Bioremediation of petroleum hydrocarbon by mixed bacteria culture of *Pseudomonas aeruginosa* and *Brevibacterium sp.*

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**Abstract.** The intention of this research is to overcome pollution by petroleum using bioremediation method with the help of hydrocarbonoclastic bacteria. The first step in this research was to characterize petroleum as pollutants and cultivate mixed culture bacteria, i.e. *Pseudomonas aeruginosa* and *Brevibacterium sp.* The second step was degradation test with growth medium of SMSS (Stone Mineral Salt Solution) with variations of pH and concentration. It was conducted in a 300 ml Erlenmeyer series with composition of 80% media, 5% petroleum, and 10% bacteria placed in an incubator shaker with a rotation speed of 200 rpm for 7 days. Based on the results from degradation test with growth medium of SMSS, obtained results that TPH (Total Petroleum Hydrocarbon) degradation efficiency is higher at a pH of 8 that is equal to 28.47%. In the variation of concentration, the highest degradation efficiency occurs at concentration of 20% oil which is reduced oil viscosity up to 22.00%. This research proves that *Pseudomonas aeruginosa* and *Brevibacterium sp.* can degrade hydrocarbon composition in petroleum with an efficiency of up to 20%.

1. **Introduction**

Crude oil is a highly complex mixture that contains hydrocarbon compounds, which are organic compounds of carbon and hydrogen compound molecule. Crude oil exploration can produce negative effects on the environment, one of them caused by oil spillage. Crude oil can be dangerous because it contains toxic and carcinogenic hydrocarbon that would kill existing organism in an area. Hydrocarbon and its constituent components’ degradation and fission can biologically and non-biologically occur. Biologically; it can be caused by microorganism activities such as fungi, bacteria, and yeast, meanwhile non-biologically; it can be caused by vaporisation and dissolution [1]. Soil pollution problem caused by crude oil can be solved on several ways such as by using physics-chemical method (for example: in-situ burning, adsorption, and washing oil) and biological method. Physics-chemical method application commonly requires a long and complex process and a high cost. That is why biological method has become a better alternative because of its effectiveness and efficiency with a lower cost and less pollution problem. A commonly used biological method is bioremediation.

Bioremediation is an effort to repair environmental condition by using biologic bacteria activity to degrade and lower toxicity of various pollutant compounds [2]. Bioremediation can be classified into two classes which are in-situ bioremediation and ex-situ bioremediation. In in-situ bioremediation, degradation process happens naturally according to environmental situation so that the process highly dependent on degrading microorganism’s survivability. Because of that, in this research an ex-situ...
bioremediation was chosen because it is easier to control environmental condition (in this case are pH and pollutant concentration), so that the degradation can be processed faster.

Based on various researches on crude oil bioremediation this method is proven effective to lower TPH (Total Petroleum Hydrocarbon) rate contained in crude oil. Based on performance test, R122-BN5 carbonoclastic bacteria is able to lower TPH from 6.8% into 1% (0.82%) in 32 days [3]. Based on the description, this research is required to determine optimum environmental condition that provides highest efficiency in TPH degradation process.

Hydrocarbon compound degradation process can be enacted by utilizing hydrocarbonoclastic bacteria that is able to degrade hydrocarbon compound and utilize the compound as carbon and energy source needed for its growth. This bacterium is able to decompose crude oil component and produce several bio-products such as gas, fat acid, surfactant, and biopolymer during crude oil degradation [4].

In this research, a mixed culture bacterium that contains of Pseudomonas aeruginosa and Brevibacterium sp. will be utilized. Bacteria will be cultivated on Media Stone Mineral Salt Solution (SMSS) because it is highly suitable for hydrocarbonoclastic microorganism [5]. SMSS contains Mg and Mn ions that function as acceptor used when oxygen level is low during biodegradation process and as accelerator of extra-cellular enzyme such as lipase that can help mono-oxygenation in hydrocarbon biodegradation process [6].

Based on previously conducted research, Pseudomonas aeruginosa possesses a good growth rate when utilizing crude oil as carbon source. The growth of this bacteria experience an exponential phase started from hour-0 until reaches its maximum cell number in hour-36. The other bacteria, which is Brevibacterium sp. owns rhlB and psoA genes that responsible in biosurfactant synthesize as a bio-product of crude oil degradation [7]. Based on the explanation, this research is aimed to acknowledge optimum condition that provides the highest efficiency of TPH degradation process.

2. Research methodology

2.1. Crude oil characterization
Crude oil sample was obtained from PT Pertamina EP Asset 5 in Takaran, Northern Borneo Field. TPH rate of the crude oil was measured. TPH rate was analyzed by using GC-MS (Gas Chromatography Mass Spectrometry) method. Analysis was conducted in Oil and Gas institution’s’ (LEMIGAS) laboratory in Jakarta.

2.2. Mixed culture bacteria cultivation
Pseudomonas aeruginosa and Brevibacterium sp. bacterium was cultivated in a SMSS (Stone Mineral Salt Solution) medium to obtain required bacteria as degradation agent. Cultivation was conducted by using series of 250 ml Erlenmeyer flask with 250 ml of SMSS media composition, 20 ml of Pseudomonas aeruginosa bacteria and 20 ml of Brevibacterium sp. bacteria. The bacteria were heated on 300C temperature with pH of 7, in shaker incubator speed of 200 rpm. This process was conducted until it reaches exponential phase which is 7 days.

2.3. Degradation test with pH optimization in SMSS liquid media
Cultivation was conducted in series of 300 ml Erlenmeyer, in 80% of SMSS (Stone Mineral Salt Solution) media, 5% of crude oil, and 10% of bacteria with shaker incubator speed of 200 rpm. During pH optimization, a blank sample that contains no pollutant was produced for every experiment. pH optimization conducted with variation of 5, 7, and 9. Meanwhile pH adjustment was conducted by adding NaCl and H2SO4. Cultivation was conditioned on a 350C temperature and 7 days of contact days.

2.4. Degradation test with concentration optimization in SMSS liquid media
Cultivation was conducted in series of 250 ml Erlenmeyer in 80% of SMSS (Stone Mineral Salt Solution) media and 10% of bacteria with shaker incubator speed of 200 rpm. During temperature
optimization, a blank sample that contains no pollutant was produced for every experiment. Concentration optimization was conducted with a variation of 10%, 15%, and 20% in normal pH condition and 7 days of contact days.

3. Results and discussion
Pseudomonas aeruginosa and Brevibacterium sp. bacteria are able to degrade hydrocarbon compound in crude oil. This ability can be seen from Total Petroleum Hydrocarbon (TPH) decrease in 7 days on various pH and crude oil concentration. Parameter used to determine the best treatment in pH optimization is TPH degradation level and mixed culture bacteria growth rate. pH optimization was conducted by 3 different pH variations of 7, 8 and 9. The results are shown in Table 1.

Table 1. TPH degradation level in bioremediation process in different pH conditions.

| pH | Original TPH (g/100mL) | Final TPH (g/100mL) | Degradation Level |
|----|------------------------|---------------------|-------------------|
| 7  | 14.50                  | 10.80               | 24.97%            |
| 8  | 14.50                  | 10.37               | 28.47%            |
| 9  | 14.50                  | 11.58               | 20.12%            |

![Figure 1](image1.png)

Figure 1. TPH degradation level in bioremediation process in different pH conditions.

Treatment that provides best growth and degradation is in pH level of 8. This situation can be seen from high TPH degradation level if compared with any other pH levels. This pH level is able to degrade TPH as much as 28.47% (Table 1). TPH value decrease shows degradation process. Hydrocarbon compound contained in crude oil act as carbon source in bacteria growth, meanwhile non-hydrocarbon compound act as additional nutrition required in its growth [8,9]. Based on the result, it can be concluded that the pH level is the most suitable one if compared with any other pH levels. Environmental condition with low pH range salinity approaching 8 can escalate mineralization process. A Suitable pH will accelerate biodegradation process. Cellular function, membrane transport, and catalyst reaction balance is highly affected by pH, so that it would influence crude oil polluted compounds’ growth and degradation levels [10].

Besides TPH degradation level, mixed culture bacteria growth rate is also showing best growth rate in pH of 8. Mixed culture bacteria growth curve pattern in different pH condition is shown in Figure 2.
The next step is by doing a treatment to achieve largest viscosity decrease efficiency in various crude oil concentration variations of 10%, 15%, and 50%. Table 2 shows crude oil’s viscosity result from t0 until t7 (days).

Table 2. Viscosity value (Cst) in bioremediation process on different crude oil’s concentrations.

| Crude oil concentration | t 0 (day) | t 7 (day) | % decrease |
|-------------------------|-----------|-----------|------------|
| 10%                     | 12.60     | 10.88     | 13.65%     |
| 15%                     | 12.45     | 10.29     | 17.35%     |
| 20%                     | 12.68     | 9.89      | 22.00%     |

Based on the result of the research, we can see the crude oil viscosity decrease in every concentration variation (Figure 3). The highest viscosity decrease percentage happens in 20% crude oil concentration, which is 22%. Viscosity decrease happens due to simple fractions formation because of degradation process. Viscosity decrease shows that a process was on going, which is a degradation process [5].

Figure 2. Mixed culture bacteria growth curve pattern in different pH condition on 35°C temperature.

Figure 3. Viscosity value decrease (Cst) in bioremediation process on different crude oil’s concentrations.
The Degradation process produces a large number of simpler fractions. The large number simpler fractions cause crude oil viscosity decrease. CO2 was also formed as another result of degradation process, which will react with a part of crude oil fraction that would causes crude oil expansion and lower its viscosity [4,6].

4. Conclusion
Mixed culture bacteria that contains of Pseudomonas aeruginosa and Brevibacterium sp. bacteria is able to survive in SMSS (Stone Mineral Salt Solution) media. Research also proves that mixed culture bacteria can degrade crude oil on pH conditions of 7, 8, and 9 on crude oil concentrations of 10%, 15%, 20%. This situation can be seen by Total Petroleum Hydrocarbon (TPH) rate and viscosity decrease in crude oil samples.

Optimum pH condition that produces best degradation level is pH 8 with TPH degradation as much as 28.47%. Crude oil concentration that gives highest viscosity efficiency decrease is crude oil with 20% concentration. Mixed culture bacteria utilize crude oil as carbon source or energy source on its growth. A further research on crude oil bioremediation by mixed culture Pseudomonas aeruginosa and Brevibacterium sp. bacteria is required to obtain a higher degradation level on a shorter period of time.

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