

THE HYDROCARBON LOSSES ON OIL CONTAMINATED SOIL BY LANDFARMING BIOREMEDIATION: A LABORATORY STUDY

by
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ABSTRACT

Bioremediation, as a relatively new technology, is expected to be able to remediate the hydrocarbon contaminated soils which are often found in almost all areas of the petroleum industries' activities.

The bioremediation experiment had been conducted in a laboratory scale. The oil uncontaminated soil and paraffinic oil samples are taken from an oil field. The soil is mixed with more than 5% hydrocarbon concentration in order to obtain an artificial oil contaminated soil.

Indigenous microorganisms can grow on this oil contaminated soil. During incubation times, microbial cells grow rapidly, then followed by drastic decline of hydrocarbon contents on soils. It indicates that the indigenous microorganisms are capable to degrade petroleum hydrocarbon. On the addition of the organic fertilizer treatment, the highest degradation of hydrocarbon occurs. During four months of treatment, the hydrocarbon content on soil decreases more than 54%.

I. INTRODUCTION

Entering the 21st the century, the petroleum contamination is still one of the major problems in the industrialized countries. It is liable to contaminate various sites, such as soils, surface and ground waters, sediments, and air. Under some conditions, the oil contamination can be hazardous to work around. This is due to more its volatile products which constitute a fire or even an explosive hazard. The petroleum compounds which are often chronically toxic to humans, especially the aromatic compounds, are routinely found in the most common contaminants. A remediation treatment is an attempt to solve the problem of petroleum contaminated sites.

A bioremediation is a treatment, whereby the microorganisms or microbial processes can be used to detoxify and to degrade the environmental contaminants. The implementation of bioremediation depends ultimately on the activities of microorganisms. The degradation of the organic pollutants in natural environments is remediated primarily by two groups of microorganisms: bacteria and fungi. In the majority of bioremediation systems, bacteria are currently commonly used. Bacteria represent a widely diverse group of prokaryotic organisms with ubiquitous distribution throughout the biosphere. They are found in all environments containing living organisms. This does not imply, however, that all strains of bacteria are found in all environments.

Bacteria are very small organisms, having size is typically between 1 and 10 μm in size, and morphologically simple. Biochemically, however, bacteria show an amazing

metabolic versatility. They have several characteristics which make them a successful group of microorganisms. These characteristics include rapid growth and metabolisms, genetic plasticity, and the ability to adjust easily to a variety of environments. Their characteristics, as mentioned above, are pertinent factors which make these microorganisms so useful in bioremediation.

Bioremediation as a new technology can remediate the oil contaminated soils. Many petroleum products are often readily consumed by bacteria in the soil resulting the petroleum contaminated sites, in effect, remediate themselves. This type of petroleum contaminated sites is generally easier and cheaper to be bioremediated compared to the sites contaminated with hazardous wastes. Although bioremediation is considered as relatively a new technology, but the microorganisms activities had been used routinely for the treatments and transformation of waste products for at least 100 years (Eckerfelder, 1989).

The natural or intrinsic bioremediation is one of such treatments which, in principle, does not require high additional cost. In this treatment, the indigenous microorganisms in the subsurface are recognized of being capable of hydrocarbon degradation, when the critical environmental factors are supporting and not being limited, especially nitrogen, phosphorous, temperature, moisture, acidity, salinity and electron acceptor (Baker and Herson, 1994).

To some extent the bioremediation is a good alternative for the remediation of petroleum contaminated soils. During the remediation process, the soil moisture content should be properly monitored to ensure that the minimum water

content is not less than 14% (Wittenbach, 1995).

The selected natural bacteria consortia prove that they have a high capacity for hydrocarbon degradation. The hydrocarbon wastes from soils, slops, and water/waste oil mixtures have been degraded up to 72,75% by the bacterial consortia isolated from soil and slop samples collected from several sites in oil fields. And this can be increased up to 82,94% in case the bacterial consortia isolated from water/waste oil mixture samples which are collected from the same oil field sites. Paraffin deposits can also be degraded by such bacterial consortia up to 36,63% (Lazar et al, 1995).

Bioremediation process can be applied either *in situ* on the contaminated fields or *ex situ* in a bioreactor. In general, the former is more effective than the latter. (Udiharto, 1998).

A pilot scale bioremediation study has been conducted in LEMIGAS, as an effort to remediate oily waste contaminated soil by microorganisms. In the beginning of treatment, the soil is enriched with nitrogen and phosphorus fertilizer. Furthermore, the soil is added with crude oil as contaminant with hydrocarbon content more than 2%. During seven weeks, the decrease of hydrocarbons content is 41%. The degradation of hydrocarbon content is supported by the indigenous bacteria (Udiharto, 1998).

II. MICROORAGNISMS FOR BIOREMEDIATION

Bioremediation activities depend on choosing the proper microorganisms, the suitable place, and the supported environmental conditions for degradation. The selected microorganisms are those bacteria or fungi which have the physiological and metabolic capabilities to degrade the petroleum hydrocarbons. In many instances, these organisms which are already present at the sites, are called indigenous microorganisms. In order, for the microorganisms, to degrade the contaminants, they must be in close proximity to the contaminants. Certain exogenous microorganisms are added, if necessary.

The number of bacteria can be increased using a process known as a binary fission. In this process, one bacterial cell will divide into two cells. The amount of time needed for a bacterial cell to divide, where the total population of bacteria will be doubled, is called the generation time. The bacteria have short generation time, which is typically measured in terms of minutes, hours, days, weeks, or months. Since the population number of bacteria doubles in every generation development, the bacterial numbers can increase extraordinarily rapid rates. Mathematically, the growth of bacterial population under nonlimiting conditions, can be expressed in terms of an exponential function.

A growing bacterial population in this manner will rapidly alter its environment. It may deplete the necessary nutrients or produce inhibitory metabolic through its pro-

ducts, ultimately causing the population growth to stop. Results of the bacterial population during incubation are plotted and known as growth curve.

Macronutrients are elements the microbial needed in relatively large quantities to support their metabolism. One of the macronutrients is phosphorous (P), which is required for normal metabolisms and population growth. Phosphorous is essential to microbial cells for the synthesis of ATP, nucleic acids, and, cell membranes. This compound is frequently limited to bacterial/microbial growth in soil environments. On nutrient medium the optimum carbon to phosphorous ratio is required for the microbial activities to degradate the contaminants (Atlas and Bartha, 1972).

The other macronutrient is nitrogen needed for the synthesis of proteins and nucleic acids. Protein serves both structural and enzymatic functions in the microbial cell. Nucleic acids (DNA and RNA) function as genetic formation of the cells. The energy from the oxidation-reduction reactions of catabolism is used by the cell for biosynthetic reactions. In these reactions, the major type of molecules found in the cells (proteins, lipids, carbohydrates, and nucleic acid) are synthesized. Besides the carbon, the elements nitrogen and phosphorous, referred to as macronutrients, are needed in large amount for the synthesis on some of these molecules (Baker and Herson, 1994).

Microorganisms involve in the degradation of xenobiotics in natural systems primarily depend on the fixed forms of nitrogen (nitrite, nitrate, ammonia, and organic nitrogen) to meet their nitrogen requirements. Nitrogen is one of the macronutrients the microbes needs to support their metabolism. The forms of nitrogen are frequently limiting for microbial populations in soils (Atlas, 1991).

Microbial growth can be limited by the abiotic environment. One of the abiotic factors is pH of environment. The microorganisms are generally limited to pH values. The optimum pH ranges from 6.0 to 8.0 with best result as close to 7.0 as possible. Dibble and Bartha (1979) had reported that a soil pH of 7.8 as an optimum value for the microbial degradation of petroleum hydrocarbons in soil. Due to production of intermediate organic acids and CO₂, the pH will decrease during bioremediation. If the pH slips much below 6.0, the bacterial activity will decelerate dramatically. In this case, the soil liming can be used to increase the soil pH.

III. METHODOLOGY

These experiments were conducted to simulate landfarming practices in the laboratory to determine the hydrocarbon degradation on oil contaminated soil. The oil contaminated soil is artificially made from a mixture of topsoil and crude oil. The uncontaminated soil and crude

oil were taken from an oil field.

Indigenous microbes will be used to degrade the petroleum hydrocarbons. In order to do that, these microbes are stimulated by adding nitrogen, phosphorous, organic fertilizer and exogenous microbes to the oil contaminated soil. The organic fertilizer and exogenous microbes used in these experiments are developed by the LEMIGAS Biotechnology Laboratory.

The bioremediation process on oil contaminated soil can be divided into four treatments. First, the oil contaminated soil is added with nitrogen and phosphorous as basic conditions. Then, the exogenous microbes are added to this basic condition of soil. After that, the organic fertilizer is also added to the basic conditions of soil. And finally, the mixture of organic fertilizer and exogenous microbes are added to the basic conditions of soil.

IV. DISCUSSION OF THE RESULTS

Besides the microbial capability, the type of petroleum and suitable environmental conditions is the important factor in biodegrading the hydrocarbon compounds. For the microbes, special macronutrients requirements include carbon, nitrogen, and phosphorous. Carbon is given by the hydrocarbons themselves. Nitrogen and phosphorous are given by chemical compounds, fertilizer or manure. Other environment parameters that may affect the conditions are acidity (pH), temperature, and soil moisture. The optimum acidity and appropriate temperature should be maintained and controlled. During the incubation, temperature is kept at ca. $30 \pm 2^\circ\text{C}$.

A. Characteristics of Crude Oil

The soil is mixed with crude oil in order to obtain a contaminated soil condition. The characteristics of crude oil used are shown in Table 1.

Table 1
Analysis results on the crude oil properties

No.	Parameter	Unit	Value
1	API gravity at 60°F		33.2
2	Specific gravity (60/60°F)		0.8589
3	Pour point	°F	90
4	Sulphur content	wt%	0.113
5	Asphaltenes content	wt%	0.361
6	Wax content	wt%	15.50

This oil does not belong to heavy crude, because the specific gravity is 0.8589 (at 60/60°F), API gravity (60°F) equals to 33.2, while wax and asphaltenes contents are 15.50 and 0.361 wt%, respectively. Based on the US Bureau of Mines Classification, this crude oil belongs to the intermediate paraffinic class.

B. Microbial Population

The study of bioremediation simulation was carried out in four kinds of medium, denoted as S₁, S₂, S₃, and S₄. The microorganisms used for hydrocarbons degradation on treatment S₁ and S₃ were indigenous microbes, while the exogenous microorganisms were added in other medium S₂ and S₄. The results of microbial population measurements on oil contaminated soil are illustrated in Figure 1.

In these four treatments, the microorganisms were found to be capable to grow on oil contaminated soil. During two months of incubation, the microbial population grew rapidly. The microbial population enters the exponential phase of growth. During this phase, the available nutrients and environment conditions are sufficient for the microbes to grow. Between two to three months incubation, the growth rate of the population is equal to the death rate of the population. Hence, there is no net increase in the number of cells. Then, the microbial population enters the stationary phase. Finally, the available nutrient is depleted and environment conditions are not sufficient for the microbes to grow anymore. Whenever the death outstrips growth, the decline of population numbers and their activity occur.

C. Nitrogen Content

The soil for simulating the bioremediation study is the top soil. In general, the nitrogen content on this soil is low. In order to obtain sufficient nitrogen, urea and diammonium phosphate are added to the soil at the beginning of the

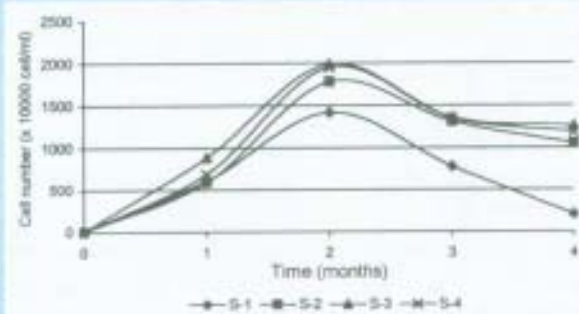


Figure 1
The growth curve of microbial on oil contaminated soil

experiments. Nitrogen contents are the total nitrogen on soil. It measured from nitrite, nitrate and ammonia content on soil. During incubation, the nitrogen content on soils is maintained at optimal condition. The nitrogen is frequency limiting for microbial population in soils, ground water, and surface water. (Atlas, 1991). The nitrogen on soils utilized by the microbes for cell growth. Hence, nitrogen content on soils is drastically decrease during two months. And this will be close to the minimum content, after three months incubation. Where the N content is minimum. On this point, the N content is near 100 ppm, and as the nitrogen sources are added to the soils, hence, the total nitrogen on the fourth month soil sampling increase.

The curves of nitrogen contents on soil are illustrated in Figure 2. The total nitrogen on soils decreases during the incubation. The decreasing nitrogen contents on soil are related to indigenous microbe activity, where some nitrogen is consumed by soil microorganisms. The nitrogen is used for the synthesis of protein and nucleic acids. The growth and activity of microbial cells always need these compounds. The decreasing microbial activity is related to the nitrogen consumed. This phenomenon is shown on oil uncontaminated soil. Under this condition, the carbon sources are limited. In this case, the microbial activity goes down, and the nitrogen consumption is low (20.8% after one month incubation). On oil contaminated soil, the cells of microbe grow well, and the nitrogen consumption is high (more than 31% after one month incubation)

D. Phosphorous Content

During the experiment of simulating the bioremediation, phosphorous content on soil is maintained more than 50 ppm. In order to have a sufficient phosphorous consumption, phosphorous compound is added to the soil at initial condition and after three month incubation. On oil contaminated soil, the phosphorous content decreases during three month incubation.

Phosphorous compounds as macronutrient are needed in large amount for the synthesis of these molecules. The microorganisms consume phosphorous compounds for synthesis ATP nucleic acid and cell membranes (Atlas R.M. and R. Bartha, 1972). The decreasing of phosphorous content and microbial growth on soil indicate that the phosphorous compounds are already consumed by microorganisms.

In initial samples, phosphorous contents on oil contaminated soil are more than 50 ppm. During the incubation, the phosphorous compounds decrease. The curve of phosphorous content declines during three month incubation. Increasing phosphorous content on soil at the fourth month caused by phosphorous compound addition at the third month, after soil sampling.

E. pH

For the landfarming simulation in the laboratory, the test is conducted on oil contaminated soil. The soil used is the top soil from an oil field area. The soil will be treated with limestone to adjust the pH. During bioremediation process, the soil pH of sample is measured. Initial soil pH is between 7.18 and 7.79. This pH is a good condition for indigenous microbial activity. During four month incubation, limestone addition to the soil pans is not necessary anymore, because limestone is needed only when the soil pH is less than 6.

After four months incubation, the results of soil pH are plotted as shown in Figure 4. This figure shows that the pH on oil contaminated soil, have a decrease trend during four month incubation. It means that during the bioremediation process the acid compounds are produced.

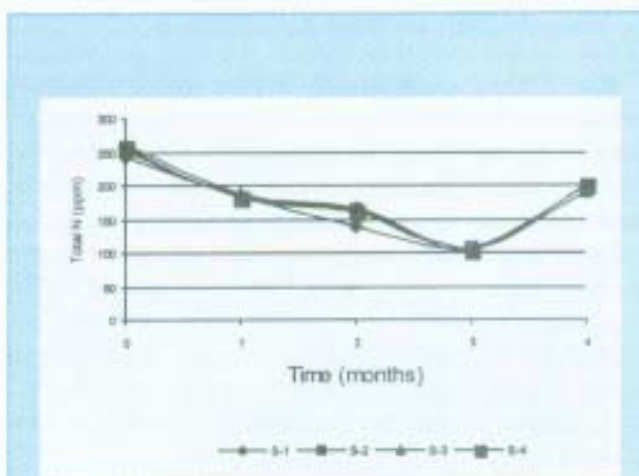


Figure 2
The curve of the nitrogen content on soil

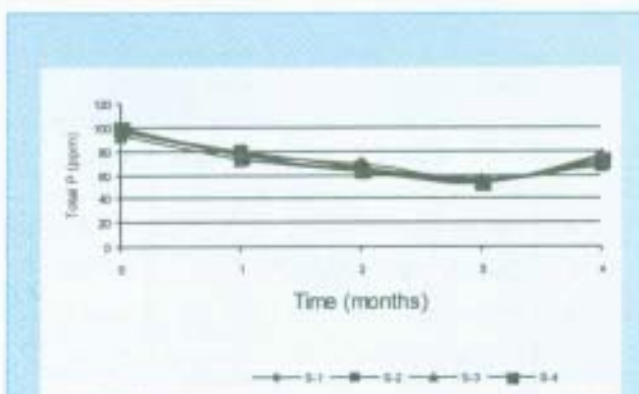


Figure 3
The curve of phosphorous content on soil

F. Hydrocarbon Content

Petroleum and petroleum products are complex mixtures of organic compounds. The majority compounds found in petroleum are hydrocarbons. These compounds are a class of organic compounds composed of carbon and hydrogen. There are three major categories of hydrocarbons: aliphatic hydrocarbons, naphthenic hydrocarbons, and aromatic hydrocarbons. These hydrocarbon compounds are degradable.

The microorganisms have the capability to degrade hydrocarbon compounds. The biodegradability of every petroleum hydrocarbon compounds is not the same. Aliphatic hydrocarbons are generally easier to degrade than aromatic compounds. Straight chain aliphatic hydrocarbons are easier to degrade than branched chain hydrocarbons. Saturated hydrocarbon are also easily degraded as well compared to unsaturated hydrocarbons. Long chain aliphatic hydrocarbons are easier degraded than short chain hydrocarbons. The optimum chain length for biodegradation is between 10 and 20 carbons.

Bioremediation of oil contaminated soil is a process of aerobic microbial oxidation of hydrocarbon contaminants to CO_2 and H_2O using naturally occurring soil bacteria. The overall process may be enhanced or accelerated by providing nutrients to bacteria, laboratory cultured bacteria, or both. Most microbial activity is carried out in an aqueous layer that exists on the surface of soil particles. This is the reason that the soil moisture content is necessary to be controlled.

The bioremediation on oil contaminated soil is done in the laboratory. The contaminant is one type of crude oil. During incubation, petroleum hydrocarbon compounds will be degraded by the native soil microbial. Since the major degradation pathway in aerobic, the microbial activity proceeds more rapidly in aerated soil than in poorly aerated soil. The delivery of oxygen to the site of microbial activity is facilitated by a porous soil. The porous soil is maintained by stirring the soil twice a week.

The basic process of bioremediation is the contaminants degradation on site by microbial activity. On petroleum contaminated soils, the bioremediation is an oxidative process degradation of hydrocarbon contaminants to CO_2 and H_2O using soil bacteria. The total hydrocarbon contents on soil indicate the contaminants degradation. Under landfarming remediation, the decrease in hydrocarbon concentration is due to volatilization of petroleum hydrocarbons and biodegradation by microbes. In general, the biodegradation of petroleum hydrocarbon is closely related to microbial population. The increase in microbial population is followed by a decrease on hydrocarbon contents.

During four month incubation, the total hydrocarbon contents decrease on all oil contaminated soil. The hydrocarbon contents drastically decrease at the first month incubation. On further incubation, the decrease in hydrocarbon contents on oil contaminated soil is very low. The curves of total hydrocarbon contents are illustrated in Figure 5. While the incubation, the hydrocarbons losses are

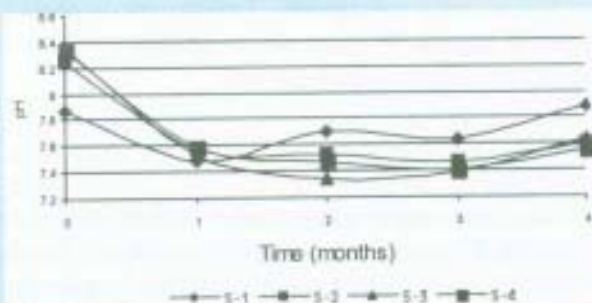


Figure 4
The curve of soil pH

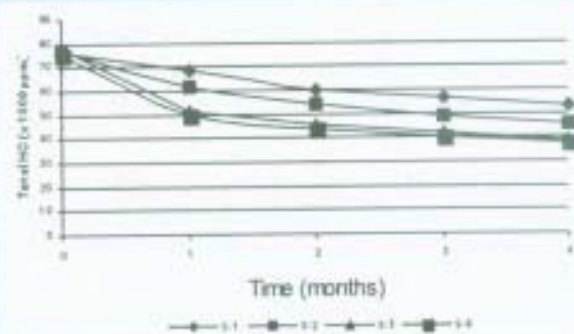


Figure 5
The curve of hydrocarbon content on oil contaminated soil

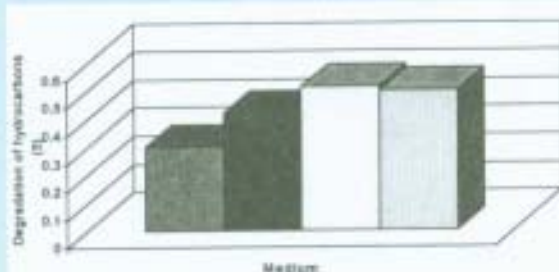


Figure 6
Percentage of hydrocarbon losses on oil contaminated soil

used for microbial growth.

The value of hydrocarbon degradation depends on the indigenous microbial activity (see Figure 6). On normal condition S1, the value of hydrocarbons degradation is 30%, after four month incubation. After the addition of exogenous microbe, the degradation of hydrocarbons increases of about 41%. If an organic supplement is added, the degradation of hydrocarbons can increase up to 54%. This hydrocarbon degradation is almost the same, if the mixture of organic fertilizer and exogenous microbes are added to the basic conditions of soil.

V. CONCLUSION

The indigenous microorganisms of soil can grow on soil contaminated by more than 5% parafinic crude oil. It means that the native microbes can be adapted to petroleum contaminated sites. On these sites, the essential nutrients for microbial growth served in such concentrations that these will be sufficient to support microbial metabolism.

N and P contents, as the essential nutrients, on oil contaminated soil decrease during the incubation time. At the first month period, the maximum lowering N content is 44.3% and the maximum decreasing P content is equal to 23.2%. Decreasing N and P contents indicates that these compounds are consumed by the indigenous microorganisms.

The indigenous microorganisms are capable to degrade petroleum hydrocarbon. It can be seen from microbial cells from the rapid grow of microbial cell, which is followed by a drastic decline of hydrocarbon contents on soil. This phenomenon indicates that the petroleum hydrocarbon can be metabolized by the native soil microorganisms.

The petroleum hydrocarbons are degradable. The highest degradation of hydrocarbon occurs, if the organic fertilizer is added to the basic conditions soil. During four months

treatment, the decreasing of hydrocarbon contents on oil contaminated soil reaches more than 54%.

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