

**THE PETROLEUM HYDROCARBON BIOREMEDIATION PERFORMANCE BY INOCULATING CONSORTIUM PETROPHYLIC AND THE CHICKEN MANURE AMENDMENT**

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**ABSTRACT**

Biodegradation of petroleum hydrocarbons in a land-treatment bioremediation system requires an effective degrading microbes and optimum system conditions. The study aimed to obtain the characteristics of the interaction between the petrophylic consortium (*Pseudomonas* sp. and *Aspergillus* sp.) and the amendment of chicken manure and to obtain the optimum dose to increase the biodegradation rate of petroleum hydrocarbons, soil pH change, and increased the population petrophylic fungi. The experiment was carried out on a greenhouse scale, using a factorial randomized block design (RBD) with two factors and three replications. The first factor was the inoculation of the petrophylic consortium consisting of: (i) without Petrophylic, (ii) 1% Petrophylic, (iii) 3% Petrophylic, (iv) 5% Petrophylic. The second factor was the amendment of chicken manure, consisting of 3 levels: (i) without chicken manure, (ii) 2% chicken manure, and (iii) 4% chicken manure. The results showed that petrophylic consortium inoculation and chicken manure amendment did not show an interaction effect on increasing the rate of biodegradation of petroleum hydrocarbons, soil pH, and increasing the population of petrophylic fungi. Likewise, the effect of independent treatment could not increase the biodegradation rate and soil pH. But, the application of chicken manure increased growth of the petrophylic fungus population during the 4-week incubation time. However, increasing Petrophylic fungi population density did not show increasing in degrading petroleum hydrocarbons. The dose of chicken manure used has not been able to supply N and P nutrients to achieve optimal C: N: P ratio conditions for degrading hydrocarbons effectively, resulting the inoculated petrophylic could not degrade hydrocarbons maximally. Compatibility between consortium microbes members as a hydrocarbon-degrading agent is an important factor for achieving effectively bioremediation performance.

**Keywords:** *Aspergillus* sp., Bioremediation, Bioremediation, Petrophylic, and *Pseudomonas* sp.

**1. INTRODUCTION**

Oil refinery production in Indonesia, based on data from [1], reports that oil production in 2019 reached 259,246.8 (in thousand) barrels, and the waste generated was around 37.38% of the production figure. Petroleum is a very complex mixture consisting of hydrocarbon compounds. The element carbon contained about 85% and hydrogen 12% [8]. The composition of hydrocarbon compounds in crude oil is not the same, depending on the source that produces the oil [24]. Hydrocarbon group compounds are divided into four groups, namely (1) saturated hydrocarbons consisting of aliphatic alkanes and alicyclic alkanes, (2) aromatic hydrocarbons consisting of aromatic monocyclic and aromatic polycyclic, (3) Resins are polar compounds

containing nitrogen, sulphur, and oxygen. (4) Asphalt is a compound that has a high molecular weight and is associated with metals [26], [14]. These hydrocarbon compounds are organic compounds that can contaminate the aquatic and soil environment. Therefore, the handling of pollutants caused by petroleum waste is a must that needs to be done. It is necessary to develop effective techniques to overcome petroleum pollutants.

Bioremediation technology is one effective, inexpensive, and environmentally friendly technology. According to [4], bioremediation is a process of utilizing microorganisms to decompose pollutants through an oxidation-reduction process. One of the techniques for applying bioremediation is using land treatment techniques. This method is one of the bioremediation techniques on the soil surface. The process requires aerobic conditions and can be carried out in-situ or ex-situ [4]. And [13] reported that, the land treatment technique is a method that is often chosen for soil contaminated with hydrocarbons because it is relatively cheaper and has the potential to be successful. The mechanism principle of bioremediation to remove petroleum that contaminates the soil environment is the process of biodegradation of hydrocarbons by petrophylic or hydrocarbonoclastic microbes. Hydrocarbon biodegradation involves monooxygenase enzymes [19]. The success rate of the biodegradation of hydrocarbons in the soil depends on the appropriate environmental conditions for microbial activity, including oxygen adequacy, moisture, soil pH, temperature, and nutrients. The biodegradation process requires oxygen which acts as an electron acceptor to accommodate excess electrons from other reactants [14].

The consortium form in mixed culture accelerates the biodegradation process ultimately. Reported by [12], oil decomposing microbes do not work individually/species but in the form of a multi-species consortium. A microbial consortium is a group of microbes that mutually benefit from one another, and each organism cannot do so separately. Biodegradation of hydrocarbons by a mixture of microorganisms is more effective than natural culture because of the complexity of hydrocarbon compounds in oil that various microorganisms can degrade. Microorganisms work synergistically by cleaving hydrocarbon compounds at different carbon chain bonds. Petroleum can be degraded by microbes such as bacteria, fungi, yeast, and microalgae [20], [3]. Many studies have reported that: low molecular weight alkanes degrade faster and mixed cultures degrade faster than pure cultures [7], [17], [23] and [6].

Petrophylic bacteria degrade the pollutant organic compounds by absorbing these compounds into their cells through the cell wall diffusion process and utilizing intracellular enzymes for cleaving pollutants substances [21]. While petrophylic fungi have a different degradation mechanism from bacteria, namely degrading enzymes secreted by fungi from their mycelia, or called extracellular enzymes. Thus, biodegradation occurs outside the fungal cells or their mycelia [21]. Fungi produce extracellular enzymes such as amylase, pectinase, and lipase enzymes. These enzymes are produced to break down complex compounds into simpler compounds. So that it can be absorbed by cells and used for growth, fungi can decompose hydrocarbon compounds with high concentrations and molecular weights in the soil, including Polycyclic Aromatic Hydrocarbons - Polycyclic Aromatic Hydrocarbons (PAHs), namely aromatic compounds containing more than two benzene rings [5]. Several microbes are known to degrade petroleum hydrocarbons (petrophylic), including *Pseudomonas* sp., *Acinetobacter* sp. ;

*Bacillus* sp., *Micrococcus* sp., and hydrocarbon utilizing fungal isolates were *Aspergillus* sp., *Candida* sp., *Fusarium* sp., *Mucor* sp., *Penicillium* sp. The study indicated that nutrient amendments could enhance the rate of biodegradation of crude oil polluted soil [24], [16]. However, the compatibility and effectiveness between species when used together still need to be tested comprehensively.

From the description above, the crucial factors that still need to be investigated in utilizing hydrocarbon-degrading microbes are the effectiveness of microbes as bioremediation agents and the selection of appropriate nutritional ingredients adapted to spur the degradation process. Nutrient content, especially soil N and P, must be available in adequate quantities to support degrading microorganisms' growth. Carbon sources can come from hydrocarbons, while nitrogen and phosphorus can be added to inorganic fertilizers. The nutrient amendment can increase the biodegradation rate of crude oil polluted soil [16]. A potential alternative material as a substitute for inorganic fertilizers is chicken manure, which supplies N and P needed by petrophylic microbes. According to [11], that applying fertilizers can increase the growth and population of soil microbes, increase soil microbial respiration and potentially increase the biodegradation of hydrocarbons in the soil. In addition, the function of chicken drum fertilizer for microbes is as the primary energy source for soil microorganism activity and as a food source for soil microorganisms.

The focus of the research was to examine the effectiveness of petrophylic consortium inoculants (*Pseudomonas* sp. and *Aspergillus* sp.) and chicken manure on the rate of Biodegradation of Petroleum Hydrocarbons, Soil pH, and population viability of petrophylic Fungi with Land treatment Bioremediation system on Inceptisols.

## 2. MATERIALS AND METHODS

The materials used in this experiment were: Inceptisols Jatinangor soil samples as land treatment media were taken compositely from the topsoil with a depth of 0-20 cm. Petroleum waste in the form of crude oil originating from Kelayan Pertamina, Cirebon. A consortium of petrophilic solid inoculants (bacteria and fungi) from the collection of the Soil Biology Laboratory, Department of Soil Science, Faculty of Agriculture, Universitas Padjadjaran. Chicken manure from Padjadjaran University fertilizer house. Potato dextrosa agar (PDA) media + 1% petroleum hydrocarbons for calculating the total population of petrophylic fungi.

### *Experiment Design*

The experimental design used was a factorial randomized block design (RAK). The first factor was the inoculation of variations in the concentration of the petrophylic consortium which consisted of 4 levels. The second factor is the application of variations in the concentration of chicken manure which consists of 3 levels, with 3 replications.

Factor I, the petrophylic consortium (A), consisted of 4 levels of treatment, namely:

a0 = without the provision of petrophylic consortium

a1 = 1% petrophylic consortium concentration

a2 = 3% petrophylic consortium concentration

a3 = 5% petrophylic consortium concentration.

Each percentage of the petrophylic consortium concentration is calculated based on the concentration of the crude oil load.

Factor II is the concentration of chicken manure (B), consisting of 3 levels of treatment, namely:

b0 = without giving chicken manure,

b1 = 2% concentration of chicken manure

b2 = 4% concentration of chicken manure.

Each percentage concentration of chicken manure was calculated based on the weight of the soil used as land treatment media.

Observations made in this study include Observations tested: The rate of biodegradation of petroleum hydrocarbons by the gravimetric method through n-hexane extract measured at the end of the experiment, calculated by the formula:

$$k = \frac{-(\ln C_x - \ln C_0)}{(t_x - t_0)} \quad 1).$$

Description :

k = Biodegradation rate; C<sub>x</sub> = Final TPH concentration; C<sub>0</sub> = initial TPH concentration; t<sub>x</sub>= observation time (end); t<sub>0</sub> = observation time (beginning).

Measurement of soil pH was measured using a potentiometric method with a pH meter which was measured at the end of the experiment, while the population of petrophylic fungi used PDA media containing 1% hydrocarbons using the Total Plate Count (TPC) method, which was measured at the end of the experiment.

***Preparation of Petroleum Contaminated Soil and bioremediation processes.***

The soil used in the experiment was 72 kg, obtained from the topsoil layer at a depth of up to 20 cm. Soil processing was carried out by turning the soil repeatedly, and then the drying process was done by air drying. Then the filtering process was carried out at a size of 2 mm. The treated soil was put into a microcosm measuring 30 cm long, 25 cm wide, and 25 cm high, as much as 2 kg per microcosm. The number of microcosms that needed to be prepared was 36 microcosms. After processing the soil, the soil was mixed with petroleum waste which is carried out by evenly mixing crude oil with a TPH concentration of 10% of 2 kg of soil weight by stirring evenly so

that a homogeneous soil contaminated with waste oil is obtained. The mixture of soil and petroleum was incubated for one day to allow the petroleum waste to unite with soil particles. The media was turned or stirred daily during the bioremediation process to provide aeration. In addition, water was given to maintain soil moisture, namely at the water content of field capacity. The bioremediation process was carried out for four weeks so that the level of degradation could be known gradually.

#### ***Chicken Manure Application.***

The chicken manure used was first carried out by chemical analysis of their content, including C-organic, N-total, and C/N ratio. After the analysis, then the manure is mixed with soil that has been contaminated with petroleum hydrocarbons which have been incubated for one day .

#### ***Petrophilic Consortium inoculation and Chicken Manure amendment .***

Petrophylic consortium inoculant were inoculated with 1%, 3%, and 5% (w/w), respectively to the crude oil concentration. Meanwhile, the concentration of chicken manure applied was 0%, 2%, and 4% (w/w) respectively to a load of petroleum waste. The microcosm containing soil contaminated by petroleum waste that had been prepared was mixed directly with each petrophil consortium treatment and chicken manure according to the treatment concentration that had been determined and mixed homogenously.

#### ***Analysis of Total Petroleum Hydrocarbon (TPH) of Hydrocarbon polluted soil***

TPH analysis was carried out at the beginning and end of the experiment, with an interval of 2 months. Before sampling, the contaminated soil was stirred first, and then the soil was sampled in a composite manner. 5 g of composite soil samples were taken and extracted using n-hexane with a 1:2 ratio between soil samples: n-hexane. The extraction process was done by shaking the contaminated soil sample, which had been added with n-hexane on a shaker with 100 rpm agitation. The extraction process was repeated three times in a row until a clear hexane solution was obtained. The extracts obtained from each solvent were combined and weighed as TPH gravimetrically [24].

#### ***Analysis of Petrophilic Fungi and Soil pH.***

The analysis was carried out using the Total Plate Count (TPC) method on the petrophilic fungus population by using PDA added by 1% Hydrocarbon media at the study's end. Furthermore, incubation was carried out for 5 to 6 days to calculate the total population. The pH analysis was carried out by the potentiometric method. This analysis used H<sub>2</sub>O and was calculated using a pH meter.

### **3.RESULT AND DISCUSSION**

#### **Chemical and Physical Characteristics of Experimental Soil.**

The soil used in this study was Inceptisols taken from the topsoil layer in Ciparanje Jatinangor land. The results of the initial soil analysis are presented in Table 1.

The results of the chemical analysis showed that the soil pH (5.98) was slightly acidic, the C-organic content (2.29%) was moderate, the CEC was low (15.9 cmol kg<sup>-1</sup>), and the soil texture was clay with 53% clay fraction, 40% dust. , and 7% sand. The clay texture can hold water and bind molecules well to support microbial growth [10]. The analysis results that Inceptisols used have a slightly acidic pH (5.98) which is suitable for microbial growth. Microbial growth is

influenced by soil pH; bacteria grow optimally at neutral pH to slightly acidic pH, if the pH is below five or above 8.5, resulting in microbes growth will be inhibited [4]. Microorganisms can use C-organic in the soil as a carbon source to produce energy, but soil analysis shows that the C-organic content in the soil used in this study is in moderate criteria so that microorganisms can utilize the carbon element from petroleum hydrocarbons which is applied in this study.

**Table 1. Results of Preliminary Analysis of Experimental Soil - Mineral Inceptisols**

No.	Parameters	Unit	Score	Criteria <sup>*)</sup>
1.	pH H <sub>2</sub> O (1 : 2,5)		5,98	slightly acidic
2.	pH KCl 1 N (1 : 2,5)		5,24	-
3.	C-Organic	%	2,29	moderate
4.	N-total	%	0,16	low
5.	C/N ratio		14,31	moderate
6.	P <sub>2</sub> O <sub>5</sub> Bray I	ppm P	4,75	low
7.	P <sub>2</sub> O <sub>5</sub> HCl 25%	mg 100 g <sup>-1</sup>	56,28	high
8.	K <sub>2</sub> O HCl 25%	mg 100 g <sup>-1</sup>	21,05	moderate
9.	Al-dd	cmol kg <sup>-1</sup>	0,10	-
	H-dd	cmol kg <sup>-1</sup>	0,41	-
10.	KTK	cmol kg <sup>-1</sup>	15,90	low
11.	Base Saturation	Al's %	19,18	very low
12.	Saturation	Cation %	2,80	very low
13.	Arrangement :			
		cmol kg <sup>-1</sup>	0,26	low
	K-dd	cmol kg <sup>-1</sup>	0,11	low
	Na-dd	cmol kg <sup>-1</sup>	2,02	low
	Ca-dd	cmol kg <sup>-1</sup>	0,66	moderate
14.	Mg-dd			
	Soil texture:	%	7	
	Sand (%)	%	40	dusty clay
	Dust (%)	%	53	
	Clay (%)			

Description: Source of criteria determination: \*) Sarwono Hardjowigeno (2014).

**Hydrocarbon Biodegradation Rate**

The analysis of variance showed that there was no interaction between the inoculation of the concentration variation of the petrophylic consortium and the application of the variation of the concentration of chicken manure on the rate of hydrocarbon biodegradation. However, further analysis showed the independent effect of the concentration of the petrophylic consortium in the hydrocarbon bioremediation process. The rates of hydrocarbon biodegradation in various treatments are listed in Table 2.



**Table 2. Table 2. Effect of Petrophylic Consortium Concentration and Chicken Manure on Hydrocarbon Biodegradation Rate in Petroleum Hydrocarbon Bioremediation during an incubation period of 4 weeks**

Treatment	Rate of Hydrocarbon Biodegradation (ppm/week)
<u>Petrophylic Concentration (A)</u>	
a0 = no petrophylic consortium	
a1 = 1% petrophylic consortium	0,0256 b
a2 = 3% petrophylic consortium	0,0244 b
a3 = 5% petrophylic consortium	0.0144 a
	0.0200 ab
<u>Concentration of Chicken Manure (B)</u>	
b0 = without chicken manure	0.0183 a
b1 = 2% chicken manure	0.0258 a
b2 = 4% chicken manure	0.0192 a

Note: The average value followed by the same letter is not significantly different, according to Duncan's Multiple Distance Advanced Test at a 5% significance level.

The inoculation of 3% petrophylic gave the lowest biodegradation rate significantly different compared to control and 1% petrophylic treatments. Meanwhile, the inoculation of without petrophylic (control treatment), 1% and 5% treatments did not increase significant effect to the biodegradation rate. This shows that the increase in inoculated petrophylic has not been able to increase the biodegradation rate of petroleum hydrocarbons that contaminate the soil; its performance is equivalent to the indigenous petrophylic community. The low performance of hydrocarbon biodegradation could be due to the four weeks of incubation of the inoculated petrophylic communities still adapting to high hydrocarbon stress conditions and experiencing decreased growth and activity. Another argument possibility is the inoculated petrophylic cannot degrade the hydrocarbons present because the enzymatic systems involved in the breakdown of the hydrocarbon chains have decreased activity.

In contrast to the results of research conducted by [7], using a microbial consortium consisting of *Pseudomonas aeruginosa*, *Bacillus* sp., *Micrococcus* sp. can degrade petroleum waste consisting of n-alkane hydrocarbon compounds with C10 to C28 for 30 days. In comparison, the results of this study during the incubation period of 4 weeks did not show an optimal biodegradation rate, indicated by a low biodegradation value and not significantly different from the control treatment. Another possibility that can be explained by the low value of the rate of hydrocarbon

biodegradation by augmentative petrophylic is the low petrophylic viability as a result of the toxic conditions of the hydrocarbons, which can result in death in the early incubation or adaptation phase. In conditions of low population density resulting the degradation rate will also be low. Reported by [7], that adding the appropriate compatible consortium of microorganisms could affect the performance of petroleum biodegradation. In addition, the enzymes produced are more varied in the type and level of decomposition, and the number of enzymes is greater than the indigenous microbes so that decomposition occurs more quickly [18].

Treatment without the provision of a petrophylic consortium showed a tendency for better results. This showed that indigenous microbes had better adaptability to the presence of petroleum hydrocarbons, so indigenous petrophylic microorganisms were more able to degrade petroleum hydrocarbons. The application of chicken manure did not show a significant increase in the biodegradation rate of petroleum hydrocarbons. Chicken manure is rich in N elements [10]. However, the experimental results showed that the effect was not significantly different (Table 2); the dose of chicken manure applied can cause this is estimated to be insufficient to contribute N and P elements for petrophylic microbial activity. Another reason that can occurred was the inoculated petrophylic still use a carbons source from chicken manure, resulting the carbons source from hydrocarbons has not been used by the augmentation petrophylic, which results in the process of using hydrocarbons as a carbon source not occurring and resulting in the biodegradation of petroleum hydrocarbons not occurring as evidenced by the rate of biodegradation which was not significantly different from the control treatment. The dose of chicken manure used has not reached the conditions to achieve the optimal C: N: P ratio for the 10% TPH hydrocarbon biodegradation process. According to some sources the, sufficient Nitrogen and phosphate availability factor in the process of hydrocarbon degradation is the main factor for maximum success. According to [8], [2], [26], microbial degrader activity to remove hydrocarbons depends on adequate nutrients supply, especially nitrogen and phosphorous. An optimal C:N:P:K ratio is approximately 100:10:1:0.1.

### **Experimental Soil pH Condition**

The results of the analysis of variance showed that there was no interaction between the application of the petrophylic consortium and chicken manure on soil pH. However, it has an independent effect due to the application of the petrophylic consortium on soil pH. Soil acidity in various treatments can be seen in Table 3.

**Table 3. Effect of petrophylic Consortium Concentration and Chicken Manure on Soil pH in Petroleum Hydrocarbon Bioremediation during an incubation period of 3 days.**

Treatment	Soil pH
Petrophylic Concentration (A)	
a0 = no petrophylic consortium	6,7333 b
a1 = 1% petrophylic consortium	6.7444 b
a2 = 3% petrophylic consortium	6.5778 ab
	6.5000 a



a3 = 5% petrophylic consortium	
<u>Concentration of Chicken Manure (B)</u>	
b0 = without chicken manure	6.6667 a
b1 = 2% chicken manure	6.6500 a
b2 = 4% chicken manure	6.6000 a

Note: The average value followed by the same letter is not significantly different, according to Duncan's Multiple Distance Advanced Test at a 5% significance level.

The pH value of the soil during the experiment is shown in Table 3, and the results showed that the 5% petrophylic consortium treatment resulted in a significantly lower soil pH value compared to the 1% petrophilic consortium application treatments and without the petrophylic consortium administration. This phenomenon could be due to petrophylic activity at high concentrations producing organic acids in higher concentrations, resulting in a decreasing soil pH. According to [22], one of the biodegradation activities of petroleum hydrocarbons are marked by a decrease in the pH of the media due to the production of organic acids resulting from microbial activity during the breakdown of petroleum hydrocarbons. While the application of chicken manure did not change the soil pH significantly. This phenomenon can be explained by the possibility that the dose of chicken manure compost used has not reached a dose that can change the pH of the soil; it is proven that without the application of chicken manure, the pH value is not significantly different.

**The Petrophiyc Fungus Population during the Hydrocarbon biodegradation**

The results of the analysis of variance showed that there was no interaction between the application of the petrophylic consortium and chicken manure on the population density of the petrophylic fungi in the hydrocarbon bioremediation process. The population of petrophylic fungi in various treatments can be seen in Table 4.

**Table 4. Effect of Petrophylic Consortium inoculation and Chicken Manure amendment in Petroleum Waste Bioremediation Process on Petrophylic fungus population (10.433<sup>3</sup>CFU/g soil) at 4 weeks incubation period.**

Treatment	Fungi Petrophylic density (10 <sup>3</sup> CFU/g soil)
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<u>Petrophylic Concentration (A)</u>		
a0 = no petrophylic consortium		
a1 = 1% petrophylic consortium	26.222	a
a2 = 3% petrophylic consortium	173.333	c
a3 = 5% petrophylic consortium	152.667	c
	72.889	b
<u>Concentration of Chicken Manure (B)</u>		
b0 = without chicken manure	10.4333	a
b1 = 2% chicken manure	78.8333	b
b2 = 4% chicken manure	135.667	c

Note: The average value followed by the same letter is not significantly different, according to Duncan's Multiple Distance Advanced Test at a 5% significance level.

Investigation result showed that petrophylic consortium inoculation affected to increase the growth of the petrophylic fungus population. Nevertheless, the higher the dose applied tended to suppress the growth of the petrophylic fungus population. This phenomenon could be caused of there was competition phenomenon for receiving carbon sources and nutrients for growth between them. Another phenomenon was caused by a negative interaction between petrophylic species, which can suppress the growth of one population or both suppress each other. Petrophylic fungi are expected to help the degradation process of hydrocarbons into simpler substrates. Nevertheless, they could not degraded hydrocarbon effectively.

The more abundant amendment of chicken manure was higher in increasing in petrophylic fungi population. In this experiment showed that the source of nutrients came from chicken manure can supply the carbon and nutrient for petrophylic fungus. This phenomenon could be cause of the nutritional content contained in chicken manure has been able to meet the nutritional needs required by petrophylic fungi for their growth, but the N and P allocated from chicken manure has not been to cover for degrading hydrocarbon process, so it is resulting the hydrocarbons biodegradation inhibited, so it suggested that the use of chicken manure for biodegradation of TPH 10% of the waste load needs to be increased until it reaches the optimum ratio to support the growth of petrophylic fungi and also for increasing hydrocarbons biodegradation process. This experiment result also showed that the petrophylic fungi or petrophylic bacteria inoculated were not able to degrade the remaining hydrocarbon compounds having a high level of toxicity and being resistant (eg. polycyclic aromatics). As reported by [24], that the indigenous microorganisms are more effectively able to degrade hydrocarbon compounds then the petrophylic augmentation.

**4.CONCLUSION**

The petrophylic consortium inoculation and amendment of chicken manure did not show any interaction effect on increasing the biodegradation rate of petroleum hydrocarbons, soil pH, and the population of petrophylic fungi. Similarly, all treatments independently of the Petrophylic dose variation and the chicken manure dose variation did not increase the biodegradation rate, soil pH. Nevertheless, the amendment of chicken manure increased significantly. However the increasing of the petrophylic fungus population was not able in increasing the hydrocarbons biodegradation rate in bioremediation process of petroleum waste during an incubation period of 4 weeks. This result showed that the effectiveness of the petrophylic consortium used has not been showing their performance maximally. This phenomenon can be caused by the dose of chicken manure applied has not been able to supply sufficient N and P nutrients to achieve optimal C: N:P ratio conditions required for petrophylic activity to degrade petroleum hydrocarbon maximally. And also the applied petrophylic consortium cannot carry out its activities to increase degrading hydrocarbons significantly. Thus, the adequacy of N and P nutrients and compatibility between members of the hydrocarbon-degrading microbial consortium in the bioremediation process are important factors that need to be prepared.

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