JOURNAL OF DEGRADED AND MINING LANDS MANAGEMENT
ISSN: 2339-076X, Volume 3, Number 2 (January 2016): 521-526
DOI:10.15243/jdmlm.2016.032.521

Research Article

Bioremediation of crude oil waste contaminated soil using petrophilic consortium and *Azotobacter sp*.

M. Fauzi[1*], P. Suryatmana[2]

[1] Soil Science Master Programme, Faculty of Agriculture, Padjadjaran University, Jl. Raya Jatinangor, Sumedang, West Java 45363, Indonesia
[2] Department of Soil Science, Faculty of Agriculture, Padjadjaran University, Jl. Raya Jatinangor, Sumedang, West Java 45363, Indonesia

* corresponding author: muhammadfauzi2791@gmail.com

Abstract: This study was aimed to determine the effect Petrophilic and Azotobacter sp. consortium on the rate of degradation of hydrocarbons, Azotobacter growth, and Petrophilic fungi growth in an Inceptisol contaminated with crude oil waste originating from Balongan refinery, one of Pertamina (Indonesia's largest state-owned oil and gas company) units in Indramayu – West Java. This study was conducted from March to April 2014 in the glasshouse of research station of the Faculty of Agriculture, Padjadjaran University at Ciparanje, Jatinangor District, Sumedang Regency of West Java. This study used a factorial completely randomized design with two treatments. The first treatment factor was Petrophilic microbes (A) consisting of four levels (without treatment, 2% Petrophilic fungi, 2% Petrophilic bacteria, and the 2% Petrophilic consortium), and *Azotobacter s*p. The second treatment factor was *Azotobacter* sp. (B) consisting of four levels (without treatment, 0.5%, *Azotobacter sp*., 1% *Azotobacter sp*., and 1.5% *Azotobacter sp*.) The results demonstrated interaction between Petrophilic microbes and *Azotobacter* sp. towards hydrocarbon degradation rate, but no interaction was found towards the growth rate of *Azotobacter* sp. and Petrophilic fungi. Treatments of a_1b_3 (2% consortium of Petrophilic fungi with 1.5% *Azotobacter* sp.) and a_3b_3 (2% Petrophilic consortium and 1.5% *Azotobacter* sp.) had hydrocarbon degradation rate at 0.22 ppm/day for each treatment, showing the highest hydrocarbon degradation rate.

Keywords : *Azotobacter sp., bioremediation, petrophilic.*

Introduction

Crude oil is the primary energy source used in transportation industries and households. The activity of crude oil industry is a series of complex process from upstream to downstream. The rapid progress in crude oil industry sector has both positive impact on the improved people's wealth and negative side effect on the environmental pollution concurrently (Haris et al, 2005).

Environmental pollution may result from crude oil exposure in soil. Crude oil or its waste is a complex mixture of organic compounds that consists of hydrocarbon and non-hydrocarbon compounds. Hydrocarbon compound is the largest component of crude oil that accounts for more than 90 %, while the rest is non-hydrocarbon compounds (Udiharto, 1996). Hydrocarbon compounds in clay can penetrate into soil layers, contaminating soil and water nearby.

Soil must be treated well in order to preserve and maintain soil health so that it can sustain plant growth. One of the attempted efforts is to maintain soil health from crude oil exploration by human. Crude oil waste, which is disposed on the soil surface, can lead to contamination of poisonous and hazardous compounds because of its hydrocarbon content. In order to curb the negative effect of crude oil contamination, green technology of bioremediation with land farming system is necessary.

One of the currently implemented waste management technologies is bioremediation technology. The advancement of this technology is due to its relatively easy implementation and affordable operational cost. Technology of

bioremediation technology has potential to be implemented in Indonesia. Tropical climate condition with abundant sun lights, high humidity, and profound microorganism diversity support the acceleration process of microbe growth to actively degrade oil (Hafiluddin, 2011).

Bioremediation is defined as technology that utilizes microbes to process pollutants through natural biodegradation mechanism (intrinsic bioremediation) or to augment natural biodegradation mechanism by adding microbes, nutrients, electron donor and/or electron acceptor (enhanced bioremediation) (Zhu et al., 2001). The common type of bioremediation technique is ex-situ technique, which is a technique that excavates contaminated soil or water and processes it in a prepared treatment area for bioremediation process. This form of treatment is safer for environment because the degrading agents used are microbes that can be naturally decomposed (Alvarez et al., 2008).

The existence of hydrocarbon-degrading microorganisms (bacteria, fungi, and leaven) are widely spread in nature. Certain microorganisms can degrade hydrocarbon compound and used it as carbon source to generate energy. Microbes use oil hydrocarbon for their growth by cutting aliphatic, cycloaliphalitic, and aromatic hydrocarbon. The mechanism of oil biodegradation is very numerous and depends on the hydrocarbon composition that it has (Brock et al., 1991). Then, hydrocarbon degrading microbes are known as Petrophilic microbes.

Petrophilic microbes are hydrocarbon-degrading microorganisms that comprise bacteria and fungi. Some effective hydrocarbon degraders in natural setting, which have been isolated, are *Pseudomonas aeruginosa*, *P. putida*, *Bacillus subtilis*, *B. cereus*, *B. laterospor* (Cybulski et al., 2003; De Carvalho and Da Fonseca, 2005) and *Azotobacter chroococcum* AC04 (Suryatmana, 2006). The fungal group that degrades polycyclic aromatic hydrocarbon commonly comes from genus of *Phanerochaete*, *Cunninghamella*, *Penicillium*, *Candida*, *Sporobolomyces*, *Cladosporium*. Fungi from group of *Deuteromycota* (*Aspergillus niger*, *Penicillium glabrum*, *P. janthinellum*, *Zygomycete*, *Cunninghamella elegans*), *Basidiomycetes* (*Crinipellis stipitaria*) are also known of their capability to degrade polycyclic aromatic hydrocarbon (Waluyo, 2005).

Hydrocarbon biodegradation process by Petrophilic microorganisms commonly includes enzymatic oxygenation activity. Although the diversity number of hydrocarbon-oxidizing enzymes is relatively smaller in Petrophilic bacteria, the enzymes are able to degrade the structure and composition of varied hydrocarbon. This is as a result of some activities of microbes as follows: (1) the majority of oxygenation activity of Petrophilic bacteria has a quite large specification in which one enzyme could work with more than one substrate, a characteristic that generally does not prevail in enzymatic reaction; (2) Petrophilic microorganisms are able to degrade hydrocarbon fast because the microbes show diverse metabolic ability to alter products of hydrocarbon oxidation into necessary substrates (Van Eyk, 1997). Petrophilic fungi possess different degradation mechanism from bacteria. Bacteria decompose organic pollutants by taking up the compounds into their cells, while fungi use degrading enzyme secreted by mycelium, or known as extracellular enzyme. *Azotobacter chroococcum* AC04 culture is a species that produces biosurfactant, but it is not the main degrader of the target contaminant compounds. Therefore, it is named co-culture AC04 (Suryatmana, 2006) later. Besides, *Azotobacter* sp. can also fix N in air.

The synergy between Petrophilic microbes and *Azotobacter* sp. in soil bioremediation process is expected to affect the growth rate of inceptisol from Jatinangor. The observed growth rate was hydrocarbon biodegradation rate, growth rate of Petrophilic microbes, and growth rate of Azotobacter sp.

Materials and Methods

This study commenced from March to April 2014 in the glasshouse of research station of the Faculty of Agriculture, Padjadjaran University at Ciparanje, Jatinangor District, Sumedang Regency of West Java. The soil that was taken as sample in the present study was an inceptisol. Completely randomized factorial design which consisted of two factors, was used in this study. The first experiment factor was Petrophilic microbes that encompass four levels, with the given treatment as follows: a_0 (control), a_1 (2% Petrophilic fungi), a_2 (Petrophilic bacteria), a_3 (2% Petrophilic consortium). The second factor was *Azotobacter* sp. that comprised four levels, with the given treatment as follows: b_0 (control), b_1 (0.5% *Azotobacter* sp.), b_2 (1% *Azotobacter* sp.), and b_3 (1.5% *Azotobacter* sp.). Total treatment was 4 x 4 = 16 treatment combination with 3 replicates, so the overall total was 48 experimental units. 10% concentration of waste load was used. The need of waste load was adjusted with initial TPH of 99.1 % with 2 kg soil media.

The experiment stages were elaborated as follows: (1) Isolation of Petrophilic microbes from crude oil waste discharged by Balongan refinery,

(2) Acclimatization of Petrophilic microbes for 60 days, (3) Production of Petrophilic microbes, (4) Initial soil analysis, (5) Preparation of soil media with TPH waste load of 10%, (6) Adding of bulking agent from oyster mushroom waste media, then it was incubated for 7 days, (7) Application/ inoculation of Petrophilic microbes and *Azotobacter* sp., (8) Maintenance, and (9) Observation of hydrocarbon degradation rate, growth of *Azotobacter* sp. and Petrophilic fungi. Data were collected from the following parameters: (1) Hydrocarbon degradation rate, (2) Growth rate of *Azotobacter* sp., and (3) Growth rate of Petrophilic fungi.

Results and Discussion

Hydrocarbon degradation rate

Hydrocarbon degradation rate is the amount of hydrocarbon molecular mass that can be degraded by hydrocarbon-degrading microbes in certain period. According to the experiment result, it can be noticed that an interaction between Petrophilic microbes and *Azotobacter* sp. occurred towards hydrocarbon degradation rate of crude oil waste disposed by Balongan refinery, Indramayu – West Java. The analysis result showed interaction between Petrophilic microbes and Azotobacter sp. towards hydrocarbon degradation rate (Table 1).

Based on the result of further experiment (Table 1), it can be inferred that treatment without Petrophilic (a_0) towards treatment without *Azotobacter* sp. or a_0b_0 (0.13 ppm/day) showed significant difference in hydrocarbon degradation towards treatment *Azotobacter* sp. as the concentration of *Azotobacter* sp. was increased in a_0b_1 (0.21 ppm/day), a_0b_2 (0.20 ppm/day), and a_0b_3 (0.19 ppm/day). However, treatments of a_0b_1, a_0b_2, and a_0b_3 were not significantly different. In concentration level of 2% Petrophilic fungi and 2% Petrophilic bacteria towards b_0 (each 0.19 ppm/day), the result showed significant difference in consortium with 1% *Azotobacter* sp. (each 0.22 ppm/day and 0.21 ppm/day), but it was not significantly different in 0.5% *Azotobacter* sp. consortium (0.19 and 0.18 ppm/day) and 1.5% *Azotobacter* sp. (0.22 and 0.21 ppm/day). Then, treatment a_3 (2% Petrophilic consortium) towards *Azotobacter* sp. for all concentrations showed insignificantly indifferent degradation rate.

Table 1. The impact of interaction between petrophilic microbes and *Azotobacter* sp. on the hydrocarbon degradation rate.

Petrophilic (A)	***Azotobacter* sp. (B)**			
	b_0 (0%)	**b_1 (0.5%)**	**b_2 (1%)**	**b_3 (1.5%)**
	TPH Degradation Rate ppm/day			
a_0 (Control)	0.13 a A	0.21 a B	0.20 a B	0.19 a B
a_1 (2% Petrophilic Fungi)	0.19 a A	0.19 a AB	0.22 a B	0.22 a AB
a_2 (2% Petrophilic Bacteria)	0.19 a A	0.18 a AB	0.21 a B	0.21 a AB
a_3 (2% Petrophilic Consortium)	0.19 a A	0.20 a A	0.19 a A	0.22 a A

Notes : Numbers marked with the same letters are not significantly different based on Duncan multiple range test on 5% degree of freedom. The capital letters are read horizontally, and the small letters are read vertically.

Treatment b_0 towards treatment without Petrophilic (a_0), 2% Petrophilic fungi, 2% Petrophilic bacteria, and 2% petrophilic consortium (0.13; 0.19; 0.19; and 0,.19 (ppm/day) respectively displayed insignificant difference in TPH degradation growth rate. Similar result was also present in treatments b_1, b_2, and b_3 towards all Petrophilic (a) treatments where the hydrocarbon degradation rate was not significantly different either. According to all treatment data, the highest hydrocarbon degradation rate was found in treatment a_1b_3 (2% Petrophilic fungi consortium with 1.5% *Azotobacter* sp.) and a_3b_3 (2% Petrophilic consortium and 1.5% *Azotobacter* sp.) where each had hydrocarbon degradation rate of 0.22 ppm/day without significant difference. On the other hand, if we look at the efficiency aspect, treatment a_0b_1 (without Petrophilic and 0.5% *Azotobacter* sp.) indicated more efficient treatment in degrading hydrocarbon compared to other treatments. This is attributed to the fact that hydrocarbon degradation process had already taken place even without adding Petrophilic microbes (0.5% *Azotobacter* sp.). Petrophilic microbes are indigenous group in soil that play a role in hydrocarbon degradation process.

Based on the aforementioned hydrocarbon degradation phenomenon, it can be learned that consortium between Petrophilic microbes and *Azotobacter* sp. is mutually beneficial in hydrocarbon degradation process. This is caused by the ability of *Azotobacter* sp. to produce biosurfactant as crude oil emulsifier, which assists the performance of bacteria and Petrophilic fungi to detoxify hydrocarbon compounds. Thus, *Azotobacter* sp. is also able to assist in utilization of intermediate compound from the hydrocarbon degrading activity of Petrophilic bacteria and fungi that affects the growth of *Azotobacter* sp. *Azotobacter* sp. also belongs to rhisozphere microorganism that exhibits distinct characteristics such as diverse metabolic capability, adaptive trait, and positive association with plan roots or other microorganisms (Daane, et al, 2001; Suryatmana, 2006).

Petrophilic bacteria and fungi have different properties in degrading hydrocarbon compounds. For instance, Petrophilic bacteria, although they can degrade various hydrocarbon compounds, have some weaknesses as what Van Eyk (1997) explained. He stated that *Bacillus cereus* type of Petrophilic bacteria does not undergo significant improvement of hydrocarbon-degrading compounds in stationary phase of the growth. This phenomenon results from change in culture condition caused by transformation substrates that form compounds, and they are oftentimes unknown and more toxic to degrading-bacteria culture.

Suryatmana (2006) asserted that hydrocarbon degradation rate is oftentimes limited by mass transfer from solid phase to liquid phase of the substrate that will be used as carbon source. In addition, according to Allen (1998), solubility level is one of the key factors that needs to receive special attention to ensure the readiness of substrate so that it can be used soon by microorganisms. According to the decree of Minister of Environment Number. 128 year 2003 on the final outcome of bioremediation, the result of bioremediation in the present study can be considered successful because the final TPH of crude oil waste hydrocarbon reached ≤ 1%. The use of *A. chroococcum* AC04 as co-culture of biosurfactant producer combined with Petrophilic can induce optimal condition for system of hydrocarbon-biodegradation process (Suryatmana, 2006).

Growth rate of petrophilic fungi

Fungi use nitrogen primarily in the form of ammonium produced by *Azotobacter* sp. in order to stimulate fungus growth and synthesis of some important cell contents including amino acid and protein (Noferdiman et al., 2008). Petrophilic fungi cannot perform their activities well to degrade hydrocarbon during the growth if the supporting nutrients essential to their life are not available in their living ecosystem in soil. This condition is illustrated in the statistical test result of the impact of Petrophilic microbes and *Azotobacter* sp. on the growth rate of Petrophilic fungi, showing there was no significant impact.

According to Table 2, it is noticeable that the independent test analysis result of Petrophilic and *Azotobacter* sp.'s impact on the growth rate of Petrophilic fungi was not significantly different either on the application of Petrophilic microbes or Azotobacter sp application.

Table 2. Impact of petrophilic consortium and *Azotobacter* sp. on the growth of petrophilic fungi.

Treatment	**Growth rate of petrophilic fungi (mg/10^2 CFU/day)**
Petrophilic (A)	
a_0 = without Petrophilic	97
a_1 = 2% Petrophilic fungi	83
a_2 = 2% Petrophilic bacteria	74
a_3 = 2% Petrophilic consortium	81
Azotobacter sp. (B)	
b_0 = without *Azotobacter* sp.	87
b_1 = *Azotobacter* sp. 0,5%	65
b_2 = *Azotobacter* sp. 1%	92
b_3 = *Azotobacter* sp. 1,5%	90

Treatment impact on the growth rate of Petrophilic fungi showed insignificant different result, whether it was the independent treatment impact of Petrophilic (A) or the independent treatment impact of *Azotobacter* sp. (B). Quantity interpretation of Petrophilic (A) in control level (without Petrophilic) unveiled higher rate of fungal growth (97 mg/10^2 CFU/day) than the growth rate in 2% level of Petrophilic fungi (83 mg/10^2 CFU/day), 2% Petrophilic bacteria (74 mg/10^2 CFU/day), or 2% Petrophilic consortium (81 mg/10^2 CFU/day). This indicates that bacterial group like *Pseudomonas fluorescens* is rod-shaped gram-negative bacteria (normally found in soil, plant, and water) can produce antibiotic compounds (antifungal), siderophore, and other secondary metabolites whose characteristics can obstruct the activity of *Fusarium oxysporum* fungi. Therefore, the antifungi produced by *Pseudomonas fluorescens* is also a key-player accounting for the suppression of Petrophilic fungi.

Similarly, the impact of treatment *Azotobacter* sp. (B) disclosed insignificant indifference in all levels. However, treatment of 1% *Azotobacter* sp. showed higher growth of Petrophilic fungi of 92 mg/10^2 CFU/day quantitatively than the level of treatment without *Azotobacter* sp. (87 mg/10^2 CFU/day), *Azotobacter* sp. 0.5% (65 mg/10^2 CFU/day), 1.5% (90 mg/10^2 CFU/day). This drop in the growth rate of Petrophilic fungi is presumably attributed to the existence of *Azotobacter* sp., which can suppress the growth of Petrophilic fungi with its antifungi.

Fungal cells in half range of dried fungal cell mass consists of carbon, which becomes an indication of the importance of carbon component in cell walls. Organic compounds can be used as structure-forming materials and energy provision for cells. Fungi can use organic materials as carbon source. Useable source of organic materials covers carbohydrate and organic acid. Carbohydrate is the most important organic material. Every fungus has different ability to use different carbon source, so it can affect the nutrient content. Hindersah and Simarmata (2004) mentioned that *Azotobacter* is one of the most important bacterial inoculants to improve nitrogen availability in soil and crop yields. Nevertheless, the result uncovered that soil nitrogen produced by *Azotobacter* sp. cannot bolster the growth of Petrophilic fungi in degrading hydrocarbon compounds in soil in terms of nitrogen supply. A plausible cause to this condition may be the competition to obtain nutrient source in soil. Anti-fungi compound produced by *Azotobacter* sp. is predicted to originate from growth hormone produced by the respective bacteria (Ridvan, 2009). Siderophore compound is produced in environment lacking of Fe ion (Adesina, 2007).

Growth rate of Azotobacter sp.

Azotobacter sp. bacteria in the present study are a group of bacteria isolated from rhisozphere soil of soybean. The rhisozphere soil has carbon source and energy for *Azotobacter* sp. that can be obtained from residual of degraded plant tissues. This genus has changeable morphology that depends on the cell age, media composition, and available substrate (Suryatmana, 2006). Nutrients in substrate is thought of not being able to supply carbon source for *Azotobacter* sp. in order to enhance the growth rate of *Azotobacter* sp. The statistical test result of the impact of Petrophilic microbes and *Azotobacter* sp. on the growth rate of *Azotobacter* sp. demonstrated no interaction. Table 3 presents the independent experiment result of the impact of Petrophilic microbes and *Azotobacter* sp. on the growth rate of *Azotobacter* sp.

The independent experiment analysis result (Table 3) revealed that the adding of Petrophilic microbes was not significantly different in increasing the growth rate of *Azotobacter* sp. An underlying reason might be that Petrophilic fungi and bacteria can only degrade substrate for their needs, so they do not produce metabolite that can be used by *Azotobacter* sp. to stimulate the growth. Furthermore, it may be the case that anti-fungi produced by *Azotobacter* sp. can affect the growth of Petrophilic fungi, so fungi cannot help *Azotobacter* sp. in terms of secondary metabolite provision. The adding of *Azotobacter* sp. (B) also delivered insignificantly different impact on the growth of *Azotobacter* sp.

Table 3. Impact of petrophilic consortium and *Azotobacter* sp. on the growth rate of *Azotobacter* sp.

Treatment	Growth rate (mg/10^5 CFU/day)
Petrophilic microbes (A)	
a_0 = without Petrophilic	34
a_1 = 2% Petrophilic fungi	31
a_2 = 2% Petrophilic bacteria	31
a_3 = 2% Petrophilic consortium	32
Azotobacter sp. (B)	
b_0 = without *Azotobacter* sp.	31
b_1 = *Azotobacter* sp. 0,5%	33
b_2 = *Azotobacter* sp. 1%	32
b_3 = *Azotobacter* sp. 1,5%	32

Notes: Numbers without letter notations mean there was no further Duncan's multiple range test because it was not significantly different based on range test in 5% level.

The difference in the growth rate of *Azotobacter* sp. is influenced by the ability of *Azotobacter* sp. in utilizing its energy source to grow and proliferate. Tarigan and Kuswandi (2010) said that one of the factors that contributes to the difference in growth rate is the ability of respective bacteria to use the available carbon source. The diversity of hydrocarbon-degrading microbes in soil can suppress the growth of *Azotobacter* sp., for a competition exists between three types of different Petrophilic microbes to obtain energy source of hydrocarbon from crude oil waste that can constraint the growth of *Azotobacter* sp.

Azotobacter sp. also requires some external factors to enhance its growth. Some of the external factors are water and oxygen. Without water and oxygen, microorganisms cannot reside in crude oil waste because microorganisms live in interphase between oil and water as well as crude oil pollutant on soil surface. Lack of water can become a

hindrance for microbes to obtain oxygen (Charlena, 2004). Thus, watering and land reversal are prerequisite to meet the need of water and aeration of *Azotobacter* sp.

Conclusion

There was an interaction between Petrophilic fungus and *Azotobacter* sp. occurred towards hydrocarbon degradation rate of crude oil waste. However, there was no interaction observed on the growth of *Azotobacter* sp. and Petrophilic fungus.

Acknowledgements

The first author thanks the Organizing Committee of the 11th Congress of Indonesian Soil Science Society, and the reviewers for their useful and inspiring comments

References

Adesina, M.F. 2007. Characterization of bacterial antagonists of *Rhizoctonia solani* and *Fusarium oxysporum* from six European soils and their potential application for biological control. Technischen Universität Carolo-Wilhelmina zu Braunschweig zur Erlangung des Grades einer Doktorin der Naturwissenschaften genehmigte. Dissertation

Alvarez, V.M., Santos, S.C., Casella, R.C., Vital, R.L., Sebastian, G.V. and Seldin, L. 2008. Bioremediation potential of a tropical soil contaminated with a mixture of crude oil and production water. *Journal of Microbiology and Biotechnology* 18 (12): 1966-1974.

Brock, T.D,, Madigan, M.T., Martinko, J.M. and Parkerm, J. 1994. Biology of Microorganisms, 7th edition. Prentice-Hall, Englewood Cliffs, NJ. :909 pp.

Charlena, 2004. Pencemaran Logam Berat Timbal (Pb) dan Cadmium (Cd) pada Sayur-sayuran. Program Pascasarjana/S3/Institut Pertanian Bogor.

Cybulski, Z., Dziurla, E., Kaczorek, E. and Olszanowski, A. 2003. The influence of emulsifiers on hydrocarbon biodegradation by *Pseudomondacea* and *Bacillacea* strains. *Spill Scince & Technology Bulletin* 8: 503–507.

Daane, L.L., Harjono, I., Zylstra, G.J. and. Haggblom, M.M. 2001. Isolation and characterization of polycyclic aromatic hydrocarbon-degrading bacteria associated with the rhizosphere of salt marsh plants. *Applied and Environmental Microbiology* 67 (6): 2683–2691.

De Carvalho, C. and Da Fonseca, M.M.R. 2005. Degradation of hydrocarbons and alcohols at different temperatures and salinities by *Rhodococcus erythropolis* DCL 14. *FEMS Microbiology Ecology* 51*:* 389–399.

Hafiluddin, 2011. Bioremediasi tanah tercemar minyak dengan teknik bioaugmentasi dan biostimulasi. *Embryo* 8 (1) : 47-52.

Haris, A; Udiharto dan M. Fierdaus. 2005. Penanggulangan Limbah Cair Kilang Minyak dengan Metode Bioremediasi. Prosiding Diskusi Ilmiah X PPPTMGB Lemigas. Jakarta.

Hindersah, R. dan Simarmata, T. 2004. Potensi rizobakteri Azotobacter dalam meningkatkan kesehatan tanah. *Jurnal Natur Indonesia* 5 (2): 127-133.

Jamilah. 2005. Potensi Bakteri Pendegradasi Hidrokarbon Minyak Bumi pada Tanah Terkontaminasi Minyak Bumi dengan Penambhaan Surfaktan. Fakultas Matematika dan Ilmu Pengetahuan Alam, IPB. Bogor.

Nababan, B. 2008. Isolasi dan Uji Potensi Bakteri Pendegradasi Minyak Solar dari Laut Belawan. Tesis Magister : Universitas Sumatera Utara.

Noferdiman, N., Rizal, Y., Mirzah, M., Heryandi Y. dan Marlida, Y. 2008. Penggunaan urea sebagai sumber nitrogen pada proses biodegradasi substrat lumpur sawit oleh jamur *Phanerochaete chrysosporium*. *Jurnal Ilmiah Ilmu-ilmu Peternakan* 11 (4): 175 – 182.

Nurhayati, N., dan Samallo, I.M. 2006. Analisis degradasi polutan limbah cair pengolahan rajungan (*Portunus pelagicus*) dengan penggunaan mikroba komersial. *Jurnal Ilmiah Fakultas Teknik LIMIT'S* 9 (1) : 1-13.

Ridvan, K., 2009. Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. *Journal of Environmental Biology* 30 (1): 73- 82.

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

Tarigan, R. dan Kuswandi. 2010. Mikrobiologi. JICA, Malang.

Thavasi, R., Nambaru, M.S., Jayalakhsmi, S., Balasubramanian, T. and Banat, I.M. 2009. Biosurfactant production by *Azotobacter chroococcum* isolated from the marine environment. *Marine Biotechnology* 11:551-556.

Udiharto, M. 1996. Peranan Bioremediasi dalam Pengolahan Lingkungan. Prosiding Pelatihan dan Lokakarya. Lembaga Ilmu Pengetahuan Indonesia. Cibinong.

Van Eyk, J. 1997. Petroleum Bioventing. A.A. Balkema, Ritternam, The Netherlands

Waluyo, L. 2005. Mikrobiologi Umum. Malang: UMM Press