Removal of crude oil by *Thiobacillus* sp. and *Clostridium* sp. at various temperatures and concentration of pollutant in liquid media

I P P Primadani, R Ratnaningsih and A Rinanti*

Environmental Engineering Department, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia

*astririnanti@trisakti.ac.id

Abstract. The activities of the petroleum mining industry in Indonesia have caused many problems by producing hazardous waste pollution that would produce dangers for plants, animals or human lives. One technique to recover environmental situation is through land farming technique. The purpose of this study is to overcome crude oil pollution in the environment by using biotechnology method through the process of enzymatic bioremediation. The parameters discussed to determine the optimal environmental conditions that provide the highest efficiency for Total Petroleum Hydrocarbon (TPH) degradation processes such as temperature with a variation of 25 – 45°C then the concentration of pollutants with a variation of 5% – 20%, and the contact time carried out in solid media with variations amounting to 3 – 21 days. After conducting research, research that produces the bacterium *Thiobacillus* sp. and *Clostridium* sp. under optimal conditions at 35°C with a pollutant concentration of 10% and a contact time of 14 days. Thus, it can be concluded that the bacterium *Thiobacillus* sp. and *Clostridium* sp. can set aside hydrocarbon compounds with a removal efficiency of 65%. So, this research needs to be done to study other physical parameters.

1. Introduction

Crude oil is an irreplaceable energy source so far. However, crude oil production and utilization can produce waste that would pollute the environment [1]. Oil-polluted land often found in oil refinery and collection locations. This pollution can occur due to oil spills during crude oil mining and transportation activities. Soil pollution by crude oil have caused environmental problem because crude oil contains various toxic hydrocarbon compounds that would endanger human and the other organisms [2].

Observation of crude oil pollution in the soil can be done through complete hydrocarbon component, known as Total Petroleum Hydrocarbon (TPH). TPH is a crude oil hydrocarbon pollutant concentration measurement in a soil sample measured with mg units of hydrocarbon for each kilograms of soil [3].

The effect of oil spillage can affect physical and mental health in affected population, which is usually seen by clinical and health disruption symptoms connected with life quality. Population or individual with a high exposure or that lives near oil spillage areas have shown low mental health level if compared with population living with low exposure of oil spillage [4].

One effort to overcome oil-spilled environment is by conducting bioremediation technique, which is an effort to recover polluted environment by exploiting organism abilities to degrade organic compounds...
Bioremediation often used because of its relatively low cost, effectiveness, efficiency, and environmental friendly nature [3,6-8].

There are two types of bioremediation technology, which are ex-situ and in-situ technologies. Ex-situ is a process by physically moving contaminated materials in a location chosen to conduct further actions. Bioreactor utilization, land farming, compost adding and any other solid treatment phase are examples of ex-situ technology, meanwhile in-situ technology is a direct treatment towards contaminant materials in polluted areas [9].

Pollution can be dealt by using several methods. Environmental pollution countermeasure method can be conducted through three types, which are physics, chemical, and biological manners. One environmental friendly technology with several advantages, for example low cost, is bioremediation if compared with chemical and physics remediation. So far, there are several definitions regarding bioremediation, which special emphasize on degradation process. Physics and chemical processes possess effectiveness from time perspective. However, these processes often cause contaminant movement.

Naturally, soil microbe is able to degrade polluting hydrocarbon compounds [10]. Short-chained hydrocarbon compound is the fastest compound degraded by soil microbe. Meanwhile a more complex hydrocarbon compound such as aromatic compound requires more time to degrade [11,12]. Factors that influence bioremediation are: microbe, nutrition, and environment. Microbe has the ability to degrade, transform and absorb pollutant compound. Microbe utilized usually comes from fungi, bacteria, or micro algae classes. The kinds of nutrition needed by microbe are carbon (C), nitrogen (N), phosphor (P) [13]. Meanwhile the most effective environmental factors are oxygen, temperature, DO, and pH. Usually, to increase aeration and to maintain soil moisture, compost or any other organic compound is added so that degradation process can be effectively conducted [14,15].

Land farming technique is a well-known low cost bioremediation technique and easy to utilize. To accelerate its bioremediation process, besides using selected carbonoclastic bacteria, the required nutrition addition is also conducted in bioremediation process [3].

Numerous researches has been conducted to prove that crude oil polluted soil can be degraded by conducting land farming method. There are also other researches that utilize land farming on crude oil polluted soil. The soil sample is mixed with clay or compost with bacteria addition (bio-augmentation) or without bacteria addition (bio-stimulation) [3]. Bacteria that utilized are consortium bacteria of horse and cow dungs. Observation result shows that bio-augmented mixed sample with compost (1:1) shows pH, water, and temperature levels that are suitable for bacteria growth (6.5, 25%, and 35°C) so it would decrease TPH value of 48%. This result of soil-compost mixture hydrocarbon analysis was obtained after 16 weeks of research [16].

In this research, land farming method is utilized, which is one of well-known low cost bioremediation techniques that is relatively easy to practice. Besides that, construction design of any other bioremediation method is not needed. This research is also important to degrade crude oil that contains hydrocarbon with *Thiobacillus* sp., and *Clostridium* sp. bacteria addition.

2. Research methodology

2.1. Stone Mineral Salt solution (SMSs) media preparation
SMSs media is a growth media used in this research. One liter of SMSs media contains 0.5 grams of CaCO₃; 2.5 grams of NH₄NO₃; 1 gram of Na₂HPO₄.7H₂O; 0.5 grams of KH₂PO₄; 0.5 grams of MgSO₄.7H₂O; and 0.2 grams of MnCl₂.7H₂O.

2.2. Crude oil preparation
Crude oil is an aliphatic and aromatic compound that is also classified as sulfur and nitrogen compound. Pollutant characteristic was conducted to acknowledge crude oil components and to acknowledge these components concentrations. Crude oil utilized as pollutant was obtained from Jatibarang refinery in Cirebon.
2.3. *Thiobacillus* sp. and *Clostridium* sp. bacteria cultivation

*Thiobacillus* sp. and *Clostridium* sp. was obtained from Universitas Trisakti, Environmental Microbiology Laboratory in Jakarta. *Thiobacillus* sp. and *Clostridium* sp. bacteria was grown in SMSs growth media with pH level of 7, in temperature of 30°C, and inserted inside shaker incubator with rotation speed of 200 rpm, until it reaches exponential phase in between 7014 days.

2.4. Temperature variation on crude oil removal

Research on temperature variations of 25°C, 30°C, 35°C, and 45°C, on pH level of 7, with 7 days of contact days was conducted by acknowledge optimum temperature that would produce highest hydrocarbon removal. As much as 10% of mixed culture bacteria were added into an Erlenmeyer flask that contains SMSs media, which further incubated in various temperatures inside a shaker incubator.

2.5. Bacteria concentration variation in crude oil removal

*Thiobacillus* sp. and *Clostridium* sp. bacteria was inserted into Erlenmeyer flask that contains SMSs liquid media. With previously obtained optimum temperature, the solution was observed for 7 days with crude oil concentration variations of 5%, 10%, 15%, and 20% as pollutant.

2.6. Total petroleum hydrocarbon content value

To obtain TPH content value, the following formulation was used:

\[
TPH \text{ Weight} = \frac{B - A}{\text{Sample weight}} \times 100\%
\]

B = Weight of oven dry boiling flask before being extracted, gr.
A = Weight of oven dry boiling flask after being extracted, gr.

3. Results and discussion

3.1. Temperature optimization in crude oil removal

Temperature can highly influence bacteria life during hydrocarbon biodegradation process. Crude oil removal research result on incubation temperature of 25°C, 30°C, 35°C, and 45°C can be seen in Figure 1.

Based on the result shown in Figure 1, each mixed culture bacteria show abilities to degrade hydrocarbon above 30% in every temperature variation. Hydrocarbon removal continues to increase along with temperature escalation from 25°C until 35°C. However, in 45°C, crude oil removal decreases into 40.6%. Therefore, we can say that temperature of 35°C is the most suitable temperature for *Thiobacillus* sp. and *Clostridium* sp. to remove crude oil pollutant with removal efficiency of 56%. The next of the research was conducted on temperature of 35°C. It seems that between temperatures of 25°C to 35°C, hydrocarbon metabolism reaches maximum level, meanwhile above temperature of 30°C enzyme activity will decrease and hydrocarbon toxicity on cell membrane will affect enzyme activities.
Based on the previously conducted research on *Pseudomonas putida* bacteria, optimum condition was occurred on between temperatures of 28 to 30°C with removal efficiency of 45.4% [3]. This research of *Thiobacillus* sp. and *Clostridium* sp. bacteria usage seems to produce a better result to remove crude oil than the ones resulted by *Pseudomonas putida* bacteria.

### 3.2. Bacteria concentration optimization in crude oil removal

After obtaining optimal temperature of 35°C, bacteria concentration optimization was conducted to acknowledge highest crude oil removal.

![Figure 2](attachment:figure2.png)

**Figure 2.** Hydrocarbon removal efficiency on various bacteria concentration.

This research was conducted in a controlled environment with temperature of 35°C, pH level of 7, with bacteria addition levels of 5%, 15%, 10%, and 20%. Crude oil removal efficiency on various bacteria concentration can be seen in Figure 2. Based on Figure 2, we can see that mixed culture bacteria addition of 5% is able to remove 52% of crude oil. After that, if 10%, 15%, and 20% of mixed culture bacteria is added, crude oil removals efficiency reach 56%, 61%, and 65%. The higher bacteria concentration addition was conducted; the higher crude oil removal efficiency was resulted (mentioned in Total
Petroleum Hydrocarbon). Thiobacillus sp. and Clostridium sp. bacteria enzymatic activities are able to remove hydrocarbon compound in the environment so that both bacteria have the potential to be involved in a further research regarding crude oil polluted soil. From test result, it was acknowledged that MM2 media can be utilized as growth media to increase three carbonoclastic numbers of R122-2.3, R122-5 and R122-BN5. R122-BN5 carbonoclastic bacteria performance test through land farming technique can lower TPH level from 6.8% to less than 1% (0.82%) in 32 days [16].

4. Conclusion
Based on the result of the research, we can conclude that Thiobacillus sp. and Clostridium sp. bacteria are able to decently grow in liquid media that contains 10% of crude oil pollutant in temperature of 35°C. Thus, removal efficiency can reach 65% if 20% of bacteria was added on a pH level of 7.

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