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PETROLEUM DEGRADATION IN SOIL BY THERMOPHILIC BACTERIA WITH BIOPILE REACTOR

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Abstract

Crude oil degradation has been carried out using biopile reactor in total petroleum hydrocarbon (TPH) concentration of 5%, 10% and 15%. The thermophilic microorganisms used from isolation result and identification are *Aeromonas salmonicida*, *Bacillus pantothenicus*, and *Stenotrophomonas maltophilia*. The biodegradation of biopile reactor is done by various concentrations of TPH, total plate count (TPC), and volatile suspended solid (VSS) per day for 30 days. The biodegradation of kinetic parameter calculated consists of μ , μ_m , Y, Y_t, Y_{obs}, K_d, K_s from TPH concentration decision, and TPC and VSS in every microorganism with t (observation time) of 0 hour to 168 hours. The crude oil separation efficiency in a biopile reactor shows that the largest separation occurs in a starting TPH concentration of 15%, which becomes 61.8% later on, followed by a starting TPH concentration of 10% and 5%, which is as much as 61% and 48.4%.

Keywords: Biopile, crude oil, degradation, kinetic, thermophilic

1. Introduction

Basically in its most simple form, nature has the ability to biologically and chemically heal itself. Nevertheless, nature's ability to degrade crude oil is less than effective due to a slower degrading speed of microorganism when compared to the amount of contamination introduced to the environment. As a xenobiotic and recalcitrant substances, crude oil proves to be difficult to degrade without the human help and current existing technology.

Biopile is an environmental repair/regeneration technology which utilizes indigenous microorganism such as bacteria and fungi to degrade organic contaminants in the soil such as hydrocarbon [1]. Degradation process as biopile is effected by oxygen transport, humidity and temperature [2]. Vidali, *in* Battelle, states that biopile is the unification of landfarming and composting, and is generally used to process hydrocarbon contaminants [3].

Temperature provides a good parameter to illustrate the variety and adaptation abilities of microbes towards the environment. Benefits of utilizing thermophilic microbes within biotechnology process consist of; a faster reaction time, less contamination risks and the less expensive cooling costs used due to the lengthy fermentation process. Extreme thermophilic bacteria possess high amounts of lipids in its membrane which functions to stabilize heat inside a bacteria cell.

In its correlation to bioremediation techniques to overcome crude oil soil contamination, the objective of this research is to determine biodegradation kinetics with TPH variations, knowing the highest crude oil trace in a laboratory scale biopile reactor that is able to degrade the crude oil mentioned.

2. Methods

Preliminary Test. Crude oil with a TPH concentration of 5%, 10% and 15% are inserted into a liquid stone mineral salt solution (SMSS) medium as an induction to microorganism growth contained in the crude oil sample. Treatment were conducted in Erlenmeyer with a total volume of 60 mL that is placed in a shaker incubator with a speed of 170 rpm, room temperature, and a pH of 6.8–7.2. Sampling is conducted in intervals of 5 days for 30 days. Sample conducted analysis is the measurement TPH concentrate tool with gravimetric method. The resulted microorganism are isolated and identified.

Bacterial Isolation. Bacterial isolation were done in 2 stages, isolation in both liquid and solid medium. Thermophilic bacterial isolation from crude oil started with added 10% crude oil in a liquid medium that is gradually heated to 150 °C temperature so as to allow the bacteria to adapt to high temperature in accordance to the starting environment of the bacteria.

Bacterial Identification. Apart from morphological identification exercise with gram coloring, identification exercise are also conducted through biochemical and API 20 NE (analytical profile index–non enterobacteraceae) methods in the Indonesian Department of Health Research and Development.

Bacterial Growth Test. Pure microorganism isolate inoculation and temperature culture are inserted into crude oil on a minimum liquid medium with a 60ml total volume. Crude oil concentrate in a created TPH are 2.5%, 5%, 7.5%, 10%, 15%. A blank (no microorganism additions) is conducted for control. Exercise is conducted on an Erlenmeyer placed on top of a shaker with a 170 rpm spin cycle, at room temperature with a pH 6.8–7.2. Samplings were done at (t) 0 hour, 6 hours, 12 hours, 24 hours, 49 hours, 72 hours, 96 hours, 120 hours, 144 hours and 168 hours. Analysis conducted is to account crude oil concentrate in a TPH through gravimetry, total enumeration with a TPC method, biomass concentrate by measuring the VSS.

Degradation test in a Biopile Reactor. Sample were taken from Babelan, Bekasi. The soil organic compound have been eliminated by heating in a furnace for 1 hours at 600 C. the soil is then mixed with crude oil in a TPH 2.5% and 15% concentrate with a total volume of 3 kg. Microorganism added were cultured microorganism with a minimum 10% (b/b) variance. Nutrients are added by providing fertilizer. Variance between TPH : N : P are 120 : 5 : 1. For control, 2 blanks are created, which are control 1 (by adding nutrition, without adding microorganism) and control 2 (without adding nutrition and without adding microorganism). A biopile reactor with a (35 X 25 X 15 cm) dimensions, in room temperature, pH 6.8–7.2, circulated with air and is mixed for each sample. Samplings are done everyday for 30 days. TPH are measured through gravimetric, whereas biomass concentrate inspection are done enumeration using TPC.

3. Results and Discussion

Isolations and Identification. From the result of isolation and identification, 3 species of thermophilic

bacteria are retained; *A. salmonicida*, *B. pantothenicus*, and *S. maltophilia*.

Bacteria consortium growth. In Figure 1, the longest exponential phase can be seen on TPH 10% for 36 hours finalizing on the 120th hour, and on TPH 20% for 66 hours finalizing on the 132nd hour. The most numerous biomass increment occurred from the 102nd hour to the 108th hour at 25% TPH, which was 2397 mg VSS/L and remaining TPH as much as 23.82% from 21.12% at the 108th hour. Crude oil degradation results by microorganism can be in the form a biomass cell, water and carbon dioxide. Swanell, *et al.* stated that on bioremediation pollutant that is toxic will be converted to non-toxic such as carbon dioxide, water and biomass cell [4].

Crude oil reduction kinetics by bacteria. Crude oil reduction kinetics by bacteria was conducted by determining 2 parameters, the growth of microorganism and crude oil concentration. Bacteria growth parameter (X) is measured as VSS to determine biomass amount and crude oil concentration parameter (S) is measured as TPH to determine subtractive amounts. The result on TPH concentration decline can be seen on Table 1.

Highest TPH decline for *A. salmonicida*, *B. pantothenicus*, and *S. maltophilia* occurs at TPH 10% which is as much as 43.1%. This decline can be sourced to a 10% crude oil concentrate non toxic nature to microorganism. As such biodegradation is dependant on the amount of crude on concentrate that will be processed. Ma, stated that hydrocarbon concentrate s a factor which effects biodegradation largest TPH decline at 10% occurs during the usage of all three bacteria [5]. This proves that hydrocarbon degrading bacteria consortium usage is more effective. Eweis, *et al* in Marquez, *et al.*, stated that the use of bacterial consortium can increase the speed of the degradation process [6]. Allen in Marques, *et al.* stated that bacterial consortium is required for bioremediation to optimize mineralization [6].

Largest TPH decline occurs at 5% TPH which was as much as 25.1%. This proves that bacterial consortium

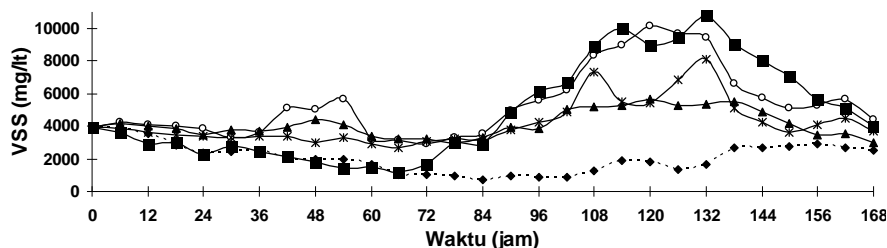


Figure 1. Consortium Growth Curve of TPH 5% (◆), TPH 10% (○), TPH 15% (▲), TPH 20% (■), and TPH 25% (*) at pH 6.8–7.2, 28 °C

can use advantage of crude oil as substrate. Not all hydrocarbon content declines are due to microorganism, decline can also be the result of vaporising. Nevertheless, decline that is the result of vaporizing are relatively small. Parameter kinetics values from each bacteria can be seen in Table 2 below.

In a single culture, a highest specific growth rate occurs in (?) *B. pantothenicus*. For maximum specific growth rate occurs to *A. salmonicida*. Largest saturation concentrate occurs to *B. pantothenicus*. Largest specific subtract utilization motion occurs to *B. pantothenicus*. A small mortality rate occurs to *A. salmonicida*

Largest specific microorganism growth in this research occurs to consortium growth as much as 0.00857–0.05551/hour with a maximum specific growth rate as much as 0.016482/hour. Effendi, test results, maximum specific growth rate on East Kalimantan crude oil

bioremediated contaminated soil as much as 0.073/hour [7]. Okoh, *et al.* research, specific growth rate on bioremediation in Nigeria as much as 0.23–0.71/day [8]. Coi, *et al.* in Marquez, *et al.* research result, maximum specific growth rate up to 0.3–0.4 hours [6].

Largest subtractive utilization motion in this research occurs on the growth of *B. pantothenicus* 0.285188–4.229334/hour. Christopher and Catherine in Okoh, research result, specific subtractive utilization rate on crude oil bioremediation as much as 0.6/hour [8].

Crude oil reduction a biopile reactor. Table 3. shows crude oil reduction efficiency in a biopile reactor. Largest TPH reduction occurs at TPH 15% for as much as 61.8% followed by TPH 10% and TPH 5% for as much as 61% and 48.4%. Crude oil degradation will be more affective on an subtractive concentrate optimized for crude oil degradation microorganism growth.

Table 1. Crude Oil Reduction

| Bakteria | TPH Reduction (%) | | | | |
|-------------------------|-------------------|------|------|------|------|
| | 5 | 10 | 15 | 20 | 25 |
| <i>A.salmonicida</i> | 47.6 | 21.3 | 27.1 | 22.1 | 18.3 |
| <i>B. pantothenicus</i> | 66.9 | 20.2 | 23.3 | 17.1 | 14.5 |
| <i>S. maltophilia</i> | 59.5 | 27.2 | 28.1 | 24.5 | 29.9 |
| Mix culture *) | 30.3 | 43.0 | 23.0 | 17.4 | 9.7 |
| Consortium bacteria **) | 25.1 | 24.4 | 12.4 | 11.7 | 15.0 |

*) Isolated bacteria: *A. salmonicida*, *B. Pantothenicus*, *S. Maltophilia*

**) Mix culture and other indigenous microorganism which are unidetified

Table 2. Crude Oil Biodegradable Kinetics Parameter Value

| Parameter Kinetika | Bakteri | | | | |
|----------------------|-----------------------|-------------------------|-----------------------|-------------------|-------------------------|
| | <i>A. salmonicida</i> | <i>B. pantothenicus</i> | <i>S. maltophilia</i> | Mix culture *) | Consortium bacteria **) |
| μ (1/jam) | 0.0065–0.0095 | 0.0031–0.1055 | 0.0038–0.0694 | 0.00012–0.0123826 | 0.00857–0.05551 |
| μ_{maks} (1/jam) | 0.008096 | 0.007038 | 0.01159 | 0.001711 | 0.016482 |
| <i>Ks</i> (mg/l) | 2648.41 | 7349.116 | 19020.93 | 289573 | 21439.57 |
| <i>Y</i> | 0.0054 | 0.0109 | 0.0173 | 0.0341 | 0.0491 |
| <i>q</i> (1/jam) | 1.200799–1.754383 | 0.285188–4.229334 | 0.219735–4.009342 | 0.00342–0.363127 | 0.174542–0.363127 |
| <i>Yt</i> | 0.0054 | 0.0109 | 0.0173 | 0.0341 | 0.0491 |
| <i>Yobs</i> | 0.00378–0.00463 | 0.003109–0.008862 | 0.004392–0.017096 | 0.00012–0.012383 | 0.009160–0.044017 |
| <i>Kd</i> (1/jam) | 0.0011–0.0041 | 0.0020–0.0946 | 0.0002–0.0521 | 0.0217–0.0338 | 0.0064–0.0405 |

*) Isolated bacteria: *A. salmonicida*, *B. pantothenicus*, *S. maltophilia*

**) Mix culture and other indigenous microorganism which are unidetified

Table 3. Crude Oil Reduction in a Biopile Reactor

| Start of TPH concentration | Reduction efficiency (%) |
|----------------------------|--------------------------|
| Blank 1 (5%) | 21.2 |
| Blank 2 (5%) | 17.3 |
| 5 (%) | 48.4 |
| 10 (%) | 61.0 |
| 15 (%) | 61.8 |

Aislabie, explains that bioremediation can be at maximum on a subtractive concentrate optimized for crude oil degradation microorganism [9].

From separation results on a blank, it can be seen that the largest TPH separations occurs at blank 1, which was added by nutrition. This proves that nutritional addition influence the amount of crude oil separation. Margesin & Schinner stated that nutrition influence hydrocarbon biodegradation levels inside the soil [10]. Kastner, *et al.* in Van Hamme, explains that adding nutrition has a profound effect in the degradation process [2].

4. Conclusion

This have been achieved, the isolation and identification of 3 (three) species of specific hemophilic bacteria which can degrade crude oil, these are; *A. salmonicida* (positive gram and ingot form), *B. pantothenicus* (positive gram and ingot form), and *S. maltophilia* (positive gram and ingot form). From the result of TPH concentrate decline, it can noted that both single culture and mixed culture can take advantage of crude oil as subtracts for its growth. Kinetics degradation values from a concentrate range of TPH 5%, 10%, 15% in room temperature with a pH of between 6.8–7.2 to mix culture of *A. salmonicida*, *B. pantothenicus*, *S. maltophilia* resulted $\mu = 0.00012\text{--}0.0123826/\text{hour}$, $\mu_m = 0.001711/\text{hour}$, $K_s = 289573 \text{ mg/L}$, $Y = 0.0341$, $q = 0.00342\text{--}0.363127/\text{hour}$, $Y_{obs} = 0.00012\text{--}0.012383$, and $K_d = 0.0217\text{--}0.0338/\text{hour}$. For bacterial consortium: $\mu = 0.00857\text{--}0.0555 \text{ 1/hour}$, $\mu_m = 0.016482/\text{hour}$, $K_s = 21439.57 \text{ mg/L}$, $Y = 0.0491$, $q = 0.174542\text{--}0.363127/\text{hour}$, $Y_{obs} = 0.009160\text{--}0.044017$, and $K_d = 0.0064\text{--}$

0.0405/hour. Crude oil separation efficiency in a biople reactor shows that the largest separation occurs on a starting TPH concentrate of 15% which was 61.8% later on followed on a starting TPH concentrate of 10% and 5% which was as much as 61% and 48.4%. In blanks the largest separation of (21.2%) occurred to the reactor that has been nutrition added. This shows that adding nutrition influences degradation levels.

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