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ISOLATION AND ACTIVITY TEST OF HYDROCARBON DEGRADING MOLD FROM THE PETROLEUM WASTE OF PT OLLOP BULA IN WEST SERAM DISTRICT

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ABSTRACT

The high activity of offshore petroleum mining causes water pollution by crude petroleum spills which damages the ecosystem. Naturally the offshore petroleum spill disappear by the microorganism activity which uses the petroleum as a source of nutrition. Molds is a microorganism which has the capability to degrade hydrocarbons because it has certain enzymes that can break down hydrocarbons into simpler forms and are not harmful to the environment. The research results found six mold isolates and all of the isolates have a good growth activity in PDA modification + petroleum waste medium. In addition, the results of the degradation activity test of mold isolates show that the isolate V is more powerful in degrading hydrocarbon.

KEY WORDS: Mold, Iisolates, Hydrocarbon, Ollop

INTRODUCTION

Petroleum is the main energy source that the world population needs. Other energy sources have not been able to replace the role of petroleum as a major energy source. This encourages the development of petroleum industry to intensify exploration, transportation and processing of petroleum (Silvia, 2009). In addition to be an energy source, petroleum is also a source of foreign exchange for the country. As an energy source, petroleum has a lot of benefits such as for industrial activities, transport and households (Karwati, 2009; Astuti, 2012; Atlas., 1981). However, the existence of petroleum can also pollute land, water, and air environments around the petroleum industry operations. The petroleum contamination may come from petroleum spills during drilling, the storage system leaks, production, disposal of waste from industrial activities, seepage from the source, refining, and transportation (Astuti, 2012; Atlas, 1981; Leahly and Colwell, 1990). Petroleum contamination can cause serious problems to coastal, river, land and environmental ecosystems near the petroleum exploration. This is because petroleum contains a contaminant which is hard to be degraded, which is hydrocarbons (Atlas, 1981; Leahly and Colwell, 1990). When these compounds contaminate the soil surface, then these substances can evaporate, swept away by rainwater, or seep into the ground and then deposited into a toxic substance that causes intoxication in living organisms, interfering the absorption of light for the photosynthesis in aquatic plants, and affect the balance of the surrounding ecosystem. This is in line with the opinion by Alexander *in* Karwati, that the presence of contaminants which are difficult to be deraded and which are toxic to the soil will interfere the growth of plants and other organisms that live in it. As a result, the quality of the environment of the living organisms becomes less good. Thus, it requires serious handling. Therefore, an appropriate, fast, effective handling is needed and which does not disturb the environment (Sudrajat *et al.*, 2015; Nugroho, 2006; Gunalan, 1993).

There are many ways that can be done to overcome petroleum contamination. In general, it can be done by, physics, chemistry and biology (Yojana, 1995). According to Doeffer *in* Shinta Sivia, the physical approach is usually used in an initial

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step to isolate quickly before the petroleum spreads. Chemical approach can be done by using a dispersant, so that the petroleum can be dispersed (Alpetri *et al.*, 2001; Listiandiani, 2011). The weakness of these approaches is that it is expensive to do, it can interfere with life in the environment, and it does not recycle.

Biological approach is an alternative for dealing with the petroleum waste without damaging the environment by making use of degrading microorganisms. The decomposition of a complex organic material into other simpler forms in the form of CO_2, H_2O, and metals with the microorganism activity that do not pollute the environment called biodegradation (Ahmad, 2009). According to Udiharto, the benefits of using such methods are economical, effective, efficient, and more environmentally friendly. Through these activities, it is expected that petroleum contaminated environment will become normal again.

Biodegradation by microorganisms can occur because these microorganisms utilize the components of petroleum. The components of the petroleum are approximately 85% carbon and 12% hydrogen (hydrocarbons), and 1-5% of nitrogen, phosphorus, sulfur, oxygen, and the metal element (Alpine, 1999). Hydrocarbon compounds that have the largest components of petroleum are used as carbon sources by certain microorganisms, while non-hydrocarbon compounds are a complementary nutrient for their growth, so that they can metabolize well. As a result of this process on petroleum is the degradation or termination of the hydrocarbon chain which is commonly called petroleum hydrocarbons biodegradation (Irawan, 2004).

MATERIALS AND METHODS

This research was conducted in three stages, stage I: isolating the mold from the petroleum waste from PT Ollop Bula; Stage II: activity test of petroleum hydrocarbon degrading mold isolates; and the third stage was the mold ability test in degrading petroleum hydrocarbons. The material used for the testing is crude oil, mold isolate obtained from the isolation of mold from petroleum *waste.* The sample used for the source of hydrocarbons was the petroleum *from PT Ollop Bula.* Ammonium sulfate $(NH_4)_2SO_4$ as nitrogen supplement, kalium dihydroxy phosphate (KH_2PO_4) as a phosfor supplement, NaOH 1N and HCl 1N, buffer pH 4 and pH 7, and sterile distilled water. Modified PDA Medium, PDA modification + petroleum medium, Potato Dextrosa Broth (PDB) modification medium, and PDB modification + petroleum medium are used to test the activity. Biodegradation Medium was for the petroleum hydrocarbons biodegradation test.

The tools used consisted of test tubes, petri dish, erlemeyer, measuring cups, glass stirrers, magnetic stirrer, bunsen, ose needle, pH meter, autoclaves, incubators, shakers, rotary evaporator, analytic scales, electric cooker, microscopes, desiccator, ose needle. The preparation of Potato Dextrosa Agar Modification medium began with fainding the right composition of peptone and potato extract, namely the composition of 1:1. The mixture of potato extract l g, pepton l g, agar 15g with sterile distilled water 1000 mL, was heated while being stirred with magnetic stirrer until homogeneous. Sterilized in autoclave at 121 ^oC for 15 minutes at a pressure of 15 lbs. For Potato Dextrosa Agar Modification + 2 mL petroleum, added with 100 mL medium with 2 mL of petroleum.

Potato extract I g, peptone 1 g with sterile distilled water 1000 mL, heated while being stirred with a magnetic stirrer until homogeneous. Sterilized in autoclave at 121 ^0C for 15 minutes at a pressure of 15 lbs. For medium Potato Dextrosa Broth Modifiation + 2 mL petroleum added with 2 ml petroleum in 100 mL of medium Potato Broth Dextrosa modification. Potato Dextrosa Broth Modification medium is prepared as basal medium, the pH is set to 7 by adding HCl 1N when alkaline or NaOH 1N when acid. Nitrogen element was added from ammonium sulfate $(NH_4)_2SO_4$ 200 ppm and phosphorus element was added from potassium dihidrophosfat KH_2PO_4 20 ppm. After that the medium was put into the erlemeyer 250 mL. Each was 100 mL and added with 2 g (2000 ppm) of petroleum. Erlemeyer was covered with cotton and sterilized with autoclaving at 121 ^oC for 15 minutes at a pressure of 15 Ibs.

The mold isolation was done by using pour agar technique. Waste sample in the bottle was shaken, then taken as much as 1 mL and made dilution by putting into 9 ml of sterile distilled water in test tubes, homogenized (10^{-1}), and then from the 10^{-1} dilution was taken 1 mL and then put in 9 ml of the second sterile distilled water. This was done until the dilution 10^{-4}, and then the suspension was pipetted 0.1 mL and dropped in Petri dish

containing PDA medium that was still liquid, and then the petri was shaken until the suspense was even. After frozen, the petri was turned upside down. To avoid the condensation water of falling, the clan was incubated for 3 x 24 hours. After that isolation was done to obtain pure cultures by placing on aslant PDA medium in a test tube.

Pure cultures contained in the test tube were planted in PDA modification + petroleum medium. The isolates that grew were assumed as the petroleum mold, because it can utilize the petroleum for its growth. This treatment was intended for selecting the mold which were considered active for reducing petroleum as nutrients and could then be used for the subsequent tests. After that, the isolates that grew were made into pure cultures by planting it on an aslant PDA medium. To identify the mold which actively degrades the petroleum hydrocarbons, activity test can be done by observing the growth rate of each mold on the hydrocarbon medium compared to that on non-hydrocarbon medium. Each test tube containing mold cultures was put into sterile distilled water as much as 5 ml and then shaken. The suspension was taken as much as1 ml and put into PDB modification + 5 ml kerosene. The medium was placed in a shaker and incubated for 24 hours. After that, the culture was ready to be used as inoculum stock.

Add 1 mL inoculum which has been activated to the Potato Dextrosa Broth (PDB) modification medium and PDB modification + 2 mL petroleum medium. At first pipetted 1 mL from each medium cla, did dilution from 10^{-1} to $10^{-3,}$ and each dilution was pipetted 0.1 mL to be implanted into each petri dish that already contained PDA modification medium and PDA modification + 2 mL petroleum medium in order to calculate the population of bacteria. The same thing was also done on each medium which had been dishakered and incubated for 24 hours at room temperature.

Each tube containing 5 mL mold cultures was entered sterile distilled water and then shaken. 1 mL suspension was taken and put into 250 mL erlemeyer containing 100 mL biodegradation medium. After that incubated with a shaker for 1 x 24 hours. The inkulum starter was then ready to be inoculated 1 mL in 100 mL biodegradation medium and incubated for three days in the shaker at room temperature at 110 rpm.

Into each treatment erlenmenyer was entered 40 mL klorofonn ($CHCl_3$), after that poured into a separating funnel. This treatment was intended for extracting for 15 minutes. After the solution was separated into two parts, the top part was a biodegradation medium, on the bottom part was the petroleum mixed with chloroform. Evaporation of the extract was carried out by using rotary evaporator at 80 ^{0}C for 60 minutes. The extracted flask extract was stored in the desiccator. After that, weighing was carried out until the weight was constant. The weight obtained was the total of petroleum hydrocarbon.

The activity test can be observed by looking at the growth rate coefficient of each mold by using the formula:

$$\mu = \frac{2,3}{tx} \log \frac{a\ tx}{b\ to}$$

Description:
m = Growth rate coefficient
a tx = Mold Population after 24 h incubation
b to = Mold Population after incubation 0 hours
2,3 = Constants
Tx = Time

The percentage of biodegradation of petroleum can be determined by the formula:

$$\%B = \frac{Tmbo - Tmbn}{Tmbo} x100\%$$

Description % B = Biodegradation percentage
Tmbo = Total initial petroleum
Tmbn = Total petroleum after incubation

The research on the biodegradation of petroleum hydrocarbons was analyzed by calculating the petroleum degradation percentage as follows [9]:

$$\%\ B = \frac{(BMo - BMn)}{BMo}\ x\ 100\%$$

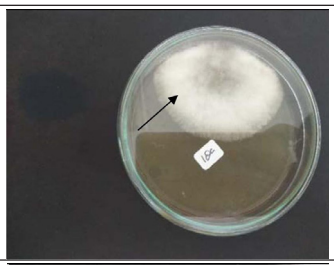
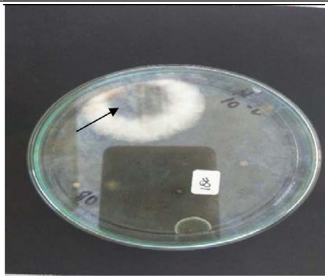
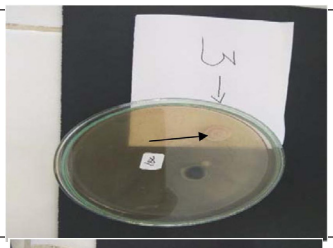
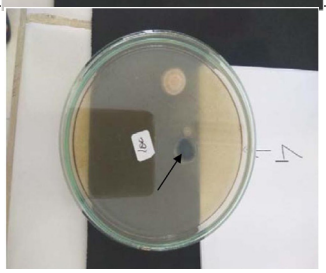
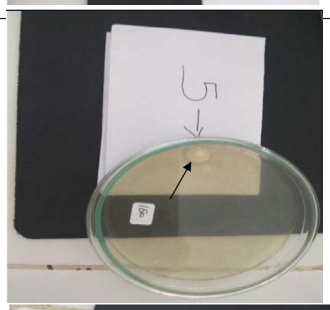
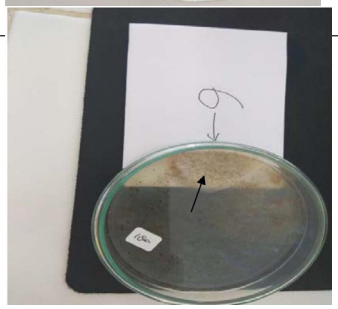
Description
% B = degradation percent (%)
BMo = initial petroleum weight (g)
BMN = final petroleum weight (g)

RESULTS

Crude petroleum waste obtained from the reservoior of PT Oll of was brought to laborartorium of mathematics and science in IAIN Ambon, and the process of preparation was carried out, and the mold was grown on the PDA modification medium. The results of isolation found 6 mold species with morphological features as follows:

Based on Table 1, six mold species that grew on

Table 1. Mold Isolate from Petroleum Waste of PT Ollop

Testing Code	Picture of mold	Typical features
I.		White colonies resemble cotton
II		Edge colony is white resemble cotton and the center of the colony is black
III		Creamy colonies resemble a hill with flat edges
IV		Colonies are black with cream-colored edges
V		Colony round, cream-colored, jagged edges and forming hills
VI		Brown colony resemble powder

PDA modification medium were found with varied shapes and colors of the colony. Before used in further tests, it is necessary to test the activity of each isolate in degrading petroleum. In order to find out the activity, each isolate was tested in medium with the addition of petroleum and medium without petroleum. The observation of the activity test was done by calculating the growth rate coefficient of mold. The growth rate coefficient of the six mold isolates in the PDB modification medium and PDB modifications added with petroleum can be seen in Table 2.

Table 2. The growth rate of mold isolates I, II, III, IV, V, and VI in thePDB modification medium and PDBB modification + petroleum

Mold Isolate Code	Growth rate coefficient per hour*(U)*	
	NB modification without petroleum	NB modification + petroleum
I.	0.178	0.196
II	0.195	0.216
III	0.182	0.290
IV	0.174	0.301
V	0.168	0.328
VI	0.172	0.298

Table 2 shows that the isolate V has the highest growth rate in NB modification + petroleum compared with isolate I, II, III, IV, and VI. The increased growth rate in the medium containing petroleum showed that the mold isolates were active in the petroleum environment and had the ability to degrade petroleum hydrocarbons. The mold was able to grow on the medium by utilizing the elements of the media and it grew better in the media containing petroleum because the mold had the ability and physiological activities to develop in the petroleum medium, so that the mold had a higher growth rate.

According to Udiharto, microorganisms which potentially and actively degrade petroleum will show high growth rate in medium containing petroleum compared with in non-petroleum medium. The ability to degrade petroleum is associated with the presence of hydrocarbons degrading enzymes, such as dehydrogenases, monooxygenases, deoksigenase and other enzymes responsible for degrading hydrocarbons that allows microorganisms to grow on petroleum (Burghal *et al.*, 2015; Elella *et al.*, 2015; Adekunle *et al.*, 2017). Each mold isolate that has the ability to grow in PDA modification + petroleum medium, was tested for its activity in degrading hydrocarbon, and the results can be seen in Table 3.

The data in Table 3 show that the capability of the mold isolate in degrading hydrocarbon is different. The most powerful mold in degrading the hydrocarbon was the isolate with the mold code V, and the weakest mold in degrading hydrocarbons was the isolate with the mold code I.

DISCUSSION

Petroleum is a very complex mixture containing 50-98% hydrocarbon and non hydrocarbon components. The content varies depending on the petroleum source. Petroleum contains carbon 83,9-86,8%, hydrogen 11.4-14%, sulfur 0.06-8.0%, nitrogen 0.11-1.7% and oxygen 0.5% and metal (Fe, Cu, Ni), 0.03%. There are four series of hydrocarbons minimum contained in the petroleum, the n-paraffin series (n-alkanes), which consists of methane ($CH_{4)}$, the asphalt which has more than 25 carbon atom (C) in its chain, iso-paraffin series (isoalkanes) contained only slightly in petroleum, the neptene series (cycloalkanes) which has the second most component after n-alkanes, and the aromatic series. The composition of the hydrocarbons in petroleum is different depending on the source of the petroleum (Ichor *et al.*, 2014).

Leahy and Rita state that the growing ability of

Table 3. Activity Test of Hydrocarbon Degradation Molds Isolate

Mold code	Early petroleum weight (g)	Final petroleum weight (g)	Degradation Percentage (%)
I.	0.27	0.13	51.85
II	0.41	0.13	68.29
III	0.58	0.16	72.41
IV	0.71	0.16	77.46
V	0.80	0.13	83.75
VI	0.91	0.16	82.42

petroleum degrading microorganisms varies depending on the microorganism adaptation to the environment. According to Higgins and Gilbert, in a petroeum polluted environment, the growth rate will be faster and the number will be bigger compared with the uncontaminated environment. This is in line with Atlas's statement that the requirements of the microbes to be in petroleum are that it has the ability to degrade petroleum components, has stable genes, high viability, rapid growth, high enzymatic activity, able to compete with indigenous microorganisms, non-pathogenic and does not produce toxic metabolites (Kamaluddeen *et al.*, 2016; Adongbede and Majekodunmi, 2016; Asadirat *et al.*, 2016).

Table 3 shows that the five isolates of mold which were isolated from petroleum waste PT Ollop Bula could biodegrade the petroleum hydrocarbons. Pelczar and Chan in general stated that microorganisms are capable of remodeling or recycling pollutants into simple compounds (Sabhan *et al.*, 2014). Biodegradation by each of these microbes can occur in the presence of bacterial growth and enzyme activity possessed by microorganisms. Through enzymatic processes, bacteria can trànsform hydrocarbon substances into simpler forms.

The difference in the percentage of petroleum hydrocarbon biodegradation in these five isolates is because each type of mold has different capabilities to metabolize hydrocarbon compounds. Udiharto said that hydrocarbons are oxidized because of the large specificity of the enzyme possessed by microba, such as monooxygenase enzyme that plays a role in oxidizing n-alkane into primary alcohol, oxygenase enzyme that plays a role in the degradation of cycloalkanes, and deoxygenase enzymes in the degradation of benzene and catechol (Swandi *et al.*, 2015; Utami *et al.*, 2017; Retno T and Mulyana, 2013).

Biodegradation is supported by aeration for oxygen supply by means of shaker and nutritional factors, namely, carbon nutrients in crude petroleum and the addition of nitrogen and phosphorus nutrients that can stimulate and provide optimal conditions for biodegradation. The optimal compotition of carbon, nitrogen and phosphor for petroleum degradation by microorganisms based on the Polybac Coorporation report is by a ratio of 100:10:1. The research by Zulkifliani using *Pseudomnnas sp. Gamma* cobalt-60 with the addition of nitrogen (ammonium sulfate) of 300 ppm and phosfor (dihydro potassium phosphate) 30 ppm gave the highest biodegradation as much as 17%, and the research by Rida, using *Pseudomona.s chlororaphis*, provide additional nitrogen, with the ratio of carbon and nitrogen 100:10 obtained the highest biodegradation as much as 51.33% (Yolantika *et al.*, 2015; Olajire and Essien, 2014). The degrading capability of this mold can also be seen from the growth rate from the growth phase until the exponential phase in biodegradation medium. Where in this exponential phase, the number of cell increases exponentially and the activity is very high.

The capability of microorganisms to degrade petroleum and its products depends on the microorganism adaptation and physiology with its environment. In addition, environmental factors will also determine the speed of biodegradation. The environmental factors affecting petroleum biodegradation are:

Oil Composition (Chemical Structure)

Biodegradation of a compound is related to the structure of the hydrocarbon compound and the concentration of the petroleum. Biodegradation of petroleum hydrocarbons by microbes occurs on saturated hydrocarbons first and is followed by aromatic hydrocarbons. High concentrations of petrolum hydrocarbons have a high toxic level, so that they can cause the biodegradation rate of petroleum hydrocarbons to decrease (Olajire and Essien, 2014; Helmy and Kardena, 2015).

Microbial Community

The composition of a microbe can greatly affect degradation process of petroleum hydrocarbons. Some of the important characteristics that must be owned by hydrocarbonoclastic microbes are such as having oksegenase enzyme and binding hydrocarbons, producing emulsifer, optimizing contact between microorganisms and hydrocarbons. Besides, the process of petroleum hydrocarbons bidegradation is influenced by the number of hydrocarbonoclastic microbes because sufficient number of microbes will produce a lot of specific enzymes that can degrade petroleum hydrocarbons (Helmy and Kardena, 2015).

Temperature

Temperature is an environmental factor that affects the biodegradation of hydrocarbon compounds, especieally toward the metabolic process and the growth rate of bacteria. In general, temperature

increases affect the activity of enzymes. Beyond the optimum temperature, the bacterial growth will be slower or there will be no growth (Mohanty and Jena, 2017).

Oxygen

According to Jordan and Payne in Sinta Silvia, microorganizms need oxygen both in the form of free oxygen obtained from air and oxygen dissolved in the water. Oxygen has a significance in petroleum biodegradation. Oxygen is used for the oxidation and respiration processes of microorganisms. Most of petroleum degrading microorganisms belong to the aerobic microorganism [28-29]. Oxygen is an important component that affects bacterial growth in the hydrocarbon environment. Oxygen is used to activate oxygenese enzymes in degrading hydrocarbons. The bacterial growth will be hampered by limited oxygen conditions. Oxygen needs can be met by aeration, that is by shaking the shaker (Mohanty and Jena, 2017).

pH

Microorganism ability to degrade hydrocarbons is also influenced by pH, as the pH determines the optimal enzyme activity. Bacteria generally has a pH of about 7.

Nutrition

The carbon element contained in petroleum is used by microorganisms for its growth. In addition to nutrients from carbon swnber, microorganisms also require additional nutrients. Additional nutrients, such as nitrogen and phosfor, can stimulate biodegradation of petroleum. The addition of ammonium sulfate [$(NH_4)_2S0_4$] as a source of nitrogen and kaliun dihidrofosfat (KH_2PO_4) as a source of phosfor is often used in experimental repetition of crude oil contamination by microorganisms. Nitrogen plays a role in the formation of amino acids, enzymes, and cell, and phosfor plays a role in the formation of amino acids, energy transport, and formation of compound in the metabolic reactions within the cells of the microorganisms (Mohanty and Jena, 2017).

CONCLUSION

6 mold isolates were obtained from the waste petroleum from PT Ollop, and each isolate had different capability to degrade petroleum hydrocarbons. The most powerful isolate in degrading hydrocarbons was the isolate code V and the weakest isolate in degrading hydrocarbons was the isolate code I.

SUGGESTION

It is expected to conduct further research, which analyzes the type of mold that is obtained and to increase the research to the molecular level testing of isolate V, as well as to do isolation and activity of the types of bacteria from the waste petroleum.

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