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The Effects of Bio-surfactant on the Oil Recovery of Oily Sludge

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Abstract

A series of experiment on surfactant enhanced bioremediation of oil refinery sludge using slurry-phase reactor have been conducted in a laboratory scale pilot plant. The pilot plant used for bioremediation process was provided with helical screw impeller to mix the oil refinery sludge and surfactant performing a slurry system. The growth of microorganisms was measured by Total Plate Count method while oil content was measured by gravimetric method. The results on soil washing of oil refinery sludge showed that the bio-surfactant produced was able to reduce the total petroleum hydrocarbon (TPH) content from 32 % (w/w) to 20 % within 24 hours. After soil washing process, the oil refinery sludge then be treated by bioremediation method using Petrophilic Microorganisms Consortia (PMC) as inoculants with the addition of 1 g/l bio-surfactant. The results showed that within an additional 29 days for bioremediation process, the TPH content could be reduced to less than 3 %. In the meantime, the same experiments using synthetic surfactant (Tween 80) resulted in reducing the TPH content to 24 % by soil washing process, and to 6.66 % by bioremediation.

1. INTRODUCTION

Oil refinery sludge may contain 30 - 40 % (w/w) of total petroleum hydrocarbon (Helmy et al., 2006). According to Krishnamurthi et al. (2007), some substances contained in the oil refinery sludge are carcinogenic and immunotoxicant so that they have to be managed properly in order to avoid negative impact on the environment, especially on human health such as neurotoxicity (Watts, 1997), DNA destruction and chromosome differences (Morelli et al., 1995), and cancer cell growths (Eweiss et al., 1998). One of the effective and environmental friendly methods used in oil refinery sludge management is using the biological agents such as petroleum hydrocarbon degrading microbes which is known as bioremediation process method. But, due to the environmental regulation for applying this method, the maximum content of total petroleum hydrocarbon (TPH) in oil refinery sludge should be 15% (w/w). So, the TPH content must be reduced to around that level before the bioremediation method can be applied. Reducing the TPH content from the oil refinery sludge can be done i.e. by means of so called soil washing using the addition of surfactant, either synthetic or bio products. The bioremediation process itself will also be enhanced or speed up by the addition of these substances.

It is well known that some bacteria are capable to produce emulsifier/surface active agents what so called bio-surfactant. According to Kosaric (1992) it has some advantages compare to synthetic products, i.e. biodegradable, low level of toxicity, biocompatible, high availability of production raw materials, low production cost especially if waste products are used as raw materials, and very useful for environmental management i.e. in oil spills handling and bioremediation of industrial wastes contaminated soils. Some researchers discovered that bio-surfactant can be produced by *Azotobacter sp*, i.e. *Azotobacter vinelandii* as published by Levisauskas et al., (2004), *Azotobacter chrococcum* (Suryatmana et al., 2006) and *Azotobacter* sp. GNC 01 as reported by (Helmy et al., 2009).

This paper will talk about the production of bio-surfactant in relation to its application for oil refinery sludge handling and its bioremediation process.

2. MATERIALS AND METHODS

2.1 *Oil Refinery Sludge*

Oil refinery sludge samples was obtained from Balongan Oil Field Indramayu Indonesia.

2.2 *Bacterial strain and Culture Conditions*

Azotobacter sp. GNC01 was used in producing biosurfactant, while the petrophilic microorganism consortia (PMC) were used in the bioremediation assay of oil refinery sludge. All bacteria were obtained from the Culture Collection of Environmental Biotechnology Laboratory-Environmental Engineering Department, Institut Teknologi Bandung, Indonesia. *A. vinelandii* was maintained at 4°C on mannitol enrichment agar slants containing (l^{-1}): 20 g mannitol, 20 g yeast extract, 20 g tryptone, and 15 g of agar. While PMC was maintained at 4°C on minimum media with addition of crude oil as sole carbon source. Sub-cultures were made to fresh agar slants every 1 month to maintain viability.

2.3 *Biosurfactant Production*

Cultures of *Azotobacter* sp. GNC01 were grown on a minimal basal medium (MB) which composed the following components (l^{-1}) of distilled water: 1.5 g of K_2HPO_4; 0.5 g of KH_2PO_4; 0.2 g of $MgSO_4$; 0.25 g of $(NH_4)_2\ SO_4$; and 20 g glucose as substrate. 10 ml Trace

Element solution was added per liter of MB medium. The compositions of this trace element (l^{-1}) are 12 g of $Na_2EDTA_2.H_2O$; 1 g of $CaCl_2$; 0.4 g of $ZnSO_4.7H_2O$; 10 g of $NaSO_4$; 0.4 g of $MnSO_4.4H_2O$; 0.1 g of $CuSO_4.5H_2O$; 0.5 g of $Na_2MoO_4.2H_2O$ (Helmy et al., 2009). The medium was sterilized by autoclaving at 121°C for 15 min. The inoculums was prepared by transferring cells grown on a slant to 250 ml Erlenmeyer flasks containing 50 ml of MB broth. Culture was incubated in an orbital shaker at room temperature, 110 rpm for 2 days. The MB containing 10^6 cells/ml was used to initiate growth using 2% (v/v) inoculums. Biosurfactant production was carried out in a 10 liter capacity of fermentor at 37°C with agitation speed of 100 rpm and aeration rate of 0.176 $ft^3.min^{-1}$ for 2 days.

2.4 *Crude Biosurfactant Isolation*

The fermentation broth was centrifuged at 13.000 rpm for 30 minute to obtain a cell free broth. After centrifugation, the supernatant was then dissolved in a 4 N hydrochloric solution and allowed to stand overnight at 4°C, followed by the biosurfactant extraction step with a chloroform solvent at room temperature (Makkar and Cameotra, 1998). The organic layer was transferred to a round-bottom flask and the aqueous layer was re-extracted two times for complete recovery of biosurfactant. The organic phases were combined yielding a viscous brown-colored crude biosurfactant product and then evaporated to remove the solvent; the residue was collected and weighted.

2.5 *Oil Refinery Sludge Washing*

A portion of the bio-surfactant produced was then used for soil washing of the Balongan Oil Refinery Plant origin oil sludge (containing 32% by weight of TPH) using a bench scale slurry reactor as shown in **Figure 1**. The reactor was provided with a helical screw impeller to mix the oil refinery sludge and surfactant performing a slurry system. This slurry consists of 30 % (w/w) oil sludge and 70 % washing liquid. (distilled water + 1 g/l bio-surfactant, Tween 80 and control). This process was carried out within 24 hours. Control was also applied using distilled water without addition of surfactant as the washing liquid. After 24 hours of soil washing, the oil parts of the slurry system which were free from the solid matrix were separated manually. The solid parts then are treated by bioremediation process in the same reactors.

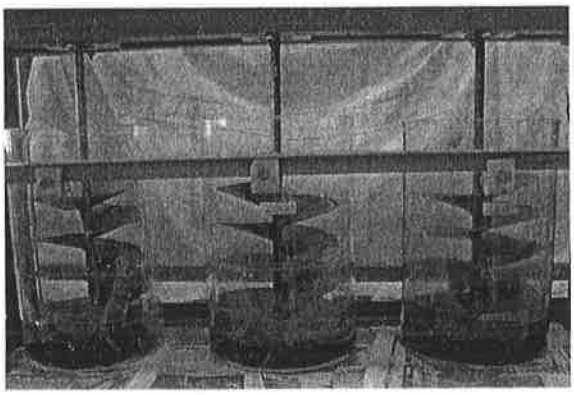

Figure 1: Bench scale slurry reactor

2.6 *Total Petroleum Hydrocarbon (TPH) Measurements*

Measurement of TPH was conducted with gravimetric method as described by Mishra et al., (2001). 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.

2.7 *Bioremediation Assay*

The bioremediation process used 5% (w/w) PMC as inoculants + 1 g/l surfactant (either bio-surfactant produced or synthetic surfactant Tween 80). The bioremediation process was continued up to 30th day. TPH concentration and growth of PMC were observed on certain time.

3. RESULTS AND DISCUSSIONS

Performing the experiments on bio-surfactant production at various G values in the reactor, at initial pH = 6.5 and temperature T = 37°C, using new strain of *Azotobacter* sp. GNC 01 as inoculants on optimum media composition, the results shown that G value effects the bio-emulsifier production as well as biomass concentration in the reactor as shown in **Table 1**.

Table 1: Bio-surfactant produced by *Azotobacter sp GNC 01* at various G values in the reactor, at initial pH = 6.5 and T = 37°C

Velocity gradient G (sec^{-1})	Biomass concentration (g/l)	Bioemulsifier produced (g/l)
186	0.83	8.44
199	1.145	11.64
212	0.970	13.10
230	0.982	9.37
246	0.982	6.83
298	0.970	6.11

3.1 Soil washing of oil refinery sludge

It had been mentioned previously that the experiments on soil washing were done to the Balongan Oil Refinery Plant origin oil sludge (containing 32% by weight of TPH). The characteristic of the oil sludge is listed in **Table 2.** Physically its form as sticky mud and have viscosity = 450 centipoises. The hydrocarbon compound contains in that oil refinery sludge are dominated by the long chain carbons, i.e. from C11 up to C44. It can be concluded that the organic phase of the oil sludge tend to have heavy petroleum residue property.

Table 2: The Characteristics of Balongan Oil Refinery Plant Origin Oil sludge

Parameter	Unit	Values
Density	g/l	878.8
Viscosity	centipoises	450
TPH content	%	30 - 33
Water content	%	2.61
Solid content	%	35.63
Volatile hydrocarbon	%	7.65
Non-volatile hydrocarbon	%	54.61
Organic C (dry weight)	%	28.49

The soil washing experiments on that oil sludge was conducted in three parallel reactors using bio-surfactant, Tween 80, and no surfactant successively in the washing liquids. The results showed that after 24 hours the TPH concentration in the oil refinery sludge decreased as shown in **Table 3**. Using 1 g/l bio-surfactant in washing liquid, after 24 hours the TPH concentration decreased to 20.2 %, while to 24 % in using 1 g/l of Tween 80, and 29.4 % in the control.

3.2 Bioremediation of oil refinery sludge

The oil sludge is attributed to two major factors controlling in its formation. First is the inorganic residue consisting of sediments, sands, scales, dust and the second is the precipitation of paraffinic wax. Since wax precipitates are sparingly soluble in crude oil, temperature changes are the reason behind wax precipitation. In addition to the above reasons, the oxidation of heavy organic material in crude oil due to climate changes or from oxidizing microorganisms and also the interruption in material balance due to losses of volatile components and the tendency of asphaltene, resin and polymeric compounds to precipitate all are the reasons of oil sludge formation.

After having 24 hours soil washing process, the released oil from the solids matrix then be separated manually. The liquid parts were drained, and into the rest solid matrix was added 5 % (w/w) of PMC inoculants for bioremediation process. The agitation by helical screw impeller was continued during the process using 1 g/l bio-surfactant, Tween 80 respectively, and control. At the 30th day of bioremediation, the process was stopped, and TPH content in each reactor was analysed. The results demonstrate that using bio-surfactant produced in the experiments was able to decrease the TPH content in the oil refinery sludge up to less than 3% or 90.80% removal within 30 days as mentioned in **Table 3**. While, using Tween 80 can reduce the TPH content up to 6,66 % or 79,2 % removal. In the meanwhile, TPH reduction in the control was 45.9 %.

Table 3: TPH content the oil sludge after 24 hours soil washing and 30 days bioremediation

Time (days)	TPH content (%)		
	Using bio-surfactant	Using Tween 80	Control
Soil washing:			
0	32.00	32.00	32.00
1	20.20	24.00	29.40
Removal Efficiency (%)	36.90	25.00	8.10
Bioremediation:			
2	20.20	24.00	29.40
4	15.84	19.70	27.04
7	12.10	16.45	26.40
15	9.60	12.58	24.20
21	4.05	9.84	21.77
30	2.96	6.66	17.31
Removal efficiency (%)	85.30	72.30	41.10
Total efficiency (%)	90.80	79.20	45.90

The surfactants affect the biodegradation process by increasing the solubility and dispersion of the compound. There are two ways in which bio-surfactant affect which is increasing the surface area of hydrophobic water insoluble substrate. Secondly is increasing the bioavailability of hydrophobic water-insoluble substances (Desai and Banat, 1997). The low water-solubility of many hydrocarbons reduces their availability to microorganisms and limits the biodegradation process. It has been assumed that bio-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 (Abalos et al., 2004). Once again, bio-surfactant can enhance growth on bound substrates by desorbing them from surfaces or by increasing their apparent water solubility.

The profile of bacterial growth during bioremediation process is shown in **Figure 2.**

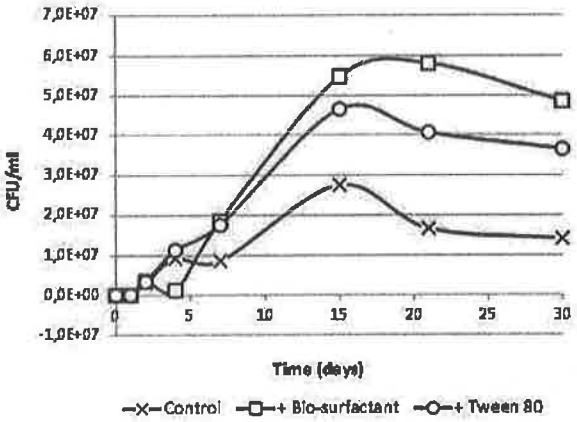

Figure 2: The profile of bacterial growth during bioremediation process

Figure 2 shows the bacterial growth during bioremediation process for 30 days of incubation, a significant growth of PMC occurred in the bioremediation system supplemented with both surfactants (bio and synthetic surfactant) also without addition of surfactant as control. This positive result suggests that bio-augmented bacteria (PMC) could degrade TPH significantly. Bio-augmentation also can be used to increase the biodegradative capabilities of the indigenous microbial population. 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 in control reactor (without addition of surfactant), the CFU values increased from $3.0x10^6$ (CFU/ml) at day 2 to $2.7x10^7$ (CFU/ml) in day 15 and decrease to $1.4x10^7$ (CFU/ml) in day 30 of incubation time. Addition of bio-surfactant enhanced the remediation process by increase biomass growth from $3.5x10^6$ (CFU/ml) at day 2 to $5.4x10^7$ and $4.8x10^7$ (CFU/ml) in the day 15 and 30 incubation time, yielding an increase of removal efficiency up to 2.18 fold. The presence of tween 80 in bioremediation system increased the removal efficiency up to 1.76 fold compared to those without addition of surfactant/control. The present of Tween 80 also increased the microbial growth from $3.4x10^6$ (CFU/ml) at day 2 to

$4.6x10^7$ (CFU/ml) in the day 15 and decreased to $3.6x10^7$ (CFU/ml) at day 30 of incubation time, respectively.

4. CONCLUSSIONS

Our findings showed that the addition of both petrophilic microorganism consortia and bio-surfactant favors the biodegradation of the oil refinery sludge. 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. The presence of bio-surfactant also stimulate the catabolism of hydrocarbon by mean of co-metabolism process since bio-surfactant are organic compound and readily degradable to microorganism. The bio-surfactant produced in these experiments are able to reduce the TPH content significantly from the oil refinery sludge that open the opportunity in oil sludge handling economically and environmental friendly.

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