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The Effect of Nutrients, Compost, and Local Bacteria in Bioremediation of Petroleum Contaminated Soil

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Abstract

Petroleum hydrocarbon contamination in soil can be overcome by chemical, physical, and biological techniques. Biological approach to cope with petroleum hydrocarbon pollution is known as bioremediation. Bioremediation research using laboratory scale land-farming technique has been carried out. The initial activity of this bioremediation study was enrichment and bacterial multiplication of indigenous bacteria in polluted area as a mixed bacterial consortium. Treatments were performed on 3 level of total petroleum hydrocarbon (TPH) at about 5, 8, and 11%, in original contaminated soil as well as biostimulation by the addition of nutrients, compost, and bioaugmentation by enriched microbes within 19 weeks. From week 4 to 19, pH of soil in the reactors was between 5.0 to 8.5 with soil temperature between 30.0-40.5°C. The moisture content fluctuated from 4.86% to 47.21%. The population of microbes performed weekly from log 8.70 to 23.76 CFU/g-dried-soil. The production of CO_2 gas fluctuated, the highest CO_2 production value achieved from C1 treatment (nutrients and compost at TPH 5%) compared with other treatments. The TPH degradation depended on treatment of initial TPH, nutrient, and compost.The largest percentage of TPH degradation were obtained from the treatment of N3 (nutrients and TPH 11%) at 52.1%. Bioaugmentation using local microbes did not affect on TPH degradation. Therefore, bioaugmentation was not needed if compost has been added.

Keywords:bacteria, bioremediation, compost, contaminated soil, nutients, petroleum hydrocarbon

1. Introduction

Petroleum contamination can occur due to spills or discharges during oil mining activities, such as exploration, exploitation, processing, up to petroleum transport and its products. Environmental pollution caused by spills or petroleum discharges causes ecosystems in the environment to be disrupted. In addition, environmental pollution caused by spills or petroleum discharges also affect the soil condition. The toxic concentration of the polluting compounds will accumulate in the soil. Total petroleum hydrocarbons (TPH) is a term used to quantify the total amount of hydrocarbon compounds found in environmental media (Pinedo *et al.* 2012). Spills of hydrocarbons into the soil and water can toxify flora, fauna that live around contaminated land, and even affecting health of human body. Therefore, the action to overcome the contamination of oil contaminated land should be carried out so that pollution is not widespread.

Handling of petroleum hydrocarbon pollution can be conducted by chemical, physical, and biological techniques. Approach with biological techniques to cope with petroleum hydrocarbon pollution is known as bioremediation. This bioremediation technique is suitable because it is environmentally friendly, simple, and inexpensive. Bioremediation is carried out by using microbes. The most important microorganisms in degrading petroleum hydrocarbon are bacteria (Atlas & Cerniglia 1995; Hamme *et al.* 2003; Yani *et al.* 2003). Not all microbes can degrade petroleum, some types of microbes are found in polluted areas. These microbes can grow and adapt to contaminated environments. Microbes isolated from contaminated oils have a high ability to degrade hydrocarbons. Several types of microbes are only able to degrade hydrocarbons in certain molecular weight ranges. Therefore, the combination of microbial or mixed cultures (consortium) will further ensure the success of petroleum hydrocarbon degradation process. In a consortium, several types of microbes work simultaneously to degrade petroleum pollutants.

In the bioremediation process of oil-contaminated sites, pollutants such as oil or other hydrocarbons are broken down into harmless compounds, such as CO_2 and water. Bioremediation activities are carried out to reduce high toxic TPH, so that the concentrations of TPH reduce to non-toxic levels. Microbial biodegradation of pollutants has intensified in recent years as mankind strives to find sustainable ways to clean up contaminated sites (Diaz 2008).

There are two main approaches in stimulating microbial growth, i.e. by bioaugmentation and biostimulation. Bioaugmentation is a bioremediation process by adding known oil-degrading bacteria to supplement the existing microbial population to contaminated soil. Meanwhile, biostimulation is multiplying and accelerating the indigenous oil degraders by providing the necessary growth environments, for example by the addition of nutrients and oxygen.

In this study, the initial activity of bioremediation was enrichment and bacterial multiplication of indigenous bacteria from polluted area. After the initial process was carried out, then bioremediation activities in the laboratory were conducted using two approaches mentioned before. Activities in the laboratory was intended to identify the most appropriate treatment through the evaluation of various conditions and additives.

1.1 Materials and Methods

The research was conducted at SEAMEO BIOTROP Bogor, West Java, Indonesia and at Forest Biotechnology Laboratory - Biotechnology Resource Research Center - Bogor Agricultural Univeristy Bogor, West Java, Indonesia.

1.2 Microbial Starter Preparation

The materials required in this study were petroleum contaminated soil, crude oil, and a consortium of microbes, urea, SP36, glucose, NaOH, technical $CaCO_3$, sea water, marine agar, broth nutrients, hexane, Na_2SO_4, silica gel, and aquadest. The contaminated soil of oil waste was obtained from the soil around the community oil wells in Lubuk Bintialo Village, Batanghari Leko District, Musi Banyuasin Regency, South Sumatra Province, Indonesia. In addition, crude oil was obtained from one of the community oil wells in Macang Sakti Village. Meanwhile, the microbial consortium was derived from oil waste and compost from cattle waste taken from the Faculty of Animal Husbandry, Bogor Agricultural University. There were three sources of local microbes taken, namely in the form of soil, water, and mud as much as 200 g per source. Soil samples were taken 5-10 cm from the surface of contaminated land, water was taken from the river closest to the oil wells, and the mud was taken from the bottom of the river. Each sample was taken from two research sites and two replications so that there were 12 microbial sources. The interval of sampling in the field up to start the microbial development in the laboratory was less than 24 hours.

The bacteria were grown in enrichment cultures in 10 L plastic containers. The enrichment media compositions consisted of 10 g/L urea, 1 g/L TSP, 100 g/L sugar, and 100 g/L inoculum. Inoculum and nutrients were dissolved in aquadest resulting in 5 L volume cultures (Yani *et al.* 2003). Aeration was given through the air hose at each container every week. At week 3, 10% v/v crude oil was added. Starting at week 3, the bacterial population was measured by the Total Plate Count (TPC) method. The pH measurements were performed two times/day (morning and afternoon) and maintained in the pH range of 6-8 using 36% HCl and 1M NaOH. If there was a decrease in culture volume, but there was still crude oil, aquadest was added to reach the initial volume. After one culture cycle was achieved, the rejuvenation of culture was carried out.

Cultures were rejuvenated on the minimal medium by adding 10% culture/culture starter (500 mL from 5 L medium), 5% biodiesel, nutrients consisting of 2 g/L urea, 1 g/L TSP, and 20 g/L sugar. The medium used was sea water (Charlena 2010). pH culture was mantained at pH 6-8. This culture was carried out for one cycle only. In cycle two, media used was minimal media without sugar.

In the second and subsequent cycles, culture was cultured in minimal medium with 5% diesel, 10% culture starter, and seawater as much as 5 L. Culture was maintained with respect to pH and diesel oil demand. Cultures are preserved in such a way that they were replicated in accordance with bioremediation requirements.

1.3 Bioremediation Method at Laboratory Scale

The material used was soil from the contaminated site with three TPH levels.These three TPH quantities were obtained by measuring the soil TPH from contaminated sites first, then added with crude oil as needed to set 5%,

8%, and 11%. The study was conducted using a mixed bacterial consortium of local enrichment and multiplication bacteria consortium as described above. The petroleum contaminated soil (8 kg) was mixed with compost (2 kg) so it weighed 10 kg and added with Urea and SP-36 fertilizer as nutrients addition (Table 1). Compost used was compost that had the composition of cow manure and husk. Aeration or oxygen supply was given by using the aerator. TPH measurement was carried out every week in the first month and every two weeks in the following month.

Table 1. Bioremediation treatments of contaminated soil at different TPH concentration, stimulated by fertilizer addition of nutrients and compost, and bioaugmented with enriched microbes

Code	Fertilizers (Urea and SP-36)(g)	Compost (kg)	Microbes (L)	Initial TPH (%)
S1	-	-	-	5
S2	-	-	-	8
S3	-	-	-	11
N1	46 and 5	-	-	5
N2	93 and 9.5	-	-	8
N3	140 and 14	-	-	11
C1	46 and 5	2	-	5
C2	93 and 9.5	2	-	8
C3	140 and 14	2	-	11
M1	46 and 5	2	1	5
M2	93 and 9.5	2	1	8
M3	140 and 14	2	1	11

The research activity with 12 treatments used only original contaminated soil (S) as well as the addition of nutrients (N), compost (C), and enriched microbes (M) in 3 level of TPH (TPH-5, TPH-8, TPH-11). This procedure was done by preparing 36 reactors for 12 treatments and 3 replications (Figure 1).

The technique used in bioremediation research of this petroleum contaminated soil was a land-farming technique. According to Azubuike*et al.* (2016), land-farming techniques are often selected for hydrocarbon contaminated soil, because they are relatively inexpensive and less equipment requirement for operation. In land-farming techniques, the oxygen demand is met by periodic stirring or reversal. The reactor was stirred evenly by reversing the soil and by wiggling the reactor every two weeks. Land-farming system used was a closed system using sealed plastic containers. The treatment of the study was to obtain an efficient mixing medium with the mentioned compositions. The TPH was measured gravimetrically using Method 3540C (Soxhlet Extraction, part of Test Methods for Evaluating Solid Waste) by US EPA (1996).

The parameters observed were TPH (%), soil pH, soil moisture content (%), total plate count (log CFU/g), soil temperature (^oC), humidity (%), and CO_2 (mg/m^3). Room temperature and air humidity in the greenhouse were evaluated three times: morning, noon, and afternoon.

1.4 Sampling and Chemical Analysis

Sampling and chemical analysis carried out can be seen in Table 2.

Table 2. Sampling Time, Parameter, and Method

Sampling Time	Parameter	Method
Daily	Temperature	AP ASTM
	CO_2	APHA (1985)
Weekly	Soil pH	ASTM D4972-01
	TPC	Cappucino & Sherman (1987)
	TPH	US EPA (1996)
	Moisture Content	Gravimetric (APHA)

2. Results and Discussion

The soil is a layer of the earth's surface that physically functions as a place to grow and develop roots, support the growth of plants, and supply water and air. The soil chemically serves as a place and suppliers of nutrients such as organic and inorganic compounds and essential elements such as N, P, K, Ca, Fe and so forth. In

addition, soil serves as a habitat for biota or organisms that participate in the supply of nutrients and additives to plants.

Soil contamination occurs because the entry of foreign objects whether intentional or not that changes the original soil environment, so that the occurrence of soil quality was deteriorated. An example of soil contamination occurs due to contamination by crude oil. Petroleum contamination soil is considered a contaminant that can reduce the productivity of the soil. Pollution can cause imbalance and if it happens continuously it will endanger the living creatures, including plants, animals and humans, that exist in nature.

Furthermore, it is important to address that petroleum contamination on the ground poses a serious threat to living creatures and therefore requires effective treatments. One way of alternative technology which is environmentally friendly, simple, and inexpensive to overcome the problem of soil pollution caused by petroleum contamination is by bioremediation.

2.1 Bioremediation Process

According to Azubuike *et al.* (2016), bioremediation is defined as the process of degrading, detoxifying, mineralizing or transforming biologically harmfull organic materials into innocuous state. Meanwhile, according to Crawford (1996), bioremediation refers to the productive use of biodegradative processes to remove or detect pollutants (usually contaminants of soil, water and sediments) that pollute the environment and threaten public health.

When bioremediation occurs, enzymes produced by microorganisms modify toxic pollutants by altering the chemical structure of the pollutant. Enzymes accelerate the process by lowering the activation energy, which is the energy required to initiate a reaction. Stages of this process include biotransformation or biodetoxification of toxic compounds into compounds that are less toxic or not toxic. In many cases, biotransformation leads to biodegradation. Degradation of chemical compounds by microbes in the environment is a very important process to reduce the levels of harmful materials in the environment. The process takes place through a series of fairly complex chemical reactions and eventually becomes a harmless and non-toxic metabolite.

Degradation of hazardous chemicals may occur in the presence of suitable microbes and available for ideal conditions for microbial growth sites, such as temperature, pH, nutrients, and oxygen. In most petroleum contaminated soils, oxygen usually is the limiting requirement for hydrocarbon biodegradation, because the bioremediation for contaminated sites are mainly based on an aerobic process. Bacteria in their breaking down of aliphatic cycle and aromatic hydrocarbons involve oxygenize enzymes for which molecular oxygen is required (Milton & Rachakonda 2005).

Bioremediation application in Indonesia refers to the decision of the State Minister of Environment Number 128 Year 2003 to regulate the procedures and technical requirements of waste treatment and petroleum contaminated soil biologically. Bioremediation can be conducted using local microbes (Kepmen LH 2003).

Bioremediation technique in this research was land-farming technique which also called land-treatment. Land-farming technique is one of bioremediation techniques that is usually done at ground level. Arrangement of soil pH and by adding water, nutrient and oxygen to this land-farming technique is needed to increase biological activity (Nugroho 2006).

Bioremediation application withland-farming technique in this experiment using laboratory scale was done for 19 weeks. During 19 weeks, petroleum hydrocarbon contaminated soil was observed using TPH measurement. Then, the moisture content in the first month is observed weekly and every two weeks for the following month, whereas for TPC and pH tests are done weekly.

Figure 1. Bioremediation process using laboratory scale land-farming technique

2.2 Total Petroleum Hydrocarbon (TPH)

The success of hydrocarbon biodegradation process depends on the TPH degradation. Treatment was performed on 3 level of TPH, ie 5%, 8%, and 11% (TPH-5, TPH-8, and TPH-11). Figure 2 shows initial and final TPH in bioremediation process at laboratory scale after 19 weeks.

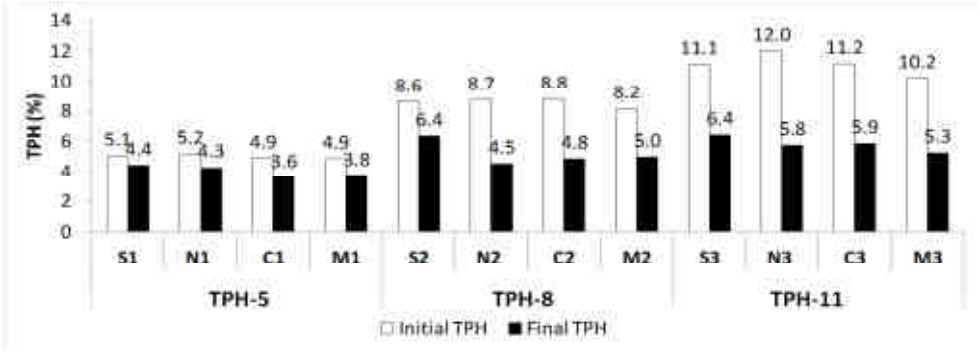

Figure 2. Initial and final TPH in bioremediation process of laboratory scale at: TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3) after 19 weeks

Figure 3 shows that the largest of TPH degradation at TPH-5 (S1, N1, C1, and M1) was obtained from C1 treatment (25.9%), which was the treatment with the addition of nutrients and compost. Compost is an added fertilizer derived from cow dung and husk. Animal waste contains bacteria. In addition, the compost contains nutrients for bacterial growth that can help bacteria degrade hydrocarbons.

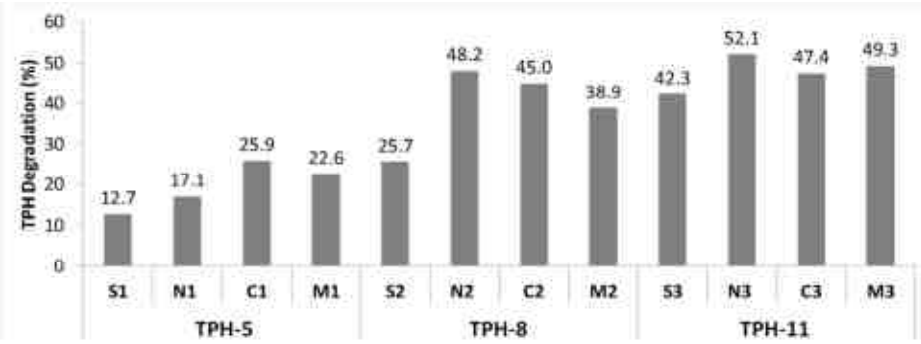

Figure 3. TPH degradation at TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3) after 19 weeks

At TPH-8 (S2, N2, C2, and M2), the largest of TPH degradation was obtained from N2 treatment (48.2%). Furthermore, for the treatment of TPH-11 (S3, N3, C3, and M3), the largest TPH degradation was obtained from treatment N3 (52.1%). For all initial TPH-5, TPH-8, and TPH-11, control soil (S) had the smallest decreased of TPH. Figure 3 also shows the higher the concentration of TPH, the higher the percentage decrease of TPH (S3 for S treatment, N3 for N treatment, C3 for C treatment, and M3 for M treatment). Based on the results obtained, it turns out that the addition of local bacteria (M) did not affect the TPH degradation, except for M3. It might be because the indigenous microbes in compost and soil grew and developed well, so the bioagumentation of enriched microbe (starter consortium) was not needed. The lowest percentage of TPH degradation in each TPH level treatment was from control treatment (Figure 3). It means that bioaugmentation and biostimulation could accelarate the biodegradation process.

Biodegradation in the land-farming techinque will be increased by aeration of the soil and nutrient addition.The maximum of TPH degradation in this study was 52.1% within 19 weeks. It was not so high TPH degradation result eventhough aeration and nutrients were already given. Aeration or oxygen supply was given by using the aerator. The reactors were also stirred evenly by reversing the soil and by wiggling the reactor every two weeks. It might be happened because the period of wiggling was not sufficient enough, therefore soil conditions did not support biodegradation of contaminants. Crude oil dervied from local area can be classified as light fraction since C_1-C_{10} (67.8%), C_{11}-C_{20} (27.1%), C_{21}-C_{30} (2.9%), and C_{31}-C_{40} (0.2%) (data not shown).

2.3 Soil pH

Figure 4 shows the change of pH value during 19 week treatment. The pH measurements performed weekly from week-0 to week-19 show that the measured pH ranged from 4.67 to 8.67. The pH value at week-0 to week-3 varied among acid, neutral, and alkaline. Subsequently in the following week i.e. week 4 to 19, pH of soil in the reactor at TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3) ranged between 6 to 8.

Cookson (1995) argued that the optimum pH for bacterial growth was 7 with a pH range between 4 to 7, while for nitrogen oxidation, a pH range is between 6 and 8. The pH value below 6 occurred at the beginning of the study. The pH value fluctuated but almost all treatments had a pH value of 6 - 8. According to Charlena (2010), degradation of hydrocarbons will occur faster if the pH is above 7. The pH measurements can show the biological activity of hydrocarbon degrading microorganisms. The degradation of petroleum will produce organic acids that cause a decrease in the pH of the medium (Hamme *et al.* 2003). The decline in pH values is thought to be caused by the activity of a bacteria that forms acid metabolites. Biodegradation of alkanes will form alcohol and it subsequently becomes fatty acids. The fatty acids will be further oxidized to form acetic acid and propionic acid, thereby decreasing the pH value of the medium (Rosenberg *et al.* 1992). Figure 4 shows there was a decrease of pH during M2 and M3 treatments.

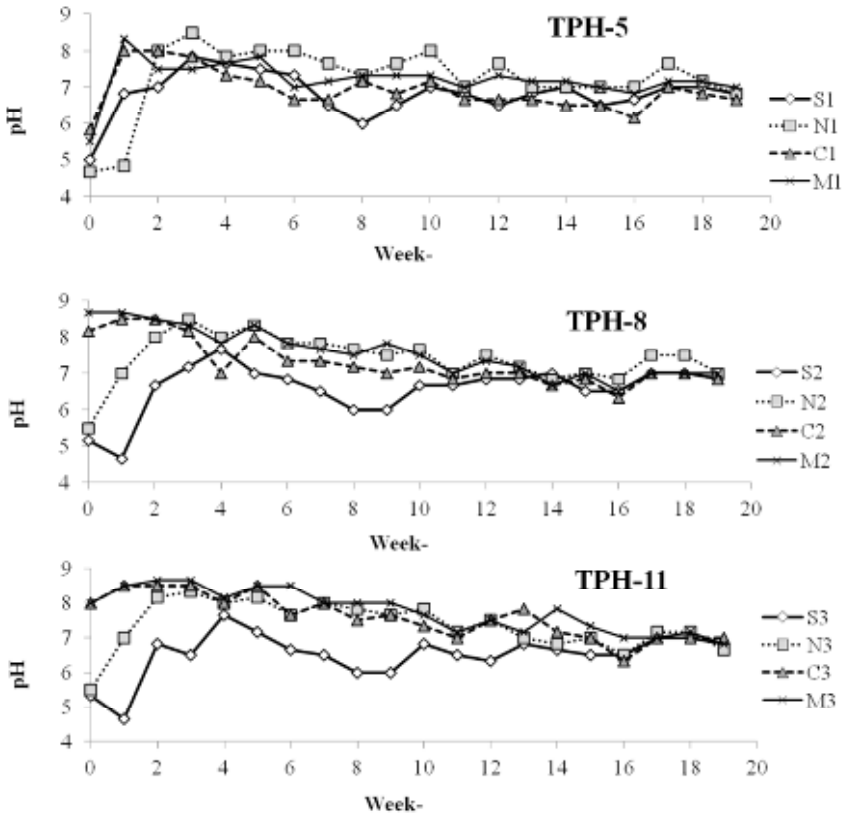

Figure 4. Change in pH in bioremediation process of laboratory scale at:TPH-5 (S1, N1, C1, M1);TPH-8 (S2, N2, C2, M2); andTPH-11 (S3, N3, C3, M3)

2.4 Soil Moisture Content

Soil moisture content is essential for metabolic activity of microbes in petroleum contaminated soil because microbes live actively in the interphase between oil and water (Udiharto *et al.* 1995). Therefore, soil moisture content affects the life and growth of microbes to run its metabolic activity (Charlena 2010). Groundwater levels measured from week-0 to week-19 showed that soil moisture content ranged from 4.86% to 47.21% (Figure 5). The soil moisture content fluctuated. Water was added when needed, because in this experiment it was attempted that the soil sample was not dry. In case the soil was dry, microbes could not live on contaminated soil. Conversely, if it was too much water level on soil samples, would cause no oxygen in soil. Optimal moisture content required for bacterial metabolism in degrading hydrocarbon has been reported at 30-90% (Dibble & Bartha 1979). Moisture content is also needed by bacteria as a nutrient solvent (Udiharto *et al.* 1995). When the value of moisture content decreased, possibly caused by the absorption of H_2O by hydrocarbon degrading microorganisms. Microorganisms in metabolism require H_2O as a reagent. In addition, the loss of water in the reactor might be due to evaporation.

Room temperature and air humidity measured were fluctuated every week (data not presented). Value of air humidity was correlated with air temperature. The higher the room temperature, the lower the air humidity in the greenhouse. Air humidity will affect to the soil moisture content in reactors. The average temperature in the greenhouse was 26.8 - 34.6ºC from a daily minimum of 23.0ºC to maximum of 47.4ºC. The average relative humidity was 50.3 - 72.1% from a daily minimum of 18% to a maximum of 91%.

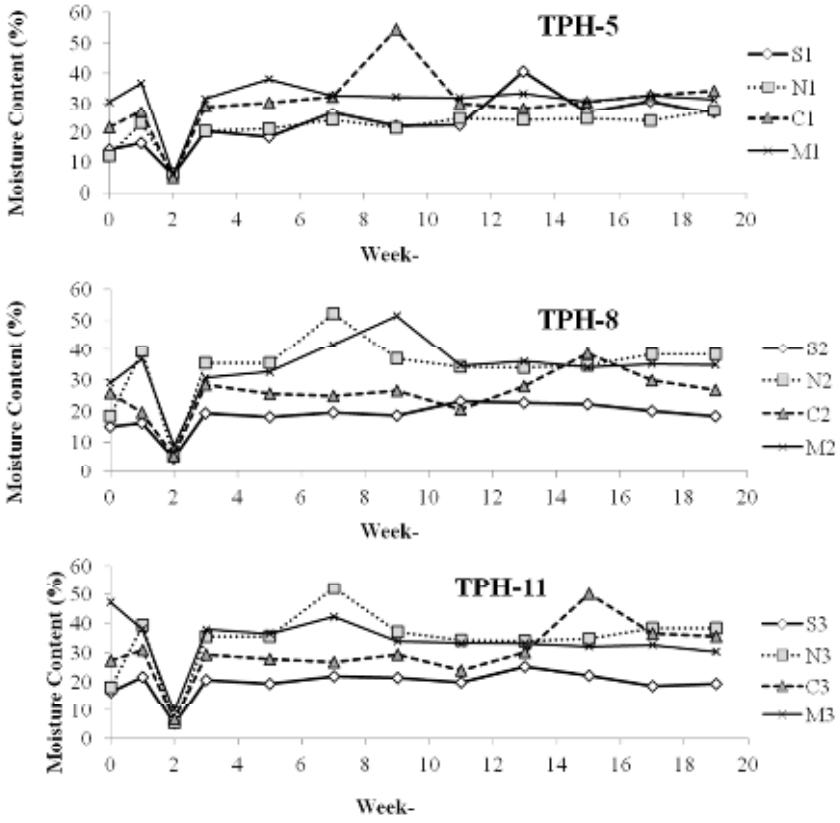

Figure 5. Changes in moisture content in laboratory bioremediation process at: TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3)

2.5 Bacterial Population (TPC)

Bacteria are important factors in bioremediation process, either indigenous bacteria or self-developed bacteria. Bacteria enables to destroy the pollutants present in the contaminated sites. Optimum bacterial cell growth will increase hydrocarbon degradation rate or decrease TPH (Charlena 2010). Total Plate Count (TPC) measurements performed every week from week-0 to week-19 show that TPC ranged log 8.70 - 23.76 CFU/g-dried-soil.

Figure 6 shows that there was an increase in bacterial growth. At TPH-5 and TPH-11, from week-0 to week-12 had an increase in bacterial population, whereas at TPH-8 increased in bacterial population starting from week-0 to week-14. An increase in bacterial population is an indication that bacteria grew by consuming carbon sources from hydrocarbons.

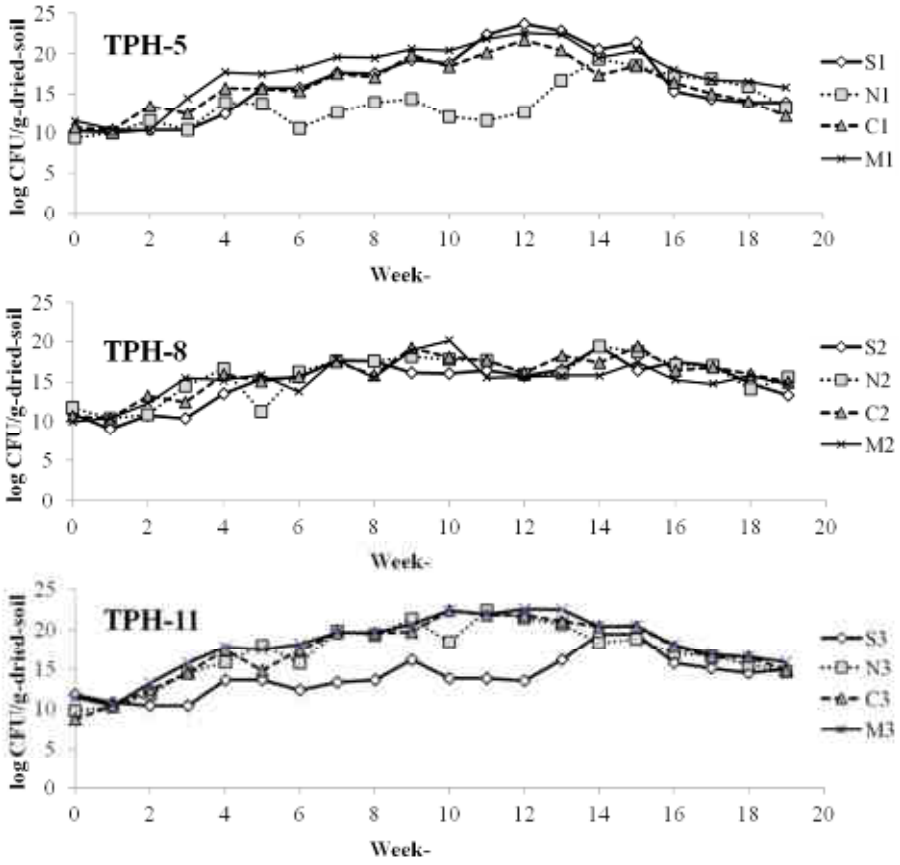

Figure 6. Bacterial population growth in bioremediation process at: TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3)

Some microorganisms used in the process of remediation are *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Cinetobacter*, *Corynebacterium*, *Flavobacterium*, *Micrococcus*, *Mycobacterium*, *Norcardia*, *Pseudomonas*, *Vibrio*, *Rhodococcus* and *Sphingomonas* (Kim *et al.* 2007; Jayashree *et al.* 2012). Brito *et al.* (2006) reported that typical bacterial groups already known for their capacity to degrade hydrocarbons, include *Pseudomonas*, *Marinobacter*, *Alcanivorax*, *Microbulbifer*, *Sphingomonas*, *Micrococcus*, *Cellulomonas*, *Dietzia*, and *Gordonia*.

The process of hydrocarbon degradation with these microbes can be increased by the addition of compost. Compost is a fertilizer derived from animal waste. Animal waste is an active ingredient that has the ability to increase porosity, an additional nutrient material for microbial growth, and as a source of microbes.

Organic waste was able to neutralize the toxic effects of the oil on the microbial population by rapid improvement of the soil physicochemical properties (Floch *et al.* 2011). The organic waste might help in improving the soil aeration and thus providing sufficient oxygen required by the microbial community which consequently favored the growth of indigenous bacteria in the soil.

2.6 Soil Temperature

Temperature has a considerable influence on petroleum biodegradation by its effect on the composition of microbial community, its rate of hydrocarbon metabolism, and its physical nature and chemical composition of the oil. The temperature will affect the physical properties and chemical properties of oil, the level of microbial hydrocarbon metabolism, as well as the microbial community composition. So, the higher the soil temperature the higher the hydrocarbon metabolism rate.

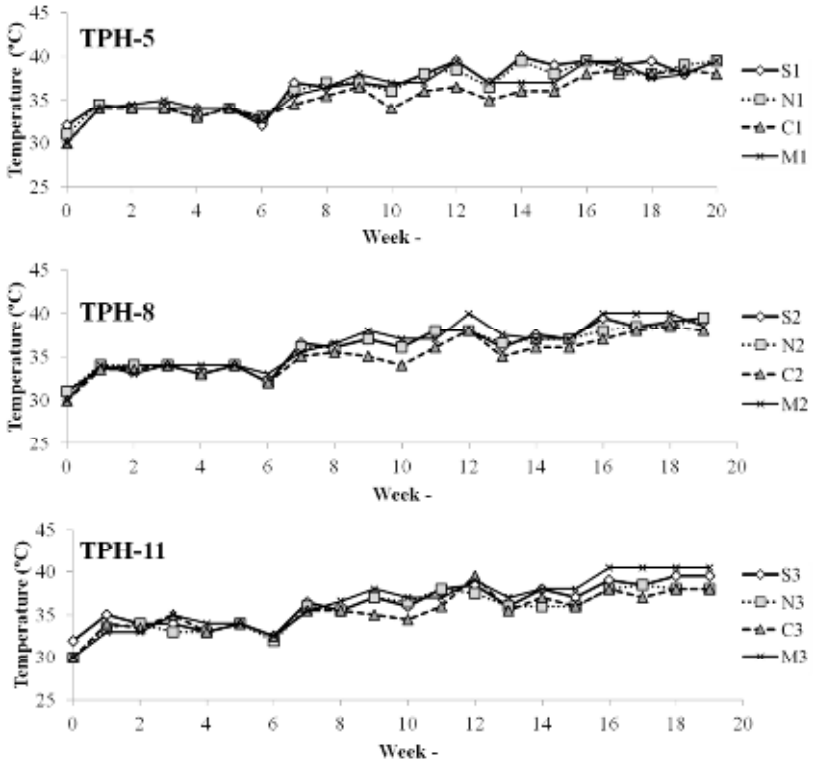

Figure 7. Soil temperature change in laboratory scale bioremediation process at: TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3)

According to Leahy and Colwell (1990), the ideal temperature for hydrocarbon degradation is between 30-40^{o}C. Above that temperature, the activity of the enzyme will decrease and the hydrocarbon toxicity of the cell membrane will be higher. In this study, the temperature fluctuates because in the process of bioremediation occurs the disconnection of hydrocarbon chains that will produce energy, so that the temperature rises. Conversely, the temperature will drop if the bioremediation process stops. At low temperatures, the viscosity of the oil will increase resulting in declining toxic short-chain alkane volatility. In addition, the solubility in the water will increase, so the bioremediation process will be hampered by the decrease of microbial enzyme activity.

Figure 7 shows that soil temperature measured from week-0 to week-19 was in the mesophilic temperature range between 30°C to 40.5°C. The temperature inside the reactor was in accordance with the desired temperature in the bioremediation process. This is in accordance with the report of Baker and Herson (1994), that the majority land restoration conducted biologically, is in mesophilic conditions.

2.7 Production of CO_2 Gas

In the biodegradation process, petroleum hydrocarbon compounds that have long chains and high molecular weight are broken down by aerobic bacteria into hydrocarbon compounds with lower molecular weights. During the biodegradation process, CO_2 gas is generated, which is an indication of the degradation process. In other words, the formation of CO_2 gas is the result of bacterial activity in degrading hydrocarbons.

The formation of CO_2 gas is caused by an aerobic process conducted by aerobic bacteria in the biodegradation of contaminated soil waste. In the biodegradation process, the alkane chain is oxidized to form alcohols, aldehydes, and fatty acids. The long chain of fatty acids is converted by coenzyme A to acetyl coenzyme A. Acetyl coenzyme A is converted to CO_2 via the tricarboxylic cycle (Atlas & Bartha 1987).

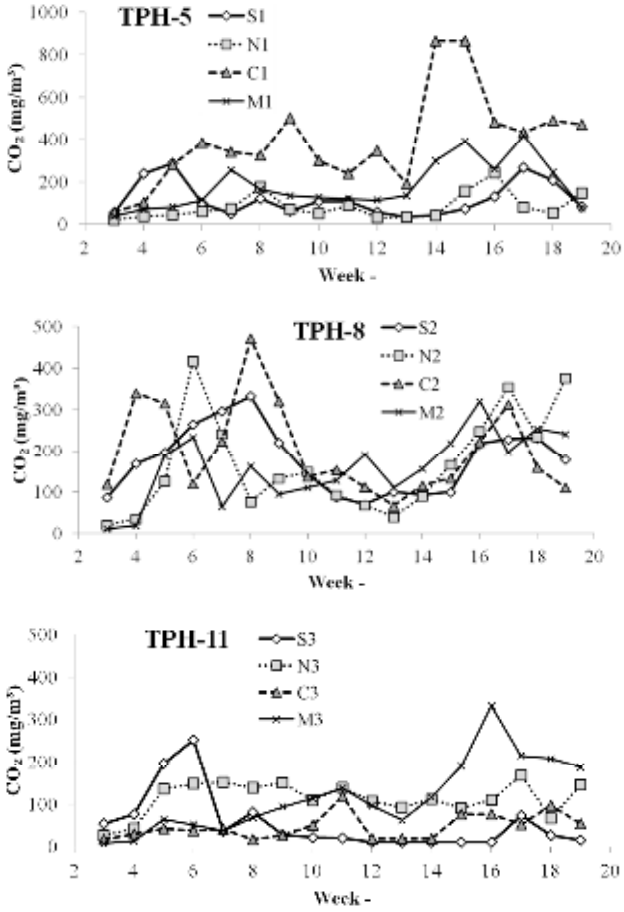

Figure 8. Production of CO_2 gas during bioremediation process at: TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3)

Figure 8 shows the production of CO_2 gas starting from week-3 to week-19 at TPH-5 (S1, N1, C1, M1); TPH-8 (S2, N2, C2, M2); and TPH-11 (S3, N3, C3, M3). CO_2 gas produced fluctuated which show an increase and decrease in CO_2 gas every week. At TPH-5 (S1, N1, C1, M1), there was an average decrease in CO_2 gas. For example, C1 (contaminated soil of TPH-5 plus nutrients and compost) increased CO_2 from week-3 to week-9 and decreased until week-13, then increased again until week-15. Overall, C1 at week-14 and 15 achieved the highest CO_2 production value compared with other treatments, whereas for TPH-8 and TPH-11 the same as TPH-5 fluctuated from week-3 to week-19.

Baptista *et al.* (2005) explained that the presence of CO_2 production indicates the presence of respiration rates in microbes, which are produced bioremediationally. Kao and Wang (2000) also explained that CO_2 gas is the result of all intrinsic bioremediation processes. Degradation of hydrocarbons is associated with respiration from microbes and the results are shown by CO_2 gas formation.

3. Conclusions and Recommendation

The results of bioremediation research using a laboratory scale land-farming technique for 19 weeks was performed. The pH changed from week-4 to week-19 between 5.0 to 8.5. The moisture content fluctuated from 4.86% to 47.21%. The population of microbes performed weekly from log 8.70 to 23.76 CFU/g-dried-soil. Biodegradation process has been indicated by CO_2 gas generated during the observation. The TPH degradation was affected to treatments of initial TPH, nutrient, and compost. The treatment of N3 (nutrients and TPH 11%) showed the highest in TPH degradation at 52.1%. The bioaugmentation using local microbes did not affect on TPH degradation. Therefore, bioaugmentation was not needed if compost has been added. It might be because the indigenous microbes in compost and soil grew and developed well, so the bioaugmentation of enriched microbes was not needed.

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