

## PERFORMANCE OF PETROFILIC CONSORTIA AND EFFECT OF SURFACTANT TWEEN 80 ADDITION IN THE OIL SLUDGE REMOVAL PROCESS

QOMARUDIN HELMY, EDWAN KARDENA and WISJNUPRAPTO\*

Water and Wastewater Engineering Research Group, Faculty of Civil and Environmental Engineering,  
Bandung Institute of Technology, Jalan Ganesha 10 Bandung, Indonesia.

\*Corresponding author: Phone: +62 817 023 5878; Fax: +62 817 023 5878; E-mails: [helmy@tl.itb.ac.id](mailto:helmy@tl.itb.ac.id)

Received: 25<sup>th</sup> July 2009; Revised: 10<sup>th</sup> October 2009; Accepted: 12<sup>th</sup> October 2009

**Abstract:** Hydrocarbons which include petroleum product, oil product also oil sludge as by product, form an important class of pollutants on a global scale. The presences of such hydrocarbons in the environment are considerable public health and ecological concerns, because of their persistence, toxicity and ability to bioaccumulate. Many technologies are employed to clean up contaminated sites including various chemical and physical methods such as thermal evaporation and soil vapor extraction. However, cleaner technologies are needed due to the environmental friendly such as microbial degradation concept. This research was investigated the performance of petrofilic consortia in degrading oil sludge and surfactant addition to enhance biodegradation process. The experiment was conducted in aerobic batch culture with minimum media. Petrofilic bacteria that grown on oil sludge as sole carbon follow the Monod kinetics which value of  $\mu_m$  and  $K_s$  was  $0.497 \text{ day}^{-1}$  and  $2,738 \text{ mg.L}^{-1}$ , respectively. Research result showed that after 105 days of incubation, the petrofilic bacteria able to remove the oil sludge up to 63.8% while addition of surfactant increase the removal efficiency up to 90.9%. These results suggest that both petrofilic consortia and biosurfactant addition stimulate the biodegradation and overcome the limitation of petroleum hydrocarbon degradation process.

**Keywords:** biodegradation, hydrocarbon, oil sludge, surfactant

### INTRODUCTION

Contamination of soils, groundwater, sediments, surface water, and air with hazardous and toxic chemicals is one of the major problems facing the oil and gas industry in Indonesia. Recent accidents attribute to oil spillages in Tarakan (East Kalimantan), Sorong (Papua), Indramayu (West Java) and Bojonegoro (East Java) should giving a due consideration of the national level.

Petroleum hydrocarbon continues to be used as the principle source of energy and hence a large global environmental pollutant. Apart from accidental contamination of ecosystem, one of the most encountered pollutants in petroleum production companies is the formation of oil sludge that is entrapped with the effluents during treatment and conditioning of the wells produced crude oil through treatment process facilities.

Common sources of these sludges are storage tank bottoms, oil-water separators, flotation and biological wastewater treatment units, cleaning of processing equipment, and soil from occasional minor spills on refinery grounds [1,2]. The composition of these sludges varies according to their origin, storage, and treatment history. Most of the oil sludge is piled up outdoor without any treatment, and poses a serious environmental problem. 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 oil sludge vaporize, leaving behind a layer of oil-containing dust of soil which blows upwards to pollute the air. Therefore, the oil sludge should be treated to prevent harm to environment. Although burning of the sludge may be simple and easily adaptable, this technique has undesirable hazard in air pollution [3]. Cleaner technologies are needed due to the environmental friendly such as microbial degradation concept [4,5].

Biodegradation is a treatment technology used to remediate a variety of contaminants, including soils contaminated with petroleum hydrocarbons. Bioremediation is an engineered process where the natural biodegradation of petroleum hydrocarbons by indigenous soil bacteria, fungi, and protozoa is accelerated. Since the vast majority of hydrocarbons in crude oils and refined products are biodegradable, and hydrocarbon-degrading microbes are ubiquitous, biodegradation can be an environmentally acceptable way of eliminating oil sludge [6]. So far, biodegradation suggests an effective method [7]. During biodegradation, hydrocarbon containing in oil sludge is used as an organic carbon source by a microbial process, resulting in the breakdown of oil sludge components to low molecular weight compounds. However, the bioavailability of weakly soluble hydrophobic compounds for microbial conversion is usually low and thus limits their degradation rate in aqueous medium [8,9]. The use of surfactants has been found to enhance degradation of crude oil [10,11] or other hydrocarbons [12]. In this paper, we investigated the performance of petrofilic consortia in degrading oil sludge and surfactant addition to enhance biodegradation process.

## **MATERIALS AND METHODS**

### **Reagents**

All chemicals were of reagent grade, purchased from Merck, J.T. Baker and Sigma Chem Co while growth media were purchased from Oxoid Ltd. Oil sludge and crude oil samples were obtained from Balongan Oil Field Indramayu and Duri Oil field Riau Indonesia, respectively.

### **Bacterial Isolate and Culture Conditions**

Isolation and characterization of bacteria isolated from several site of oil contaminated soil in Indonesia was conducted in order to find the bacteria that have an ability to metabolize petroleum hydrocarbon as it sole carbon source. Furthermore, culture of bacteria was adapted with oil sludge as sole carbon source. Bacteria that able to degrade or use oil sludge as sole carbon were isolated and then used as inoculums in this research. *Bacillus cereus* BL01,

*Pseudomonas stutzeri* BL02, *Acinetobacter* sp. BL03 and *Bacillus* sp. BL04 were successfully isolated and used in the kinetic study and biodegradation assay of oil sludge.

The experiment was conducted in batch culture and grown on a minimal basal medium (MB) which composed the following components (g.L<sup>-1</sup>) of distilled water [6]: 1.5 of K<sub>2</sub>HPO<sub>4</sub>; 0.5 of KH<sub>2</sub>PO<sub>4</sub>; 0.2 of MgSO<sub>4</sub> and 0.5 of (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>. 10 ml Trace Element solution was added per liter of MB medium. The composition of this trace element (g.L<sup>-1</sup>) are 12 of Na<sub>2</sub>EDTA<sub>2</sub>.H<sub>2</sub>O; 2 of FeSO<sub>4</sub>.7H<sub>2</sub>O; 1 of CaCl<sub>2</sub>; 0.4 of ZnSO<sub>4</sub>.7H<sub>2</sub>O; 10 of NaSO<sub>4</sub>; 0.4 of MnSO<sub>4</sub>.4H<sub>2</sub>O; 0.1 of CuSO<sub>4</sub>.5H<sub>2</sub>O and 0.5 of Na<sub>2</sub>MoO<sub>4</sub>.2H<sub>2</sub>O. Each petrofilic bacteria was maintained at 4 °C on Nutrient Agar covering with 1 drop of crude oil. Sub-cultures were made to fresh agar slants every 1 month to maintain viability.

### **Petrofilic Consortia Growth Kinetic**

To prepare the inoculums, each isolates were grown separately in 250 ml Erlenmeyer flasks containing 100 ml of the minimal medium and crude oil (1%) as sole carbon and energy source. All the isolates were grown to mid-log phase (app. 10<sup>6</sup> CFU.ml<sup>-1</sup>) and then mixed in equal proportions. The mixed culture was used as the inoculums (2%) for the growth kinetic study with oil sludge as sole carbon source at various concentrations (2,000; 5,000; 10,000; 20,000 and 25,000 mg.L<sup>-1</sup> TPH). The growth of microorganisms was measured every day with TPC (total plate count) method while culture conditions in the reactor were as follows: room temperature (27 °C), agitation at 110 rpm and pH 7.0 (adjusted with 1 N HCl-NaOH).

### **Emulsification Index (E24)**

To determine the emulsification index, Batista et al., [13] method was applied. A mixture of 1:1 between surfactant and oil sludge was agitated for about 2 minute then stabilized for 24 hour. Emulsification index (%) determined by measuring the column height of emulsified oil against its total height multiplied by 100 times.

### **Surface Tension Measurement**

Surface tension was determined using a DuNouy Tensiometer (Fischer Sci. 21) at room temperature. A 30 ml of sample was put into a clean glass vessel that was placed on the tensiometer platform. A platinum wire ring was submerged into the solution and then slowly pulled through the liquid-air interface. Between each measurement, the platinum wire ring was rinsed three times with water and three times with acetone and allowed to dry [14].

### **Total Petroleum Hydrocarbon (TPH) Measurements**

Measurement of TPH was conducted with gravimetric method as described by Mishra et al. [15]. Sample was extracted with *n*-hexane, the organic layer were pooled and dried by evaporation of solvents. After evaporation, the amount of residual TPH recovered was weighted.

### **GC-MS analysis**

Biodegradation of TPH fractions were verified by Gas Chromatography coupled to Mass Spectrometer. GC-MS analyses were performed with a Shimadzu GC-MS 5000 series. Compounds were separated on 30m x 0.25 mm GC capillary column. The column temperature was held at 60 °C for 2 min and then programmed to 320 °C at the rate of 10 °C min<sup>-1</sup>. This final

temperature was held for 2 min. Injector temperature was set at 300 °C and gas flow rate was 1.6 ml.min<sup>-1</sup>. GC eluant was subject to MS analyses with M/Z range 33-550, respectively.

### Oil Sludge Biodegradation Assay

To determine the performance of petrofilic bacteria in degrading oil sludge, a preliminary biodegradation assay developed and set up as follows:

- K1/Control-1: without addition of both petrofilic inoculums/P and surfactant/S.
- K2/Control-2: without P; add 2% (v/v) S.
- T0/Reactor-1: add 2% (v/v) P; without S.
- T2/Reactor-2: add 2 % (v/v) each of P and S.
- Oil sludge initial concentration was 100,000 mg.L<sup>-1</sup> TPH and incubation time for biodegradation assay was 105 days.

Total petroleum hydrocarbon (TPH) concentration and growth of petrofilic bacteria were observed on certain time.

## RESULTS AND DISCUSSION

### Growth kinetics determination

The aim of growth kinetic experiments is to determine the ability of isolated microorganisms in the degradation of oil sludge. The kinetics parameters to be determined are specific growth rate,  $\mu$  (time<sup>-1</sup>); maximum specific growth rate,  $\mu_{max}$  (time<sup>-1</sup>) and half saturation constant,  $K_s$  (mg.L<sup>-1</sup>). The growth of a microbial culture is a complex phenomenon composed of a number of simultaneously occurring events. The relative magnitudes of the respective rates determine what the net effect is upon the culture. The primary events are the utilization of substrate and the growth of organisms. These two events are closely related because it is only through the utilization of substrate that energy and carbon are made available for cell growth. The growth rate is referred to as a specific rate because it defines the rate of cell growth in terms of the concentration of cell present. To find the relation of cell growth and substrate utilization, petrofilic consortia were grown on oil sludge with variation in substrate concentration as described in method. Growth curve of petrofilic consortia were linearized at exponential phase resulting regression line which slope is its specific growth rate.

Specific growth rate of petrofilic consortia were shown in Table 1. The value of the kinetics parameters are very dependent upon the organism and substrate employed. If a given species of organism is grown on each of several substrates under fixed environmental conditions the value of kinetics parameters observed will depend upon the substrate. Likewise, if several pure cultures are fed the same substrate under identical environmental conditions, the value of kinetics parameters will depend upon the species of organisms.

Table 1: Specific growth rate ( $\mu$ ) and Specific growth rate due to decay ( $\mu_d$ ) of petrofilic consortia at difference substrate concentration.

Rate (day <sup>-1</sup> )	Substrate concentration (mg.L <sup>-1</sup> TPH)				
	2,000	5,000	10,000	20,000	25,000
$\mu$	0.2689 ± 0.08	0.3812±0.063	0.4705±0.011	0.5795±0.031	0.5958± 0.02
R <sup>2</sup>	0.8194	0.9191	0.8292	0.9675	0.9059
$\mu_d$	-0.072± 0.013	-0.115± 0.01	-0.149± 0.04	-0.187 ± 0.09	- 0.217 ± 0.07
R <sup>2</sup>	0.8653	0.97	0.9494	0.9377	0.9324

Based on Table 1 above showed that specific growth rate of petrofilic consortia tend to increase as substrate concentration increased. The highest specific growth rate occurs in petrofilic culture that grown on 25,000 mg/l TPH which is  $0.5958 \pm 0.02 \text{ day}^{-1}$ . Specific growth rate due to decay ( $\mu_d$ ) or bacterial death expressed as the loss of active biomass by endogenous metabolism of living cell. The measurement of specific growth rate due to decay ( $\mu_d$ ) is similar with specific growth rate ( $\mu$ ), except for data that used was at death phase of bacterial growth curve. Plot of the linearized concentration of active biomass as a function of time give a regression line with slope is equivalent to its specific death rate. Table 1 showed that the lowest specific death rate of petrofilic consortia occur in culture that grown on substrate concentration  $2,000 \text{ mg.L}^{-1}$  TPH and increase as substrate concentration increased. This means that the death rate of viable bacteria is directly proportional to the concentration of viable bacteria in the medium.

Once data are available relating to  $\mu$  and substrate concentration, it can be used to estimate the kinetics parameter which is the monod saturation constant ( $K_s$ ) and the maximum specific growth rate ( $\mu_m$ ). The most common transformation is obtained by taking the reciprocal of both side of the Monod equation, known as the Lineweaver-Burk equation. A plot of  $1/\mu$  as a function of  $1/C_s$  (Fig. 1) give a linear regression were slope or gradient is  $K_s/\mu_m$  therefore the intercept on X is  $(-1/K_s)$  and to the Y is  $1/\mu_m$ . From linear regression yielding  $1/\mu_m = 1.596$  so the maximum specific growth rate value  $0.497 \text{ day}^{-1}$ , and the monod saturation constant,  $K_s$  value  $2,738 \text{ mg.L}^{-1}$ . It means that if  $\mu_m$  condition have achieved, increasing in substrate concentration would not take effect on specific growth rate of viable biomass anymore.

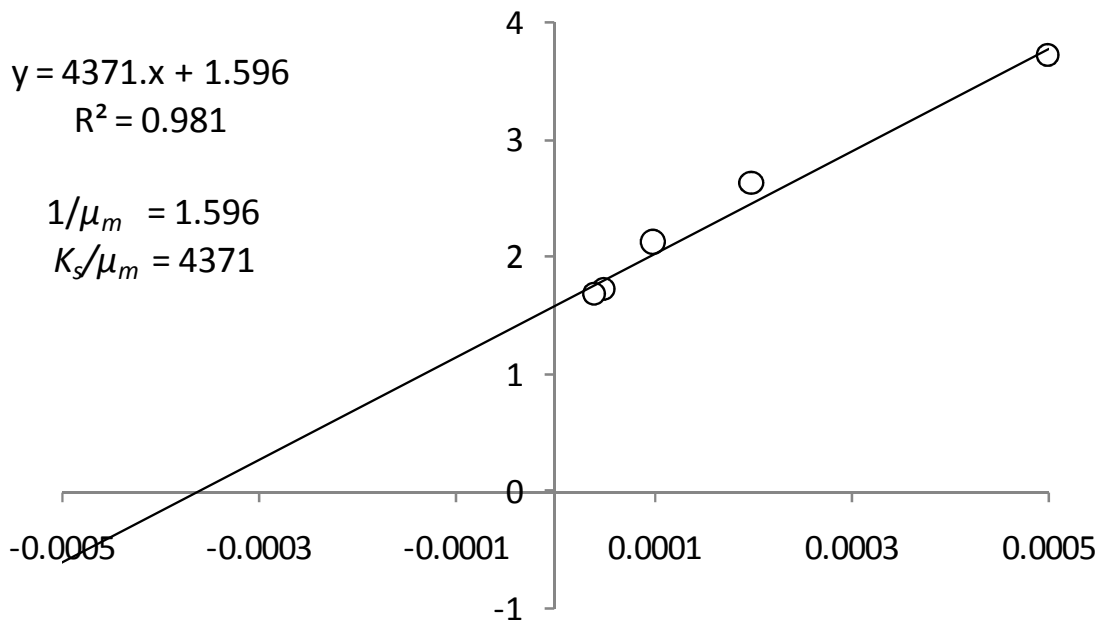


Fig. 1: A plot of  $1/\mu$  as a function of  $1/C_s$  to determine the kinetics parameters of  $K_s$  and  $\mu_m$ .

$K_s$  value showed the affinity of biomass to substrate and therefore a concentration of substrate at half of its maximum growth rate. Biomass which high affinity to substrate have a low  $K_s$  value, that mean growth rate will not effected until substrate concentration decrease to a very low value. As opposite if biomass which low affinity to substrate have a high  $K_s$  value, its mean growth rate

will be effected by the remain substrate concentration which still high. If the substrate concentration is below the  $K_s$  value thus the growth of biomass will very slow. That why the addition of substrate in the reactor at least equal to or much higher than its  $K_s$  value.

### Surfactant Enhanced Biodegradation of Oil Sludge

Surfactants are also used in emerging technologies like enhance remediation of hydrocarbon and crude oil-contaminated soils. Hydrocarbon contaminants are removed from the environment, primarily as a result of their biodegradation, which is performed by native microbial populations. Such biodegradation is known to be time-consuming and new technologies have been developed; for example the addition of surfactant help to stimulate the indigenous microbial population to degrade hydrocarbons at rates higher than those which could be achieved through addition of nutrients alone. Surfactant is a well known surface active agent that generally used in improving the viability of contaminant to the microbial attack. The surfactants affect the biodegradation process by increasing the solubility and dispersion of the compound [16]. There are two ways in which surfactant affect which is increasing the surface area of hydrophobic water insoluble substrate and secondly is increasing the bioavailability of hydrophobic water-insoluble substances. A laboratory scale of surfactant enhanced biodegradation of oil sludge was conducted. Effects of addition of surfactant Tween 80 in the biodegradation process were shown in Table 2.

Table 2. TPH removal efficiency of oil sludge biodegradation in batch reactor. K1/Control 1 without addition both Petrofilic inoculums/P and Surfactant/S; K2/Control 2 (-P, +S); T0/Reactor 1 (+P, -S); T2/Reactor 2 (+P, +S).

Biodegradation system	TPH Removal Efficiency (%) <sup>1</sup>	Increased Removal Efficiency (%)
Oil sludge biodegradation		
K1	20.1	11.7 (K1-K2)
K2	31.9	43.6 (K1-T0)
T0	63.8	27.1 (T0-T2)
T2	90.9	58.9 (K2-T2)

<sup>1</sup> means values from triplicate measurement.

The low water-solubility of many hydrocarbons reduces their availability to microorganisms and limits the biodegradation process. It has been assumed that surfactant can be used to enhance the bioavailability of hydrophobic compounds. On the other hand this low water-solubility increases sorption of compound to surface and limits their availability to biodegrading microorganisms [10]. Once again, surfactant can enhance growth on bound substrates by desorbing them from surfaces or by increasing their apparent water solubility. Figure 2 showed the microbial growth and TPH profile of control reactor. It was noticed that changes in oil sludge environmental condition from it's originally slurry phase into more aqueous phase in the reactor, triggering the indigenous bacteria in it to grow. For the total plate count measurement, the CFU values increased from  $10^{3.2}$  (CFU.ml<sup>-1</sup>) at day 0 and reach its maximum to  $10^{5.4}$  (CFU.ml<sup>-1</sup>) in the

first week of incubation. Similar pattern occurred in the control reactor-2 (without addition of petrofilic inoculants, added by 2% v/v of surfactant only). Surfactant addition make the oil sludge become more soluble in the reactor, this shown by increase in the microbial growth from  $10^{3.2}$  (CFU.ml<sup>-1</sup>) at day 0 and reach it's maximum to  $10^6$  (CFU.ml<sup>-1</sup>) in the first week of incubation. However, the degradation process of oil sludge by mean of indigenous bacteria predicted small enough throughout the experiment. TPH losses in control reactors mainly due to weathering/physical influences [17] such as temperature shift [18], shaking condition [19], volatilization of low molecular weight of hydrocarbon [20] and photo-oxidation [21]. This phenomenon confirmed the results reported by Gallego et al. [22], who observed disappearance of diesel compound in the absence of biological activity reached 11% after 45 days of incubation. While addition of synthetic surfactant and biosurfactant in the reactor increased the oil sludge degradation rate by indigenous bacteria to 12 and 21% after 60 days incubation, respectively [23].

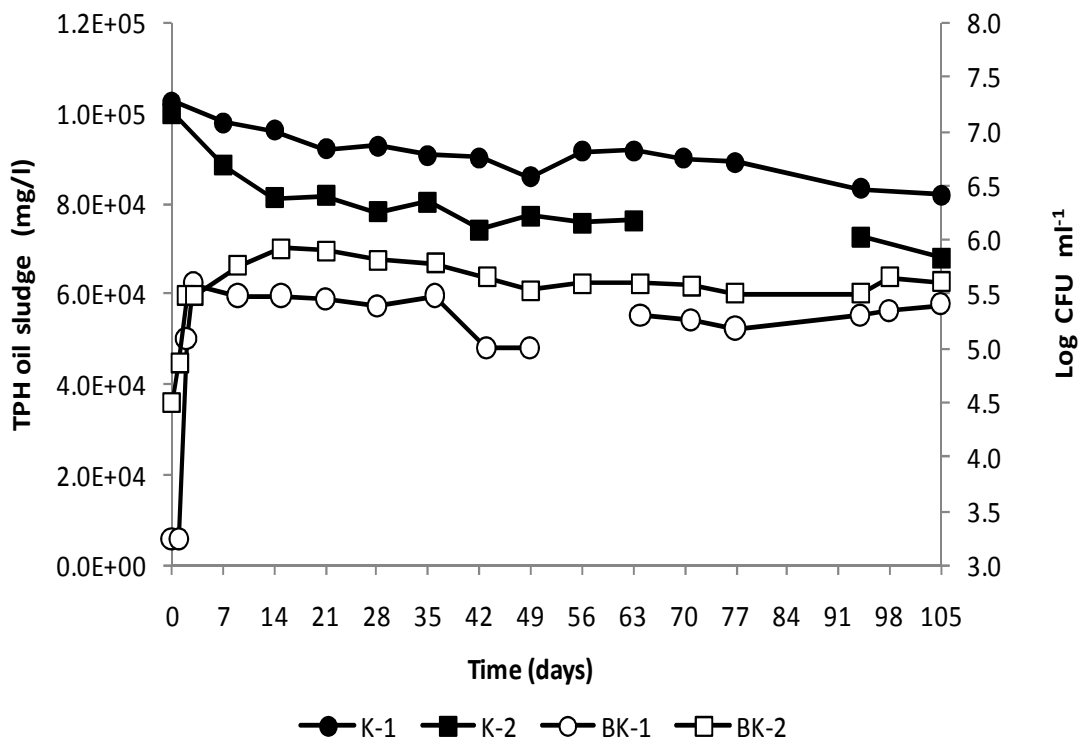


Fig. 2: The indigenous microbial growth (open symbol) and TPH degradation (solid symbol) profiles in batch control reactor system with 0 (circle/K-1) and 2 (square/K-2) % v/v of tween 80 addition at 27°C.

Figure 3 shows that after 105 days of incubation, a significant reduction of TPH (63.8%) occurred in the biodegradation system supplemented with petrofilic consortia/T-0. This positive result suggests that bio-augmented bacteria could degrade TPH significantly. Bioaugmentation also can be used to increase the biodegradative capabilities of the indigenous microbial population. Compared with control reactor/K1, addition of petrofilic consortia increased the

removal efficiency up to 43.6%. Non biological degradation (physical transformation) also occurred in the process; however the biological transformation dominated the process based on the growth of bacteria observed during the process. For the total plate count measurement, the CFU values increased from  $10^{6.5}$  (CFU.ml<sup>-1</sup>) at day 0 to  $10^{7.5}$  and  $10^{6.5}$  (CFU.ml<sup>-1</sup>) in the first week and day 105 respectively. The presence of tween 80 in biodegradation system (T-2) increased the removal efficiency up to 27% compared to those without addition of surfactant/T-0. The present of surfactant also increased the microbial growth from  $10^{6.5}$  (CFU.ml<sup>-1</sup>) at day 0 to  $10^{8.9}$  (CFU.ml<sup>-1</sup>) in the first week of incubation and  $10^{8.3}$  (CFU.ml<sup>-1</sup>) at day 105. Similar result by Whang et al. [5], that examined the effect of biosurfactant (rhamnolipid) to diesel/water degradation from 0 to 80 mg.L<sup>-1</sup> significantly increases biomass growth and diesel biodegradation percentage from 1000 to 2500 mg VSS.L<sup>-1</sup> and 40-100%, respectively.

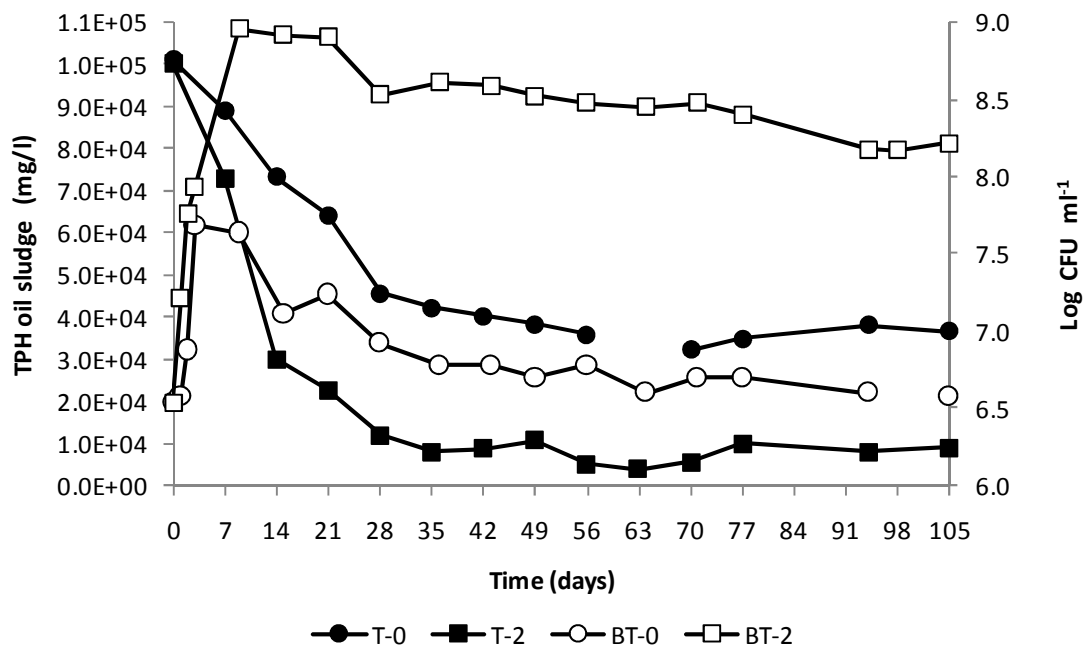


Fig. 3: The petrofilic consortia growth (open symbol) and TPH degradation (solid symbol) profiles in batch reactor system with 0 (circle/T-0) and 2 (square/T-2) % v/v of tween 80 addition at 27°C.

Our findings show that the addition of both petrofilic consortia and surfactant favors the biodegradation of the oil sludge. We choose 2% (v/v) concentration of tween 80 because more surfactants are required in soil/slurry-water system that might be expected to reach the CMC (Fig. 4) in systems utilizing clear water. It was supported by Kim et al. [24], in their study on removal of PAHs using nonionic surfactant that found the solubility of the PAHs was proportional to the concentrations of the surfactants when above the CMC while below or near the CMC, additional surfactant did not enhance the solubility of the PAHs. The limiting condition in the degradation of hydrocarbon and other PAH is their insolubility, thus decreasing the efficiency and rate of degradation. This limitation can be overcome either by addition of surface-active compounds surfactant to the growing culture, thus making hydrocarbons more water-soluble and available for

the cell to degrade, or by production of its own surfactant by the augmented organisms to facilitate uptake.

Kinetic study resulting in  $K_s$  value of 2,738 mg.L<sup>-1</sup>, which mean petrofilic consortia have the affinity to utilized substrate approximately to its  $K_s$  value. However, biodegradation assay of oil sludge showed the lowest concentration of oil sludge after 105 days incubation time was 9.130 mg.L<sup>-1</sup> (Reactor T-2). Oil sludge concentration tend insignificantly decrease after 60 days of incubation until the end of treatment (105 days). Most of the oil sludge compounds are insoluble in water systems and their bioavailability is limited by their sorption onto the soil matrix, while low molecular weight hydrocarbons are partially soluble in water and hence are used easily by petrofilic microorganisms. Other reason as reported by Atlas and Bartha, [25] was nutrient deficiency (nitrogen and phosphor) in the system that becomes limiting factors in biodegradation. Theoretically, 150 mg of nitrogen and 30 mg of phosphor are needed in order to convert 1 g of hydrocarbon compound to be material cell of bacteria. Moreover, toxic and recalcitrant compound such as PAHs in the oil sludge are persist and difficult to be degraded.

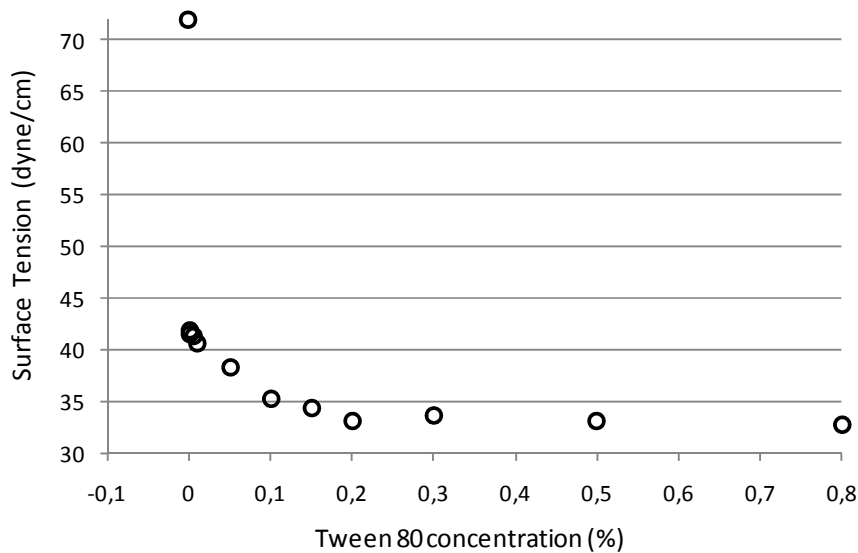


Fig. 4: Surface tension versus concentration of Tween 80 in minimal medium (pH 7), the CMC value was 0.2%.

GC-MS analyses of hydrocarbon compounds in the oil sludge explain the biodegradation assay result. Hydrocarbon compounds of oil sludge sample at day 0 constitute of 49 detectable compounds while at day 105 constitute of 21 compounds (Table 3). The removal of targeted hydrocarbon compounds observed including pristane as result of the biodegradation process (Fig. 5).

Table 3: Abundance and composition of hydrocarbon compounds in the oil sludge sample.

Compounds	Day 0		Day 105	
	Abundance	Composition (%)	Abundance	Composition (%)
Aliphatic	35	82.84	10	47
Aromatic	2	3.05	-	-
PAHs	12	14.11	11	53
Total	49	100	21	100

Chromatogram profiles (GC-MS) of TPH extract from oil sludge revealed that the n-alkanes are the most biodegradable components of the saturated fraction of petroleum product while the groups of PAHs are more recalcitrant. This has been attributed to the branched and ring chemical structure also toxic properties of PAHs [10].

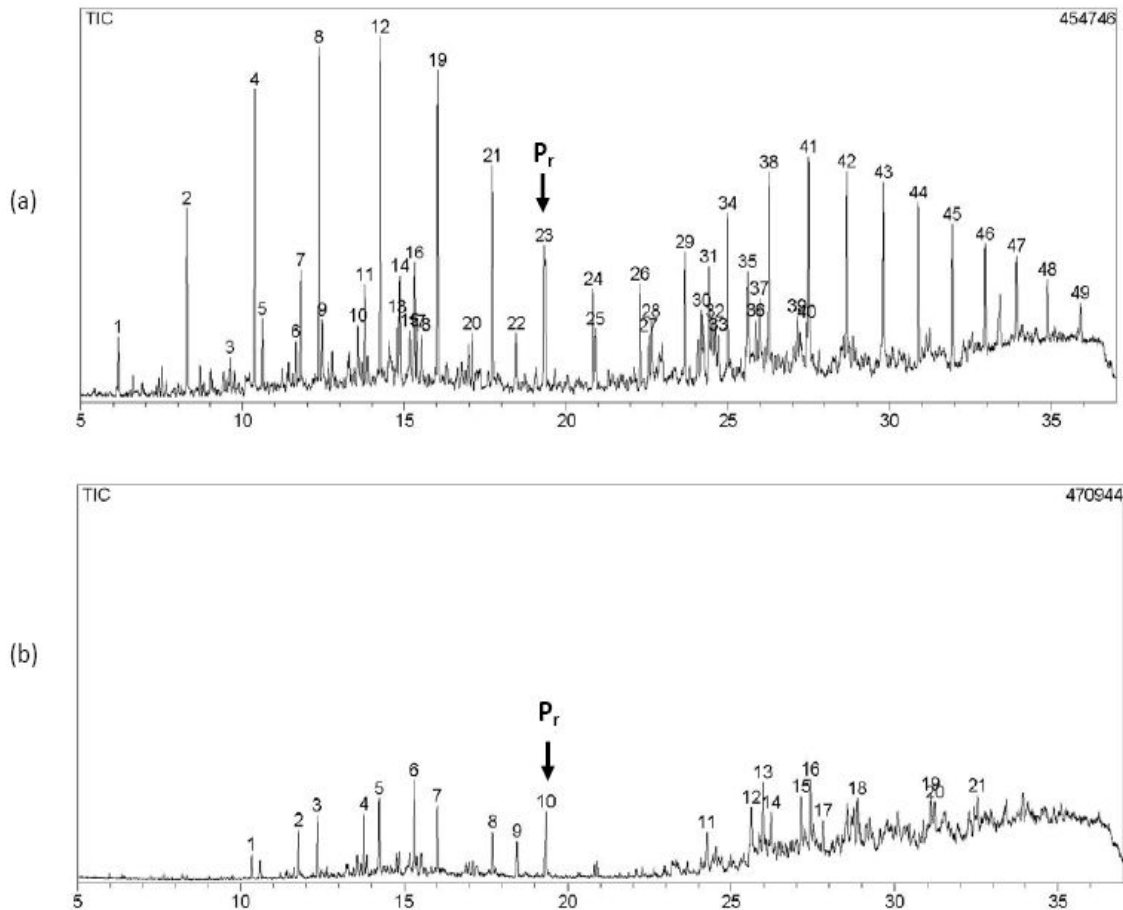


Fig. 5: GC-MS chromatogram profiles of oil sludge sample at day 0 (a) and day 105 (b) of reactor T-2; Pr, pristane.

## CONCLUSION

Referring to the Indonesian State Ministry of Environment (Decree no. 128/2003), it was mandatory to manage a safe treatment of these wastes (oil sludge) and also disposed off in an environmental friendly manner. It was regulated that the final concentration of TPH must less than  $10.000 \text{ mg.kg}^{-1}$ . Based on the data presented in this paper indicate that the petrofilic consortia have the capability to degrade oil sludge to comply with the regulation above. The petrofilic consortia have a quiet low  $K_s$  value ( $2,738 \text{ mg.L}^{-1}$ ) which means that petrofilic consortia were effective to degrade the oil sludge to a final low concentration. Effect of addition of surfactant in the biodegradation process indicated the enhancement of both degradation process and growth

of petrofilic bacteria. TPH removal efficiency increased from 63.8% up to 90.9% as the addition of Tween 80 surfactant to the process.

**Acknowledgments:** This research was supported and financed by ITB and Directorate General of Higher Education (DIKTI) Government of Indonesia. QH thanks P. Suryatmana and S. Hidayat for their contribution in part of this research. The authors also gratefully acknowledge Prof. Naoyuki Funamizu, Laboratory of Engineering for Sustainable Sanitation, Hokkaido University for facilitating QH in the research fellowship program in Japan.

## References

1. Dibble, J.T and R. Bartha. 1979. Effect of Environmental Parameters on the Biodegradation of Oil Sludge. *Applied and Environmental Microbiology*, 37:729-739.
2. Mrayyan, B and M.N. Battikhi. 2005. Biodegradation of total organic carbons (TOC) in Jordanian petroleum sludge. *Journal of Hazardous Materials B120*: 127–134
3. De-qing, S., Z. Jian., G. Zhao-long., D. Jiang., W. Tian-li., V. Murygina and S. Kalyuzhnyi. 2007. Bioremediation of Oil Sludge in Shengli Oilfield. *Water Air Soil Pollut.*, 185:177-184.
4. Helmy, Q., P. Suryatmana., E. Kardena and Wisjnuprpto. 2006. Effect of Surfactant Addition on Oil Sludge Biodegradation by Petrofilic Bacteria. The 3<sup>rd</sup> International Conference on Environmental Tecnology and Management. Bandung-Indonesia. Proceeding; Bi-O-04: 1-5.
5. Whang, L-M., P-W.G. Liu., C-C. Ma and S.S. Cheng. 2007. Application of biosurfactant, rhamnolipid and surfactin for enhanced biodegradation of diesel contaminated water and soil. *Journal of hazardous materials*, doi:10.1016/j.jhazmat.2007.05.063.
6. Helmy, Q., E. Kardena and Wisjnuprpto. 2008. Kinetic Study of Oil Sludge Biodegradation by Petrofilic Bacteria. The 2<sup>nd</sup> South East Asian Technical University Consortium (SEATUC) Symposium. Bandung-Indonesia. Proceeding, pp: 193-197.
7. Boopathy, R. 2000. Factors Limiting Bioremediation Technologies. *Bioresource Technology*, 74: 63-67
8. Genouw, G., F. de Naeyer., P. van Meenen., H. van de Werf., W. de Nijs and W. Verstraete. 1994. Degradation of oil sludge by landfarming-a case-study at the Ghent harbour. *Biodegradation*, 5:37-46.
9. Vasudevan, N and P. Rajaram. 2001. Bioremediation of oil sludge-contaminated soil. *Environment International*, 26: 409-411.
10. Abalos, A., M. Vinas., J. Sabate., M.A. Manresa and A.M. Solanas. 2004. Enhanced biodegradation of Casablanca crude oil by a microbial consortium in presence of rhamnolipid produced by *Pseudomonas aeruginosa* AT10. *Biodegradation*, 15:249-260.
11. Urum, K and T. Pekdemir. 2004. Evaluation of biosurfactant for crude oil contaminated soil washing. *Chemosphere*, 57: 1139-1150.
12. Olivera, N.L., M.G. Commendatore., A.C. Moran and J.L. Esteves. 2000. Biosurfactant enhanced degradation of residual hydrocarbons from ship bilge wastes. *Journal of industrial microbial. biotechnol.*, 25: 70-73.
13. Batista.S.B., A.H. Munteer, F.R. Amorim, & M.R. Totola. 2006. Isolation and characterization of biosurfactant/ bioemulsifier-producing bacteria from petroleum contaminated sites. *Bioresource Technology*. 97: 868–875.
14. Sarubbo, L.A., C.B.B. Farias., & G.M.C. Takaki. 2007. Co-Utilization of Canola Oil and Glucose on the Production of a Surfactant by *Candida lipolytica*. *Current Microbiology*, 54: 68–73
15. Mishra, S., J. Jyot., R.C. Kuhad and B. Lal. 2001. Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge contaminated soil. *Applied and environmental microbiology*, 67: 1675-1681.
16. Desai, J.D & I.M. Banat. 1997. Microbial Production of Surfactants and Their Commercial Potential. *Microbiology and Molecular Biology Reviews*, 6 (1): 47–64.

17. Barakata,A.O., Y. Qian., M. Kim., dan M.C. Kennicutt. 2001. Chemical characterization of naturally weathered oil residues in arid terrestrial environment in Al-Alamein, Egypt. *Environment International*, 27: 291–310
18. Swannell, R.P.J., K. Lee and M. McDonagh. 1996. Fields Evaluations of Marine Oil Spill Bioremediation. *Microbiology Rev.* 60: 342-365.
19. Oudot, J., F.X. Merlin dan P. Pinvidic. 1998. Weathering rates of oil components in a bioremediation experiment in estuarine sediments. *Marine Environmental Research* 45: 113–125
20. Venosa, A.D dan X. Zhu. 2003. Biodegradation of Crude Oil Contaminating Marine Shorelines and Freshwater Wetlands. *Spill Sci. Technol. Bulletin*, 8: 163-178
21. Atlas, R.M and R. Bartha. 1998. *Microbial Ecology. Fundamental and Applications.* Benjamin Cummings Sci. Pub., CA, pp. 556-598.
22. Gallego, J.L.R., J. Loredó., J.F. Llamas., F. Vazquez and J. Sanchez. 2001. Bioremediation of diesel-contaminated soils:Evaluation of potential in situ technique by study of bacterial degradation. *Biodegradation* 12: 325-335.
23. Rocha, C and C. Infante. 1997. Enhanced oily sludge biodegradation by a tension-active agent isolated from *Pseudomonas aeruginosa* USB-CSI. *Appl. Microbiol. Biotechnol* 47: 615-619
24. Kim, I.S., J.S. Park and K.W. Kim. 2001. Enhanced biodegradation of polycyclic aromatic hydrocarbons using nonionic surfactants in soil slurry. *Applied Geochemistry*, 16:1419-1428.
25. Atlas, R.M., R. Bartha. 1973. Stimulated Biodegradation of Oil Slicks Using Oleophilic Fertilizers. *Environ. Sci. Technol.* 7:538-541