SCIENTIFIC CONTRIBUTIONS OIL AND GAS
Vol. 39, Number 1, April 2016: 4 of 5

RESEARCH AND DEVELOPMENT CENTRE FOR OIL & GAS TECHNOLOGY LEMIGAS

Journal Homepage:http://www.journal.lemigas.esdm.go.id

BIOREMEDIATION IN PETROLEUM CONTAMINATED SOIL TREATMENT USING PLANT-MICROORGANISMS COMBINATION (Case Study: Reduction Level of TPH and BTEX in Bioremediation Process)

BIOREMEDIASI PADA PENGOLAHAN TANAH TERKONTAMINASI MINYAK BUMI MENGGUNAKAN KOMBINASI TUMBUHAN-MIKROORGANISME (Studi Kasus: Penurunan Kadar TPH dan BTEX pada Proses Bioremediasi)

Cut Nanda Sari[1]**, Tyas Putri Sativa**[2]**,** and **Setyo Sarwanto Moersidik**[2]

[1]"LEMIGAS" R & D Centre for Oil and Gas Technology
Jl. Ciledug Raya, Kav. 109, Cipulir, Kebayoran Lama, P.O. Box 1089/JKT, Jakarta Selatan 12230 INDONESIA
Tromol Pos: 6022/KBYB-Jakarta 12120, Telephone: 62-21-7394422, Faxsimile: 62-21-7246150
E-mail: ekobl@lemigas.esdm.go.id; E-mail: ln@lemigas.esdm.go.id
[2]Environmental Engineering Study Program, Civil Engineering Departement,
Faculty of Engineering Universitas Indonesia, Depok 16424, Indonesia

First Registered on March 21st 2016; Received after Correction on March 27th 2016
Publication Approval on: April 29th 2016

ABSTRAK

Tumpahan minyak bumi baik pada lingkungan akuatik maupun darat sangat merugikan manusia maupun lingkungan karena senyawa hidrokarbon yang terkandung di dalamnya yang dapat membahayakan ekosistem dan keseimbangan lingkungan serta merupakan senyawa yang karsinogenik bagi manusia dan hewan. Oleh karena itu tindakan remediasi perlu dilakukan, salah satunya adalah dengan metode kombinasi mikroorganisme dan tumbuhan. Tujuan penelitian ini adalah untuk menganalisis pengaruh beberapa perlakuan yang diterapkan terhadap penyisihan kadar TPH dan BTEX serta pengaruhnya terhadap faktor lingkungan dalam proses remediasi. Pada penelitian ini, bioremediasi dilakukan dengan menggunakan 4 perlakuan yang berbeda yaitu pemberian kompos (C), tanaman dan kompos (P), mikroorganisme dan kompos (B), dan tanaman dan mikroorganisme kompos (BP), terhadap tanah dengan kadar minyak 5% dan 10% selama 5 minggu. Dari hasil penelitian, berikut hasil pengujian TPH berturut-turut pada tanah terkontaminasi 5%: 2,10% (C); 1,31% (B); 1,66% (P); dan 0,68% (BP) dan hasil pengujian TPH berturut-turut pada tanah terkontaminasi 10% adalah 3,30% (C); 2,54 (B); 3,91% (P); dan 3,31% (BP). Persentase degradasi TPH tertinggi pada tanah terkontaminasi minyak 5% terdapat pada perlakuan BP yaitu sebesar 87,1%, sementara pada tanah terkontaminasi minyak 10% persentase penyisihan TPH terbesar ada pada perlakuan penambahan bakteri yaitu sebesar 76,19%. Persentase penyisihan BTEX pada perlakuan BP di tanah terkontaminasi minyak 5% sebesar 68,35% persentase penyisihan BTEX pada perlakuan B di tanah terkontaminasi minyak 10% sebesar 84,91%. Berdasarkan uji statistik, baik pada tanah terkontaminasi 5% maupun 10%, degradasi TPH mempengaruhi nilai pH secara signifikan karena $p < 0,05$ namun degradasi TPH tidak mempengaruhi nilai suhu karena $p > 0,05$.

Kata Kunci: *Acinetobacter baumannii;* Crude Oil; *Bioremediasi; BTEX; Fitoremediasi;* Ryegrass; *TPH.*

ABSTRACT

Oil spills, in both aquatic and terrestrial environments, are very detrimental to people and the environment due to hydrocarbon compounds that are contained in oil which are not only be harmful for the balance of the ecosystem and the environment but also carcinogenic to humans and animals. Therefore remediation needs to be done. One of the methods is by using a combination of microorganisms and plants. The aim of this research is to analyze the influences between several different treatments that are applied for TPH and BTEX removal in the process of remediation. In this research, bioremediation was conducted by using four different treatments which are: by adding compost (C), plants and compost (P), microorganisms and compost (B), and compost, plants and microorganisms (BP), to soil with oil content of 5% and 10%. The following test results of TPH in soil contaminated with 5% oil content are: 2.10% (C); 1.31% (B); 1.66% (P); and 0.68% (BP). The TPH test results in soil contaminated with oil content of 10% are: 3.30% (C); 2.54 (B); 3.91% (P); and 3.31% (BP). The highest percentage of TPH degradation in contaminated soil of 5% oil content was found in BP treatment (87.1%), while in the contaminated soil of 10% oil content the largest TPH removal percentage is by the treatment of adding bacteria (B) which is 76.19%. BTEX removal percentage in 5% oil contaminated soil in BP treatment is 68.35% while in 10% oil contaminated soil with B treatment the removal percentage is 84.91%. Based on statistical tests, both on contaminated soil with 5% and 10% oil content, TPH degradation significantly affects the pH value as $p < 0.05$ but TPH degradation does not affect temperature values as $p > 0.05$.

Keywords: Acinetobacter baumannii; Crude Oil; Bioremediation; BTEX; Phytoremediation; Ryegrass; TPH.

I. INTRODUCTION

Petroleum is the source of energy that is consumed the most by the entire world. Based on BP statistical review (2015), total global consumption of oil in 2014 is 92 Mb/d and projected to reach 109 Mb/d by 2035. The potential of national oil and gas is still large, so it is clear that further exploration will be undertaken. However, it could not be denied that the petroleum industries have the potential to harm the environment from various spills when they occur. Based on mass balance of oil and gas flow in Pusdiklat Migas oil refinery, oil lost due to evaporation, spillage, and scattered during production process is approximately 0,4% or 108,38 barrels per month or 17.232,42 litre per month (Anonymous 2011).

Petroleum components consist of many hydrocarbon compounds which can be measured as TPH (Total Petroleum Hydrocarbon), while the volatile aromatic hydrocarbon compound can be measured as BTEX (benzene, toluene, ethylbenzene, and xylene). The existence of hydrocarbon pollutants is not only detrimental for humans but also the environment. Components of petroleum aromatic compounds are carcinogenic for both humans and animals. In addition, hydrocarbon compounds affect soil chemical constituents, activities and the population of soil microorganisms (Leme et al. 2012).

Therefore, remediation of oil contaminated land is needed so that the oil can be immediately removed or/converted into harmless compounds that will not pollute the environment. One of the remediation methods is by a combination of plants-microorganisms. The interaction between a plant and microorganisms is a mutualism relationship, in which the plant provides root exudates consisting of nutrient, enzymes, and oxygen for microorganisms in rhizosphere in order to increase the population of microorganisms and accelerate degradation process while the inoculation of microorganisms can increase resistance of the plant against contaminants and accelerate the aclimation and the formation of biomass (Muratova et al. 2009).

The aim of this study was to analyze the effect of the addition of compost, microorganisms, plant cultivation, and the combination of both plants and microorganisms on biodegradation percentage of TPH and BTEX in petroleum contaminated soil and to analyze the influence of TPH and BTEX degradation processes on environmental factors, namely pH and temperature.

Theoritical Overview

Petroleum is a mixture of hydrocarbon compounds that occur naturally, which is generally in liquid form, which may also include compounds of sulfur, nitrogen, oxygen, metals, and other elements (ASTM D4175). The chemical composition of oil from different production region and even from a specific formation, can be very diverse.

The main source of oil contamination typically includes tank leaks, the rupture of the pipe, the land disposal of petroleum waste, and accidents or intentional spills, in the process of extraction and refining (Zhang et al. 2010; Mohsenzadeh et al. 2010). Oil contaminated soil is not suitable for agricultural, residential, and recreation use. Moreover, it can be a threat to the sustainability of ecosystems and human health, and significantly affect the chemical properties of the soil and the population, as well as the activity of soil microorganisms (Leme et al. 2012). Moreover, oil spills can reduce and degrade the function of the soil, the productivity of the soil, pollute the environment, lead to degraded land, and can affect the smell and taste of ground water (Sulistyono et al. 2012). Hydrocarbon's hydrophobic nature reduces the ability of plants and microorganisms to absorb water and nutrients from the soil (Nie et al. 2011).

Bioremediation is a remediation technique using microorganisms to degrade pollutants from a complex to a simpler structure with the help of the enzymes, so that the pollutants are not toxic and hazardous. The bioremediation method is a way of getting the safest oil for the environment (Munawar 2007). Phytoremediation is a green technique that appears to remediate soil and water contaminated with toxic pollutants, with a biological plant and engineering strategy (Susaria et al. 2002).

A combined strategy of phytoremediation and bioremediation microbes makes for a more successful approach to remediate contaminants, particularly organic compounds (Gerhardt et al. 2009). The inoculant bacteria improves plant resistance to contaminants and pathogens, stimulates the degradation of contaminants, and increases the rate of acclimation and the formation of plant biomass. Plants increase the population of, and the potential degradation by, microorganisms because plants release root exudate and the growth of a plant's roots can improve soil structure. According to Chaundry et al. (2005), various enzymes that degrade contaminants can be found in plants, fungi, endophytic bacteria, and bacterial colonies roots, which include peroxidase, dioxygenase, P450 monooxygenases, laccase, phosphatases, dehalogenase, nitrilase, and nitroreduktase.

Ryegrass (*Lolium multiflorum*) is used in this research because it has the resilience and the ability to grow in diesel contaminated soil (Kaimi et al. 2006). Ryegrass also shows the effective dissipation of diesel oil at the beginning of the experiment and can reduce concentrations of TPH contained in diesel oil. Ryegrass has been widely used in phytoremediation of contaminated soil PAH because it has a fibrous root system and a large root surface area (Yu et al. 2011). Grasses have fibrous root systems that make a large root surface area and may penetrate the soil to a depth of 3 meters (Yousaf et al. 2010). The bacteria used is *Acinetobacter baumannii* strain ATCC 19606 which is widespread in nature and can be found in water, soil, living organisms, and even from human skin. It is oxidase-negative, non-motile, aerobic, and visible in a microscope as gram negative cocobacilli. Some species of this genus had a role in the biodegradation of some pollutants such as polychlorinated biphenyls and chlorinated, amino acids, phenols, benzoic, crude oil, acetonitril, and the removal of phosphates or heavy metals (Abdel-El-Haleem 2003).

The combination of plant and bacterial inoculation was essential to achieve the optimum degradation process, but this combination can be affected by other rhizosphere microorganisms and environmental conditions. In order to create an efficient remediation, what has to be done is not only to keep bacteria and ensure colonization could survive, but also to be metabolically active in the degradation process of hydrocarbons.

II. METHODOLOGY

The study involved experimental research using pots as a reactor and was conducted at the Laboratory of Process, Technology, Research, and Development Center for Oil and Gas Technology (LEMIGAS), Cipulir, Jakarta, and also at the Laboratory of Environmental Engineering Study Program, University of Indonesia, and at Central Forensic Laboratory.

A. Contaminated Soil

Soil samples were taken from the Faculty of Engineering, University of Indonesia and were as much as 12 kg which had passed ASTM sieve No. 10 or 2 mm to ensure that the soil is homogeneous (there are no plant remains). Petroleum that was usedwas from LEMIGAS.

B. Bacteria and Ryegrass Seed

The bacteria used in this experiment was *Acinetobacter baumannii* strain ATCC 1960 from Department of Chemical Engineering, University of Indonesia, Depok. The number of bacteria added to contaminated soil was 10% (v/w) with initial inoculum of 2.6 x 107 CFU / mL.

Ryegrass seeds (*Lolium multiflorum*) were first sterilized by soaking them in a 5% solution of sodium hypochlorite (NaOCl) for 2 minutes, then they were soaked in 70% ethanol for 2 minutes and resuspended in sterile water 3 times. Before being planted into the soil, ryegrass seeds needs to be germinated on filter paper first, and after 10 days, transplanted germinated seeds were put into a pot of approximately ± 300 seeds.

C. Pot Experiment

This experiment was divided into 4 treatments: the addition of *Acinetobacter baumannii* strain ATCC 1960 (B); the addition of ryegrass (P); the addition of both ryegrass and *Acinetobacter baumannii* (BP); and the addition of 15% (w/w) compost (C). Urea and NPK fertilizers were added to soil as nutrients for microorganisms, in a certain number, based on calculations, to meet the levels of C: N: P optimum ratio of 100: 10: 1. Humidity was kept in ± 50%, in order that the plants can grow, so all pots were watered twice a day. Soil samples were be taken at intervals of 7, 14, 21, 28, and 35 days using injections as a replacement for cork borer.

D. Pot Design

Eight plastic pots were used, with a diameter of 25 cm from plant store in Bogor.

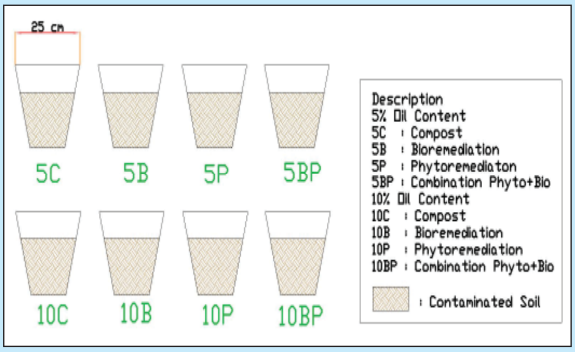

Figure 1
Pot Design.

E. Testing Parameters and Statistical Analysis

A statistical test was conducted using SPSS 22, with regression and correlation analysis between the influence of TPH degradation and environmental factors namely pH and temperature, using one-way ANOVA (anaysis of variance) to test the difference in treatment given which are the addition of compost (C), plants and compost (P), bacteria and compost (B), and plants, bacteria and compost (BP) against a reduction in the level of TPH, and determine the removal constant rate of TPH (k).

Table 1
Research parameters

No	Parameter	Standard Measurement	Interval Time
1	pH	SNI 03-6787-2002	Every 7 days
2	Temperature	SNI 06-6989.23-2005	Every 7 days
3	TPH Concentration	US EPA 9071b	Every 7 days
4	Microorganisms Population	*Bacteriological Analytical Manual*	Every 7 days
5	Benzene, Toluene, Ethylbenzene, Xylene	US EPA 5035	In the beginning and in the end

III. RESULT

A. TPH (*Total Petroleum Hydrocarbon*)

Hydrocarbon compounds decreases as a function of time in the four treatments, in which the highest percentage of biodegradation in the soil contaminated with oil content of 5% is a combination of plants - microorganism (BP) > bacteria (B) > plant (P) > compost (C). In soil contaminated with oil content of 10%, the highest percentage of biodegradation is the addition of bacteria (B) > combination plants-microorganism (BP) > compost (C) > plant (P). This following graphs illustrate the TPH test results.

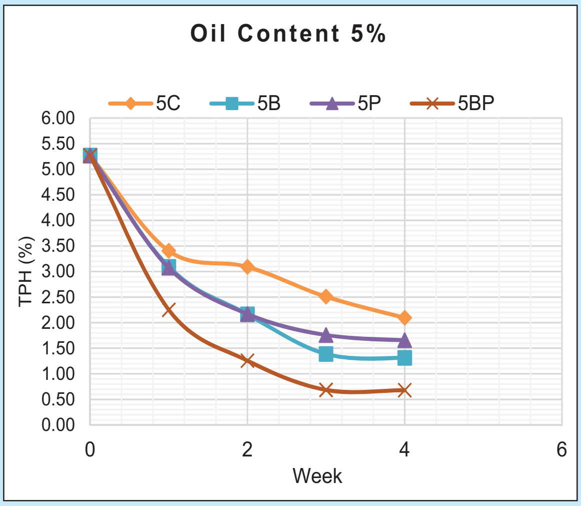

Figure 2
TPH chart in contaminated soil with 5% oil content.

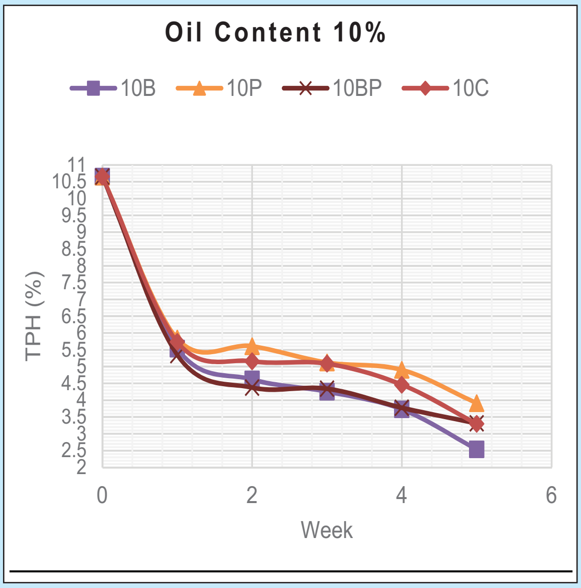

Figure 3
TPH chart in contaminated soil with 5% oil content.

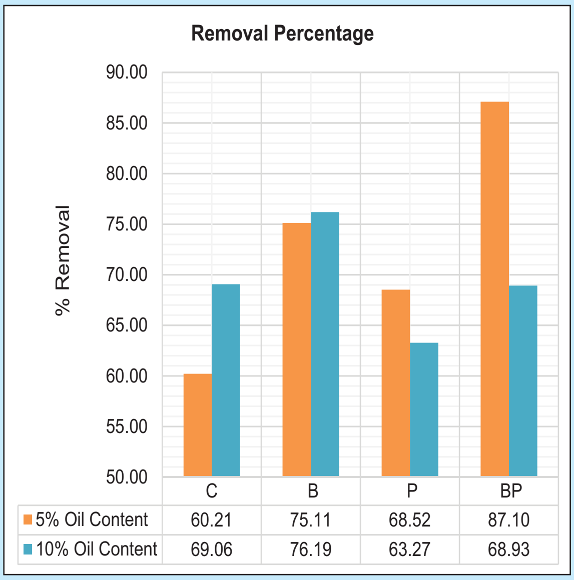

Figure 4
Removal percentage TPH chart.

B. TPH Constant Removal Rate

The constant removal rate of TPH is the extent of removal of hydrocarbons compounds in petroleum contaminated soil as afunction of time. In this study, the value of 'k' can be used to estimate the time required to degrade hydrocarbons in petroleum contaminated soil.

Table 2
Constant removal rate

Treatment	Removal Constant (day^{-1})
5C (Compost)	0,0307
5B (Bacteria)	0,0512
5P (Plant)	0,041
5BP (Bacteria+Plant)	0,0755
10C (Compost)	0,0271
10B (Bacteria)	0,0345
10P (Plant)	0,0229
10BP (Bacteria+Plant)	0,0281

C. BTEX (*Benzene, Toluene, Ethylbenzene, Xylene*)

A BTEX test was conducted on two samples that have the most optimum results in TPH degradation, namely 5BP and 10B.

Table 3
BTEX test result

BTEX	% Concentration			
	Initial BTEX		Final BTEX	
	5%	10%	5BP	10B
Benzene	2,03	6,01	-	-
Toluene	-	-	0,825	0,97
Ethylbenzene	-	-	-	-
Xylene	0,75	0,42	0,055	-
Total	2,78	6,43	0,88	0,97

D. Enumeration of Microorganisms

The test results of microoganisms enumeration has fluctuations and is influenced by the treatment and environmental factors. The following table is the calculation of results of microorganisms enumeration.

E. Environmental Factors

Environmental factors such as pH and temperature affect the degradation rate of hydrocarbons. The higher its temperature, the higher the rate of the chemical in a biochemical reaction in general. Temperature will affect the microbial population and the rate of degradation of hydrocarbons. Temperature affects the physical properties and chemical composition of the oil, the rate of metabolism

Table 4
The Results of microorganisms population calculation

Pot	Population of Microorganisms				
	Day -				
	10	17	24	31	38
5C	Can not be calculated (($>300 \times 10^4$)	$4,75 \times 10^5$	Can not be calculate ($<10^4$)	$5,25 \times 10^3$	-
5B	$4,3 \times 10^6$	$2,5 \times 10^7$	Can not be calculate ($<10^4$)	1×10^4	-
5P	Can not be calculated ($>300 \times 10^4$)	$4,2 \times 10^6$	1×10^4	$3,4 \times 10^4$	-
5BP	$7,1 \times 10^6$	$1,38 \times 10^7$	$1,93 \times 10^6$	$1,45 \times 10^5$	-
10C	Can not be calculated($>300 \times 10^4$)	$1,22 \times 10^7$	5×10^4	9×10^4	$8,5 \times 10^3$
10B	Can not be calculated ($>300 \times 10^6$)	$2,23 \times 10^8$	$1,32 \times 10^7$	$2,34 \times 10^8$	Can not be calculated ($>300 \times 10^8$)
10P	Can not be calculated ($>300 \times 10^4$)	$3,5 \times 10^6$	$2,5 \times 10^4$	$1,38 \times 10^4$	Can not be calculated ($>300 \times 10^7$)
10BP	$1,6 \times 10^5$	5×10^4	1×10^4	4×10^4	$2,25 \times 10^5$

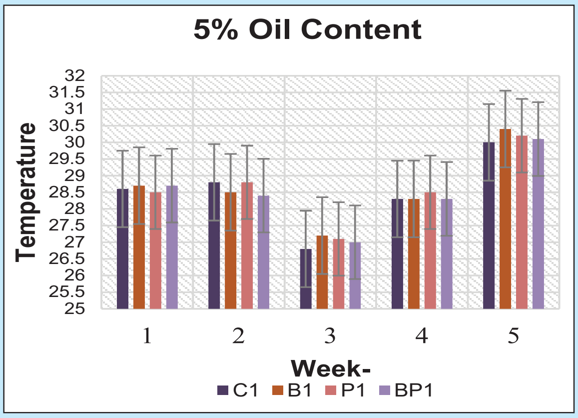

Figure 5
Temperature chart in contaminated soil with 5% oil content.

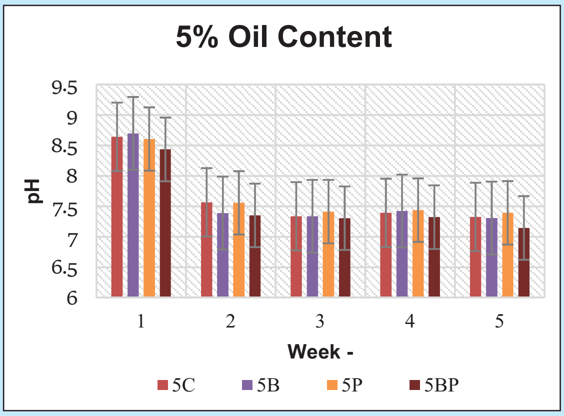

Figure 7
pH chart in contaminated soil with 5% oil content.

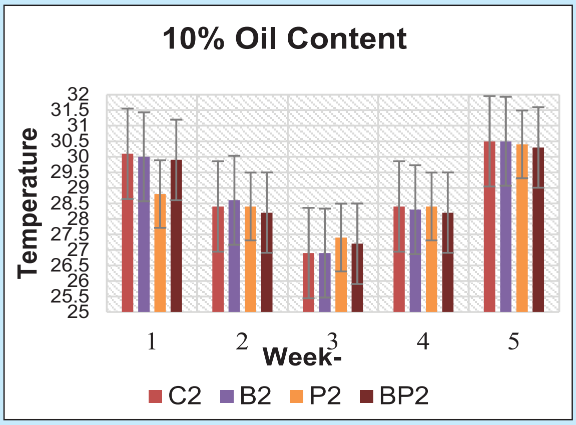

Figure 6
Temperature chart in contaminated soil with 10% oil content.

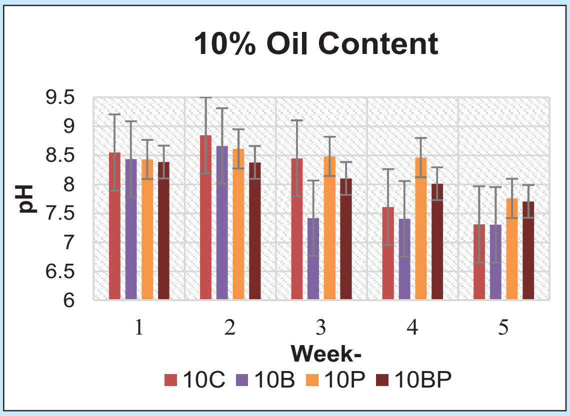

Figure 8
pH chart in contaminated soil with 10% oil content.

of hydrocarbons by microorganisms, and the components of microorganisms. The pH affects the ability of microorganisms to control cell function, cell membrane transport, the reaction equilibrium, and the proton motive force for energy production in cells. In general, microorganisms are living at a pH which is close to neutral.

IV. DISCUSSION

Ryegrass conditions continued to decline (increasingly withered) from week to week, until in the fifth week all ryegrass in all planted pots had died. This is due to high levels of contaminants that inhibit plant growth and reduce the ability of plants to absorb water and nutrients from the soil. The existence of high oil concentration in the soil causes chlorosis on the leaves so that there is no green vegetation near the end of the experiment. The number of leaves also decreases in the high concentration of crude oil (Langer et al. 2010). Light crude oil either in low or high concentrations (1-10%) contains toxic compounds which can prevent normal growth and germination of plants in the soil. This can reduce plant biomass, chlorosis, and reduce the length of roots and shoots (Minoui et al. 2015). Around 30000 ppm (3%) is an appropriate level that can be tolerated by plants. High concentration (more than 50000 ppm of TPH) of low solubility constituents can be toxic and/or not bioavailable (EPA, 2012).

Final TPH percentage on the 5% oil content contaminated soil based on composting (C), bacteria (B), plants (P), and a combination of bacteria and plants (BP) treatment were 2.10%; 1.31%; 1.66% and 0.68% respectively. Contaminated soil with a combination of phytoremediation and bioremediation treatment generate the lowest final TPH percentage from 5.26% to 0.68% (6800 ug/g) in 4 weeks and achieved the largest removal percentage which is 87.10%. This indicates that the mutualism symbiotic between plants and microorganisms in the rhizosphere positive happened, that bacteria increased the resistance of plants against contaminants, stimulating the degradation of contaminants, and increased the rate of acclimation and the formation of biomass. Further, the plants increased in number and there was also an increase in the potential degradation of microorganisms, because plants can emit roots exudates and the root growth of plants can improve

Figure 9
Ryegrass Condition In The First Week.

soil structure (Muratova et al. 2009). Some organic compounds are released through root exudates (phenolic, organic acids, alcohol, and protein) and are used as a source of carbon and nitrogen for microbial growth that can assist the process of degradation of organic compounds (Salt et al. 1998). Secretions in the form of organic compounds can help the growth and improve microbial activity in the rhizosphere which in turn increases the rate of contaminants degradation. Plants (vegetation) act to immobilize soluble contaminants in water, improve soil structure stability, and create a favorable environment for microorganisms to degrade the contaminants (Shaw and Burns 2007).

The difference on TPH reduction between phytoremediation treatment (5P) and bioremediation treatment (5B) in 5% oil content is not so significant, but the final results of pots with additional bacteria (bioremediation) is better than the pot planted with ryegrass (phytoremediation). This is because the plant died in the third week, so that the degradation of the petroleum was not optimal. In addition, the degradation of the 5B pot was faster because of the agitation treatment in the 5B pot which results in better air circulation so that the bacteria tend to get more oxygen. Basically, *Acinetobacter* is an aerobic bacteria, therefore the more they get oxygen, the more they grow and breed and the increase in microorganisms leads to a higher rate of petroleum degradation. The smallest TPH reduction occured in 5P pot with the addition of compost treatment, which happened because compost does not contain any hydrocarbon degrading bacteria and the use of compost is just as a bulking agent, so there is no treatment that can significantly degrade petroleum.

In the 10% oil contaminated soil, the final TPH percentage of composting (C), bacteria (B), plants (P), and a combination of bacteria and plants (BP) treatment were 3.30%; 2.54%; 3.91% and 3.31% respectively. Unlike the best results in the degradation of TPH with 5% oil content which is the pot with a combination of plants and microorganisms, in contaminated soil with 10% oil content, the best result of TPH degradation is in the pot with the addition of bacteria (10B) treatment with the final removal percentage being 76,19%. This happened because plants could not live on the 10% oil content, so that ryegrass does not affect the process of degradation. In the 10B pot, TPH percentage decreased significantly because of the agitation that makes better circulation of oxygen leading increased the growth of microorganisms.

The results of the TPH constants removal rate calculation (k) in 5% contaminated soil with oil content is greater than the 10% contaminated soil. This indicates that the degradation of hydrocarbons is more optimal in soil contaminated with 5% oil content rather than 10% oil content. These results are similar to Tang et al. (2010) which found that the most optimum bioremediation occurs at levels of TPH 5%. This likely occurred because basically most of the bacteria was still able to tolerate 5% oil content so that the degradation process can still occur. Moreover, TPH constant removal rate is also influenced by the plants, where ryegrass in the 5% contaminated soil still grew until the third week so that the symbiotic mutualism in the rhizosphere was still occuring.

Based on the results that were obtained from BTEX calculation, the initial and final BTEX concentrations decreased quite significantly, especially in 10B pot. The biodegradation percentage of BTEX in the 5BP pot that represents biodegradation in 5% contaminated soil is 68.35% and the 10B pot that represent biodegradation in 10% contaminated soil is 84.91%. Setyawan and Nanto (2011) state that, BTEX biodegradation percentage depends on the concentration of bacteria that exist. The greater the concentration of bacteria, the higher the percentage of BTEX biodegradation. The biodegradation percentage in 10B pot was higher than the 5BP pot because of the greater amount of bacteria that was present in pot 10B than in the bacteria in the pot 5BP.

In contaminated soil with high levels of TPH, the number of bacteria usually decreased because hydrocarbons can affect the population of bacteria and bacterial community structure (Tang et al. 2010). In contrast to 5% contaminated soil, soil with 10% oil content had a higher bacterial amount at the end of the study. In the fourth week, the total number of bacteria decreased except in the 10B pot. Increased microbial populations at higher crude oil concentrations, showed that the presence of crude oil in the soil can prevent rapid evaporation of water from the soil, so that the contaminated soil is always wet in the soil micro-environment. In addition, in the soil with a high concentration of crude oil, the presence of water in the micro-environment can also prevent the spreading of crude oil non-polar toxic material into the micro-environment that strengthen bacteria, but on the other hand, the absence of sufficient oxygen reduced the degradation of crude oil by bacteria in soil with high concentrations of crude oil. In contrast, at lower concentrations of crude oil, the possibility of decreasing soil moisture is high due to the rapid evaporation of water. Soil moisture is one of the most important elements for the growth and reproduction of microorganisms (Al Mailem et al. 2010; Ayu et al. 2011). In all pot with cultivated plants, the microbial population is higher than in non-vegetation pots, as shown in the rhizosphere, where the population of soil microbial increases (Maqbool et al. 2013).

The pH soil testing results are very diverse, but still within the range of pH that are in accordance with the standard quality of the Ministerial of Environmental Decree No. 128 of 2003 for petroleum degradation, which is 6-9. The graph shows that 5BP always has a pH value lower than the three other pots, then followed by 5B, 5P, and 5C respectively. In the contaminated soil with 10% oil content, the results varied, but the pH result in 10B pot shows the lowest pH value over several weeks followed by 10 BP, 10C, and 10P.

The reduction of pH value indicates the activity of microorganism. Most of bacteria grow in neutral or slightly alkaline pH. pH affect the cellular function of microorganisms, membrane transport, and the equilibrium of the reaction (Cookson, 1990 in Sugoro, 2002). The reduction in the pH value is caused by the activity of bacteria that formed acid metabolites. Biodegradation of alkanes that contained in the oil will form alcohol and then established fatty acids. Alkane fatty acid degradation products will be further oxidised to form acetic acid and propionic acid, thus lowering the pH value of the medium (Nugroho 2006). The aerobic degradation of alkanes by *Acinetobacter* sp. used alkane monooxygenases to convert hydrocarbons into alcohol.

The temperature of contaminated soil on both levels of oil content are varied and depend on air ambient. But the temperature test results on the eight pots are still within the range of temperatures that can be tolerated by microbes to perform its activities, which is in the range 25-45^{o}C.

Based on One-Way-ANOVA statistical tests on soil contaminated with 5% oil content, the results show that there were no significant differences in the final TPH result between all the treatments given because the significance value of all are more than 0.05 ($p > 0.05$). However, after further analysis tests were conducted which compare the relationship between treatments that influence final TPH results, there was a significant difference among the combination between plants with microorganism (5BP) treatment because the significance is 0.038 ($p < 0.05$). On the other hand, in the contaminated soil with 10% oil content, there were no significant differences between final value of TPH with all treatments and relationship between treatments as well.

Moreover, correlation and regression analysis was also conducted to analyze the relationship between parameter studies and the influence of TPH results to them. Based on the result of this analysis, in both the 5% and 10% petroleum contaminated soil, the final percentage of TPH has a strong influence and impact on pH because $p < 0.01$, but has no influence on temperature values because $p > 0.05$. In addition, the influence of TPH percentage 0n the pH and temperature value in 5% petroleum contaminated soil is 52.5% while 37.4% was influenced by other factors. The influence of TPH percentage against pH and temperatures value in 10% petroleum contaminated soil is 62.6%. Furthermore, based on a regression double linear test in both the 5% and 10% petroleum contaminated soil, it is concluded that pH was more influenced by the TPH result compared to the temperature variable (due to p>0,01).

IV. CONCLUSIONS

Some treatments that were given in soil contaminated with 5% and 10% of oil content, namely the addition of compost (C), microorganisms (B), planting of plants (P), and combinations of both plant and microorganisms (BP) succeeded in reducing the level of TPH and BTEX contained in the petroleum contaminated soil. In 5% petroleum contaminated soil, the combination of microorganisms and plants (5BP) generated the largest TPH removal percentage value of 87.1%. This happened due to the symbiotic mutualism between the plants and microorganisms. On the other hand, in 10% petroleum contaminated soil, the addition of microorganisms (10B) generated the highest removal percentage, which was 76.2%. A combination between microorganisms and plants are not effective in soil contaminated with oil content of 10% because the plants can not survive in contaminated soil with a high level of oil content. Moreover, the percentage degradation of BTEX in the combination treatment of microorganisms and plants in 5% petroleum contaminated soil is 68.35%, whereas the addition of bacteria treatment in 10% petroleum content of contaminated soil (10B) has 84.91% removal of BTEX.

The degradation process of TPH and BTEX affect the environmental factors namely pH and temperature. There were no significant differences between TPH result percentage and all treatments given in both the 5% and 10% petroleum contaminated soil. Furthermore, based on the correlation and regression analysis, it showed that TPH affect the value of pH significantly with $p < 0.05$, while the value of TPH does not affect temperature since the value of $p > 0.05$.

SUGGESTIONS

a. Need to do further research with more variation on initial oil content, such as levels of oil of 1%, 3%, 5% and 7%, so it can be seen more clearly in which level of oil content the plants are unable to grow effectively and the most optimum degradation of the TPH.

b. Phytoremediation is usually effective in soil which is not more than 3% of oil content, while the amount of oil spilled in the field often exceeds 3%. Accordingly there needs to be further research on the combination of bioremediation and phytoremediation in the series formation, which means bioremediation has to be done first until the contaminated soil reach the oil content that can be tolerated by plants, then phytoremediation applied as tertiary treatment.

c. Microorganisms which are added to the soil in this experiment were exogenous microorganisms and there were no indigenous microorganisms, while indigenous microbes itself actually can degrade hydrocarbons as well, so in the further research, isolation, acclimatization, and identification of bacteria in contaminated soil need to be done so that the type and potency of indigenous microorganisms could be known.

ACKNOWLEDGEMENTS

This study was supported by Laboratory of Process, Technology, Research, and Development Center for Oil and Gas Technology (LEMIGAS), Central Forensic Laboratory, and Civil and Environmental Department University of Indonesia.

REFERENCES

Abdel-El-Haleem, D. (2003). Acinetobacter: Environmental and Biotechnological Applications. *Academic Journal*, 71-74.

Al-Mailem, D., Sorkhoh, N., Al-Awadhi, H., Eliyas, M., & **Radwan, S.** (2010). Biodegradation of crude oil and pure hydrocarbons by extreme halophilic archaea from hypersaline coasts of the Arabian Gulf. *Extremophiles*, 321-328.

Ayu, K., Toyama, T., & **Mori, K**. (2011). Bioremediation of crude oil by white rot fungi Polyporus sp. S133. *J Microbiol Biotechnol 21*, 995-1000.

Chaudhry, Q., Blom-Zandstra, M., Gupta, S., & **Joner, E.** (2005). Utilising the synergy between plants and rhizosphere microorganisms to enhance breakdown of organic pollutants in the environment. *Environ. Sci. Pollut. Res. 12*, 34-48.

Gerhardt, K., Huang, X., Glick, B., & **Greenberg, B.** (2009). Phytoremediation and Rhizoremediation of organic soil contaminants: potential and challenges. *Plant Science*, 20-30.

Kaimi, E., Mukaidani, T., Miyoshi, S., & **Tamaki, M.** (2006). Ryegrass enhancement of biodegradation in diesel-contaminated soil. *Environmental and Experimental Botany 55*, 110-119.

Leme, D., Grummt, T., De Oliveira, D., Sehr, A., Renz, S., Reinel, S., Marin-Morales, M. (2012). Genotoxicity assessment of watersoluble fractions of biodiesel and its diesel blends using the Salmonella assay and the in vitro MicroFlow kit (Litron) assay. . *Chemosphere 86*, 512-520.

Maqbool, F., Wang, Z., Malik, A., Pervez, A., & **Bhatti, Z.** (2013). Rhizospheric biodegradation of crude oil from contaminated soil. *Advan Sci Lett 19*, 2618-2621.

Mohsenzadeh, F., Nasseri, S., Mesdaghinia, A., Nabizadeh, R., Zafari, D., Khodakaramian, G., & **Chehregani, A.** (2010). Phytoremediation of Petroleum-polluted Soils: Application of Polygonum aviculare and Its Root-associated (penetrated) Fungal Strains for Bioremediation of Petroleum-polluted Soils. *Ecotoxicology and Environmental Safety 73*, 613-319.

Munawar, Mukhtasor, & **Surtiningsih, T.** (2007). Bioremediasi Tumpahan Minyak Mentah Dengan Metode Biostimulasi Nutrien Organik di Lingkungan Pantai Surabaya Timur. *Berk. Penel. Hayati*, 91-96.

Muratova, A. Y., Golubev, S., Wittenmayer, L., Dmitrieva, T., Bondarenkova, A., Hirche, F., Turkovskaya, O. (2009). Effect of the polycyclic aromatic hydrocarbon phenanthrene on root exudation of Sorghum bicolor (L.). *Moench. Envirron. Exp. Bot. 66*, 514-521.

Nie, M., Zhang, X., Wang, J., Jiang L. F., Yang, J., Quan, Z., Li, B. (2009). Rhizosphere effects on soil bacterial abundance and diversity in the Yellow River Deltaic ecosystem as influenced by petroleum contamination and soil salinization. *Soil Biol. Biochem. 41*, 2535-2542.

Nugroho, A. (2006). Biodegradasi 'Sludge' Minyak Bumi Dalam Skala Mikrokosmos. *Makara Teknologi 10 (2)*, 82-89.

Pusdiklat Migas. (2011). *Laporan Arus Minyak Pusdiklat Migas Cepu Tahun 2011.* Cepu.

Salt, D.E., R.D. Smith and **I. Raskin.** 1998. Annual Review Plant Physicology and Plant Molecular Biology: Phytoremediation. Annual Reviews. USA. 501-662.

Setyawan, E. Y., & **Nanto, R. D.** (2011). *Bioremediasi Lahan Terkontaminasi Minyak Bumi Dengan Menggunakan Bakteri Bacillus cereus Pada Slurry*

Bioreaktor. Surabaya: Institut Teknologi Sepuluh Nopember.

Sugoro, I. (2002). *Bioremediasi 'Sludge' Limbah Minyak Bumi Lahan Tercemar Dengan Teknik 'Land Farming' Dalam Skala Laboratorium*. Bandung: ITB.

Sulistyono, S. M. (2012). Kajian Dampak Tumpahan Minyak dari Kegiatan Operasi Kilang Minyak Terhadap Kualitas Air dan Tanah (Studi Kasus Kilang Minyak Pusdiklat Migas Cepu). *Jurnal Ekosains Vol. IV*, 2.

Susaria, S., Medina, V., & **McCutcheon, S.** (2002). Phytoremediation: an ecological solution to organic chemical contamination. *Ecol. Eng. 18*, 647-658.

Tang, J., Wang, R., Niu, X., Wang, M., Chu, H., & **Zhou, Q.** (2010). Characterisation of the rhizoremediation of petroleum-contaminated soil: effect of influencing factors. *Biogeosciences*, 3961-3969.

Yousaf, S., Andria, V., Reichenauer, T. G., Smalla, K., & **Sessitsch, A.** (2010). Phylogenetic and Functional Diversity of Alkane Degrading Bacteria Associated with Italian Ryegrass (Lolium multiflorum) and Birdsfoot Trefoil (Lotus corniculatus) in a Petroleum Oil-Contaminaterd Environment. *Journal of Hazardous Materials*, 523-432.

Yu, X., Wu, S., Wu, F., & **Wong, M.** (2011). Enhanced dissipation of PAHs from soil using mycorrhizal ryegrass and PAH-degrading bacteria. *Journal of Hazardous*, 1206-1217.

Zhang, Z., Zhou, Q., Peng, S., & **Cai, Z.** (2010). Remediation of Petroleum Contaminated Soils by Joint Action of Pharbitis nil L. and Its Microbial Community. *Science of the Total Environment*, 5600-5605.

http://www.egr.msu.edu/tosc/akron/factsheets/fs_pdf.pdf diakses pada 9 Desember 2014, pukul 23.24.

http://www.epa.gov/teach/chem_summ/BENZ_summary.pdf diakses pada 16 November 2014, pukul 23.11.

http://www.esdm.go.id/berita/56-artikel/4586-peluang-investasi-migas-di-indonesia.pdf diakses pada 4 Desember15.16.