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Research Article

BACTERIAL CONSORTIUM UTILIZATION IN DEGRADATION OF PETROLEUM FROM PETAPAHAN, RIAU

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ABSTRACT

The process of petroleum degradation using the right combination of microbes in a mixed culture will guarantee the success of the petroleum degradation process. In a consortium, several types of bacteria works together to degrade oil-polluting compounds in accordance with the specificity of the substrate. This study used a collection of hydrocarbonoclastic bacterial isolates using three consortia namely consortium I using 7 isolates, consortium II using two new isolates and consortium III using the group of *Pseudomonas* bacteria. Test of oil degradation level was based on the degradation level parameters on Total Hydrocarbon Petroleum (TPH) and calculated by gravimetric method. The Chemical Oxygen Demand (COD) test was calculated using the Dichromate Reflux Technique Standard method and analysis of petroleum compounds which had been degraded was done by GC-MS method. From the research that has been carried out using three consortiums, consortium 1 was showed the best result, which used 7 different types of isolates. Consortium 1 is the best consortium in degrading petroleum with a TPH level of 77.70 % and a COD reduction of 89.83 %. The results of GC / MS analysis of mixed culture by using 7 bacterial isolates contained 4 compounds that were 100 % degraded, namely 7- methyltridecane, tetradecana, 1-eikosane, and triacontane.

Keywords: mixed culture, consortium, hydrocarbonoclastic bacteria, petroleum, Petapahan, Riau

INTRODUCTION

Petroleum is the main source of energy for human life in the world. Aside from being an energy source, petroleum is also a raw material for various activities such as engine lubricating oil, solvents, plastics, fibers, detergents, pharmaceuticals and cosmetics[1]. However, these conditions will increasing the environmental pollution, both in the terrestrial and aquatic environments that came from activities and the rest of the tank cleaning, the leakage of pipes and spills during the transportation process[2]. The physical and chemical characteristics of petroleum, make it difficult to degrade so it has the potential to reduce the quality, function and aesthetics of the polluted environment and disrupting the ecological balance. Pollution caused by heavy oil spills in the environment can be recovered in various ways. One of them is through a biological approach that is relatively cheap, effective, efficient and environmentally friendly. This method is called as bio remediation, which utilizes living things as biological agents for the recovery of polluted environments. Specifically, this method is carried out microbiologically, namely using microbes, especially bacteria that have the ability to utilize heavy oil as a carbon source and energy for cell metabolic activity which is then converted to CO_2, H_2O and biomass.

One method to manage and use waste is by using a biological agent called bio remediation. Bio remediation is a process of recovery (remediation) of an area that contaminated with organic and anorganic waste by utilizing organisms. Management by using organisms is an an cheap, effective and environmentally friendly alternative to overcome the petroleum waste. Bio remediation also caused the waste degradation to produce stable and non-toxic final compounds, but this method requires a longer time compared to physical or chemical methods[3]. Often a type of microbial is only able to degrade hydrocarbon compounds in a certain molecular weight range. Therefore the right microbial combination in a mixed culture will better guarantee the success of the degradation process of oil polluting compounds. In a consortium, several types of microbes work together to degrade oil pollutants according to the specificity of the substrate.

Bio degradation of hydrocarbon compounds such as petroleum usually requires the cooperation of more than one species of bacteria. This condition was because petroleum is formed from many different hydrocarbon compounds and bacteria can only use hydrocarabon in a certain range. The difference in the ability of bacteria to use hydrocarbon compounds can be used to maximize the bio degradation process, therefore the characterization for the bacteria ability in degrading hydrocarbon compounds is necessary[4].

According to Rahman *et al.* (2003); Al-awadhi *et al*. (2002); and Ciawi *et al*. (2000) the process of petroleum degradation using mixed cultures gave better results when compared to using a single isolate. In this study, the ability of petroleum degradation to use bacterial mixed culture (consortium) was observed and analyzed. The bacteria used were obtained from the petroleum mining area in Petapahan, Riau. Based on studies that have been carried out, seven best isolates in degrading oil were obtained, namely IMB-05, BMI-07, BMI-09, BMI-10, BMI-11, BMI-12, BMI-15[8]. Potential ability of hydrocarbonoclastic bacteria (hydrocarbon degradation) isolated from the consorstium of bacteria derived from petroleum needs to be analyzed through a

series of studies, so that it can be used as a bio remediation agent to overcome environmental pollution. Therefore, the examination of the degradation process using a mixture of bacteria culture (consortium) need to be done to see the level of petroleum degradation.

MATERIALS AND METHODS

Hydrocarbonoclastic bacterial sources

The source of bacteria used came from the stock of isolates that had been isolated by Sayuti *et al*. (2018) which isolated hydrocarbonoclastic bacteria from petroleum, Petapahan, Riau. The bacterial isolates used were IMB-05, IMB-07, IMB-09, IMB-10, 1MB-11, 1MB-12, IMB-15.

Inoculum production of hydrocarbonoclastic bacterial isolate

The source of inoculum was made by inoculating 2 oz of bacterial isolates in 9.8 ml of liquid SMSS medium which was added with 0.2 ml of crude oil (petroleum) in a 25 ml Erlenmeyer, then shakewith agitation 120 rpm for 72 hours at 28^{o}C. After 72 hours the bacterial inoculum was added to the production medium of liquid SMSS which was added with petroleum then through the test phase of a consortium (mixed culture) of hydrocarbonoclastic bacteria[8].

Petroleum degradation using consortium (mixed culture) of hydrocarbonoclastic bacterial

The ability test of a mixed culture of hydrocarbonoclastic bacteria was done by comparing the mixture of bacterial groups consisting of:

- Mixed culture group I, using 7 bacteria isolates (IMB-05, IMB-07, IMB-09, IMB-10, IMB-11, IMB-12 and IMB-15)
- Mixed culture group II, using 2 isolates from new isolate group(IMB-01 and IMB-12)
- Mixed culture group III, using 2 isolates from*Pseudomonas* group(IMB-09 and IMB-15). In each mixed culture, the inoculum of 1 ml was added to each hydrocarbonoclastic bacteria, then 2 ml of oil was added and liquid SMSS medium was added to reach volume of 100 ml.

Observation Parameter

Degradation level total petroleum hydrocarbon

Measurement of the degradation level was carried out using the gravimetric method. This method was carried out by extracting 5 ml of sample using benzene, pentane and diethylether with a ratio of 3: 1: 1 as much as 5 ml. The oil obtained then weighed to find out the amount of oil contained in the sample. The level of degradation was measured by the following formula[9].

$$\text{Degradation } \% = \frac{A - B}{A}$$

Annotation
A: Total petroleum hydrocarbon (TPH) at the beginning
B: Total petroleum hydrocarbon (TPH) at the end

Chemical oxygen demand (COD)

COD measurement was carried out to determine the total chemical concentration found in the waste before and after bio remediation. Measurementwas done using the Dicromate Reflux Technique Standard method. The COD content was determined by the following calculation [10].

$$\text{COD}\left(\text{mg}/\text{l}\right) = \frac{(a-b).(N).8000}{v}$$

Annotation:
a: ml $Fe(NH_4)_2(SO_4)_2$ used for from
b: ml $Fe(NH_4)_2(SO_4)_2$ used for sample
N: normality of $Fe(NH_4)_2(SO_4)_2$
v: sample volume

Analysis of compound composition

Analysis of the compound composition in petroleum was done using GC / MS. The purpose of this analysis was to determine the composition and type of compounds contained in the sample before and after bio remediation. The instrument used was HP-5890 GC type with FID detector and 300^{0}C temperature, GC column was glass capillary (30 m long and 0.25 mm diameter) with a pressure of 100 kPa and column flow 1.6 ml / minute, while the sample carrier gas that will be analyzed is helium.

RESULTS AND DISCUSSION

Mixed culture test of hydrocarbonoclastic isolate TPH and COD degradation levels of hydrocarbonoclastic bacteria mixed culture

In this study, 3 additional bacterial consortiums were made from bacteria of the same genus, namely Consortium 1 (IMB-5, IMB7, IMB 9, IMB-10, IMB-11, IMB-12, IMB-15); Consortium 2 (IMB- 5 and IMB - 15); and Consortium 3 (IMB-9, IMB-15). This test was done to prove that the mixed culture treatment can increase the degradation activity of hydrocarbonoclastic bacteria. Observation results can be seen in Table 1.

Table 1: TPH and COD Results from Mixed Culture of Petroleum Degrading-Bacteria

No.	Code	TPH (g/100 ml)	COD (g/100 ml)	TPH Degradation (%)	COD Decrease (%)
1	Control	42,81	15,29	0,00	0,00
2	IMB1+ IMB12	11,87	1,75	72,27	88,54
3	IMB 9 + IMB 15	12,95	1,76	69,76	88,47
4	7 Isolates	9,55	1,56	77,70	89,83

Table 1 shows the level of degradation of petroleum by 3 different consortiums. The biggest petroleum degradation is shown by bacterial concentration consisting of 7 bacterial isolates. The use of a bacterial consortium in the process of petroleum bio remediation will affect the process of petroleum degradation. This is because each bacterial species needs a specific substrate to degrade the entire constituent component of petroleum. According to Asadirad (2016), there are two possibilities in bacterial mixed culture (consortium) that can affect the bioremediation process, namely synergism and antagonism, the synergism process of mixed culture bacteria can improve the bioremediation process and vice versa.

The complex composition of petroleum compounds was causeda single species of microorganism cannot degrade the entire constituent component of petroleum. Some bacteria has mutually benefit interaction in the form of a consortium where the consortium plays a role during the process of petroleum degradation. Consortium of petroleum degrading-bacteria is more effective in degrading petroleum than single culture bacteria. Petroleum is a complex mixture of hydrocarbon compounds, while each type of bacteria has specific enzymes that work on certain substrates so that they have limited ability to degrade. Therefore, each type of bacteria will alternately dominate the consortium in accordance with the hydrocarbon fraction that can be utilized[12].

Bio degradation of petroleum using mixed cultures is determined by measuring the metabolism of one single hydrocarbon, usually compounds that are rapidly metabolized such as alkanes. The microbial degeneration community in nature is found together with a complex hydrocarbon mixture, some of which affect the metabolism of other hydrocarbons. Some of the hydrocarbons will be oxidized when the microbes use another fraction[13]. The degradation ability of a microbial species is limited only to the range of certain hydrocarbon compounds, but some types of microbes will work together in degrading petroleum according to the specificity of the substrate [14].

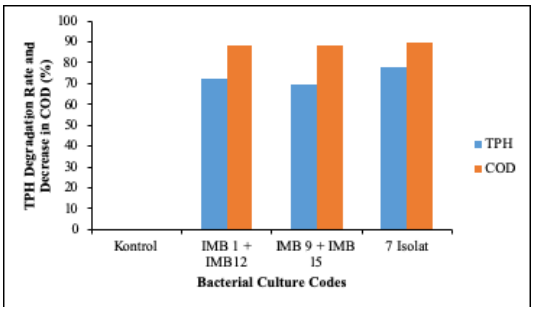

Figure 1: Level of TPH and COD degradation by Bacterial Consortium

Figure 1 shows the results of the ability test of 3 types of bacteria consortium in degrading hydrocarbons and COD. The TPH (Total Petroleum Hydrocarbon) value is the total value of petroleum hydrocarbons contained in petroleum. The decline in TPH value was caused by the degradation of pollutants by bacteria. Bacteria are able to degrade petroleum components through their metabolic activity because of their ability to oxidize hydrocarbons and turned them into a source of carbon and energy. From the consortium that has been carried out, the best results were shown by consortium 3 consisting of 7 bacterial isolates with a decrease in TPH of 77.70 % and COD of 89.83 %. This is because in a joint state between isolates there was a synergistic collaboration to produce enzymes that can break down hydrocarbon structures. Another possibility is because the consortium isolates produce enzymes that are more variable in type and level of decomposition as well as more amount than single isolates. Sumarsono (2011) stated that the process of bio degradation of hydrocarbon compounds to be perfect is impossible to do if only involving one type of bacteria, but always carried out by a collection of bacteria that interact synergistically in the form of a consortium. According to Alexander (1977), percentage of degradation of petroleum hydrocarbons in the process bio remediation was related to the enzymatic degradation process carried out by bacteria. Hydrocarbonoclastic bacteria produce monoxidase and dioxygenase enzymes which play a role in degrading petroleum hydrocarbon components into a simpler fraction.

The decrease in COD concentration was caused by bacterial metabolism that utilizing substrates other than hydrocarbons such as fatty acids and other compounds that influence the decrease in COD concentration. The decrease in COD concentration also occurs due to the degradation of TPH and other compounds contained in petroleum. There are several factors that determine the quality of degradation done by bacteria consortium, including nutrition, competency to compete for nutrientsand enzymes produced by each bacterial isolate.

Bacillus and *Pseudomonas* produces oxidase enzymes to utilize hydrocarbons as nutrients in their growth. There are two kinds of oxygenase enzymes, those are mono-oxygenase and dioxygenase. Mono-oxygenase plays a major role in the degradation of aliphatic hydrocarbons, whereas dioxygenase in alicyclic hydrocarbons. Both plays role in the initial stages of degradation, when the insertion of oxygen molecules into the hydrocarbon structure. In n-alkanes, the insertion can occur in the terminal methyl group as well as in the subterminal methyl group. N-alkane is oxygenated to alcohol, then carboxylic acid. If an organic compound has degraded to its acidic form, the subsequent degradation reaction takes place through the continuous separation of two carbon units. This reaction is a common reaction to living cell metabolism and known as oxidation beta sequence. The name of reaction was because oxidation occurs in the methyl beta group in n-alkanes to ketones. The degradation reaction of other hydrocarbon compounds such as alkene, branched, alicyclic and aromatic hydrocarbons is done with the same principle. The differences lies in microbial preferences during the biodegradation process as well as the specific biochemical pathways and enzymes involved [17].

Degradation Level of Petroleum Compounds Using GC-MS Analysis

Decreasing of the oil degradation level by mixed culture was analyzed using GC-MS. The results of the analysis showed that in the 3 types of mixed cultures the petroleum degradation by bacterial isolates was really happened. This can be seen from the comparison between the control and the treatment given (Figure 2). The occurrence of petroleum degradation can be seen from the emergence of peaks of 3 types of mixed cultures when compared with controls. Carbon chain loss showed on the peak number 7 and peak number 28. At this peak, the activity of enzymes that play a role in breaking the double bonds of hydrocarbons was happened. Mixed culture activity also showed the enzymes activity in degrading petroleum and some types of bacteria work together to break the double bonds of hydrocarbons. These microbes fulfill their needs for carbon sources and their energy by using materials such as hydrocarbons which are very small in water. There are three ways for transporting hydrocarbons into microbial cells in general, namely the interaction of cells with dissolved hydrocarbons in the water phase, direct contact (attachment) of cells with the surface of hydrocarbon droplets larger than microbial cells through active diffusion or transport processes, and cell interactions with droplets hydrocarbons emulsified or solubilized by bacterial cells[18].

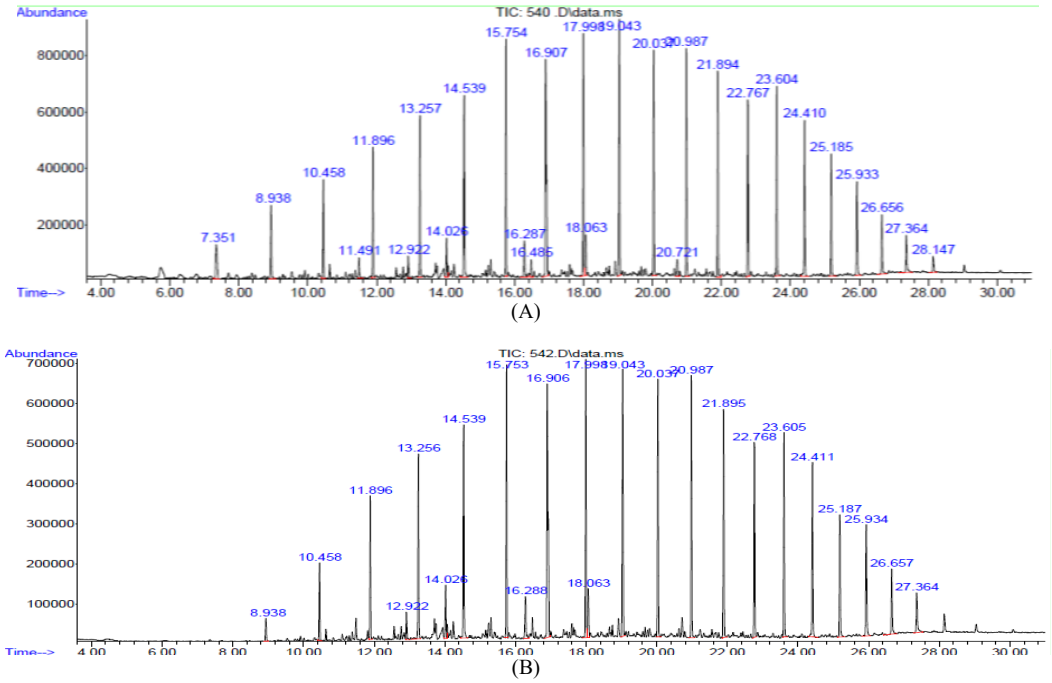

Figure 2: Results of GC-MS Petroleum Analysis
(A) Control; (B) Mixed Culture of 7 Isolates

GC-MS results showed that the mixed culture was through bio degradation process. The carbon chain which was disconnected by bacterial activity showed that the peak at retention time 11.491 with a peak height of 68983 in the control was 0 in 7 isolates with a degradation rate of 100 %. Then at a retention time of 20.721 the peak height in the control became 0 in 7 isolates. Retention time 16.485 showed peak height of 56282 in the control. Finally, 100 % degradation occurred at retention time of 28.147 with a peak height of 52590 in the control to 0 after addition of 7 bacterial isolates. The lost compounds from the analysis were7-methyl tridecane, tetradecane, 1 eikosane and triacontane (Table 2 and Figure 2).

Table 2: Analysis of GC-MS Mixed Culture of Hydrocarbonoclastic BacteriaUsing 7 Isolates

Retention time(minute)	Peak height		Degradation level (%)	Compound name	Chemical formula
	Control	**7 isolates**			
	118731	0	100,000	Decane	$C_{10}H_{22}$
8,938	242193	52113	78,483	Decane	$C_{10}H_{22}$
10,458	313812	170190	45,767	Decane	$C_{10}H_{22}$
11,491	68983	0	100,000	7-Methyltridecane	$C_{13}H_{28}$
11,896	405692	314986	22,358	Tridecane	$C_{13}H_{28}$
12,922	67530	59185	12,357	Decane	$C_{10}H_{22}$
13,257	526670	430446	18,270	Tetradecane	$C_{14}H_{30}$
14,026	124803	121315	2,795	2,6,10-Trimethyltridecane	$C_{16}H_{34}$
14,539	633077	530425	16,215	Pentadecane	$C_{15}H_{32}$
15,754	698458	558742	20,003	Hexadecane	$C_{16}H_{34}$
16,287	125049	103304	17,389	Pentadecane,2,6,10-trimethyl	$C_{18}H_{38}$
16,485	56282	0	100,000	Tetradecane	$C_{14}H_{30}$
16,907	767099	612759	20,120	Heptadecane	$C_{17}H_{36}$
17,998	760726	609283	19,908	Octadecane	$C_{18}H_{38}$
18,063	134174	115616	13,831	Hexadecan,2,6,10,14-tetramethyl	$C_{20}H_{42}$
19,043	811878	600863	25,991	Nonadecane	$C_{19}H_{40}$
20,037	685238	549032	19,877	Eicosane	$C_{20}H_{42}$
20,721	54311	0	100,000	1-Eicosane	$C_{20}H_{42}$
20,987	725555	583401	19,592	Heneicosane	$C_{21}H_{44}$
21,894	675747	527974	21,868	Docosane	$C_{27}H_{56}$
22,767	639562	496164	22,421	Octadecane	$C_{18}H_{38}$
23,604	588829	441606	25,003	Tetracosane	$C_{24}H_{50}$
24,41	507894	395751	22,080	Pentacosane	$C_{25}H_{52}$
25,185	384155	294764	23,270	Hexacosane	$C_{16}H_{34}$
25,933	317882	253781	20,165	Heptacosane	$C_{27}H_{56}$
26,656	190426	148196	22,177	Octaacosane	$C_{28}H_{58}$
27,364	123203	93483	24,123	Nonadecane	$C_{19}H_{40}$
28,147	52590	0	100,000	Triacontane	$C_{30}H_{62}$

Table 2 shows the results of GC / MS analysis from mixed culture of hydrocarbonoclastic bacteria where in an eight minute retention time the petroleum degradation by bacteria activity was happened for four times. This can be seen between the initial hydrocarbon compounds (control) hydrocarbon decane compounds with the chemical formula $C_{10}H_{22}$ which occurs at a rate of 100 % degradation, namely tridecane, 7-methyl with the chemical formula $C_{10}H_{22}$. At retention time 20.721 there was 100 % degradation and 1-eicosane compounds with the chemical formula $C_{20}H_{42}$ was formed. Degradation of hydrocarbons as much as 100 % also occurred at retention time 28.147 which formed $C_{30}H_{62}$ compound. It turned out that 7 isolates of hydrocarbonoclastic bacteria had different abilities in degrading hydrocarbon compounds for 28 minutes using GC-MS analysis.

According to Desai and Vyas (2006) bio degradation some bacteria and groups of bacteria have been known to have the ability to degrade hydrocarbon compounds, such as *Pseudomonas* sp. that able to degrade saturates compound, monocyclic aromatic hydrocarbon compounds can be degraded by *Pseudomonas* sp. and *Bacillus* sp., polycyclic aromatic hydrocarbon compounds can be degraded by the *Pseudomonas* sp. and *Bacillus* sp. and resin compounds can be degraded by the *Pseudomonas* sp.

Gas chromatography - mass spectroscopy (GC-MS) is one combined technique to determine a method of analyzing a mixture of chemicals. Gas chromatography can separate mixed components and mass spectroscopy characterizing each component produced. If the GC conditions (oven temperature, type column, etc.) are the same, a given compound will come out of the column with almost the same retention time. By knowing the retention time of a compound, we can make several assumptions about the complex identity of the identified compounds[20]. Observation of microbial activity in degrading petroleum sludge was done by cutting the long chain aliphatic hydrocarbon components and transforming aromatic hydrocarbon compounds, so that the petroleum sludge will show changes in the composition of the constituent hydrocarbon fractions[21].

From the Table 2 it can be seen that petroleum hydrocarbons were degraded by bacteria ranging from C_{10} to C_{30}. This results means, the petroleum content was dominated by paraffinic and olefin groups. The highest percentage of degradation by the culture of 7 isolates was 100 % which occurs in hirdocarbon decane, 7 methyl tridecane and 1 - eikosane. This condition showed that in these compounds, carbon chains were eaten out by bacteria for use in metabolism. Conversely, bacterial isolates haddifficulties in degrading the hydrocarbons with branched chains. From Table 2, it also can be seen that the highest degradation rate for branch chain hydrocarbons was 17 % at 2,6,10 trimethylenes. This is because the synergism of the enzymes produced by each bacterium was not strong enough to break the hydrocarbons with branched chain.

CONCLUSION

From the research that has been carried out using three consortiums, consortium 1 was obtained using 7 different types of isolates. Consortium 1 was the best consortium in degrading petroleum with a TPH level of 77.70 % and a COD reduction of 89.83 %. Results of GC / MS analysis of mixed culture using 7 bacterial isolates showed 4 compounds which 100 % degraded, namely 7-methyltridecane, tetradecane, 1-eikosane, and triacontane.

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