SCIENTIFIC CONTRIBUTIONS OIL AND GAS
Vol. 39, Number 3, December 2016: 6 of 8
RESEARCH AND DEVELOPMENT CENTRE FOR OIL & GAS TECHNOLOGY LEMIGAS
Journal Homepage:http://www.journal.lemigas.esdm.go.id
ISSN: 2089-3361 (Print) e-ISSN: 2541-0520 (Online)

EFFECTS OF PETROFILIC MICROORGANISMS AND BULKING AGENT ON HYDROCARBON'S BIODEGRADATION EFFICIENCY

EFEK MIKROORGANISME PETROFILIK DAN BULKING AGENT TERHADAP EFISIENSI BIODEGRADASI HIDROKARBON

Zulkifliani[1], Pujawati Suryatmana[2], Annisa Rosalina Sylvia[2], and **Syafrizal[1]**
[1,4)]"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/KBY-Jakarta 12120, Telepon: 62-21-7394422, Faxsimile: 62-21-7246150
Email: zulkifliani@lemigas.esdm.go.id; Email: syahrizal@lemigas.esdm.go.id;
[2)]Program Studi Agroteknologi, Fakultas Pertanian, Universitas Padjadjaran
Jl. Raya Jatinangor Km. 21 Jatinangor, Sumedang 45363
Email: annisarosalinas@yahoo.com

First Registered on October 7th 2016; Received after Correction on December 12nd 2016
Publication Approval on: December 30th 2016

ABSTRAK

Bioremediasi adalah metode yang dapat digunakan untuk mengurangi jumlah polutan atau zat beracun yang merusak lingkungan melalui penggunaan mikroorganisme untuk menghilangkan kontaminan. Tujuan dari penelitian ini adalah untuk menentukan dampak dari mikroorganisme petrofilik dan bulking agent pada peningkatan efisiensi biodegradasi hidrokarbon, kadar air tanah, laju pertumbuhan bakteri petrofilik dan Azotobacter vinelandii. Sembilan perlakuan dalam penelitian ini adalah konsorsium petrofilik (Pseudomonas spp., Actinomycetes sp., dan jamur petrofilik) dan Azotobacter vinelandii (biosurfaktan), serta penggembur (arang sekam padi dan limbah baglog Jamur Tiram). Desain percobaan yang digunakan adalah rancangan acak kelompok dengan tiga ulangan, berjumlah 27 unit eksperimental (microscosmos). Hasil aplikasi dari mikroorganisme petrofilik dan bulking agent adalah perlakuan terbaik untuk meningkatkan efisiensi biodegradasi hidrokarbon, laju pertumbuhan bakteri petrofilik, dan kadar air tanah. Pada perlakuan konsorsium petrofilik nilai efisiensi biodegradasinya mencapai 83,9%.

Kata Kunci: *azotobacter vinelandii, bioremediasi, efisiensi biodegradasi hidrokarbon, petrofilik*

ABSTRACT

Bioremediation is a method which can be used to reduce the amount of pollutants or toxic substances that damage the environment through the use of microorganisms to remove the contaminants. The purpose of this research is to determine the effects of petrofilic microorganisms and bulking agent on the enhancement of hydrocarbon's biodegradation efficiency, soil water content, growth rate of petrofilic bacteria and Azotobacter vinelandii. Nine treatments in this research were a petrofilic consortium (Pseudomonas spp., Actinomycetes sp., and petrofilic fungi) and Azotobacter vinelandii (biosurfactan), as well as bulking agent (rice husk charcoal and baglog waste of Oyster Mushrooms). The experiment design used was a randomized block design with three replications, so there were 27 experimental units (microscosmos). The result was that the application of petrofilic microorganisms and bulking agent is the best treatment to enhance the efficiency of hydrocarbon's biodegradation, the growth rate of petrofilic bacteria, and soil water content. The petrofilic consortium treatment with the highest value of biodegradation efficiency reached 83,9%.

Keywords: azotobacter vinelandii,bioremediation, biodegradation efficiency of hydrocarbons, petrofilic

L. INTRODUCTION

Oil can seep into the ground and cause the closing of the supply of oxygen and poison soil microorganisms. Moreover, it can also cause the death of soil microorganisms. Soil microorganisms poisoned by these hydrocarbons can interfere with the development and survival of plants, because soil microorganisms act as providers of nutrients that are also active in the decomposition of litter and in the process of weathering of organic matter (Rao 1994).

Hydrocarbons and photochemical oxidants in the air react with each other. Air pollution by hydrocarbons along with pollutants NO_x then with the free oxygen in the air form Acetyl Peroxy Nitrates (PAN) together with CO and ozone to form photochemical smog. Prodjosantoso and Tutik (2011) stated that this photochemical smog can damage crops as indicated by the pale color of the leaves and the cell surface of dead leaves.

One alternative that can solve hydrocarbon contaminated soil is to use bioremediation techniques. Bioremediation aims to mineralize contaminants, namely by changing the chemical compounds that are harmful to less harmful the carbon dioxide or some other gas, inorganic compounds, water, and materials needed by degrading microbes to proliferate (Eweis et al. 1998).

Myers and Williford (2000), stated that the land treatment system is one of the ex- situ bioremediation techniques, in which soil contaminated activated by being tilled and moved from its original site so as to allow the ground to interact with the climate like at contaminated sites. Bioremediation techniques developed in Indonesia generally are a land treatment system. This system involves adding a bulking agent and biosurfactant with the use of agents that support the process of bioremediation.

The use of bulking agent in the land treatment system aims to maintain the porosity of the soil, moisture, and is a source of nutrients in the soil (Imaddudin 2011). The basic materials for a bulking agent that is easily found in nature is rice huskcharcoal and baglog waste of oyster mushroom. In addition, a bioagent can increase the population density of petrofilic microbes when the bioremediation process is carried out. Petrofilic microbial growth can be supported by the use of biosurfactant agents in the process of biodegradation of petroleum waste which serves to reduce the interface tension between oil and water (Vater et al. 2002). One of microbes that can be used as a biosurfactant is *Azotobacter sp*.

Given biosurfactant and bulking agent in the process of bioremediation is the best way to optimize the process of biodegradation by petrofilic microorganisms, research is needed on the effect of the application of petrofilic microorganisms and bulking agent to increase the efficiency of biodegradation of hydrocarbons.

II. METHODOLOGY

A. Experiment Design

The experiment design used was a randomized block design. The treatments in this research were a petrofilic consortium *(Pseudomonas spp., Actinomycetes sp.,* and petrofilic fungi*)* and *Azotobacter vinelandii* (biosurfactant), as well as bulking agent (rice husk charcoal and baglog waste of Oyster Mushrooms). Total units in this treatment were nine treatments and three replications, so there were 27 experimental units (micros-cosmos) in total.

B. Culture Mediums dan Preparation

The medium used for the production of culture media petrofilic consortium are NB (Nutrient Broth) for petrofilic bacteria (*Pseudomonas sp.*), PDB (Potato Dextrose Broth) for petrofilic fungi, and actinomycetes liquid for *Actinomycetes sp*. Media in *Azotobacter vinelandii* the mineral medium (1,5 g K_2HPO_4, 0,5 g KH_2PO_4, $MgSO_4$ 0,5 g in 1000 mL of distilled water) with a glucose concentration of 2 % at pH 7 is sterilized at a temperature of 12˚C for 15 minutes as the media for the fermentation process to produce biosurfactant. Media is stored at a temperature of 28°C for three days and each inoculated with as much as 10% of the volume of media with agitation of 100 rpm for 7 days of incubation.

C. Microorganisms

Bacterial isolates petrofilic and *Azotobacter vinelandii* obtained from stock culture agar slant had previously been isolated in soil at the Biological Laboratory Department of Soil Science and Land Resources, Faculty of Agriculture, University of Padjadjaran.

D. Preparation of Soil

Soil used in this study is Jatinangor Ultisols. Tillage is done by repeatedly stirring, and subsequently undergoing a drying process in order to be dried off for three days. Then soil that has undergone the drying process is screened with a 2 mm sieve. After processing the soil, then mix the soil with petroleum waste homogeneously. Mixing soil and waste oil with concentrations of TPH (Total Petroleum Hydrocarbons) amounted to 10% of 2 kg of soil media, in order to obtain soil contaminated petroleum waste homogeneously. A mixture of soil and petroleum waste were incubated for one day to make a homogeneous oil waste with soil.

E. Hydrocarbon Biodegradation Studies

The concentration of rice husk charcoal and baglog waste of oyster mushroom is applied at a concentration of 1% of the weight of the soil with a bulking agent requirement calculated for each microcosm of approximately 20 g. Inoculant petrofilic consortium and *Azotobacter vinelandii* inoculated into the microcosm that already contain the soil and mix of petroleum waste as prescribed treatment has been calculated each dose of 2% (v/v) mixture petrofilic consortium and *Azotobacter vinelandii* (1:1) per concentration waste load. Maintenance is carried out from soil treatment that has been contaminated with sewage load that is 10 % of the initial weight of the soil and have added petrofilic consortium, *Azotobacter vinelandii*, and bulking agent that has been homogenized, then stirring is performed every day.

F. Total Petroleum Hydrocarbon Analysis

Observation of the hydrocarbons degradation efficiency analysis of TPH (Total Petroleum Hydrocarbons) is using the Test Method for Evaluating Solid Waste, US EPA 9071 B.

G. Total Plate Count Analysis

Analysis of the petrofilic consortium (Total Plate Count) used Basal media + 1% of the hydrocarbons to petrofilic bacteria (*Pseudomonas sp*.) and *Azotobacter vinelandii* used selective media such as Vermani + 1% hydrocarbons. TPC analysis is performed on each point of observation is T-0 (H-0), T-1 (H-7), T-2 (H-14), T-3 (H-28), and T4 (H-42). Furthermore, the incubation was for 5-7 days to calculate the total population. The data were statistically analyzed using SPSS. If there is any real effect then a further test is needed using Duncan's multiple range at 5% significance level.

III. RESULTS AND DISCUSSION

A. Soil Water Content

The treatment of petrofilic micro-organisms and bulking agent to produce a value sufficient soil water content was optimum. Based on the statistical test, the average of soil water content showed that the petrofilic consortium + rice husk charcoal (H) was the best treatment compared to the control treatment (A) and *Azotobacter vinelandii* (B) with a value of 17.50 % (Table 1). However, treatment of the petrofilic consortium + rice husk charcoal (H) was not significantly different from other treatments.

Using less water would hinder mass transfer into cells degrading bacteria. Otherwise, circumstances of excess water can inhibit the oxygen flow rate which can slow down the degradation process. Therefore, the water content in this study belong optimum water content enough that no more than 20 %. This is supported by the statement by Fermiani (2003), that the water content in the bioremediation process should be at the optimum condition, that is 10-25 % in order to ensure the process of oxygenase by petrofilic micro-organisms goes well. The average of soil water is not significantly different, because in the process of aeration or stirring it is done every day with the same environmental conditions during the research so that evaporations process occurred.

It can happen because the mixing process is less homogenous, so that less water content is adsorbed

Table 1
Impact of petrofilic microorganismsand bulking agent against average of soil water content

Treatment	Water Content (%)
A = Control	15,15a
B = *Azotobacter vinelandii*	15,44a
C = Petrofilic Consortium	16,24ab
D = Rice Husk Charcoal	15,73ab
E = Baglog waste	15,98ab
F = *Azotobacter* + Rice Husk Charcoal	16,52ab
G = *Azotobacter* + Baglog Waste	16,14ab
H = Petrofilic Consortium + Rice Harcoal	17,50b
I = Petrofilic Consortium + Baglog Waste	16,30ab

Notes: The average value followed by the same letter are not significant according to Duncan's Multiple Range Test up at the level of 5 %.

by the soil or by soil microbes and evaporates into the air. Hydrocarbon compounds will be degraded naturally which is caused by environmental factors, although the rate of degradation is running slowly. The factors include evaporation, emulsified in water, adsorbed on the solid particles, immersed in water and biodegradable by microbes (Nugroho, 2006). However, water levels which led to evaporation can indirectly lower the concentration of hydrocarbons, especially on low-molecular compound and the compounds are usually toxic. Therefore, with the same environmental conditions, the average of soil water content which were not significantly different among treatment but is considered quite optimum for the biodegradation process.

B. Hydrocarbon's Biodegradation Efficiency

The treatment of petrofilic micro-organisms and bulking agent has a significant effect on the efficiency of hydrocarbon's biodegradation. Figure 1 shows that the process of biodegradation of petroleum hydrocarbons using petrofilic consortium (C) in the first week were significantly different compared to the control treatment (A). Treatment of petrofilic consortium (C) has an effect on the efficiency of biodegradation within one week, with the biodegradation efficiency value being 24,08%. At week-6 at the end of the observation, petrofilic micro-organisms and bulking agent were able to improve the efficiency of hydrocarbon's biodegradation. Statistical analysis showed that an increase in the efficiency of hydrocarbon's biodegradation at week - 6 where the petrofilic consortium treatment (C) with the highest value of bio-degradation efficiency reached 83,9%.

This is presumably because the treatment petrofilic consortium (C) applied to soil contaminated with petroleum waste is able to adapt to the toxic conditions and is capable of utilizing hydrocarbons as a source of nutrients. The petrofilic consortium have a good metabolism rate and have undergone genetic adaptation to hydrocarbons (Bento et al. 2003). Moreover, the abundance and diversity of petrofilic microorganisms in nature (indigenous) also has a linear relationship with the increase of efficiency degradation of hydrocarbons (Retno and Nana 2013). The treatment of petrofilic consortium comprising petrofilic bacteria, *Actinomycetes sp*., *Azotobacter vinelandii* and petrofilic fungi were assumed to have synergistic activity of microorganisms with each other to degrade hydrocarbons. Performance mutually synergistic among them occurred when there was a microorganism that can only parse a particular substrate after degraded (as metabolites) in advance by other microorganisms. As well the use of *Azotobacter vinelandii* in petrofilic consortium can help the process of degradation of hydrocarbons.

This is because *Azotobacter vinelandii* can produce biosurfactant to emulsify oil and water interface so that other petrofilic microorganisms can grow between the interphase. *Azotobacter vinelandii* has its own unique properties that are adaptive, diverse metabolic capacity, and can be positively associated with other soil microorganisms (Suryatmana 2006). This serves to help petrofilic microorganisms accelerate the process of biodegradation of hydrocarbons.

However, the effect of treatment was not significantly different of petrofilic consortium with several other treatments such as sewage treatment baglog waste oyster mushroom (E) and petrofilic consortium + baglog waste of oyster mushrooms (I), with a value of 81,62% and 81,25%. Baglog waste treatment applications with oyster mushrooms make a positive contribution to improving the efficiency of hydrocarbon allegedly due to biodegradation of organic matter content as well as high macro and micro nutrients baglog waste of oyster mushrooms. The content of the materials and energy sources form cell components and the development of microbial cells, especially petrofilic microbial (Suharni et al. 2008).

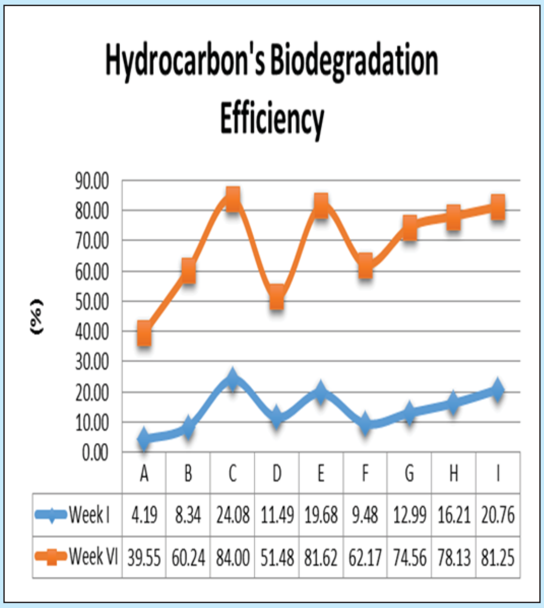

Figure 1
Hydrocarbon's biodegradation efficiency.

C. Growth Rate of PetrofilicBacteria (Total Plate Count)

The treatment of petrofilic micro-organisms and bulking agent have a significant effect on the growth rate of petrofilic bacteria. Table 2 shows that the growth rate of petrofilic bacteria in baglog waste of oyster mushroom treatment (E) was significantly different from the control treatment (A) and a consortium + rice husk charcoal (H). This is in line with the value of hydrocarbon's biodegradation efficiency high enough from the first week until the last week analysis that was the waste treatment baglog oyster mushroom (E).

The effect of baglog waste of oyster mushrooms treatment provides the highest value to the growth rate of petrofilic bacteria, this is allegedly because baglog waste of oyster mushrooms contains residues of nutrients such as cellulose, hemicellulose, lignin, protein, and vitamins that assist the petrofilic bacteria to grow and degrade pollutants such as hydrocarbons (Khammuang and Sarnthima 2007; Subyakto et al. 2009). According to Retno and Nana (2013) the higher of growth rate of petrofilic bacteria, since the population of bacteria that dominate petrofilic in bioremediation process and has proved able to adapt to extreme conditions by using compounds with the contained in waste oil as a source of nutrients, especially carbon to metabolize.

Table 2
Impact of petrofilic microorganisms and bulking agent against growth rate of petrofilic bacteria

Treatment	Growth rate (log 10^4 CFU/hari)
A = Control	9 A
B = *Azotobacter Vinelandii*	18 Abc
C = Petrofilic Consortium	31 Bc
D = Rice Husk Charcoal	19 Abc
E = Baglog Waste	37 C
F = *Azotobacter* + Rice Husk Charcoal	12 Ab
G = *Azotobacter* + Baglog Waste	31 Bc
H = Petrofilic Consortium + Rice Charcoal	9 A
I = Petrofilic Consortium + Baglog Waste	20 Abc

Notes: The average value followed by the same letter are not significant according to Duncan's Multiple Range Test up at the level of 5%.

D. Growth Rate of Azotobacter vinelandii (Total Plate Count)

Statistical test results and treatment applications of petrofilic microorganisms and bulking agent have no significant effect in increasing the growth rate of *Azotobacter vinelandii* (Table 3).

Meanwhile, the use of petrofilic microorganisms and bulking agent have no significant effect in increasing the growth rate of *Azotobacter vinelandii*. It indicates that the competition between the three types of petrofilic microorganisms and indigenous microbes to *Azotobacter vinelandii* to obtain carbon sources namely hydrocarbons derived from petroleum waste, resulted in cell regeneration from *Azotobacter vinelandii* blocked or working less than optimal (Fauzi and Suryatmana 2016). Moreover, they are believed to be resistant hydrocarbon compounds and toxic, so *Azotobacter vinelandii* is not able to adapt and will use it as an energy source (Van Eyk 1997).

Table 3
mpact of Petrofilic microorganisms and bulking agent against growth rate of *azotobacter vinelandii*

Treatment	Growth rate (log 10^4 CFU/hari)
A = Control	25
B = *Azotobacter vinelandii*	13
C = Petrofilic Consortium	18
D = Rice Husk Charcoal	14
E = Baglog waste	11
F = *Azotobacter* + Rice Husk Charcoal	17
G = *Azotobacter* + Baglog Waste	18
H = Petrofilic Consortium + Rice Harcoal	8
I = Petrofilic Consortium + Baglog Waste	16

Notes: The average value followed by the same letter are not significant according to Duncan's Multiple Range Test up at the level of 5%.

IV. CONCLUSION

There is a petrofilic consortium treatment which is consistently able to increase the efficiency of the biodegradation of hydrocarbons. Applications bulking agent such as baglog waste of oyster mushrooms can affect the bacterial growth rate of petrofilic bacteria.

REFERENCES

Bento, F.M., Flavio, A.O.C., Benedict O.,Willian T.F., 2003. Bioremediation of Soil Contaminated by Diesel Oil. *Braz. J. Microbiol.* vol.34 suppl.1 São Paulo.

Eweis, J.B., S.J. Ergas, D.P.Y. Chang, and **E.D. Schroeder,** 1998. Bioremediation Principles. *McGraw-Hill, Boston, MA*.

Fauzi, M dan **Pujawati, S.,** 2016. Bioremediasi Limbah Minyak Bumi Menggunakan Konsorsium Petrofilik dan *Azotobacter Chroococcum* Untuk Meningkatkan Laju Degradasi Hidro-karbon, Pertumbuhan *Azotobacter*, dan Jamur Petrofilik. Program Studi Agroteknologi Fakultas Pertanian Universitas Padjadjaran. Jurnal Ilmu Tanah Indonesia.

Fermiani, F., 2003. Pengolahan Tanah Terkontaminasi Minyak dengan Teknik Bioremediasi di Lapangan Minas, PT. Caltex Pasific Indonesia. Dalam Prosiding Seminar Bioremediasi dan Rehabilitasi Lahan Sekitar Pertambangan dan Perminyakan. Pusat Kajian Sumber Daya Pesisir dan Lautan, Institut Pertanian Bogor. Bogor.

Imaddudin, F., 2011. Hubungan Antara Laju Konsentrasi Oil and Grease dan Bulking Agent (Sekam Padi dan Bintaro) pada proses Bioremediasi. Teknik Sipil dan Lingkungan, Institut Pertanian Bogor. Bogor.

Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia, 2013. Status Lingkungan Hidup Indonesia. Jakarta : Kementerian Lingkungan Hidup dan Kehutanan Republik Indonesia.

Khammuang S. dan **Sarnthima R.,** 2007. Laccase from Spent Mushroom Compost of *Lentinus polychrous* Lev. and its potential for Remazol Brilliant Blue R decolorization. *Biotechnology* 6: 408–413.

Lasari D. P., 2010. Bakteri Pengolah Limbah Minyak Bumi yang Ramah Lingkungan.http://www1.esdm.go.id/berita/artikel/56-artikel/3507-bakteri-pengolah-limbah-minyak-bumi-yang-ramah-lingkungan. [Diakses pada 07 Juli 2015]

Myers, T. E., and **Williford, C. W**., 2000. Concepts and technologies for bioremediation in confined disposal facilities. DOER Technical Notes Collection (ERDC TN-DOER-C11), *U.S. Army Engineer Research and Development Center, Vicksburg, MS*.

Nugroho, B., 2000. Upaya Mengatasi Pembalakan Ilegal. Suatu Gagasan Untuk Mengatasi Masalah Pembalakan Ilegal di Hutan Alam Produksi. Disertasi Program Pasca Sarjana IPB. Bogor.

Nugroho, A., 2006. Biodegradasi sludge minyak bumi dengan skala mikroorganisme: simulasi sederhana sebagai kajian awal bioremediasi *landtreatment*. Markara.Teknologi 10(2) :82-89.

Prodjosantoso dan **Tutik, R.,** 2011. Kimia Lingkungan (Teori, Eksperimen, dan Aplikasi). Kanisius (Anggota IKAPI). Yogyakarta.

Rao, N.S.S., 1994. Mikroorganisme Tanah dan Pertumbuhan Tanaman. Universitas Indonesia Press. Jakarta.

Retno, T. dan **Nana, M**., 2013. Bioremediasi Lahan Tercemar Limbah Lumpur Minyak Menggunakan Campuran *Bulking Agents* yang Diperkaya Konsorsia Mikroba Berbasis Kompos Iradiasi. Pusat Aplikasi Teknologi Isotop dan Radiasi - BATAN. Jakarta.

Suharni, Nastiti S.J., dan **Ningsih S.E.S.,** 2008. Mikrobiologi Umum. Universitas Atmajaya. Yogyakarta.

Suryatmana, P., 2006. Biodegradasi Hidrokarbon Minyak Bumi dengan Penambahan *Azotobacter chroococcum* AC04 sebagai Bakteri Penghasil Biosurfaktan. Bandung. Disertasi Institut Teknologi Bandung. Bandung.

Van Eyk, J., 1997. Petroleum Bioventing. A.A. Balkema Rotterdam and Brookfield, Vermont.

Vater J, Kablitz B, Wilde C, Franke P, Mehta N, and **Cameotra SS.,** 2002. Matrix assisted laser desorption ionization-time of flihgt mass spectrometry of lipopeptide biosurfactant in whole cell and culture filtrates of *Bacillus subtilis* C-1 isolated from petroleum slude. *J. Appl. Environ. Microbiol* 68: 6210-6219. *Microbiol* 68: 6210-6219.