EFFECT OF OIL BACTER, OIL FUNGI AND ALBIZIA *(PARASERIANTHES FALCATARIA* L. NIELSEN)-MYCCHORHIZA ON PHYTOREMEDIATION OF OILY SLUDGE CONTAMINATED SOIL

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Abstract

Phytoremediation is a promising technology for the clean-up of oily sludge petroleum-contaminated soils, especially in the tropics where climatic conditions favour plant growth and microbial activity and where financial resources are limited. The objective of this work was to test for their ability to stimulate consortium bacteria (oil bacter : Pseudomonas sp, Alcaligenes sp and Flavobacterium sp (m1) and consortium of fungi (oilfungi: Aspergillus sp1, Aspergillus sp2, Penicillium sp1 (m2) in soil contaminated with 30% and 35% (w/w) of oilysludge. After 1 months composted then planted with tropical legume plant species Albizia (Paraserianthes falcataria L. Nielsen)- mycchorhiza that could be used for phytoremediation. PAH (Polyaromatic Hydrocarbons) and heavy metals (Cd, Cr, Cu, Ni, Pb, Zn,) content were analyzed after 2, 6 and 8 months of phytoremediation in greenhouse experiments. Our results showed that after 8 months of phytoremediation, oil bacter performed better than oil fungi in reducing PAH in 30% of concentration oily sludge. Oil bacter treatment effectively decreased Pb (77,16%),Cu (68,56%), Cd (60,44%), Cr (76,24%) levels in 30%, while oil fungi only lowered Zn (87,17%) and Ni (71,32%) at concentration of 35% of oily sludge phytoremediation contaminated soil. The population of bacteria and percentage of infection mycorrhiza at the albizia roots of the waste 30% are higher than at inoculated by fungi, which is 76.67%. Growth of albizia (Paraserianthes falcataria L. Nielsen) was determined in oily sludge-contaminated soil with inorganic fertilizer. Resulted in the greatest reduction in PAH, heavy metals levels across the plant treatments following the 8 months of study. The studies demonstrated that oil bacter were considerably important for phytoremediation of oilysludge -contaminated soil-

Key words: bacter and fungi

INTRODUCTION

In Indonesia, oil refinery industries produce up to 1.2 million barrels per day with 150 thousand tons of waste per year. Most of moreover, 37.500 tons of this waste are considered hazardous to the environment (Santosa, 2009).

Oily sludge is the residual of oil distilled from crude oil processing companies, including hazardous and toxic waste, if referring to the rules of the Ministry of Environment Ministerial No. 18 and 33 year of 2009 (Ministry of Environmental Republic of Indonesia, 2009). This regulation clearly states that no activities are allowed to pollute the environment and all of the hazardous and toxic that are generated must be processed prior to the disposal. Bioremediation considered as a method to manage petroleum waste and contaminated soil by the process of decomposition of biological waste by utilizing microorganisms (Minister stipulation no.128 year of 2003). Based on our research bacteria and fungi obtained from the isolated oily sludge was *Pseudomonas sp, Alcaligenes sp, Flavobacterium sp.,* and *Aspergillus sp*1, *Aspergillus sp*2, *Penicillium sp*1 and *Penicillium sp*2.

Research in situ bioremediation of oily sludge contaminated soil by biostimulation of indigenous microbes (*Pseudomonas phosphoreum*) through adding manure was conducted at the Shengli oilfield in Northern China (Wuxing Li, 2010). After bioremediation

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thinly scattered wild plants, such as *Eleusine indica* (L.) Gaertn. and *Echinochloa crusgalli* (L.) Beauv, etc., were observed. Resulting from the substantial reduction of oil content in the soil due to microbial biodegradation.

Ismail, (2009) observed that the effluents from petroleum refineries contain a diverse range of pollutants including heavy metals. They are also contain oil and grease, phenols, sulphides, dissolved solids, suspended solids and BOD-bearing materials. An overview of heavy metal removal from industrial effluents with emphasis on biological methods is given. For instance, heavy metals removal in effluents from a petroleum refinery by water hyacinth were applied with restricted successful rate for the case of iron and zinc.

Phytoremediation is a further development of bioremediation techniques by utilizing the ability of various plants together with microorganisms to restore land or water bodies which are polluted. The principle of this technique is based on an optimization of indigenous microorganisms and plant root system to degrade the target compound in pollutant. Albizia plants (*Paraserianthes falcataria* L. Niesen) can be associated either with endomycchorhiza in extreme environments, critical nutrients, or water.

Oilysludge are characterized by the presence of recalcitrans, poliaromatic hydrocarbons (PAHs) and metal content. Analyses in year of 2009 showed that the oilysludge contains 30 types of PAHs with a chain of C (carbon) ranged from C12-C43 with concentration of Cu (91.9 ppm), Pb (u.d), Zn (251.9 ppm), Ni (264.2 ppm), Cd (0.215 ppm) and Cr (15.8 ppm). While the analyses year of 2000, oilysludge contained 66% of Total Petroleum Hydrocarbon (TPH), 43.56% to 60% of oil and grease, 0.86% of asphaltent, Cd (0.14 ppm), Cr (2.96 ppm), Cu (3.58 ppm), Ni (58.21 ppm), Pb (7.90 ppm) and Zn (73.53 ppm). Indicating that every refinery contain different characteristics of pollutant depending of drilling location.

MATERIAL AND METHOD

This study was conducted in Oilysludge concentration (L) with two levels ie, 30% (L1), 35% (L2), and the Consortium of Bacteria and fungi (M) with two levels with a consortium of bacteria *Pseudomonas sp, Alcaligenes sp and Flavobacterium sp* (m1) and consortium of fungi *Aspergillus sp*1, *Aspergillus sp*2, *Penicillium sp*1 (m2). Soil samples were taken at the time of planting (week-0 planting) to be analyzed first, and then at week 8, 24 soil samples were taken back to analyzed, for the final analysis of soil samples taken at week 32 after phytoremediation.

The main parameters to be measured in this study is the content of PAH (mg^{-1}. g^{-1}.%) and heavy metal content (ppm), and supporting parameters were total plate count (TPC) of microorganisms ($CFU.ml^{-1}$) and percentage of mycorrhizal infection (%).

Material

1. Consortium of bacteria *Pseudomonas sp, Alcaligenes sp and Flavobacterium sp* (m1) and consortium of fungi *Aspergillus sp*1, *Aspergillus sp*2, *Penicillium sp*1 (m2)
2. Oilysludge with two levels of concentration i.e, 30% (s1) and 35% (s2)
3. Albizia *(Paraserianthes falcataria* L. Nielsen)-mycchorhiza
4. Sands and Soil
5. Fertilizers

Methods

a. Treatment of Bacteria and Fungi

Preparation of starter medium with a mixed culture into a solid waste composting (oily sludge) with a concentration of 2% mixed with sand, soil sterilized. Starter medium was stored in bottles at 300 g, and was made by inserting isolate of bacteria (m1) and fungi (m2), this inoculum allowed to stand for ± 1 week.

Implanted medium starter in to treatment 30% and 35% oily sludge mixed with sand and soil 2:1 in the pot, composted for one month with inoculum and then planted with Albizia-mycorrhizal as phytoremediation for 2, 6 and 8 months.

b. Treatment of the plant

Seedling and mycorrhizal inoculation. Seedling media consisting of soil and sand with a ratio of 2:1 was inserted into a plastic with size of 5cm x 10cm, then sterilized. Albizia Seeds were soaked in hot water (±80^{o}C) for approximately one minute. Afterwards the seeds were re-soaked in cold water for about 24 hours, then were drained. Furthermore, seeds were planted in conjunction with albizia mycorrhizal 300g / 5 kg of mycorrhizal propagules in seedling media (1 plants require mycorrhizal propagules 15g).

Planting and Plant Maintenance Albizia

One-month-old plants were moved into a planting medium composting 30% and 35%. Plants were then maintained by watering regularly.

c. Determination

Determination of Polyaromatic Hydrocarbons (PAH)

Determination of the PAH content was conducted by adding 3 gr of samples into the beaker glass. Afterwards HCl was added until pH <2, then were stirred until smooth. Furthermore, anhydrous sodium sulfate was added into the sample to dry, then was wrapped with paper incorporated into soxhlet device, prior to extraction with methylene chloride until thick. Moreover, samples were analyzed using instruments Gas Chromatography Mass Spectrophotometer (GCMS) (Damiri, 2004).

Determination of heavy metal

Determination of heavy metal content was conducted by dissolving as much as 0.5 gram of sample in 10 ml of distilled water, (HCl and Nitric Acid 3: 1) in a beaker and were heated until dry. The samples were added 5 ml of nitric acid and were heated until late. When the samples were cooling down, 50 ml of aquadest was added. Afterwards, samples were centrifuged for 10 minutes at 3000-4000 rpm. Supernatant was taken then the content of heavy metals were measured using Atomic Absorption Spectrophotometry (AAS) (Damiri, 2004).

Determination of Degree of Infection mycchorhiza. Determination of the degree of mycorrhizal infection was carried out to observe the association between plants and mycorrhizae, than to find the resistance of mycorrhizae to critical lands.

Sample Root Isolation

Roots samples were taken from the plants aged 3 months. The roots were washed until there is no soil attached.

Sample Staining Roots –

Plant roots that have been washed were cut along 1cm and were soaked in a bottle with 10% KOH solution and were heated for 30 minutes at 90ºC. Afterwards, 10% KOH solution of each film discarded bottles and roots were rinsed 3 times by using distilled water. Subsequently, roots were soaked in 1% HCl solution for 5 minutes. Furthermore, a solution of HCl 1% was thrown back and the roots were soaked in a dye (Fuchsin), settling for 1-2 days and then ready to observe.

Observation Sample Roots

10 pieces of plant roots that have been coloring in the glass object were provided. Then, a little amount of distilled water was added and covered with glass cover objects. Three replication was made for each treatment. Furthermore, samples were observed under light microscope.

RESULTS

a. Content of Polyaromatics Hydrocarbon

Based on analysis by GC / MS (Gas Chromatography / Mass Spectrophotometry), prior to treatment identified types of PAH in oily sludge. In this study 6 types of hydrocarbon compounds were identified, namely: Heptadecane, n-Nonadecane, n-Heptacosane, Pentadecane ,2,6,10,14-tetramethyl-(CAS) pristane, Hexadecane 2, 6, 10, 14-tetramethyl, and Phenanthrene 2,5-dimethyl. Analysis of PAH content (%) during phytoremediation experiment showed that PAH levels decrease as represented with a total area of hydrocarbons (Fig. 1.)

Forthwith, preliminary test showed that there are six compounds contained in oily sludge with a carbon chain ranged from C16H14 to C20H42. Subsequently after composting bioremediation treatment for 2 months, a large decreased in hydrocarbon levels was observed up to 90%. However, PAH in the medium increased up to 17% with carbon chain ranged from $C_8H_8N_2S$ to C_4OH_{28}. The medium treated by phytoremediation using albizia plants (*Paraserianthes falcataria* (L.) Nielsen) mycorrhizal for 6 months. This treatment allowed to increase the level of hydrocarbons in the medium, which was also followed by multiplication in total population of microorganisms.

8 types of hydrocarbon compounds in oily sludge were identified. While after composting for 2 months 9 hydrocarbon compounds were identified with a carbon chain range from C19 to C24. Furthermore, After 6 months of phytoremediation, 28 hydrocarbon compounds were identified (Appendix 2). Then after 8 months of phytoremediation, 14 hydrocarbon compounds were identified with the range from C7 to C33 of carbon chain (Appendix 3). Changes was observed in the range of carbon chains which became longer and the number of compounds identified during the phytoremediation process showed that there is a breaking from carbon chains complex to a more simple carbon chains during the process of phytoremediation.

Compounds of PAH contained up to 45 is greatly increased and more varied types of carbon chain C5H11CL to C40H28, on the other hand hydrocarbon levels was steeply decreased until 90% after 8 months of phytoremediation.

Compounds contained in the medium increased with 8 compounds ranged from CL to C40H64 C3H4 with a new compound that has not existed before.

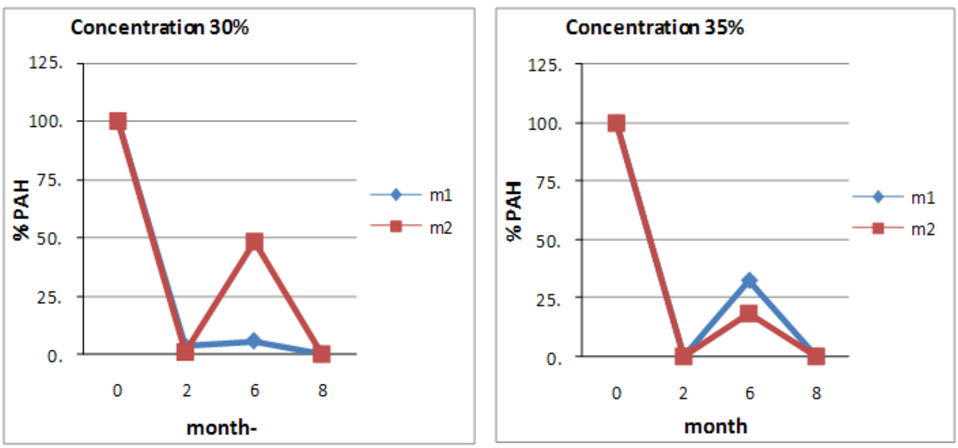

Fig. 1 Comparison of the addition of bacteria consortium (m1) and fungi consortium (m2) of the oilysludge 30% and 35% on PAH content (%) during phytoremediation

The carbon content in the treatment medium reduced progressively, and carbon chains became more simple to reach C3H4.

The decrease pattern of polyaromatics hydrocarbons (PAH) was not uniform. This was due to some degradation of PAH compounds and with a number of smaller rings, so that will take effect at the time measured (Maharani, 2003). Decreased of PAH levels may due to the activity of a variety microorganism contained in the medium. Activities of microorganisms in degrading of hydrocarbon content is unbelievably supported by nutrients that can increase the metabolism of microorganisms.

The existence of myccorhiza inoculation on the medium of the treatment favoured the decrease of hydrocarbon levels, because myccorhiza secrete enzymes which can produced by exoenzyme hydrocarbons. As confirmed by Donnelly & Fletcher (1994), that by enzyme released mycorrhizae, aromatic compounds in the oily sludge medium can be degraded. Hyphae of mycorrhiza also bind pollutants in the medium, so that contaminants do not inhibit the activity of hydrocarbon degrading microorganisms (Donnelly & Fletcher, 1994; Subiska, 2001).

The Content of Heavy metals

In the present study, there were 6 types of heavy metals were observed: Cu (copper), Pb (lead), Cr (chromium), Zn (zinc), Ni (nickel), and Cd (cadmium). Heavy metal content was measured during 8 months of experiment, on

0 (before composting), month 2 (after composting), on the 6 and 8 month after phytoremediation.

Percentage decrease of Cu was the greatest among the media with the addition of oil fungi on medium 30%, at 68,56%.

Pb significantly decreased at 30% of waste quantities in each media with the addition of a consortium of bacteria and fungi, at 77,16% and 75.40%. Percentage decrease in Zn concentration in media markets, the treatment with the addition of a consortium of fungi with a rate of 35% waste, as much as 87.17%. The largest decrease of Ni content occurred in the treatment with the addition of a consortium of bacteria on the amount of waste 35%, as much as 71.32%.

Cd levels decreased in all treatment media. The highest percentage decline occurred in the media with the addition of a consortium of bacterial treatment of waste levels of 30%, as much as 60.44%. The decrease of Cr content on the amount of waste 30% showed the same dose with 35% of waste, for treatment with the addition of a consortium of bacteria and fungi were 76.24% and 76.75%, respectively. At 30% amount of waste, fungi relatively appeared with more population. Similarly, a consortium of bacteria, on the 6th month of phytoremediation, fungal populations on both the dose of waste increased and then declined at month 8 after phytoremediation.

Cu (copper)

Percentage reduction in Cu levels were the greatest among the media with the addition of microorganism on the amount of waste consortium 30%, at 68,56% as shown in fig. 2.

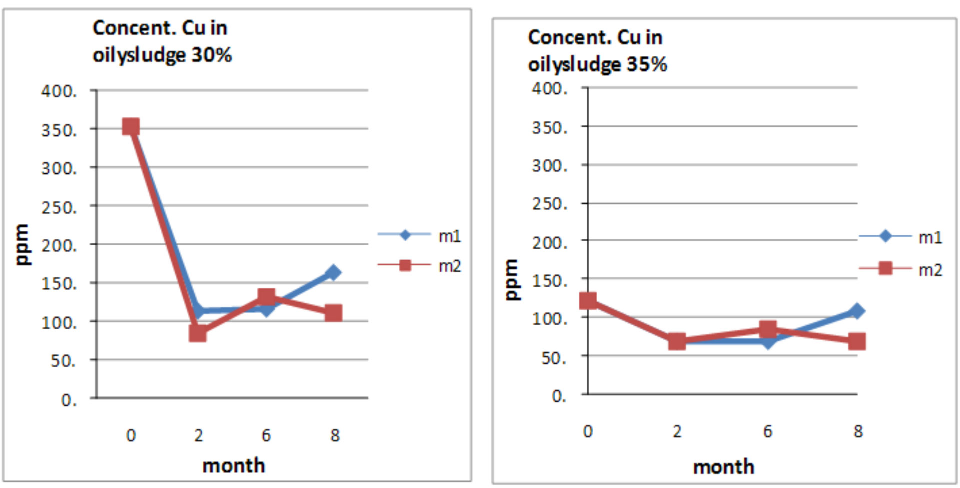

Fig. 2 Comparison of the addition consortium of bacteria (m1) and consortium of fungi (m2) on the amount of oilysludge 30% and 35% on Cu content during phytoremediation

In natural soil generally contain between 2-40 ppm of Cu (total). Soils containing more than 200 ppm of Cu is considered not to be used in agricultural land.

Pb (lead).

Result analysis of Pb showed that Pb levels decreased at a relatively high dose of 30% with the addition of consortium bacteria and fungi, at 77,16% and 75.40% (Fig. 3). However, the consortium of bacteria and fungi with a rate of 35%, respectively indicated that the consortium of bacteria reduce Pb levels greater than the consortium of fungi.

The decrease of heavy metal content in the media indicates a transfer or use metals by microorganisms and albizia mycorrhizal. According Vijayaraghavan & Sang Yun (2008), bacteria and fungi is a heavy metal biosorbent agent. The fungi of *Aspergillus* and *Penicillium* and bacteria Pseudomonas in the group reported as agent of potential biosorbent of heavy metals. Mechanism of heavy metal biosorption by microorganisms is a complex process, consisting of: metal transport through the cell membrane, ion exchange, and production of organic acids by microorganisms. Ion exchange occurs between ions in the cell membrane (polysaccharide) with metal ions in the form of bivalent (Kuyucak & Volesky, 1998 in Ahalya, *et.al*, 2003). Ion exchange process is done either by bacteria or fungi in heavy metal binding. In addition, the microorganisms produce organic acids (citric, oxalic, gluonnic, fumaric, lactic, and malic), which allows for chelating toxic metal. These organic acids help dissolve the metal compound and irrespective of the surface. Metals are absorbed by the carboxyl group of polysaccharides contained in the cell membranes of microorganisms (Ahalya, *et al*., 2003).

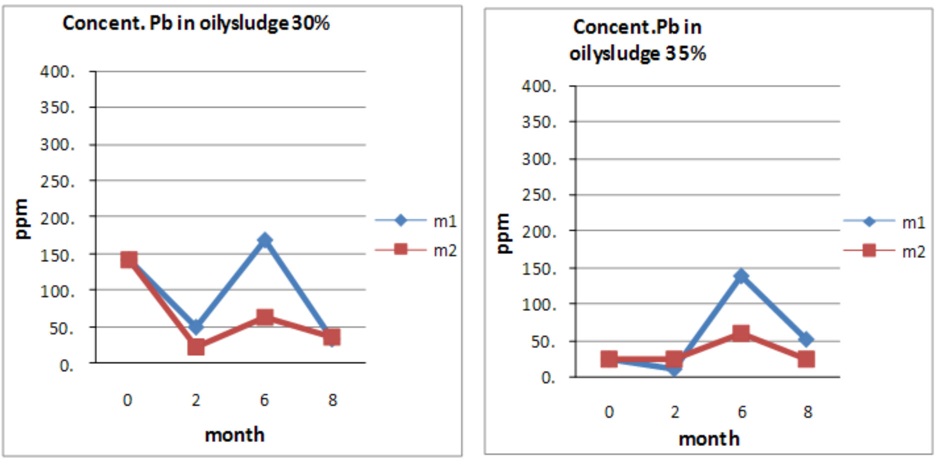

Fig. 3 Comparison of the addition consortium of bacteria (m1) and fungi (m2) on oilysludge 30% and 35% on Pb during phytoremediation

In this study chelating substances derived from organic acids produced by microorganisms may cause of the increase of Pb. Pb is the limited element availability for plants and needed in small amounts. However, the presence of Pb can be improved by adding a chelate to the soil material.

Decreased levels of heavy metals is also influenced by the existence of a symbiosis with the endomycchorhiza in the roots. Endomycchorhiza known to bind heavy metals in the carboxyl group in the cortex of host plants, the sheath cell wall polysaccharides and the hyphae (Suryatmana, *et al*, 2006).

Cr (Chromium)

The decrease of Cr content on the oilysludge 30% showed greater results than on the waste 35%, treatment with addition of a consortium of bacteria and fungi were 76.24% and 76.75% , respectively, as shown in Figure 4.

According to Sarwono (1994), the reaction of the acidic soil, micro elements would be easily soluble are nutrients required by plants in very small amounts, and it becomes toxic if are present in too large quantities. For examples of micro elements are Mn, Fe, Zn, and Cu.

Condition soil with acidic pH causing heavy metals contained in the medium becomes soluble and actively absorbed by plants. Heavy metals are absorbed, according to Connell & Miller (1995), can cause toxic to the plant. Excessive heavy metal content which can cause decreased growth, reduced crop productivity, and can cause death. The mechanism of metal toxicity by Ochiai in Heryando (1994), divided into 3 categories, namely:

- Block or impede the work of functional groups of biomolecules is essential for biological processes, such as proteins and enzymes.

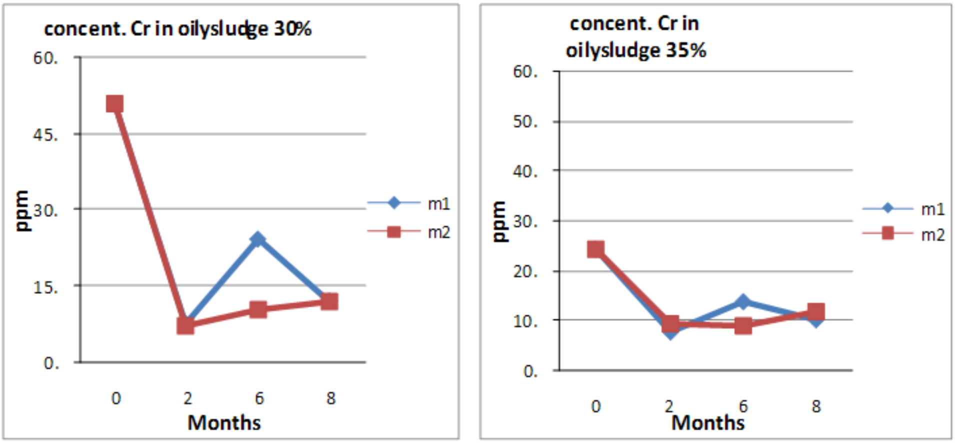

Fig. 4 Comparison of the addition consortium of bacteria (m1) and fungi (m2) on oilysludge 30% and 35% on Cr content during phytoremediation

- Replacing essential metal ions contained in the related molecule
- Hold modifications or changes in shape of the active clusters owned by biomolecules.

State of contaminated soil toxic heavy metals will inhibit the supply of plant nutrients. This situation, according to Setiawati *et al* (2003), is very beneficial to mycorrhizae, because it explores the role of mycorrhiza in phosphorus in the soil through hyphal external more effective at low soil phosphorus content. Similarly, Fitter & Hay (1991), confirmed that on nutrient-deficient soil phosphorus, mycorrhizal plants usually grow better than plants without mycorrhizal inoculation. But the opposite will happen on the ground well supplied with phosphate, the plants can show that level of infection is low.

According to Hidayat (1995), mycorrhiza can increase plant growth because it can increase the absorption of nutrients by plants. Endomycorrhiza that infect the host plant root system will produce intensively interwoven hyphae, so that mycorrhizal plants would be able to increase its capacity to absorb water and nutrients. Smooth hyphae size would allow the hyphae infiltrate the soil pores which are very small (micro), so that the hyphae can absorb water under conditions of very low water content (Subiksa Kilham, 2002). With the role of mycorrhiza in assisting the absorption of water and nutrients, the plant cells will rapidly grow and develop, so as to enhance the growth of plant height.

In addition to phosphorus, Dwidjoseputro (1994) showed that mycorrhizae can enhance the absorption of some nutrients such as N, K, Mg, Fe, Mn, Cu, and Zn, which are materials that play a role in the formation of chlorophyll. Moreover, chlorophyll will increase the photosynthesis process that will affect both the number of leaves and leaf surface area. This indicated that the inoculated mycorrhizal plants Albizzia is more effective in absorbing heavy metals. Galli in Khan *et al* (2000), states that mycorrhizae play an important role in protecting plant roots from toxic elements, among which heavy metals.

According Subiksa (2002), the mechanism of protection against heavy metals and toxic elements by mycorrhizal can through filtration effect, chemically deactivate, or accumulation of these elements in the hyphae of fungi. Mycorrhizal inoculated plants have the ability to suppress uptake of Pb. As confirmed by Suryatmana *et al* (2003), mycorrhizae are known to bind these metals and compounds in the carboxyl group pektak (hemicellulose) on the contact surface between the matrix and the mycorrhizal host plant, the sheath cell wall polysaccharides and hyphae.

Zn (Zinc)

Zinc levels decreased during phytoremediation. with the addition of a consortium of fungi in oilysludge 35% up to 87.17% as shown in Figure 5.

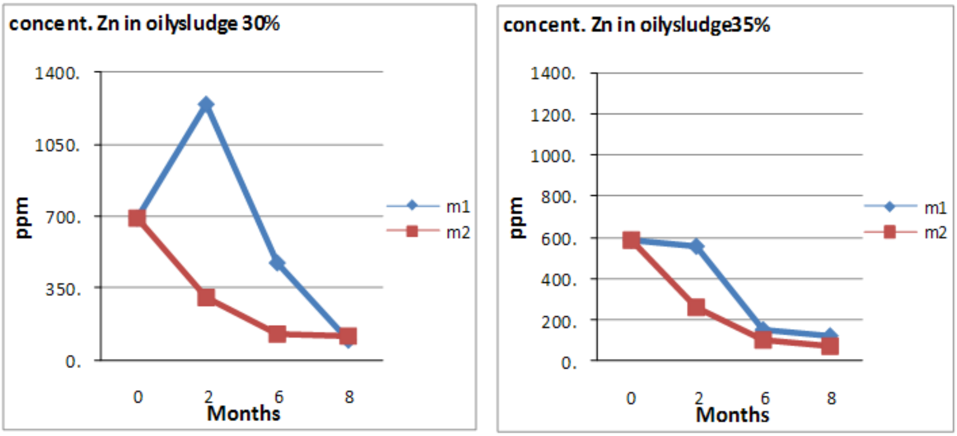

Fig. 5 Comparison of the addition consortium of bacteria (m1) and fungi (m2) on oilysludge 30% and 35% on Zn content during phytoremediation

Ni (Nickel)

Nickel content in the medium decreased after phytoremediation for 8 months with the addition consortium bacteria on oilysludge 35% amount 71.32% as were shown in Figure 6.

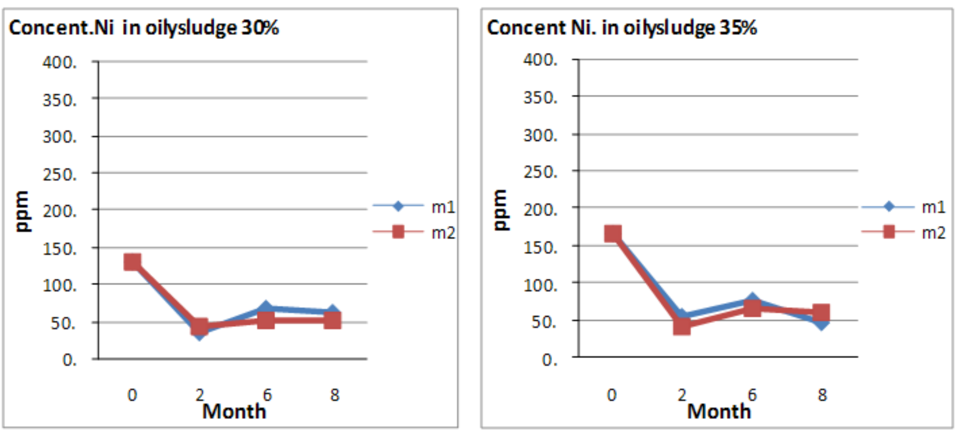

Fig. 6 Comparison of the addition consortium of bacteria (m1) and fungi (m2) on oilysludge 30% and 35% on Ni content during phytoremediation

Cd (Cadnium)

Results of analysis showed that levels of cadmium in all media had the highest percentage of reduction in the addition consortium of bacterial treatment of oilysludge levels of 30%, amount 60.44% as were shown in Figure 7.

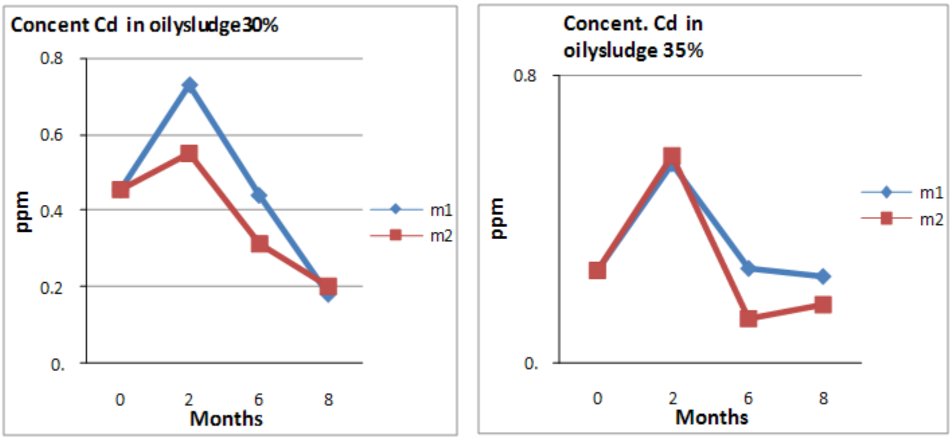

Fig. 7 Comparison of the addition consortium of bacteria (m1) and fungi (m2) on oilysludge 30% and 35% on Cd content during phytoremediation

Figure 7 showed that the levels of Cd on two and eight months after phytoremediation were decreased in both media. Furthermore, the medium with addition of consortium of fungi is relatively better in Cd levels as compared to the addition of consortium bacteria.

Total Plate Count (TPC) and infection percentage of mycchorhiza

Differences in populations of bacteria on 35% of oilysludge are relatively visible than at 30%. While on the 6 month of phytoremediation, bacterial population increased in both concentration then decreased after 8 months of phytoremediation as were shown in Figure 8 and 9.

The percentage of infection mycchorhiza at the albizia root and a consortium of bacteria inoculated from the waste at 30% was greater than inoculated by fungi, which was 76.67%. While in the concentration of 35% media inoculated with a consortium of fungi, the percentage of infection root of albizia endomycchorhiza is relatively higher as compared to inoculated by consortium bacteria.

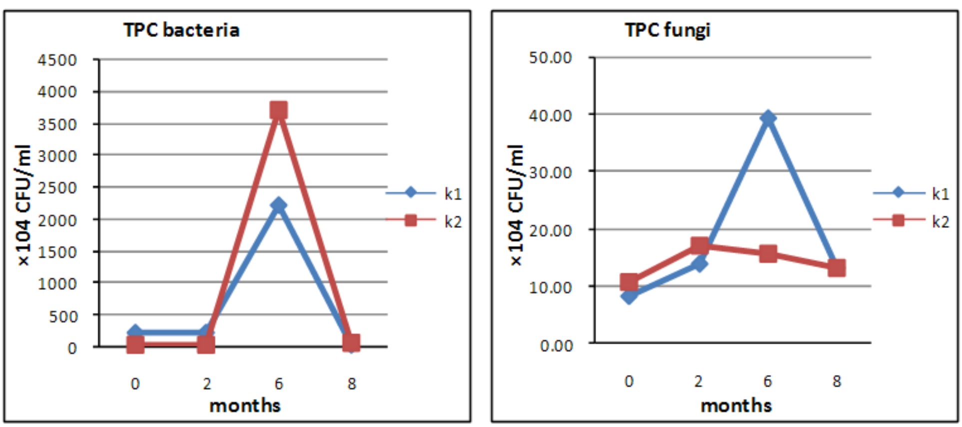

Fig. 8 Comparison total population of bacteria and fungi on oilysludge 30% (k1) and 35% (k2) during phytoremediation

Indigenous and exogenous microorganisms can cooperate and create positive interaction in polihydrocarbon degradation. According to Ward *et al.*, (1992), the addition of degrading microbial inoculation enhance biodegradation activity. as *Aspergillus* and *Penicillium sp.* degraded the carbon chain of PAH.

The ability of microbes in degrading the hydrocarbon chain, beginning with the dissolution of hydrocarbons in the liquid phase produced by microbes in the form of siderophore (biosurfactant) between oil and microbes (Rosenberg *et al*, 1979).

Microbial growth in hydrocarbon often followed by emultion of carbon sources that are not soluble in the medium, due to the extracellular agent which is formed during the fermentation of hydrocarbons (Zajic *et al*, 1977).

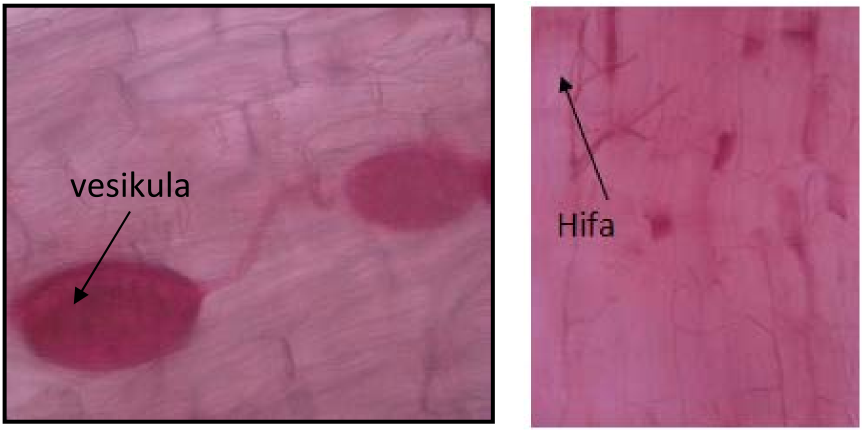

Fig 9. Colonization of endomyccorhiza in Albizia roots

According Suryatmana *et al* (2003) and Subiksa (2002), mycorrhizae are able to adapt to the soil containing a high metal, soil acidity and high aluminum content, which is not a limiting factor for mycorrhizal fungi, but it is a problem for plant growth.

CONCLUSIONS

1. Oil bacter and oil fungi can potentially reduce 90% of polyaromatics hydrocarbons's level and heavy metals in the phytoremediation of petroleum waste (30%; 35%) with albizia-mycorrhizal in different value and treatments
2. Oil bacter reduce levels of heavy metals Zn (87.17%) and Cd (60.44%) in phytoremediation of petroleum waste (30%) with albizia-mycorrhizal
3. Oil bacter reduce levels of Ni (71.32%) in phytoremediation of petroleum waste (35%) with albizia-mycorrhizal
4. Oil fungi reduce levels of Cu (68.56%) and Cr (76.24%) in phytoremediation of petroleum waste (30%) with albizia-mycorrhizal
5. Oil fungi reduce level of Pb (77.16%), in phytoremediation of petroleum waste (35%) with albizia-mycorrhizal

REFFERENCES

[1] Ahalya, N., Ramachandra, T.V., and RD. Kanamadi. 2003. Biosorption of Heavy Metals. *Research Journal of Chemistry and Environment vol.7 (14).*

[2] Alloway BJ. 1995. *Heavy Metal in Soils*. London: Blackie Academic and Professional.

[3] Alpentri, Juli N dan Septoratno S. 2001. *Evaluasi Kemampuan Isolat Jamur dari Salah Satu Sumur Minyak di Minas dalam Mendegradasi Minyak Bumi*. Prosiding Simposium Nasional IATMI 2001.

[4] Amer, RA, Mahmoud MN, and Ehab RE. 2008. Biodegradation of Monocyclic Aromatic Hydrocarbons by Newly Isolated *Pseudomonas* Strain. *Journal of Biotechnology Vol. 7 (4): 630-640*

[5] American Society of Microbiology. 2002. Mycobacterium. USA. http://archive.microbelibrary.org/ASMOnly/Details.asp?ID=370 (diakses tanggal 26 Desember 2010)

[6] Anas I. 1997. *Bioteknologi Tanah*. Laboratorium Biologi Tanah, Jurusan Tanah, Bogor : Fakultas Pertanian IPB.

[7] Crawford, R.L. dan Crawford, D.L. 1996. Bioremediation : Principles and Application. Cambridge University Press
[8] Effendi AJ. 2006. Treatability Test of Oil-Contaminated Soil Using Bio-Augmented Bacteria. *Journal of Infrastructure and Built Environment Vol.II no.2*
[9] Hakim N, Nyakpa MY, Lubis AM, Nugroho SG, Saul MR, Diha MA, Hong GB, Bailey HH. 1986. *Dasar-dasar Ilmu Tanah*. Lampung : Universitas Lampung.
[10] Harder, E. 2004. The Effects of Essential Elements on Bioremediation. Little Flower Academy
[11] Heitkamp, MA and Carl EC. 1989. Polycyclic Aromatic Hydrocarbon Degradation by *Mycobacterium* sp. In Microcosms Containing Sediment and Water from a Pristine Ecosystem. *Journal of Applied Environmental Microbiology Vol. 55, No.8 p. 1968-1973*. American Society of Microbiology.
[12] Leahy JG and Colwell RR. 1990. *Microbial Degradation of Hydrocarbons in The Environment*. Microbial Reviews, 53(3), 305-315.
[13] Leitao AL. 2009. Potential of *Penicillium* Species in The Bioremediation Field. *International Journal of Environmental Research and Public Health Vol. 6, p : 1393-1417*
[14] Mandri T and Lin. 2007. Isolation and Characterization of Engine Oil Degrading Indigenous Microorganism in Kwazulu-Natal, South Africa. *African Journal of Biotechnology Vol. 6 (1), pp. 023-027*
[15] Mangkoedihardjo S. 2002. Waterhyacinth Leaves Indicate Wastewater Quality. *J. Biosains, 7 (1): 10-13*.
[16] Mathur N, Bohra JSS, Quaizi A and Vyas A. 2007. Arbuscular Mycorrhizal Fungi: A Potential Tool for Phytoremediation. *Journal of Plant Sciences 2 (2):127-140*
[17] Mengel K & Kirkby EA. 1987. *Principles of Plant Nutrition*. 4th Edition. International Potash Institute. Bern. 687 h.
[18] Munir E. 2006. *Pemanfaatan Mikroba dalam Bioremediasi : Suatu Teknologi Alternatif untuk Pelestarian Lingkungan*. Medan : USU.
[19] Moriya K. 1993. *Miccroorganism Belonging to The Genus* Flavobacterium*, Hydrocarbon Emulsifier and Solubilizer and Separation For Organic-Solvent Tolerant Microorganism*. Japan Marine Science and Technology.
[12] Plantamor. 2009. *Albasia*. http://www.plantamor.com/index.php?plant=51 (diakses pada 29 Juni 2009)
[13] Rossiana N. 2005. *Bioremediasi Lumpur Minyak Bumi dengan Zeolit dan Mikroorganisme serta Pengujiannya dengan Tanaman Sengon* (Paraserianthes falcataria *L. Nielsen*). Bandung : Disertation Program Pascasarjana Unpad
[14] Sallehin A, Hussaini, and Apun K. 2008. *Bioremediation of Crude Oil Sludge Contamination*.http://www.innovations-report.com/html/reports/environ ment_sciences/report-113199.html (diakses pada 29 Juni 2009)
[15] United States Environmental Protection Agency (USEPA). 2005. *Use of Field-Scale Phytotechnology for Chlorinated Solvents, Metals, Explosives and Propellants, and Pesticides*. USA : Office of Solid Waste and Emergency Response Technology.
[16] Verloo M. 1993. *Chemical Aspect of Soil Pollution.* ITC-Gen Publication series No.4 : 17-46
[17] Vidali, M. 2001. *Bioremediation : An Overview. Pure and Applied Chemistry*. p 1163-1172
[18] Vijayaraghavan, K. and Yeong-Sang Yun. 2008. *Bacterial Biosorbent and Biosorption*. Biotechnology Advanced 26 (2008) 266-291.
[19] Wright, SF. and Nichols Kristine . A. 2002. Glomalin : *Hiding Place for a Third of The World's Stored Soil Carbon*. USDA Agricultural Research Service. http://www.ars.usda.gov/is/ar/archive/sep02/soil0902.htm (diakses pada tanggal 25 November 2010)
[20] Wu Y, Teng-Teng H, Jing-Shen L, Khalid M, Tong-Wang H, Zhong H. 2009. Removal Benzo[a]pyrene by a Fungus *Aspergillus* sp. BAP14. *World Journal Microbiology of Biotechnology Vol. 25 : 1395-1401*