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Effect of Provisioning Bacterial Isolates and NP Fertilization on Total Microorganism and Degradation Level of Contaminated Inceptisol Soil

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ABSTRACT

A study on the influence of provisioning bacterial isolates and NP fertilization on total microorganism and degradation level of hydrocarbon has been conducted to improve land damages due to the pollutant. Isolates of *Pseudomonas fluorescens* bacteria were delivered into inceptisol soil and that of the soil by fertilizating the nitrogen-phosphorus with N low, N medium, N high, as well as P low, P medium and P high of dosages. The results showed that the bacteria and fertilizers significantly influenced total microorganisms and degradation level of hydrocarbons. The total bacteria increased significantly in the polluted soil due to both the bacterial isolates and fertilizers. Consequently, the hydrocarbon content decreased significantly in the bacteria provisioned soil compared with the soil without bacterial isolates. Thus, the bacterial delivery into the hydrocarbon polluted soil has significantly enhanced the degradation level of the pollutant. Continuously, when fertilizing the polluted soil with fertilizers of nitrogen-phosphorus, it also successfully reduce the hydrocarbon content in the polluted soil. This result assumed that the isolates bacteria and the fertilizers effectively improve the damages of polluted soil. Moreover, there was a very significant interaction between the isolates bacteria with the fertilizers. The best results for reducing hydrocarbon in the inceptisol soil was achieved with the medium dosage of nitrogen-phosphorus fertilization.

Key words: Hydrocarbon, inceptisol, fertilizer, nitrogen, phosphorus

INTRODUCTION

Crude oil as one of the hydrocarbons spills frequently either in land or territorial waters. The spill can cause the environmental pollution in various ways. Natural disasters and accidents on the processing unit and the transport of oil can cause contamination of soil and reduction of the land productivity. Today, the decreasing of land productivity because of pollutants such as hydrocarbons is directly related to the activity and the decrease of agricultural output. Not only that but also it can spoil the environment. Currently in the cases of environmental pollution more and more were found. The pollution of soil caused by the spills of crude oil or hydrocarbon is the main problems around the world (Atlas and Bartha, 1987). In Asia, decreasing land productivity because of soil, water and air pollution are the main problem in improving agriculture productivity, especially for ensuring food security (Wassmann *et al.*, 2009). Indonesia, as one of disaster-prone countries and the users of crude oil (hydrocarbons), it is necessary to find ways and methods that are appropriate to handle contaminated soil, particularly pollution caused by hydrocarbons (Sartika, 2005).

As one pollutant of organic compounds, unless we handle it well, exploration and production of petroleum and natural disasters will have a potential to pollute environment. The examples of the prone lands contaminated hydrocarbon are the soils located along the coastal lines or coastal area. The kind of the soil is generally dominated by the entisol and inceptisol soil. Therefore, the main focus on remediation land is also conducted by considering what type of the contaminated soil. As we know, bioremediation technique is one of the ways often used to remediate the polluted land. The technique is applied by combining microorganisms and plants (Salt *et al.*, 1995; Gladstones *et al.*, 1998; Wenzel *et al.*, 1999). Employing soil microorganisms can serve to participate in the remediation of hydrocarbon-contaminated soil. In addition, the majority of products in the hydrocarbon molecule may provide a source of carbon and nitrogen for microbial growth (Van Hamme *et al.*, 2003).

Hydrocarbon degradation in soil is influenced by several factors such as temperature, water content, soil organic matter, soil biota and nutrient supply or fertilization (Bragg *et al.*, 1994; Wright *et al.*, 1997; Margesin *et al.*, 2000). Furthermore, Lin *et al.* (1999) reported that fertilizer application or administration of nutrients could increase the population growth of marsh plant growth, soil microbial population, increased soil microbial respiration and demonstrate the potential to enhance biodegradation of hydrocarbons in the soil.

Several studies have showed that bioremediation with fertilizers or nutrients in the soil could increase the degradation of hydrocarbons in the soil. Bioremediation with fertilizer application could significantly reduce the total targeted normal hydrocarbons and total targeted aromatic hydrocarbons (Lin *et al.*, 1999). Additionally, Sartika (2005) approved that biodegradation of crude oil (hydrocarbons) were easily degraded by microorganisms. The concentration of petroleum hydrocarbons or petroleum hydrocarbon index decreased between 21 to 44% within 14 days after giving fertilizer.

However, the main problems in improving the soil-contaminated soil is how to investigate the characteristics of the land as contaminated material and find the appropriate method and also not to create new problems. Beside that, the used method should be with a low-cost assembly technology and environmentally friendly. The use of fertilizers, plants and bacteria in polluted soil isolates proved that they could stimulate the growth of microorganisms which degrading hydrocarbons in contaminated soil (Simonich and Hites, 1995; Walworth *et al.*, 1997; Siciliano and Germida, 1998; Susarla *et al.*, 2002; Siciliano *et al.*, 2003).

Thus, the purpose of this study was to assess the bioremediation techniques to enhance degradation of hydrocarbons in contaminated soil by using bacterial isolates and fertilizers accordingly. In detail the purposes that we achieve are: (a) studying how the effect of the bacterial isolates given to the total hydrocarbons and total microorganisms that could degrade hydrocarbons in the inceptisol soil is, (b) determining the effect of N and P fertilization or nutrient delivery to the development of microorganisms and the degradation of hydrocarbons in contaminated soil and (c) investigating the interaction between the effects of inceptisol soil with bacterial isolates given by N and P fertilization to the total of hydrocarbon degrading microorganisms and the total of hydrocarbon degradation in contaminated soil.

MATERIALS AND METHODS

Soil preparation, provisioning bacterial isolates and fertilization: The experiment was carried out by using inceptisol soil that provisioned with bacterial isolates and without bacterial isolates. The prepared soil was mixed with 13.3 g kg^{-1} hydrocarbons (crude oil produced by the State Owned oil and gas company of Indonesia called PERTAMINA). The soil characteristics were performed and showed in Table 1. Then, the mixed soil was given with fertilizer N as NH_4NO_3 and P as KH_2PO_4 with different doses. Doses of fertilizer N and P were tested as follows: N low (50 mg kg^{-1}), N medium (100 mg kg^{-1}), N high (1000 mg kg^{-1}), P low (25 mg kg^{-1}), P medium (50 mg kg^{-1}), P high (500 mg kg^{-1}). The standards NP fertilization were determined by previous research (Syafruddin *et al*., 2010). The isolates of *Pseudomonas fluorescens* bacteria were inoculated into the mixed soil with amount of 10 ml kg^{-1} soil. Continuously, each of the treated soil of 100 g was put into a bottle and incubated in an oven with a temperature of 30°C and 30% Water Holding Capacity (WHC). Finally, the soil was mixed every two days (agitation) with a special stirrer.

The experimental design: The two factors experiment was designed as a Completely Randomized Design (CRD) with three replications. The first factor was bacterial isolates in two levels: inceptisol soil without bacterial isolates and with bacterial isolates. The second factor was NP fertilization in ten levels (without fertilizer, N low, N medium, N high, P low, P medium, P High, N+P low, N+P medium and N+P high) as the standards mentioned above.

The parameters observed: After six weeks of provisioning with bacterial isolates, hydrocarbons (HC: heptane-extractable hydrocarbon concentrations) extracted and performed HC analysis by Gas Chromatography (GC). The hydrocarbon detection conducted between C10-C40 (adopted from ISO 16706:2004). Furthermore, the total number of microorganisms were measured in the soil is by using method of NA (Nutrient Agar) to calculate the Colony Forming Units/g soil.

Statistical analysis: Analysis of variance (ANOVA) with the two factors would be used to determine significant differences between the treatments of provisioning bacterial isolates (total microorganism) and the fertilizers by using Tukey HSD test at level 5% ($p<0.05$; HSD-test).

Table 1: Characteristics of the inceptisol soil

Parameter	Value	Methods
pH H_2O	7.22	pH 1:2.5
pH KCl	6.21	pH 1:2.5
C-organic (%)	2.04	Walkley and Black
N total (%)	0.18	Kjeldahl
P av (ppm)	9.23	Bray II
K (meq/100 g)	0.26	NH_4OAc pH 7
KTK (meq/100 g)	20.21	NH_4OAc pH 7
Texture	Silty clay loam	Pipette
Sand (%)	13.72	
Silt (%)	56.28	
Clay (%)	30.00	

RESULTS

The effect of bacterial isolates to the total hydrocarbons in inceptisol soil: Results of the analysis range of the F-test showed that the factor of bacterial isolates significantly affected on the content of hydrocarbons in the inceptisol soil. The average content of hydrocarbons in the soil can be seen in Table 2. The results showed that the hydrocarbon content of the inceptisol soil with bacterial isolates was lower compared with the soil without bacterial isolates. Therefore, the provision of bacterial isolates effectively reduced the total hydrocarbons in the contaminated soil.

The effect of fertilization to total hydrocarbons in soil: Results showed that the Nitrogen-Phosphorus fertilization was very significantly affected on the content of hydrocarbons in the inceptisol soil (Table 3). Therefore, the fertilization effectively reduced the total hydrocarbons in inceptisol soil. In the fact, the fertilizer with a higher content of N and P is not efficient to reduce the content or total of hydrocarbons in the contaminated soil.

Interactions between bacterial isolates and fertilization to total hydrocarbons: There is a very real interaction between the factors of bacterial isolates and fertilizers (Table 2). The content of hydrocarbon in inceptisol soil without bacterial isolates has a close connection with the treatment of fertilizers. In the inceptisol soil with bacterial isolates, we also successfully reduced hydrocarbon content. The result showed that the soil with bacterial isolates and medium NP fertilization gave the lowest total hydrocarbon compared with other treatments. The condition is very different from the inceptisol soil without bacterial isolates.

The effect of bacterial isolates to the total microorganisms: The provision of bacterial isolates significantly affected on the total content of microorganisms, *Pseudomonas fluorescens*, in

Table 2: Average of the total of hydrocarbon and microorganism in the inceptisol soil without and with bacterial isolates

Provision of bacterial isolates	Total of hydrocarbon (µL kg^{-1})	Total of microorganism ($\times 10^4$ CFU kg^{-1})
Inceptisol soil without bacterial isolates	272.80^{b}	2093.50^{a}
Inceptisol soil with bacterial isolates	24.73^{a}	4594.33^{b}
Tukey HSD test (p<0.05)	0.17	7.17

Values followed by same letters within same column are not significant at p<0.05 by Tukey HSD test

Table 3: Average of the total of hydrocarbon and total of microorgamism in various fertilizations of nitrogen, phosphorns and the combination of the both fertilizers

Fertilization	Total of hydrocarbon (µL kg^{-1})	Total of microorgamism ($\times 10^4$ CFU kg^{-1})
Without fertilizer	649.05^{i}	879.58^{a}
N low	62.68^{b}	2117.50^{b}
N medium	62.49^{b}	2591.67^{e}
N high	86.09^{c}	3354.58^{i}
P low	56.30^{a}	2551.67^{d}
P medium	111.07^{e}	3363.33^{i}
P high	113.63^{f}	2694.17^{g}
N+P low	118.53^{h}	2633.33^{f}
N+P medium	117.47^{g}	5331.67^{k}
N+P high	110.36^{d}	2891.67^{h}
Tukey HSD test (p<0.05)	0.43	28.32

Values followed by same letters within same column are not significant at p<0.05 by Tukey HSD test

Table 4: Average of the total of hydrocarbon and the total microorganism in inceptisol soil

	Total of hydrocarbon (μL kg^{-1})		Total of microorganism ($\times 10^4$ CFU kg^{-1})	
Fertilization	Inceptisol soil without bacterial isolates	Inceptisol soil with bacterial isolates	Inceptisol soil without bacterial isolates	Inceptisol soil with bacterial isolates
Without fertilizer	1266.59^{iB}	31.51^{cA}	1423.33^{cA}	1510.00^{aB}
N low	101.73^{bB}	23.62^{bA}	1213.33^{bA}	2506.67^{bB}
N medium	101.72^{bB}	23.25^{bA}	1203.33^{bA}	4453.33^{cB}
N high	140.37^{cB}	31.81^{cA}	1178.33^{aA}	5303.33^{gB}
P low	89.03^{aB}	23.57^{bA}	1476.67^{dA}	5003.33^{dB}
P medium	198.56^{eB}	23.58^{bA}	3803.33^{hA}	5013.33^{dB}
P high	203.63^{fB}	23.63^{bA}	1683.33^{eA}	5113.33^{fB}
N+P low	213.50^{gB}	23.57^{bA}	1853.33^{gA}	5016.67^{deB}
N+P medium	223.98^{hB}	10.96^{aA}	5273.33^{iA}	7003.33^{hB}
N+P high	188.93^{dB}	31.80^{cA}	1826.67^{fA}	5020.00^{eB}
Tukey HSD test (p<0.05)	0.61		15.06	

Values followed by the same uppercase in total of hydrocarbon at the same row and different columns are not significant at Tukey HSD test (p<0.05), Values followed by the same lowercase in total of microorganism at the same columns and different rows are not significant at Tukey HSD test (p<0.05)

inceptisol soil. The average content of the microorganisms in the soil can be seen in Table 3. The highest total of microorganisms is found in inceptisol soil with bacterial isolates. It is significantly different from the soil without bacterial isolates.

The effect of fertilization on the total of microorganisms in soil: The fertilization factor has a very real effect on the total content of microorganisms in the inceptisol soil (Table 4). The highest total of microorganisms is found in the medium N+P fertilization and is significantly different with the others. We found that in inceptisol soil without bacterial isolates, the nitrogen fertilizer is not effective to stimulate the activity of microorganism. However, the phosphorus fertilizer is successfully to enhance the total microorganism in the soil. In other way, we discovered that in inceptisol soil with bacterial isolates, both fertilizers could increase the activity of soil microorganism (Table 4).

Interactions between bacterial isolates and fertilization to total microorganisms: Results of the analysis range of the F-test shows that the provision of bacterial isolates gives very real effects to the total content of microorganisms in the soil (Table 2). In inceptisol soil without bacterial isolates, we found a connection with the treatment of fertilizers. The population of the microorganism increased by the fertilizer, especially nitrogen and phosphorus. We also successfully to enhanced the total numbers of organism in the inceptisol soil with bacterial isolates. The result of isolates soil bacteria with medium N+P fertilization is the highest total of the microorganism compared with other treatments and are significantly different from other fertilization treatment.

DISCUSSION

When provisioning bacterial isolates and medium dosage of nitrogen-phosphorus, the numbers of microorganism in inceptisol soil were successfully increased. Moreover, the content of hydrocarbon in polluted soil was also decreased significantly. A study carried out in line with Unterbrunner *et al.* (2007) was stated that the degradation of hydrocarbons in the soil increased with the use of plants and the application of P fertilizer. Contribution of vegetation and fertilizer P effectively increased microorganisms in the soil. The high numbers of microorganisms in the soil encouraged hydrocarbon degradation due to the microorganisms use hydrocarbons as a source of food and energy. Thus, the total microorganisms in the soil can reduces the content of hydrocarbons in contaminated soil.

Another research was carried out in line with what has been investigated by Lin *et al.* (1999) and Van Hamme *et al.* (2003) who concluded that the application of fertilizer or provision of nutrition enhanced degradation of hydrocarbons in polluted soil due to the occurrence of rapid plant growth and the increase of microbial population levels in the soil. Additionally, the activity of microorganisms showed the large number of microbes that degraded hydrocarbons in the soil. Furthermore, several studies (Pritchard and Costa, 1991; Wright *et al.*, 1997; Bragg *et al.*, 1994; Margesin *et al.*, 2000) suggested that N and P were the limiting factor in the process of hydrocarbon degradation in contaminated soil. Ideally, N and P giving with the right dose were supported by other factors likely to increase the total and the performance of soil microorganisms in degrading hydrocarbons in contaminated soil.

Interactions that occurred between the N and P fertilization by providing bacterial isolates (Table 3, 4) to total microorganisms and total degradation of hydrocarbons were consistent with the research that has been carried out before (Ijah and Okang, 1993; Ubochi *et al.*, 2006). In addition, Sartika (2005) found that fertilization was offset by using plants had ability to reduce the oil concentration. The concentration of petroleum hydrocarbon index (IPH) was around 21-44% within 14 days after treatment.

The quick and high growth of microorganisms in the provisioned *Pseudomonas fluorescens* isolates (Table 2) showed that indigenous microorganism was not effectively reduced hydrocarbon in polluted soil. Therefore, we should be given inoculation of microorganisms from the outside (exogenous) both the form of the mixture and the single bacterial culture that could potentially degrade the pollutants in the contaminated soil (Johnson *et al.*, 1996; Pathak *et al.*, 2008). The results of this study also consistent with previous research conducted by Donnelly *et al.* (1994) that showed the bacterium *Pseudomonas* sp. was one bacterium that could be used in hydrocarbon remediates contaminated soil. Margesin and Schinner (1997) reported that the degradation of petroleum could be conducted by using bacteria such as *Pseudomonas fluorescens* because of the microorganism was able to describe the components of petroleum. The evidence was related to ability of the bacterium to oxidize hydrocarbons and made hydrocarbons as electron donors. As like the other microorganisms, this bacterium participated in the cleanup of oil spills with oil oxidizes into CO_2 gas.

In terms of fertilizer use, the study is also consistent with what was observed by Coulon *et al.* (2004) which stated that the addition of N and P enhanced the development of hydrocarbon degrading microorganisms and simultaneously encouraged the degradation of hydrocarbons in contaminated soil (Table 2). Several previous researchers expressed the same results that showed the addition of nutrients as N and P optimized degradation of hydrocarbons in contaminated soil. Increasingly, there was a match with the development of microorganism and the total hydrocarbons in the soil. The large numbers of microorganisms enhanced degradation level of hydrocarbon in polluted soil (Walworth and Reynolds, 1995; Braddock *et al.*, 1997; Ayotamuno *et al.*, 2006).

CONCLUSIONS

The provisioning bacterial isolates and NP fertilization significantly enhanced the total microorganisms and the degradation level of hydrocarbon in contaminated inceptisol soil. The medium dosage of the NP fertilization not only successfully increased the total of microorganism but also reduced the hydrocarbon in the soil. However, the NP fertilizer dosage depends on provisioning bacterial isolates to degrade the total of hydrocarbon. The best interaction was found in provisioning bacterial isolates and medium dosage of nitrogen and phosphorus fertilization.

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