HAYATI Journal of Biosciences December 2010
Vol. 17 No. 4, p 155-160
EISSN: 2086-4094

Available online at:
http://journal.ipb.ac.id/index.php/hayati
DOI: 10.4308/hjb.17.4.155

Kinetics of Petroleum-Contaminated Soil Biodegraded by An Indigenous Bacteria *Bacillus megaterium*

BAMBANG YUDONO[1*], MUHAMMAD SAID[2], SABARUDDIN[3], ADIPATI NAPOLEON[3], MARYATI BUDI UTAMI[1]

[1]Department of Chemistry, University of Sriwijaya, Jalan Raya Palembang Km. 32, Indralaya 30662, Sumatera, Indonesia
[2]Department of Chemical Engineering, University of Sriwijaya, Jalan Raya Palembang Km. 32, Indralaya 30662, Sumatera, Indonesia
[3]Department of Soil Science, University of Sriwijaya, Jalan Raya Palembang Km. 32, Indralaya 30662, Sumatera, Indonesia

Received November 19, 2009/Accepted December 27, 2010

Bioremediation of petroleum sludge was conducted by using land-farming method in micro scale and by applying an indigenous bacteria *Bacillus megaterium*. The samples were from PT. Pertamina Musi Banyuasin district of South Sumatra. The research aim was to evaluate the performance of the bacteria in degrading petroleum sludge. The rate of the biodegradation process was determined by using differential method and the data analyses show that the reaction order is 0.74. Then, the rate of biodegradation constant was determined by using an integral method assuming that the biodegradation process was a first reaction order. From the calculation, it was revealed that the biodegradation reaction constant was 0.0204/day. The bioremediation-kinetics model is y = -0.0204X + 2.0365, and by using this model the bioremediation process could be ended after 99.83 days. The qualitative analysis was carried out by using GC-MS to investigate the components of compounds changed during the bioremediation process. The results show that the *B. megaterium* could degrade 99.32% of alkane compounds.

Key words: bioremediation, degradation, microbia, *Bacillus megaterium*, petroleum

INTRODUCTION

The generation of oil-contaminated soil during the oil production processes has been increasing by thousands of tons every year in South Sumatra (Yudono *et al*. 2009). Parts of the contaminated soil are dehydrated-oil sludge, separated from the mixture of oil, water and soil. Most of the sludge is piled up outdoor next to the production site without any treatment, and poses serious environmental problems. The hydrocarbons in the sludge penetrate from the top soil into the subsoil slowly, presenting a direct risk of contamination to subsoil and groundwater. On the other hand, the light hydrocarbons in the sludge vaporize, leaving behind a layer of oil-containing dust which is blown upwards to pollute the air. These contaminations of soil, water and air pose serious risks for the environment and human population. The environmental Indonesian regulation called UU no. 23/1997 and PP No. 18/1999 stated that the petroleum sludge pollutants are included as hazardous material. It could not be kept too long, at maximum of 90 days these materials should be treated into non hazardous material. The initial total petroleum hydrocarbons (TPH) concentration of treated soil is not more than 15% and the final TPH concentration of residue should be less than 1% (Mursida 2002).

Handling petroleum pollutants can be done in many ways; physically by using burning process even though it will cause air pollution. Chemically by using dispersant such as non ionic detergent the pollutants will be bounded with dispersant which then percolated into the water basin. This precipitation is, however, so difficult to degrade that it will be hazardous for natural life. Chemically and physically pollutant handlings are suitable to reduce petroleum pollutants in water surface. Therefore, the oil sludge should be treated properly to prevent harm to environment. Although burning of the sludge may be simple and easily adaptable, this technique has undesirable hazard in air pollution. Bioremediation of the oil sludge is believed to be an efficient, economic and versatile alternative to physiochemical treatments if sufficient space and time are available (Jackson *et al*. 1996; Venosa *et al*. 1996; Salanitro *et al.* 1997). Acceptance by the general public is another major advantage of this technology (Skladney & Metting 1993). Indigenous microorganisms can utilize the total petroleum hydrocarbons (TPH) of crude oil as source of carbon and energy and break them down to simpler non-toxic compounds such as CO_2 and H_2O, in a process called demineralization.

Bioremediation takes a long time as the degradation efficiency of the bacteria is considerably low under natural conditions (Del'Arco & de Franca1999; Chaîneau *et al*. 2003). Therefore, some engineering processes such as the addition of nutrients, watering, tilling and the addition of appropriate microbial flora are necessary to improve the rate of hydrocarbon biodegradation (Vasudevan & Rajaram 2001; Barathi & Vasudevan 2003). Many efforts had been carried out to remedy petroleumsludge by using culture of microbes in South Sumatra in an industrial scale

*Corresponding author. Phone: +62-711-580335, Fax: +62-711-580056, E-mail: yudonob@hotmail.com

(Yudono *et al*. 2009). However, the time needed was still too long (around 240 days). Therefore, the main aim of this research is to improve the degradation rate of pollutant by using land-farming method.

Land-farming method is one of bioremediation methods for treating the petroleum sludge which was conducted on small scale. The samples were taken from a South Sumatra petroleum-containing pit disposal station, PT Pertamina oil fields. The *B. megaterium* bacteria was isolated and selected from the contaminated soil. This bacterium is one of the major microorganisms responsible for biodegradation of petroleum hydrocarbon (Atlas & Cerniglia 1995; Alexander 1999; Boonchan *et al*. 2000).We evaluated the performance of the *B. megaterium* bacteria in degrading petroleum sludge. It was calculated as the decreasing rate of TPH concentration per time unit. Furthermore, the degraded components of the sludge were analyzed by using GC-MS.

MATERIALS AND METHODS

Site and Experiment Scale. Bioremediation experiments of the petroleum- sludge were undertaken on small scale of 25 kg with the ratio of 1:100 from the actual bed of field scale process. The thickness of the dehydrated sludge in the prepared bed was 10 cm.

Pretreatment of the Petroleum Sludge. The sludge collected from the storage pit was put into the prepared bed. The sludge had heavy clay texture and low oxygen diffusivity. In order to enhance the aeration and the water-holding capacity of the sludge, organic, and inorganic bulking materials (wood particles and sandy soil) were added. The content of wood particles in the sludge was 10.0% (w/w) and that of sand was 10% (w/w). Urea was provided as a nitrogen source, and potassium dehydrogenate phosphate was used as a phosphorus source. The ratio of C, N, and P in the sludge was 100:10:1 after the addition of the fertilizers. The initial TPH concentrations were 4.18, 6.60, 9.82, 10.87, and 13.42% diluted from the main contaminated soil sample (71.16%).

Bioremediation Process. *B. megatorium* was obtained from petroleum -sludge-contaminated soils produced in petroleum-sludge pit of PT Pertamina South Sumatra Indonesia (indigenous bacteria). The bacteria was isolated and selected from the mixed culture, was then purified and enriched in BHMS medium consisting of $Mg_2SO_4 \cdot 7H_2O$ 0.2 g/l, $CaCl_2$ 0.02 g/l, KH_2PO_4 1 g/l, K_2HPO_4 1g/l, NH_4NO_3 1 g/l, $FeCl_3$ 0.05 gr/l dissolved in 1 l aquadest. The amount of inoculums applied was approximately 10% of treated soil. Over the course of the experiment, the land-farming cells were tilled twice a week to maintain high level of oxygen in the sludge. Water was added after tilling to maintain a moisture level of 40% in the sludge.

Determination of Reaction Order by Using Differential Method. The general formula of the reaction rate that can describe the rate of TPH reduction is:

$$r = \frac{dC}{dt} = -kC^n \quad (1)$$

$$\ln r = -\ln k + n \ln C \quad (2)$$

where r: reaction rate (concentration unit/time unit), t: time (day), C: remaining TPH concentration (mg/l) at any time, n: reaction order, and k : first order kinetic constant (1/day).

In Equation1, it is assumed that the microbial concentration remains constant over the entire experimentation period. Therefore, the effect of microbial concentration on the kinetics constant can be neglected. If the data from Figure 1 are inserted into Equation 2 and plotted into graph ln r vs ln c described as Figure 2, it will be straight line graph with the slope is n and the intercept is ln k.

Determination of Rate Reaction Constant by using Integral Method. In the kinetics, the change in concentration with time is followed from the start of reaction, $[C]_0$ at t = 0 to $[C]_t$. If the rate of reaction is assumed following the first order of reaction, these are the limits between which the integral is taken from $[C]_0$ to $[C]_t$

$$\int_{[C]_0}^{[C]_t} \frac{d[C]}{[C]} = -k\int dt \quad (3)$$

$$\ln \frac{[C]_0}{[C]_t} = kt \quad (4)$$

where $[C]_0$ is the initial concentration (mg/l) or TPH_0.

In order to experimentally calculate the kinetic constant k, the equation 4 can be derived into equation 5 as followed

$$\ln[TPH] = -kt + \ln[TPH]_0 \quad (5)$$

Then the data were plotted as ln [TPH] vs t, the reaction constant can be determined as the slope of the graph.

The Chemical and Biological Monitoring. The initial and final states of samples from the bioremediation process were performed by using a Gas Chromatography-Mass Spectrometry (GC-MS), and The Most Probable Number method was used to monitor the number of bacteria in the sludge samples (Mesarch & Nies 1997).

Analytical Methods and Data Analysis. The petroleum sludge was sampled at different stages of bioremediation. Five samples were taken for treatment as described diagrammatically in the Figure 1. The oil content in the sludge was determined gravimetrically in the amount of TPH extracted by diethyl ether (Christofi *et al.* 1998; Capelli *et al.* 2001).

The differential method of data analysis was used to determine the reaction order, and the integral method of data analyzes was used to determine the constant of reaction rate.

RESULTS

Microbial growth on pollutant mixture is an important aspect of bioremediation treatment. However, efforts to develop mathematical models for mixed substrate kinetics were limited. When individual microbial species are considered, simple competition for the growth substrate is the only interaction included (Rugner *et al.* 2006). Here, the results are presented using *B. megaterium* growing

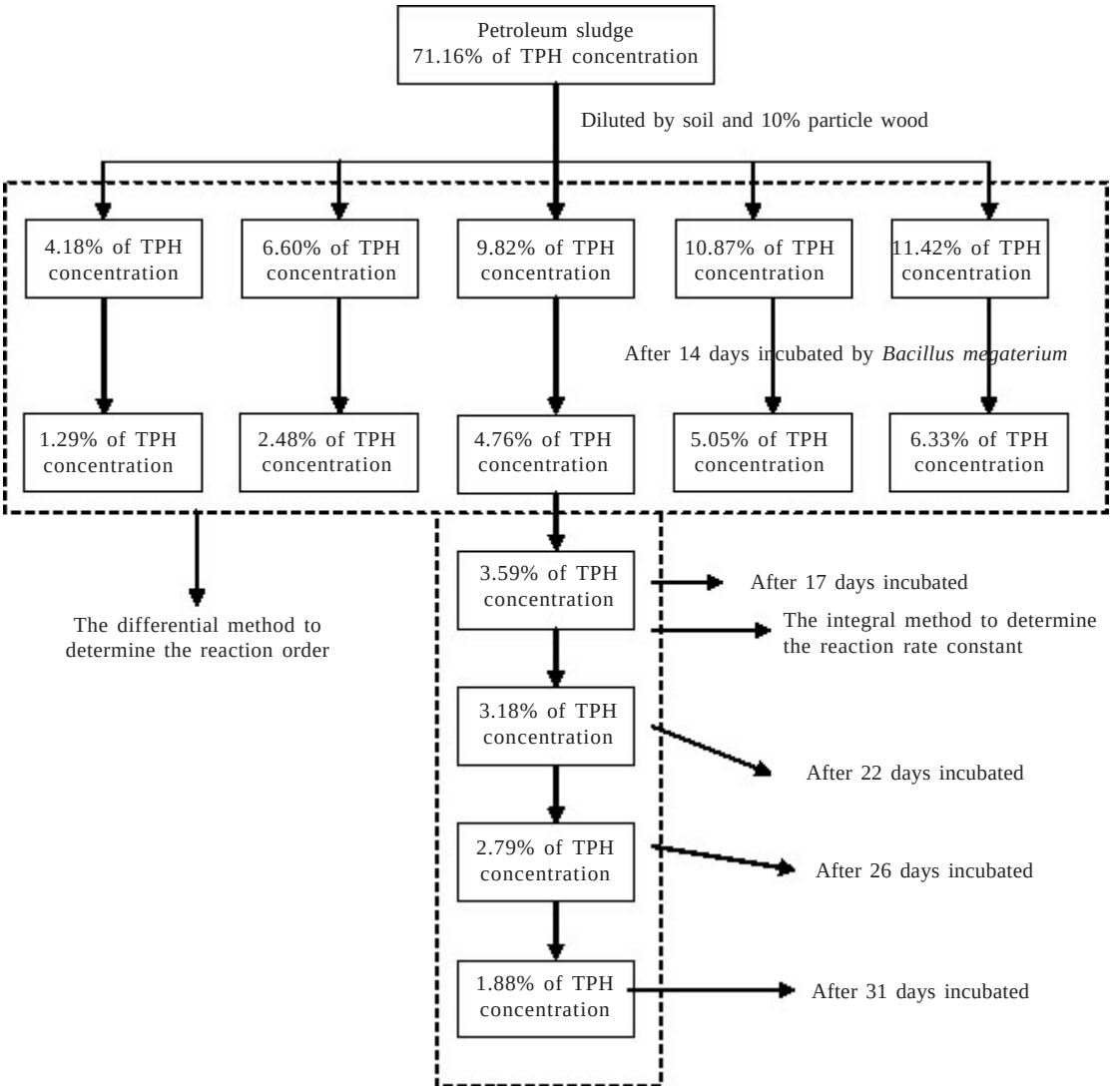

Figure 1. Diagram of The differential method and integral methods of kinetics.

individually on crude oil and using mathematical models to describe these results by using Differential Method and Integral Method.

Mathematical Model by using Differential Method. TPH was calculated to the total of hydrocarbon content in the sample. The TPH analysis was conducted by using extraction and gravimetric methods. The initial concentrations of samples were set as 4.18, 6.60, 9.82, 10.87, and 13.42% w/w. After 2 weeks inoculation, the decreasing TPH concentrations of each sample were measured and the results were 1.29, 2.48, 4.76, 5.05, and 6.33. The data was studied to determine the reaction order of biodegradation of petroleum sludge by using differential method as described in the equation 2. The data showed in the Table 1 then it was plotted into a graph ln r vs ln c as described at the Figure 2 as a linear graph, the equation is y = 0.7437X – 2.6476. The slope of the graph is 0.7437 which described as the reaction order and the intercept as ln k. Experimentally, the differential method is simpler than the integral method to describe the kinetics model, it is only 2 set data needed; initial and final concentrations with 2 weeks interval time. These are completely systematic, and eliminate the necessity of making guesses as to possible orders. It gives the order and rate constant direct from one graph.

Table 1. Biodegradation of Petroleum sludge after 14 days process

TPH Concentration (%)					
Initial condition (C_0)	After 14 days (C)	ΔTPH	r	ln C_0	1n r
4.18	1.29	2.8797	0.2056	1.43	-1.5818
6.60	2.48	4.1194	0.2942	1.88	-1.2234
9.82	4.76	5.0545	0.3610	2.28	-1.0188
10.87	5.05	5.8157	0.4154	2.38	-0.8785
13.42	6.33	7.0830	0.5059	2.59	-0.6814

$r = \Delta TPH/\Delta\tau$.

Mathematical Model by using Integral Method. The initial concentration of samples was 9.82%; it was inoculated with *B. megaterium* bacteria. During 14, 17, 22, 26, and 31 days observation, the decreasing TPH concentrations of samples were 4.76, 3.59, 3.18, 2.79, 3.72, and 3.29%, respectively. The first-order kinetics is said to be valid if a linear relationship is achieved upon plotting the logarithmic part of Equation 5 versus time. Analysis

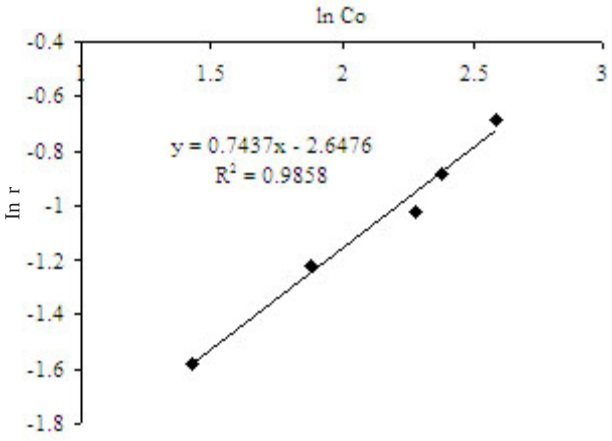

Figure 2. Gaphic ln r vs ln C to determine reaction order and rate constant by using Differential Method.

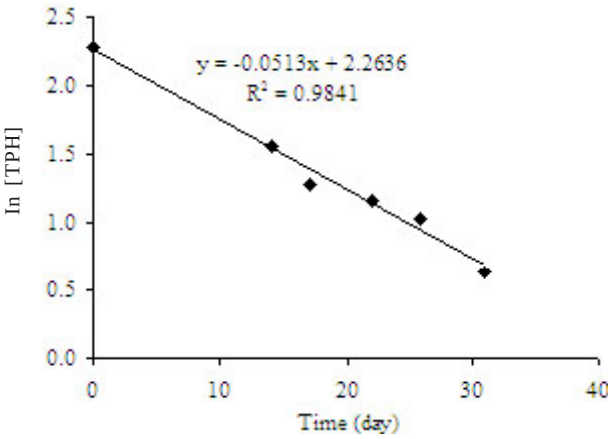

Figure 3. ln TPH vs Time to determine the constant of reaction rate by using Integral Method. The reaction order is assumed as first reaction order.

of the rates of hydrocarbon removal showed that most compounds obeyed first-order kinetics. The slope of the line represents the first-order kinetics constant k. By using the first order reaction equation;

$$\ln TPH = -kt + \ln TPHo$$

the data was plotted ln TPH vs t. The graph is shown in Figure 3. The slope of graph is -0.0204/day, it represents the rate reaction constant. The intercept of the graph is 2.0365. So the equation of reaction rate is y = -0.0204 x + 2.0365. The progress of bioremediation process can be predicted by using this chemical kinetics equation, for example to reach the TPH concentration below 1%, the bioremediation process will take place as long as 99.83 days. These results are well fitted in a great extent with the results achieved in previous studies (Hutchins *et al.* 1991).

GC-MS Analyzes. The changed composition of compounds from initial to final conditions after bioremediation process was identified by using GC-MS. Figure 4 and 5 show initial and final compositions, respectively. Every peak in the chromatogram represents a component of compound in the petroleum sludge, and the peak area represents the concentration of the component. The identical retention time in the both chromatograms show the identical compounds. The different shape of the peaks area is caused by the bioremediation process. The predicted compounds were drawn from MS Library. The data analyses were conducted at every identical retention time. The chromatograms show that *B. megaterium* bacteria could almost completely degrade alkanes compounds such as $C_{13}H_{28}$, $C_{15}H_{32}$, $C_{20}H_{42}$, $C_{24}H_{50}$, and $C_{28}H_{58}$ as seen in the Table 2. The data show that the *B. megaterium* bacteria could effectively degrade the long chain hydrocarbon compounds. However, it is

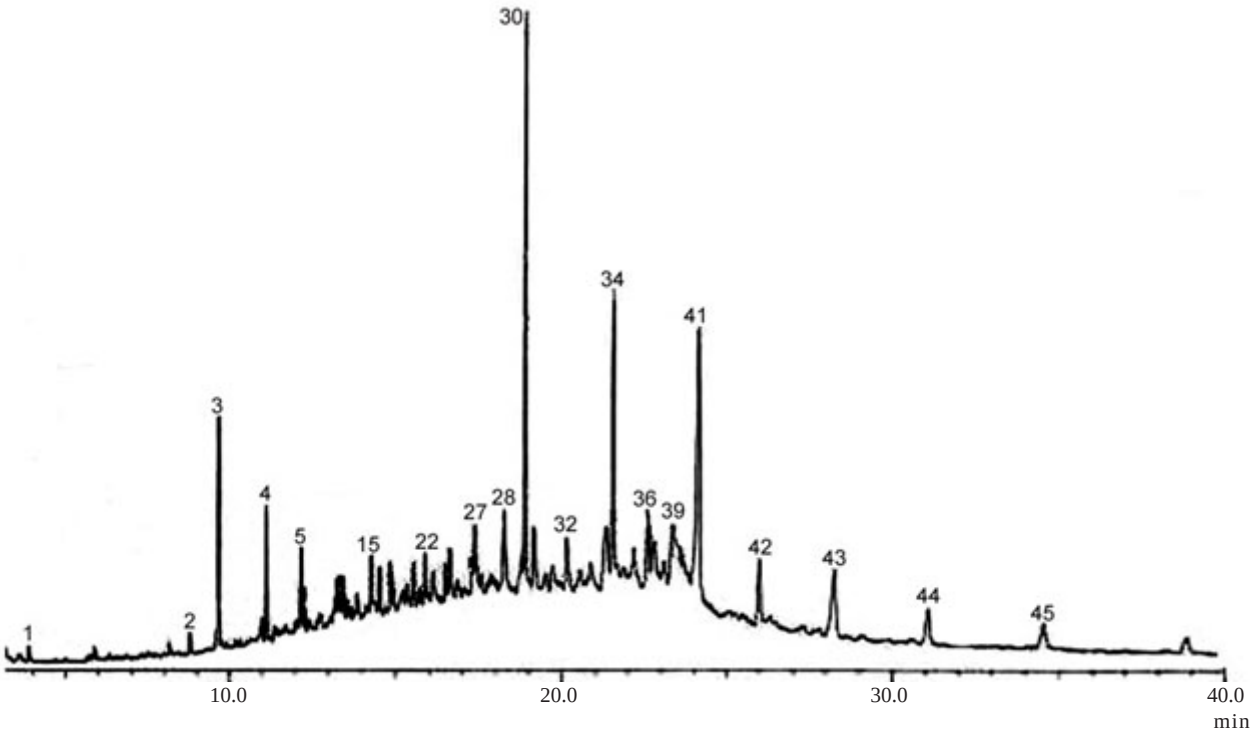

Figure 4. Chromatograph of contaminated soil in initial condition before incubated by *Bacillus megaterium* bacteria

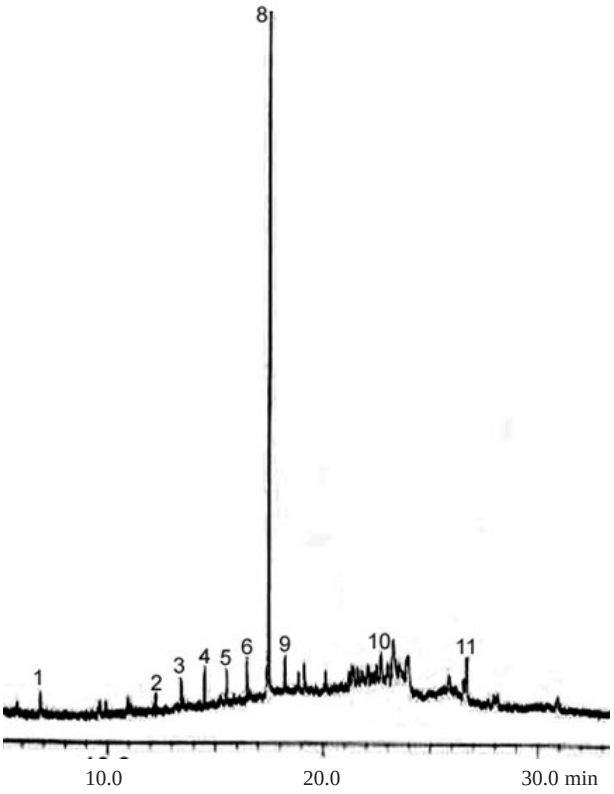

Figure 5. Chromatograph of contaminated soil after 31 days incubated by *Bacillus megaterium* bacteria.

Table 2. The GC-MS data analyzes

Suggested compounds	Retention time	Peak area		% Decrease in peak area
		Initial	Final	
$C_{15}H_{32}$	9.67	2371814	0	100
$C_{20}H_{48}$	11.12	1237247	0	100
$C_{16}H_{34}$	14.53	356315	52724	85.20
$C_{28}H_{58}$	16.49	397884	41460	89.58
$C_{13}H_{28}$	24.15	4963140	0	100
Percent of decreasing peak area average =				94.96

needed to investigate closely the structure of the compounds degraded during the bioremediation process by using more detail separation technique.

DISCUSSION

Monitored natural-attenuation is a remediation method relying on natural biodegradation processes that decreases concentration of the contaminating substances in the environment over time. When the method is used as a remediation strategy, it has to be demonstrated that the degradation processes are taking place. Degradation data are also needed in modeling based impact assessments (Rugner *et al.* 2006). Demonstrating in-situ biodegradation of contaminant is, however, often challenging, especially at heterogeneous sites where representative time series demonstrating the decrease in contaminant concentrations are difficult to obtain. Therefore, experiments in controlled laboratory may have to be performed.

Microbial degradation-rates have been showed for several specific compounds, often using microbial cultures, laboratory microcosms or with mixed cultures in the field. The degradation for any compound in any specific habitat varies depending on the prevailing microbial community as well as on highly variable environmental factors such as temperature, pH, soil moisture, other C sources, presence of inhibiting compounds as well as on the properties of compounds in the contaminant (Alexander 1999; Moreels *et al.* 2004). For this reason, degradation rates reported in literatures may not reflect the degradation rates at a specific site, and therefore the degradation rates at contaminated sites should always be investigated on a case-by-case basis (Rugner *et al.* 2006). This research has investigated the use of a single cultured bacteria *B. megaterium* to degrade the petroleum sludge, the results showed that *B. megaterium* could degrade alkane compounds effectively. By using the kinetics model, *B. megaterium* could degrade petroleum pollutan with TPH concentrations from 9.82 into below 1% after 99.83 days.

ACKNOWLEDGEMENT

We thank Directorate General Higher Education for financial support of this competitive research grant. We would like to acknowledge Department of Chemistry, FMIPA, University of Sriwijaya for providing the laboratory facilities, Department of Biology, FMIPA, University of Sriwijaya for microbial taxonomy assistance, and PT Pertamina Sumbagsel for providing petroleum sludge samples.

REFERENCES

Alexander M. 1999. Biodegradation and Bioremediation. New York: Acad Pr.

Atlas RM, Cerniglia CE. 1995. Bioremediation of petroleum pollutants. *Bio Sci* 45:332-338.

Barathi S, Vasudevan N. 2003. Bioremediation of crude oil contaminated soil by bioaugmentation of Pseudomonas fluorescens NS1. *J Environ Sci Hlt, Part A—Toxic/Hazardous Substances & Environmental Engineering* 38:1857-1866.

Boonchan S, Britz ML, Stanley GA. 2000. Degradation and mineralization of high-molecular-weight polycyclic aromatic hydrocarbons by defined fungal bacterial co cultures. *J Appl Environ Microbiol* 66:1007-1019.

Capelli SM, Busalmen JP, Sanchez SR. 2001. Hydrocarbon bioremediation of a mineral base contaminated waste from crude oil extraction by indigenous bacteria. *Int Biodeterioration & Biodegradation* 47:233-238.

Chaîneau CH, Yepremian C, Vidalie JF, Ducreux J, Ballerini D. 2003. Bioremediation of a crude oilpolluted soil: biodegradation, leaching and toxicity assessments. *Water, Air, and Soil Pollution* 144:419-440.

Christofi N, Ivshina IB, Kuyukina MS, Philp JC. 1998. Biological treatment of crude oil contaminated soil in Russia. London: Geological Society.

Del'Arco JP, de Franca FP. 1999. Biodegradation of crude oil in sandy sediment. *Int Biodeterioration & Biodegradation* 44:87-92.

Hutchins SR, Sewell GW, Kovacs DA, Smith GA. 1991. Biodegradation of aromatic hydrocarbons by aquifer microorganism under denitrifying, conditions. *Environ Sci Technol* 25:68-76.

Jackson AW, Pardue JH, Araujo R. 1996. Monitoring crude oil mineralization in salt marshes: Use of stable carbon isotope ratios. *Environ Sci Technol* 30:1139-1144.

Mesarch MB, Nies L. 1997. Modification of heterotrophic plate counts for assessing the bioremediation potential of petroleum contaminated soils. *Environ Technol* 18:639-646.

Moreels D, Bastiens L, Ollevier F, Merckx R, Diels L, Springael D. 2004. Evaluation of the intrinsic methyl ter-butyl ether (MTBE) biodegradation potential of hydro carbon contaminated subsurface soil in batch microcosm system. *FEMS Microbiol Ecol* 49:121-128.

Mursida. 2002. Consortium microorganism vs. oil sludge. *Oil plus*: 15 April-14 Mei: 48-51.

Rugner H, Finkel M, Kaschl A, Bittens M. 2006. Application of monitored natural attenuation in contaminated land management-a review and recommended approach for Europe. *Environ Sci Pol* 9:568-576.

Salanitro JP, Dorn PB, Huesemann MH, Moore KO, Rhodes IA, Jackson LMR. 1997. Crude oil hydrocarbon bioremediation and soil ecotoxicity assessment. *Environ Sci Technol* 31:1769-1776.

Skladney GJ, Metting FB. 1993. Soil microbial ecology. 2nd Ed. New York: Marcel-Dekker.

Vasudevan N, Rajaram P. 2001. Bioremediation of oil sludge-contaminated soil. *Environ Int* 26:409-411.

Venosa AD, Suidan MT, Wrenn BA, Strohmeier KL, Haines JR, Eberhart BL. 1996. Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. *Environ Sci Technol* 30:1764-1775.

Wrenn BA, Venosa AD. 1996. Selective enumeration of aromatic and aliphatic hydrocarbon-degrading bacteria by a most-probable number procedure. *Canadian J Microbiol* 42:252-258.

Yudono B, Said M, Pol Hakstege, Suryadi FX. 2009. Kinetics of indegenous isolated bacteria *Bacillus mycoides* used for ex-situ bioremediation of petroleum contaminated soil in PT Pertamina Sungai Lilin South Sumatera. *J Sust Develop* 2:64-71.