The Application of Biosurfactant Produced by Azotobacter sp. for Oil Recovery and Reducing the Hydrocarbon Loading in Bioremediation Process

Merry Sianipar, Edwan Kardena, and Syarif Hidayat

***Abstract*—Biosurfactant is potentially used to enhance oil recovery from oil sludge. The exopolysaccharide-formed biosurfactant is produced from Azotobacter sp. using 2 w/v% glucose sources. The oil sludge taken from company X and biosurfactant is agitated under the speed of 150 rpm and temperature of 20-25℃. The ratios of oil sludge and biosurfactant were varied by 1:1, 2:1 and 3:1. After 7 h agitation, the mixture was stood alone for 12 hours to obtain the 3 separated phases among oil (top), water (middle) and solid (bottom). The role of biosurfactant as surface tension reducer successfully separated among oil, water, solid from the oil sludge. The amounts of oil recovered from oil sludge are 55.95%, 51.76% and 25.57% for ratio 3:1, ratio 2:1, and ratio 1:1, respectively. Further, the slurry phase bioremediation method under 120 rpm of impeller velocity was applied to treat the bottom formation (slurry) from previous process. Both 5 w/v% Petrofilic bacteria (Unidentified Mix Culture) as a degrader agent and 5 w/v% fertilizer as nutrient were added in the first day of bioremediation process. Under controlled 10-40% solid concentration, 5.5-9.5 pH, 20-40℃ (mesofilic condition), the total petroleum hydrocarbon (TPH) was successfully decreased until 6.83%, 3.48%, and 2.11% for slurry of ratio 1:1, ratio 2:1, and ratio 3:1 in 35 days.**

***Index Terms*—Azotobacter sp., biosurfactant, oil recovery, slurry Phase bioremediation.**

I. Introduction

Oil sludge caused from oil spill during transportation, separator tanks, cleaner tanks, and spray coolers is a pollutant that should be strictly concerned in petroleum industry. The oil sludge that cannot be returned to the earth by injection is only treated by mixed it with the soil from stockpile containing TPH.

Thus, the mixture soil is drying under the sunrise which are non-environmentally treatment leading to the damage of the ecosystem life. In this study, the issue of environment protection in the oil company X as one of the largest oil exploration companies in Indonesia is our focus. Based on laboratory measurement, the oil sludge resulted by the company X contained TPH concentration over 20% which is indicating the presence of high loading pollutant which extremely impacts the environmental sustainability. Therefore, we offered an integrated approach for handling the oil sludge problem as environmental concerns: oil recovery process in the first step (pretreatment) followed by an ex-situ bioremediation process with slurry phase method in the second step (see Fig. 1). The oil recovery concept was offered due to the high concentration of TPH indicating the oil contained in oil sludge is still high. Therefore, it will be potentially economic. Furthermore, a pretreatment was applied to decrease the loading process of bacteria for bioremediation. This study used biosurfactant-produced Azotobacter sp. for pretreatment as enhancing oil recovery agent [1], [2]. Biosurfactant which basically produced from bacteria or microorganism [1], [3], [4] shows environmental compatibility and high activity at extreme temperatures, pH and salinity [5] is effective in decreasing the surface tension [6], critical middle concentration, interfacial tension in both aqueous solutions and hydrocarbon mixtures [7]

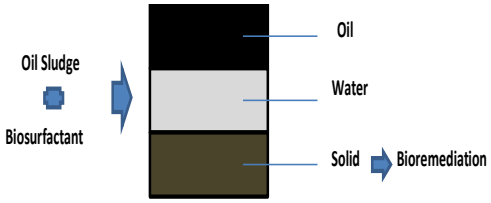

Fig. 1. Research concept.

II. Materials and Methods

A. Oil Sludge Initiation

Oil sludge sample was taken from company X in Indonesia (see Fig. 2). Company X itself is an oil and gas company which produces heavy oil characteristic. Therefore, the oil contained in oil sludge was characterized as heavy oil which is indicated by the existence of three strong organic hydrocarbon including Pristine (nC_{17}), Phytane (nC_{18}) and Botryococcane (nC_{28}). Furthermore, the oil sludge characterization included: 25.02% of TPH (Total Petroleum Hydrocarbon), 7.38 of pH, 45-69% of moisture content, 867.8 kg/m^3 for oil sludge density at 25℃, 450 centipoises (25℃) for viscosity, BTex analyse (EPA 8260B): 0 µg/L of benzene and ethylbenzene & xylene, 8.19 µg/L of toluena. We presumed that the existed toluene can be degraded in bioremediation process since *Pseudomonas* and *Achromobacter* which were reported as toluene degrader [7]

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are included in our Petrofilic bacteria consortium.

Fig. 2. The oil sludge sample from company X.

B. Biosurfactant and Petrofilic Bacteria Preaparation

The biosurfactant (see Fig. 3) was produced in our laboratory in accordance to our group previous reports [2], [8], [9]. Firstly, the Azotobacter sp. isolation was acclimated in Ashby Media using manitol as the main carbon source [10]. Thus, Ashby Media as the selective media was agitated in 100 rpm speed for 72 h at 37°C which resulted in 10^6 cell/mL density bacteria. The Ashby Media (1 L DI water) contained mannitol (15 g), K_2HPO_4 (0.5 g), $MgSO_4.7H_2O$ (0.2 g), $CaSO_4$ (0.1 g), NaCl (0.2 g), and $CaCO_3$ (5 g). Secondly, the acclimated Azotobacter sp. was proliferated and put into Basal Minimum Media to produce expolysaccharide-formed biosurfactant using 2% w/v glucose as carbon source. The basal minimum media (1 L DI water) contained K_2HPO_4 (1.5 g), KH_2PO_4 (0.5 g), $(NH_4)_2SO_4$ (0.4 g), $MgSO_4.7H_2O$ (0.2 g), 10 mL trace element contained $Na_2EDTA.2H_2O$ (12 g), $FeSO_4.7H_2O$ (2 g), $CaCl_2$ (1 g), $ZnSO_4.7H_2O$ (0.4 g), $NaSO_4$ (10 g), $MnSO_4.4H_2O$ (0.4 g), $CuSO_4.5H_2O$ (0.1 g), and $Na_2MoO_4.2H_2O$ (0.5 g) in 1 L DI water. The medium was sterilized in autoclave with temperature of 120℃ and pressure of 15 psi for 20 min. Furthermore, the bacteria used in slurry phase bioremediation process was Petrofilic bacteria (Unidentified Mixed Culture) developed by the Environmental Biotechnology of Environmental Engineering of ITB [2], [9]. The Unidentified Mixed Culture bacteria was isolated consortium included Pseudomanas stuzeri BLO2, Bacillus cereus BL01, Bacillus sp. BL04, and Achinetobacter sp BL03.

Fig. 3. Biosurfactant produced by Azotobacter sp.

C. Oil Recovery and Bioremediation Preparation

The ratio variations between biosurfactant and oil sludge were 3:1, 2:1 and 1:1. The mixture was agitated in the agitator reactor under 150 rpm speed, 7 hours and at room temperature (20-25℃)[1]. Moreover, for bioremediation process, slurry phase bioreactor (see Fig. 4) was used and set in the range of 100-200 rpm speed as optimum mixture speed [11]. The dose ratio of additional Petrofilic bacteria was 5w/v% [12].

D. Materials Characterization

As In this study, the total petroleum hydrocarbon (TPH) concentration was the main parameter used to observe the amount of recovered oil and the TPH decrease during both oil recovery as well as bioremediation process. TPH was analyzed by extraction gravimetric (modification of EPA methods 164 Rev A). 15 ml vial was heated in oven at 105℃ for 1 h, then, put into desiccator for cooling. The cooled vial was weighted called (a). Hexane was put into the erlenmeyer as much as two times volume of the sample to be examined. The weight of the sample was called (c). The erlenmeyer was stirrered at 150 rpm for 2 h in orbital shaker. The erlenmeyer was stood alone for 1 h until formed 2 separate phases. The top phase which is the solution of N-Hexane and extracted oil put in 15 ml vial. The filled vial was heated in a water bath at 70℃ to evaporate the N-hexane. Further, that vial was cooled back at desiccator and weighted (b). The equation to calculate TPH based on extraction gravimetric method is shown on Equation 1. Moreover, to describe the oil behavior of sample during process, the oil fingerprint of sample was measured (GC-5890). Furthermore, the characteristics of recovered oil were investigated using oil fingerprint (GC-5890), and density & API number (hydrometer method-ASTM D4052). In addition, other parameters such as solid concentration, pH and Temperature were checked to observe the optimum condition during treatment. Solid concentration was expected in the range of 10-40% to enhance the bidegradation process [11]. Meanwhile pH and temperature was determined using pH/Temperature meter (model 410A, 110 VAC).

$$TPH(\%) = [(b-a)/c] \times 100 \quad (1)$$

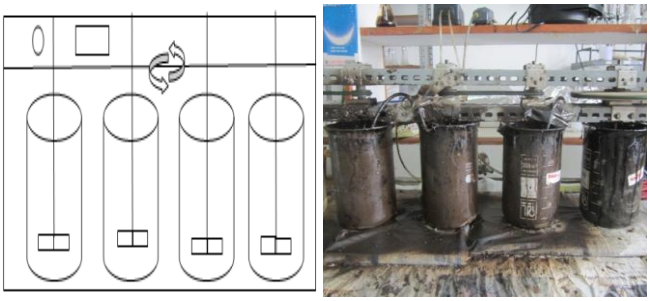

Fig. 4. Slurry phase bioreactor.

III. Results and Analysis

A. Recovered Oil Characterization

The separated phase formed after mixing biosurfactant and oil sludge were shown on Fig. 5. The recovered oil was found at 140 ml for ratio 3:1, 130 ml for ratio 2:1 and 64 ml for ratio 1:1. The highest oil recovery was achieved by the ratio 3:1 while the lowest was achieved by ratio 1:1. It was caused by the stronger separation given by the increase of biosurfactant amount. Moreover, since the results of ratio 2:1 revealed insignificant difference comparing with the results of ratio 3:1, we presumed that the ratio 2:1 was the optimum ratio in oil recovery process. Furthermore, the characteristic of oil showed that the recovered oil characteristic was not affected by the biosurfactant. The oil fingerprint and API & density number data of the oil recovered showed the heavy oil characteristic [13] as the original characteristic oil of Company X (see Fig. 6, Table I and Fig. 7).

Fig. 5. The separated phase formed after mixing biosurfactant and oil sludge at the ratio 1:1 (Left) and the ratio 2:1 (Right) with following oil on the top, water in the middle and solid in the bottom.

TABLE I: THE QUALITY OF RECOVERED OIL

Parameters	Unit	Ratio 1:1	Ratio 2:1
Density	g/cm3	0.93225	0.93539
Actual Temperature	℃	40	40
Rho API 60°F-C	g/cm3	0.9482	0.9512
API 60 °F-C	-	17.59	17.1
SG 60 °F-C	-	0.9491	0.9521
Pour Point	°F	40	35

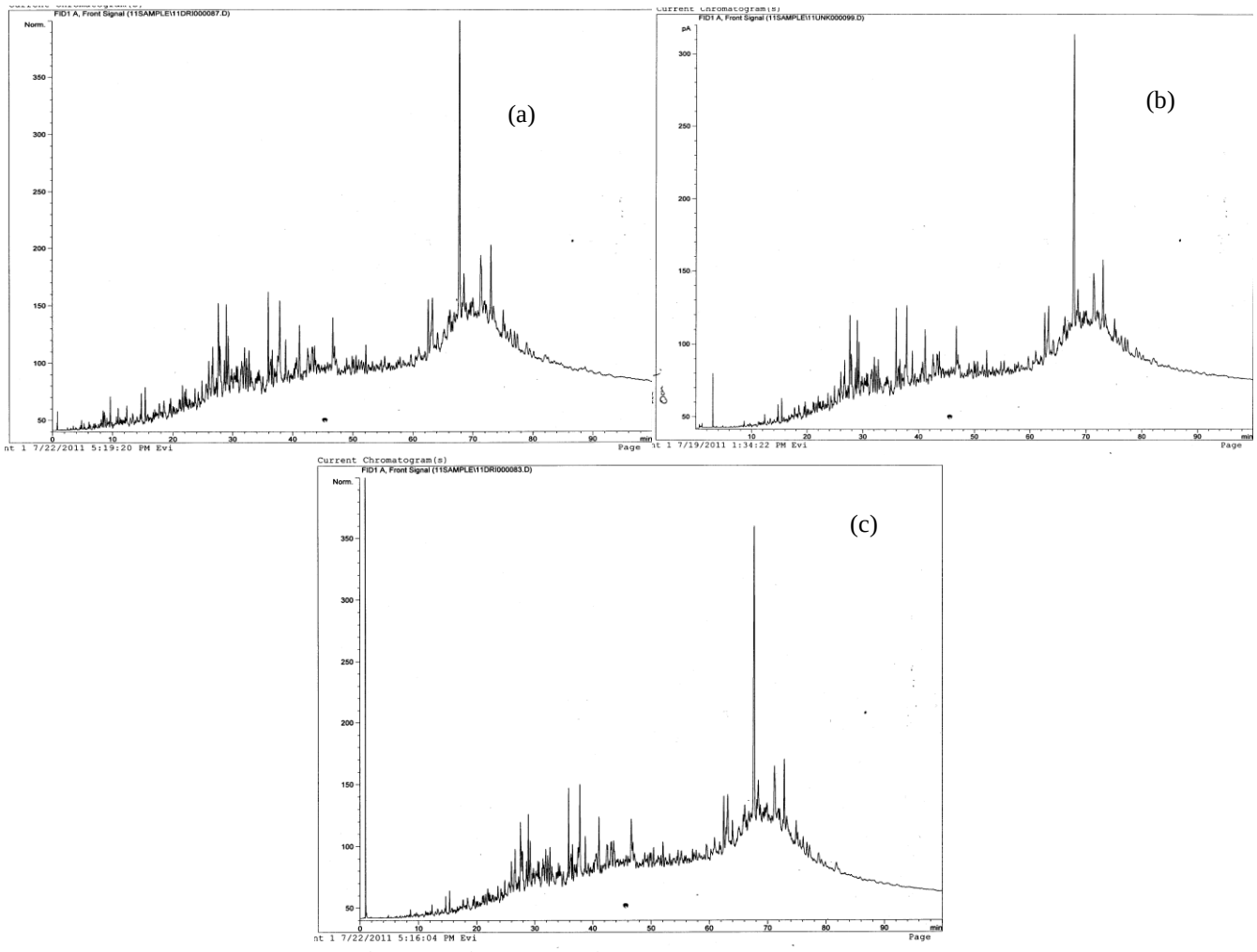

Fig. 6. The oil fingerprint of recovered oil from ratio 1:1 (a), ratio 2:1 (b), and Company X (c).

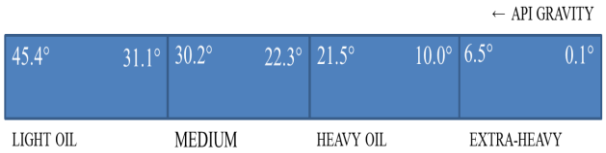

Fig. 7. API standardization referred to American Petroleum Institute [13].

A. Solid Phase (Slurry) Characterization and Slurry Phase Bioremediation Treatment

The bottom formations (solid phase) were characterized and found at 18.6% TPH for ratio 1:1, 12.1% TPH for ratio 2:1, and 11% TPH for ratio 3:1. It was consistent with the amount of recovered oil data showing the ratio 3:1 and ratio 2:1 had higher oil recovery than ratio 1:1. Moreover, the pH of slurry, around 5.6-5.9 pH for all ratios, was decreased from the initial condition, i.e., pH 7.38, due to the work of bacteria in [11] during the oil recovery process. Furthermore, the high moisture (%) of solid phase result, more than 60% moisture, for all ratios, indicated that the liquid concentration was highly contained on solid phase called slurry. Therefore, the slurry phase bioremediation was chosen to treat solid phase. Furthermore, during bioremediation, the total petroleum hydrocarbon (TPH) was decreased for pretreated slurry (see Fig. 8). Moreover, the pristine, phytane and botryococcane identified as strong organic hydrocarbon, was successfully treated in bioremediation process described by oil fingerprint results (see Fig. 9). The decrease of TPH was caused by two factors: bacteria in bioremediation process and the biosurfactant role. The role of Unidentified Mixed Culture bacteria or Petrofilic bacteria strongly biodegraded the hydrocarbon oil in slurry [12]. Moreover, the percentages of organic compound contained in solid (see Fig. 10) showed that bacteria preferred eat the light fraction first than the heavy fraction before. In addition, the smallest portion of light

fraction especially at initial condition was caused by the light hydrocarbon vaporize [1]. The form of dried slurry after bioremediation process and without bioremediation process (control) could be seen on Fig. 11.

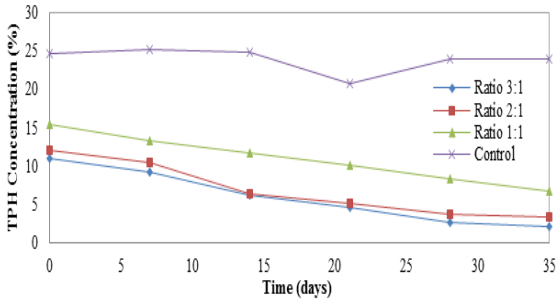

Fig. 8. TPH decrease during bioremediation process and oil fingerprint of slurry after bioremediation process field.

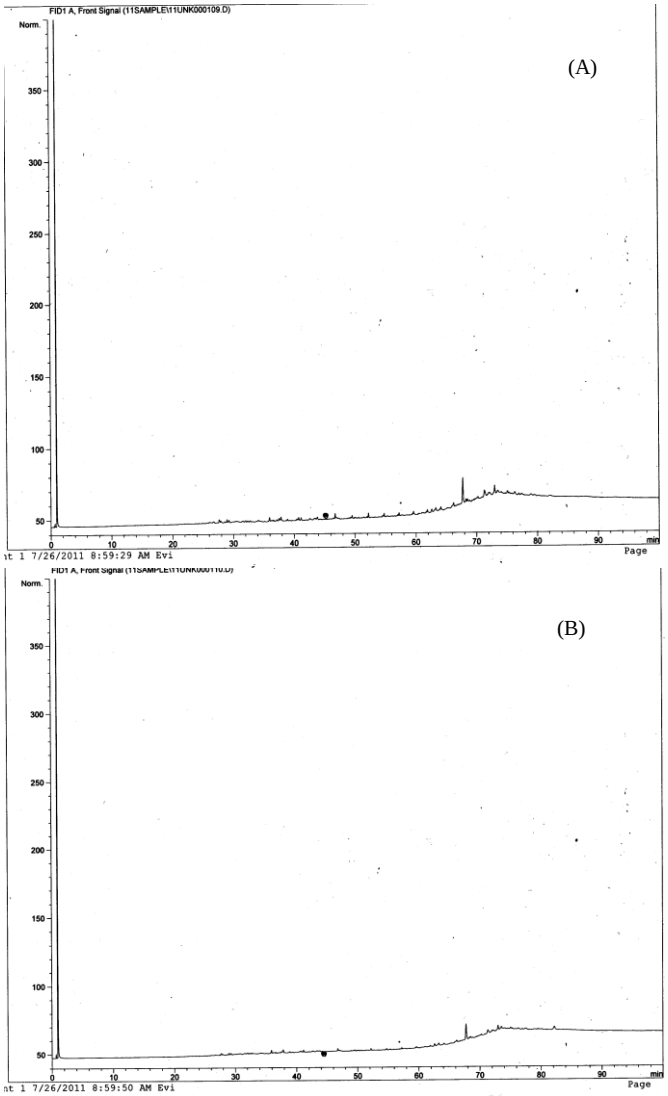

Fig. 9. The oilfingerprint of slurry after bioremediation process for Ratio 1:1 (A) and Ratio 2:1 (B).

Furthermore, the use of biosurfactant in pretreatment was presumed to give influence to the oil degradation. As emulsifier agent, biosurfactant gave arise soluble oil that led to the easiness for bacteria in degrading the hydrocarbon (bioavailability) [14]. Moreover, it was showed that the more used biosurfactant, the more increase the bioavailability of hydrophobic compound [14], [15]. Controlling the solid concentration, temperature, and pH in slurry phase bioreactor (see Fig. 12) also contributed for successful biodegradation [11], [16].

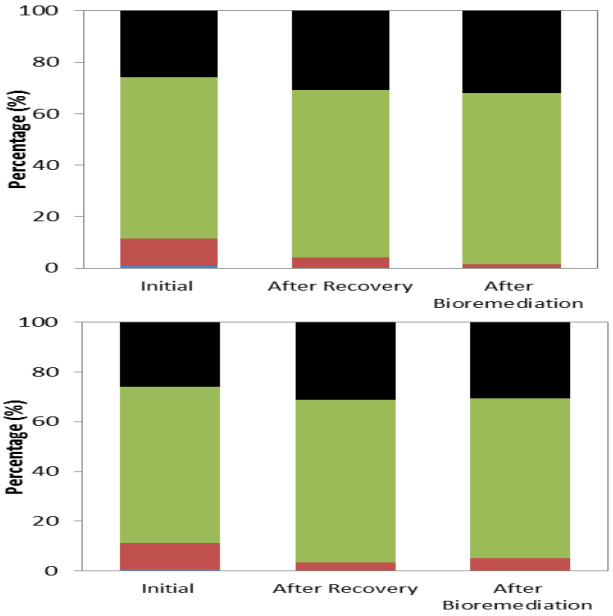

Fig. 10. The percentage of organic compund composition in solid (C6-C9, blue; C10-C24, red; C15-C28, green; C29-C36, black) from Ratio 1:1 (Top) and Ratio 2:1 (Bottom).

Fig. 11. The digital photos of the dried slurry after bioremediation process (left to right: Control, Ratio 1:1, Ratio 2:1, Ratio 3:1).

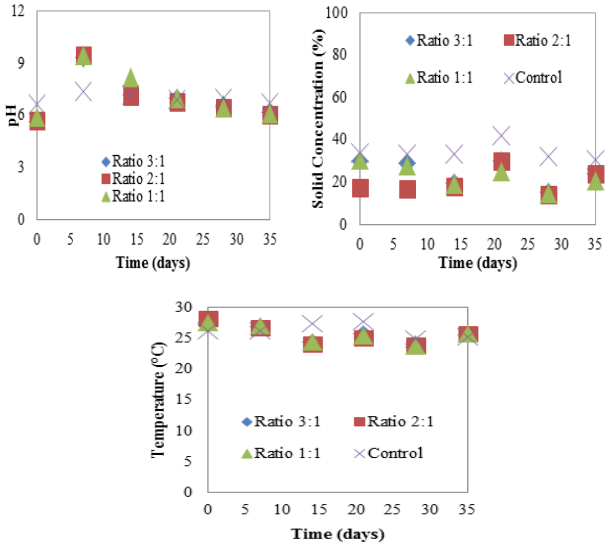

Fig. 12. Solid concentration, temperature, and pH of slurry during bioremediation process.

IV. Conclusion

Biosurfactant produced by Azotobacter sp. was found to be an oil emulsifier to separate oil, water, and solid from oil sludge. Moreover, biosurfactant is feasible in recovering oil from oil sludge without bothering the quality of recovered oil. Ratio 2:1 was the optimum dose ratio in the oil recovery process. Furthermore, the decreased TPH of recovered solid from the pretreatment process was further treated in the slurry phase bioremediation process using Petrofilic bacteria. The

order of TPH decrease was ratio 3:1 > ratio 2:1 > ratio 1:1.The Petrofilic bacteria plays role in decreasing the TPH of the slurry. Considering the feasibility and performances, we recommended the ratio 2:1 as the optimum ratio for obtaining the optimum oil recovery and bioremediation performance.

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