Interaction of *alcaligenes* sp, *bacillus* sp, and household waste compost for biodegradation of hydrocarbon in soil-contaminated lubricant oil

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**Abstract.** The purpose of this research is to obtained the best interaction between bacteria and compost in the process of hydrocarbon bioremediation in soil-contamination lubricant oil. *Alcaligenes* sp. and *Bacillus* sp. from our previous study is applied in this study. We used 3%, 6% and 9% household waste compost as the biostimulant sources. Biodegradation process is indicated by total petroleum hydrocarbon (TPH) value and the quality of bioremediated soil is checked by pH value. TPH and pH value is measured in days of 15th and 30th. This study showsthat interaction of *Alcaligenes* sp and *Bacillus* sp consortium and 6% household waste compost gave the best TPH value (27.62%) in biodegradation process after 15 days. However, on days of 30th, the best result is shown by the interaction of *Alcaligenes* sp and 9% household waste compost. The TPH value after 30 days of incubation is 30.54%. The soil’s pH is decrease from 7.98 to 5.79 respectively. It is concluded that interaction of *Alcaligenes* sp and household waste compost can increase degradation rate of hydrocarbon in soil-contaminated lubricant oil.

1. Introduction

Petroleum is the main energy source used in transportation and household appliance [1]. One of the petroleum derivative products is lubricating oil that is used to reduce friction on the surface of the machine [2]. According to Minister of Environment Decree no. 128 of 2003, waste lubricant oil is categorized as hazardous and toxic waste materials. Waste lubricant oil containing polycyclic aromatic hydrocarbon (PAH). When it is accumulated in human body can be fatal for our body. Moreover, used lubricant oil can pollute land and oceans and kill microorganisms in the environment [3].

Considering of the large risks of used lubricant oil for humans and the environment, it is necessary to make efforts to recover the polluted environment. One of the most effective efforts is the biological improvement known as bioremediation. Bioremediation is an environmental recovery technique utilizing organisms that have the ability to break down, utilize or absorb hazardous materials into non-hazardous products[4]. One of the bioremediation techniques is biostimulation. Biostimulation is an effort to increase the ability and growth rate of microorganisms by providing a source of nutrients that can support degradation activities[5]. The use of household waste compost as biostimulant can provide
elements of N and P that are needed by hydrocarbonoclastic bacteria. The bacteria used in this study are *Alcaligenes* sp and *Bacillus* sp which were previously examined *in vitro* \[^{[6,7]}\]. In this study we use degradation of TPH as an indicator for bioremediation \[^{[8]}\]. We also measure pH value to see the activities of bacteria in bioremediation. Bacteria can work optimally with a pH range of 6.5 to 7.5 \[^{[9]}\].

2. **Materials and Methods**

This study is complete randomized design in factorial. The first factor is group of bacteria and the second factor is compost concentration. Group of bacteria are *Alcaligenes*, *Bacillus* and consortia (*Alcaligenes* and *Bacillus*). The concentration of compost used are 3%, 6% and 9%.

2.1. **Bioremediation Setting and Biostimulation**

500 g of soil is sifted and mixed with 25 ml of used oil and stirred until homogeneous. The concentration of used oil on the soil is 5%. This mixture is left at room temperature for 48 hours to volatilize the toxic components of the oil \[^{[10]}\]. On the next two days compost was added to the treated soil and stirred evenly. The mixed soil is then put into polybags. The soil moisture is controlled to 60% by watering twice a day. 26 ml of bacterial isolates were then poured into each polybag.

2.2. **TPH measurement**

4 g of soil and 4 ml of n-hexane are poured into the centrifuge tube. The mixture is then centrifuged at 10,000 rpm for 15 minutes, 4°C. 2 ml of the supernatant was transferred into cuved. Furthermore, it is read by a spectrophotometer at a wavelength of 272 nm. TPH degradation is calculated by formula:

\[
\text{Degradation} = \left(\frac{O_i - O_f}{O_i}\right) \times 100\%
\]

*Oi* = initial concentration (mg/L)

*O* = final concentration (mg/L)

2.3. **pH measurement**

Soil pH was measured on days 15 and 30 of the treatment. 1 g of soil was mixed with 9 ml distilled water pH 7. The mixture was briefly vortexed and filtered. Filtered water is measured by a calibrated pH meter.

2.4. **Data Analysis**

TPH dan pH data was analyzed with two tailed ANOVA and DNMRT for advanced analysis.

3. **Result and Discussion**

3.1. **Result**

3.1.1. **TPH Degradation.** TPH degradation was measured on days 15 and 30 of the treatment. Data on degradation of TPH on day 15 is presented in Table 1.

| Group of Bacteria | Compost Percentage |
|-------------------|--------------------|
|                   | 0                  | 3       | 6       | 9       |
| *Alcaligenes* sp  | -                  | 25.26   | 26.38   | 30.54   |
| *Bacillus* sp     | -                  | 26.37   | 27.36   | 29.88   |
| Consorssium       | -                  | 26.75   | 30.47   | 27.95   |
| Negative control  | 18.28              | -       | -       | -       |

Based on Table 1, it can be seen that the bacteria that have the best degradation ability are the consortium in the compost percentage of 6% (27.62%). The lowest degradation occurs in the activity of *Alcaligenes* sp. and compost percentage 3% (23.48%). The degradation of TPH in the negative control soil was 16.65%.
Table 2. The percentage of Total Petroleum Hydrocarbon at day 30 after treatment

| Group of Bacteria | Compost Percentage |
|-------------------|--------------------|
|                   | 0                  | 3 | 6 | 9 |
| Alcaligenes sp    | -                  | 25.26<sup>a</sup> | 26.38<sup>b</sup> | 30.54<sup>b</sup> |
| Bacillus sp       | -                  | 26.37<sup>a</sup> | 27.36<sup>b</sup> | 29.88<sup>b</sup> |
| Consorsium        | -                  | 26.75<sup>a</sup> | 30.47<sup>b</sup> | 27.95<sup>b</sup> |
| Negative control  | 18.28              | - | - | - |

Based on Table 2, it can be seen that bacteria which have the best degradation ability are Alcaligenes sp. and 9% compost percentage with degradation results of 30.54%. The lowest degradation is Alcaligenes sp. and 3% compost percentage with degradation results of 25.26%. The degradation value of TPH in the negative control soil was 18.28%.

3.1.2. pH Value. Pengukuran pH dilakukan pada hari ke-15 dan 30. Hasil pengukuran pH pada hari ke-15 disajikan pada Tabel 3.

Table 3. pH Value on day 15 after treatment

| Treatment Groups                     | Mean of pH value on day 15 |
|--------------------------------------|-----------------------------|
| Alcaligenes sp. and 3% of compost    | 6.38                        |
| Alcaligenes sp. and 6% of compost    | 6.39                        |
| Alcaligenes sp. and 9% of compost    | 6.58                        |
| Bacillus sp. and 3% of compost       | 6.48                        |
| Bacillus sp. and 6% of compost       | 6.47                        |
| Bacillus sp. and 9% of compost       | 6.46                        |
| Consorsium and 3% of compost         | 6.36                        |
| Consorsium and 6% of compost         | 6.50                        |
| Consorsium and 9% of compost         | 6.62                        |
| Positive control                     | 5.79                        |
| Negative control                     | 7.66                        |

The highest pH value on day 15 was obtained from the treatment of the Alcaligenes sp and Bacillus sp. and compost 9% (6.62). The lowest pH value was obtained in the treatment of the Alcaligenes sp and Bacillus sp. and 3% compost (pH 6.36).

Table 4. pH Value on day 30 after treatment

| Group of Treatment               | Mean of pH value on day 30 |
|----------------------------------|-----------------------------|
| Alcaligenes sp. and 3% compost   | 6.05                        |
| Alcaligenes sp. and 6% compost   | 6.16                        |
| Alcaligenes sp. and 9% compost   | 6.02                        |
| Bacillus sp. and 3% compost      | 6.06                        |
| Bacillus sp. and 6% compost      | 6.11                        |
| Bacillus sp. and 9% compost      | 6.03                        |
The highest pH value showed by the Alcaligenes sp. and compost 6% group (6.16). The lowest pH value was obtained in the consortium group of Alcaligenes sp and Bacillus sp. and compost percentage of 3% (5.79).

3.2. Discussion

After 15 days of treatments, it was known that the consortium of Alcaligenes sp and Bacillus sp. and 6% compost is the best treatment for degrading TPH (27.62%). This degradation is better than negative controls which were not given bacteria and compost was only 16.65%. Degradation in treated soil is higher than control soil because of the ability of hydrocarbonoclastic bacteria to consume carbon in the soil derived from used lubricatingoil and break it down into harmless substances[c][11]. Hydrocarbonoclastic bacteria have the ability to attach to hydrocarbons, and produce emulsifiers, and have a mechanism to liberate (desorption) from hydrocarbons. In addition, the bacteria synthesizes hydrocarbon oxidizing enzymes and their plasmids are mutated. Chromosome and plasmid mutations affect the process of breaking down hydrocarbon molecules[c][12].

Another factor that influenced the rate of degradation is the household waste compost biostimulants. Biostimulant contains nitrogen and phosphorus macronutrients that are needed by microorganisms. About 3% of the dry weight of microbial cells is nitrogen and phosphorus[c][13]. Compost added to the treatment is used as source of nutrients of microbes as well as to increase porosity so that bacteria can get oxygen easier to decompose the waste[c][14]. The availability of carbon, nitrogen and phosphorus in the treated soil causes the increasing number of bacteria and their ability to degrade hydrocarbons compared to the negative control soil.

On day 30 it was known that the best degradation occurred in the treatment with Alcaligenes sp. and 9% compost 30.54%. Data show that Alcaligenes sp are more able to survive and actively carry out the degradation process of hydrocarbons contained in used oil compared to Bacillus sp. Alcaligenes sp looks easier to adapt to new environmental conditions that are more acidic due to degradation than Bacillus sp. There are no significant different of household waste compost concentration between 6% and 9%. However, they were significant different with 3% concentration[c][12]. TPH reduction efficiency in 21 days is 39%.

In this study pH value is influenced by bacteria activities. pH values decreased along the treatment. The decreasing of pH value comparable with day of treatment. Interestingly, it was not comparable to the concentration of compost in the bacteria mixture. 3% of compost caused the lowest pH value (6.36) whereas 6% of compost just can decrease pH value until 6.62 in day 15 of treatment. Bacteria activities seems caused decrease the pH value than compost concentration. The same features observed in day 30 of treatment (Table 4). However, bioremediation process takes place well at pH 6.5 – 8[c][15]. A decrease in pH indicates good bacterial activity[c][16]. This condition is due to the accumulation of organic acids (especially gluconate acid, pyruvate, citrate, and succinate) formed from organic metabolism[c][17].

In bioremediation process, bacteria produce hydrocarbon-digestive enzymes. The side products of the activity of these enzymes are organic acids and ammonia. Organic compounds cause a decrease in pH in the soil, while ammonia can cause an increase in pH in the soil. When the soil is well bioremediated, the tendency of pH on the soil is neutral[c][18]. The extremely pH values caused negative impact to the speed of hydrocarbon degradation. The acidity of pH can be overcome by adding calx or sulfur to the treatment. Calx is needed to raise the pH, whereas sulfur is needed to reduce pH.
However, the US Department of Environmental Conservation states that in several studies reported the use of calx can harm microbes, and magnesium can be used instead[14].

4. Conclusion
It is concluded that compost concentration influence the rate of TPH degradation. However, there is no significant different in group of bacteria for the same process. There is no interaction between group of bacteria and compost for TPH degradation. Bacteria activities decrease pH value, but it is still in the normal range.

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