reduced oil concentration up to 47%. While, *R. erythropolis* with application cell rate 1×10^8 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

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their physiological state, the chemical composition of the oil, its concentration, temperature, water, etc. Thus, at a concentration of 0.001 $mg \cdot dm^{-3}$ 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 CO_2, H_2O and biomass (Dhar *et al*., 2014). Microorganisms have capability 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 biodegradate 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

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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 (1×10^6 cells/ml); (B): Indigenous isolation bacteria (1×10^8 cells/ml); (C): *R. erythropolis* (1×10^8 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.

$$\Sigma \text{ cells/ml} = \mathbf{\Sigma \text{ cell count}} \times \mathbf{250.000} \times \boldsymbol{n} \times \boldsymbol{a}$$

Information:

Σ cells/ml : Total bacteria (cells/ml)
n : dilution factor
a : trypan blue volume

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Analysis of Removal Lubricant Oil Levels

Analysis of removal oil was done at day 0 and final incubation. Added 5 ml of 3 NHCl 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 Na_2SO_4 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:

$$Level\ of\ used\ oil\ (g) = W2 - W1$$

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).

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.

Table 1. Water Quality Parameters

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

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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

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media, room temperature, and photos sun 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 $\times 10^8$ cells/ml) increased of total bacterial up to 1.95 $\times 10^9$ cells/ml. While, *Pseudomonas aeruginosa* in treatment A increased up to 1.28 $\times 10^9$ cells/ml and treatment B up to 1.33 $\times 10^9$ 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).

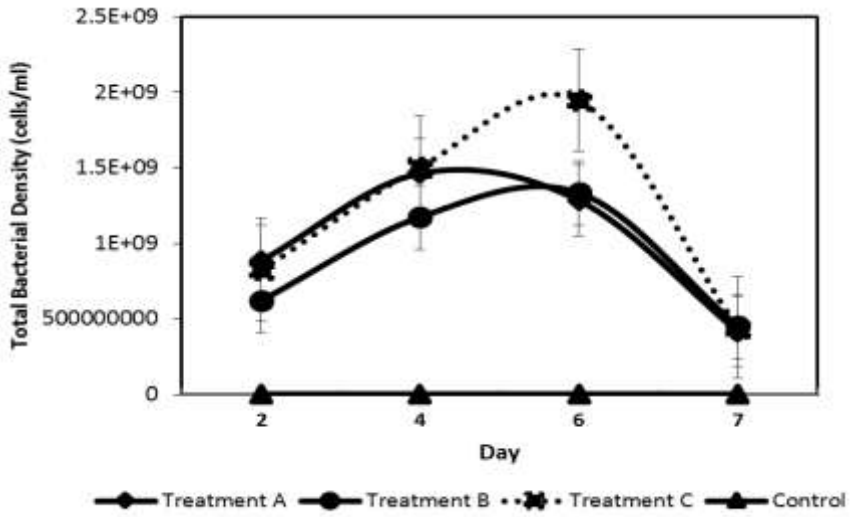

Figure 1. Bacterial Cells Growth (*n=2*)

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.

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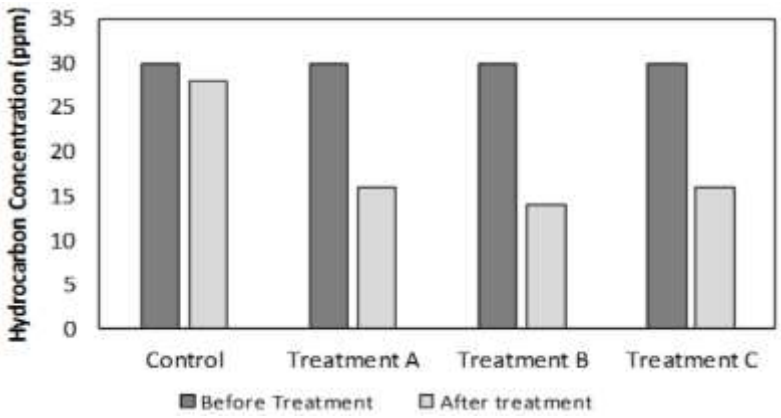

Figure 2. The Percentage of Degradation Lubricant Oil

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 CO_2 and energy. If through the fatty acid αoxidation pathway it will be converted directly into CO_2 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

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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.

VERY GOOD IDENTIFICATION	
Strip	API 20 NE V7.0
Profile	- + - - + - + - - - + + - - - - - - - - ?
Note	ID.NOT VALID BEFORE 48 HOURS

Significant taxa	**% ID**	**T**	**Test against**			
Pseudomonas aeruginosa	99.8	0.67	CITa 91%			

Next taxon	**% ID**	**T**	**Test against**			
Pseudomonas oryzihabitans	0.1	0.34	PNPG 10%	GLUa 76%	MANa 75%	GNTa 76%

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.

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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 hydrocarbonclastic 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

Tests of Between-Subjects Effects

Dependent Variable:TPH

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	374.667[a]	3	124.889	187.333	.005
Intercept	2400.000	1	2400.000	3.600E3	.000
Perlakuan	108.000	2	54.000	81.000	.012
Kepadatan	266.667	1	266.667	400.000	.002
Error	1.333	2	.667		
Total	2776.000	6			
Corrected Total	376.000	5			

a. R Squared = .996 (Adjusted R Squared = .991)

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).

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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 1×10^8 cells/ml (53%), *Rhodococcus erythropolis* with a density of $1x10^6$ cells/ml (47%) and *Pseudomonas aeruginosa* with a density of 1×10^6 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.

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