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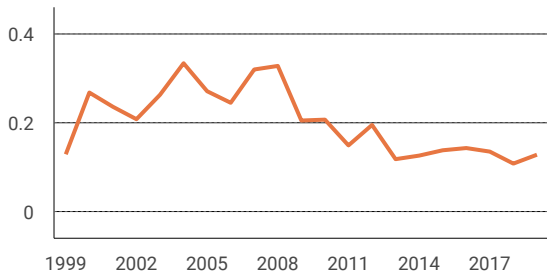
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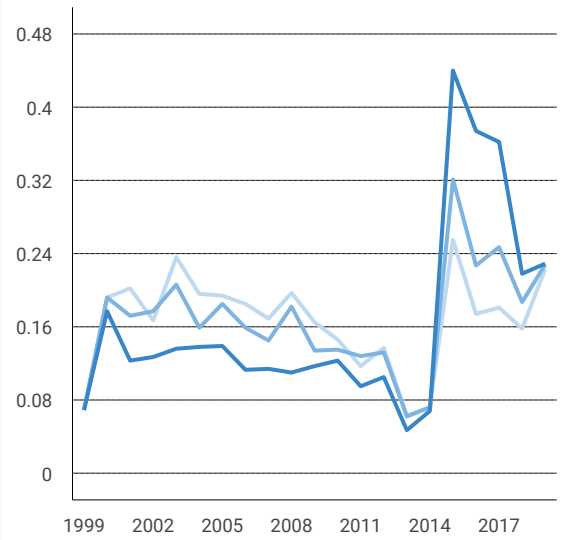
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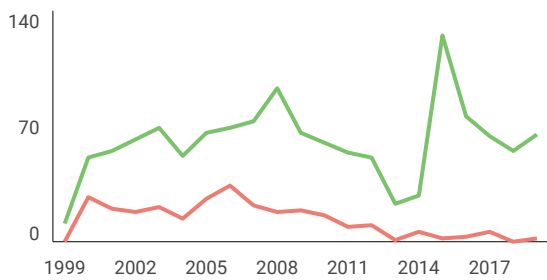
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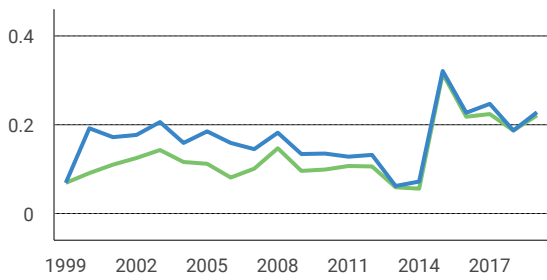


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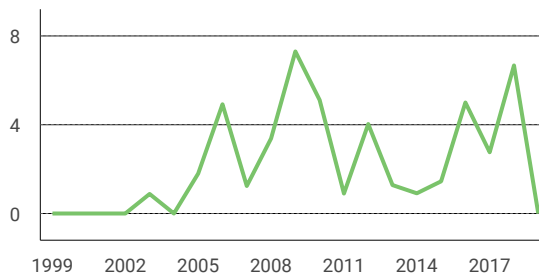


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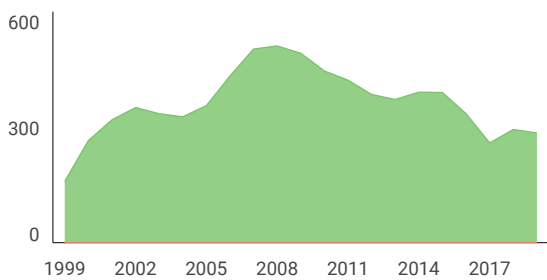
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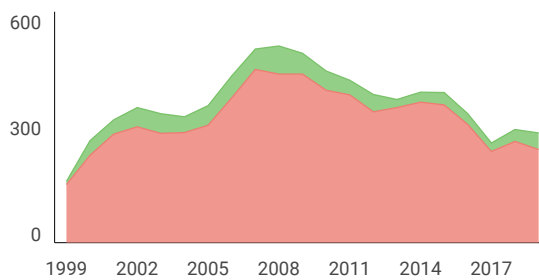
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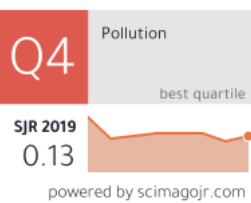
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## THE EFFECT OF POLLUTANT CONCENTRATION ON OIL SLUDGE BIODEGRADATION USING BACTERIAL CONSORTIUM IN BIO-SLURRY REACTOR

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### ABSTRACT

Bio-slurry reactor is one method of oil sludge treatment technology. Its application in the field requires a study of the exact comparison between pollutant content and hydrocarbonoclastic microbes, and this is still very poorly done. This study aims to determine the effect of pollutant concentration on Total Petroleum Hydrocarbon (TPH) and Total Plate Count (TPC) during biodegradation of oil sludge. Biodegradation by bio-slurry method was done by dissolving the pollutant into the liquid phase with a ratio of 1:9. Variations of pollutant concentrations were 20%, 30%, and 40% (v/v). Bacterial consortium consist of *Bacillus* sp., *Micrococcus* sp., *Pseudomonas* sp., and *Acinetobacter* sp. in the same ratio was used of 5% (v/v). Biodegradation was conducted at  $\pm$  30°C for 14 days incubation with aeration. Variation of pollutant concentration gave effect on reduced of TPH and total amount of bacteria. Treatment with 20% pollutant concentration and addition of bacterial consortium was able to reduce hydrocarbon oil sludge up to 0.3 g/g. Percentage of biodegradation was obtained 58.9% and total bacteria was obtained log 17.5 CFU/mL after 14 days incubation. The pH conditions during biodegradation ranged from 5.0 to 7.2. Proportion between pollutants and microbial concentrations determines the success of biodegradation.

**KEY WORDS :** Oil Sludge, Biodegradation, Bacterial Consortium, Bio-Slurry Reactor

### INTRODUCTION

Petroleum industry activities such as drilling, refining, production processes and transportation generally produce oil waste which can pollute the environment. One of the oil industry wastes is oil sludge. Oil sludge is petroleum in the form of a thick mixture of sediment, water, oil, and hydrocarbons which is usually present at the base of oil sludge storage tanks and oil refinery waste treatment plants (Ubani *et al.*, 2013). Oil sludge contains hydrocarbon compounds such as benzene, toluene, ethyl benzene and xylene (BTEX) and polyaromatic hydrocarbons (PAHs) which are difficult to decompose naturally in the environment (Ni'matuzahroh *et al.*, 2014) and have carcinogenic,

mutagenic and potentially immunotoxic (Bayoumi, 2009). Therefore, oil sludge needs to be managed and processed in accordance with hazardous and toxic waste management standards to prevent the spread and absorption of petroleum into the environment.

One of the management of petroleum waste is bioremediation by using microorganisms as degrading agents for oil sludge hydrocarbons. The use of microbes to degrade oil sludge hydrocarbons effectively converts pollutants into compounds that are more environmentally friendly and require relatively low costs (Makadia *et al.*, 2011). One of the biological and existing treatment of oil sludge waste can be done using a bio-slurry reactor. A bio-slurry reactor is an oil sludge treatment tank in which there

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are 5-50 (w/v) pollutants in the liquid phase (Hu *et al.*, 2013). The bio-slurry reactor is equipped with a stirrer aerator containing oil and water sludge at a certain ratio and added oxygen, microbes, and nutrients in it (Nano *et al.*, 2003). Bio-slurry easily facilitates microbial contact with pollutants which in this case are hydrocarbons in the liquid phase.

One of the factors that influence biodegradation speed is the ratio between pollutants and concentrations of hydrocarbon degrading microbes and incubation time. Very low petroleum concentrations cause higher water solubility so it is easy to use by microbes. Conversely, if the concentration of oil is too high, it can lead to the death of microbes that play a role in the biodegradation process due to its toxic nature (Atlas, 1992). Meanwhile, according to Zahroh (2014), the addition of a bacterial consortium and the incubation time affect oil sludge degradation. Research that reveals an appropriate comparison between pollutants and microbes and the length of incubation is still rare. Based on the background above, it is necessary to do research on oil sludge biodegradation with variations in pollutant concentrations and incubation time in the bioslurry reactor. The addition of a consortium, nutrients and aeration to the bio-slurry reactor is expected to help accelerate the process of biodegradation of oil sludge hydrocarbons. Indicators of the success of biodegradation of oil sludge hydrocarbons in bio-slurry reactors are marked by changes in pH, increase in the number of microbes (CFU/mL) and a decrease in the level of hydrocarbon residues in oil sludge (g/g).

## MATERIALS AND METHODS

### Research Design

This research was a laboratory experimental study that uses a completely randomized design of 6x5 factorial patterns with three replications. The treatment consisted of two factors: pollutant concentration (oil sludge) (K) and incubation time (H). In the control reactor no bacterial consortium (-) was added, while the treatment reactor was added with a bacterial (+) consortium. The following are the details of the treatment carried out can be seen in Table 1.

### Production of Bacterial Consortium

The bacterial consortium consisted of *Micrococcus* sp., *Pseudomonas* sp., *Acinetobacter* sp., and *Bacillus*

**Table 1.** Research design

Pollutant concentration (K)	Incubation time (H)				
	H0	H3	H7	H10	H14
K20(-)	H0K	H3K	H7K	H10K	H14K
	20-	20-	20-	20-	20-
K30(-)	H0K	H3K	H7K	H10K	H14K
	30-	30-	30-	30-	30-
K40(-)	H0K	H3K	H7K	H10K	H14K
	40-	40-	40-	40-	40-
K20(+)	H0K	H3K	H7K	H10K	H14K
	20+	20+	20+	20+	20+
K30(+)	H0K	H3K	H7K	H10K	H14K
	30+	30+	30+	30+	30+
K40(+)	H0K	H3K	H7K	H10K	H14K
	40+	40+	40+	40+	40+

sp. which is a hydrocarbonoclastic isolate collection culture of Microbiology Laboratory Universitas Airlangga. Each pure isolate was inoculated in Nutrient Agar (NA) media and incubated for 24 hours at room temperature ( $\pm 30^\circ\text{C}$ ). Culture were then used to make a bacterial consortium. A total of 4 fresh cultures each were inoculated on Nutrient Broth (NB) media and then incubated for 24 hours at room temperature ( $\pm 30^\circ\text{C}$ ) above an shaker incubator of 120 rpm. Bacterial cell density was measured using a spectrophotometer with a wavelength of 600 nm and arranged to reach 0.5 Optical Density (OD = 0.5). The consortium was obtained by mixing the four isolate cultures with a ratio of 1: 1.

### Design of Bio-slurry Reactors

The bio-slurry reactor is made of glass material which is equipped with a cover at the top. The reactor has a capacity of 1500 mL. The reactor is equipped with an aerator with a capacity of 2L/min which functions to supply oxygen in the reactor during the biodegradation process. Oxygen was channeled through hoses. Before using, the bio-slurry reactor was sterilized using an oven for 12 hours at  $150^\circ\text{C}$ . The reactor design is illustrated in Fig. 1.

### Biodegradation Test Using a Bio-slurry Reactor

Oil sludge came from PT. Pertamina Balongan, Indonesia. Oil sludge was regulated so that it has a concentration of 20%, 30%, and 40% (w/w) in sterile sand that has been sieved before using mesh 200. Biodegradation tests were carried out by making a comparison between pollutants and liquid phases of 1: 9 with a total volume of 1000 mL. The liquid



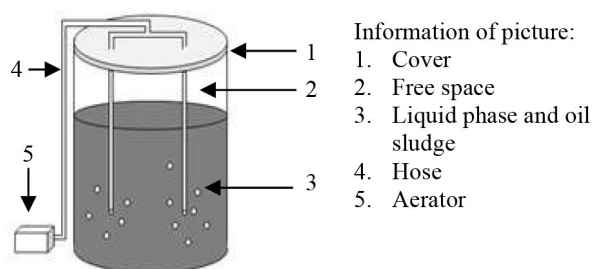


Fig. 1. Design of Bio-slurry reactor

phase consists of sterile distilled water plus 2% (v/v) molasses. The bacterial consortium was applied to each reactor as much as 5% (v/v). The aeration process lasts 24 hours for 14 days. Biodegradation evaluation was carried out on 0, 3<sup>rd</sup>, 7<sup>th</sup>, 10<sup>th</sup>, and 14<sup>th</sup> days.

#### Measurement of biodegradation parameters

In this study, the biodegradation parameters evaluated were the total number of bacteria, the levels of Total Petroleum Hydrocarbon (TPH), the percentage of biodegradation, and the pH carried out using a pH meter.

Determination of the total number of bacteria was carried out using the Total Plate Count (TPC) method. A total of 1 mL of the liquid phase from each reactor was taken and dissolved into physiological saline solution for further dilution until the cell density reached 30 - 300 CFU/mL. 1 mL of the solution from the last three dilutions was placed in a petri dish and poured using NA medium ( $\pm 35^\circ\text{C}$ ), then the microbial medium was allowed to solidify and incubated for 24 hours at room temperature ( $\pm 30^\circ\text{C}$ ). The number of microbes in each reactor and the day of observation can be used as an indicator of the growth of the original consortium and bacterial bacteria of oil sludge. TPH measurements were carried out using the SNI 06-6989.10-2004 gravimetric method. The extraction process is carried out using a separated funnel. A total of 40 mL samples from each reactor were poured into a separated funnel then extraction was carried out with N-hexane organic solvents at a ratio of 1: 1, i.e. 40 mL of sample and 40 mL of N-hexane with two stages of addition (20 mL - 20 mL). The substrate was then homogenized by shaking the solution for 15 minutes and then allowed to separate the liquid phase and the organic solvent phase. The liquid phase and the phase of the organic solvent formed are separated and placed on different bottles. If an emulsion is formed, the

emulsion was cracked using 10 mL ethanol. Extracts (hydrocarbons in organic solvent) were separated using rotary evaporator by evaporating N-hexane at a temperature of 60-69°C, the remaining hydrocarbons in the funnel evaporator are then weighed and obtain TPH values. The remaining hydrocarbon components were analyzed using GC-MS to find out what hydrocarbon components were decomposed in this study. Biodegradation percentage is obtained through calculation with the following formula:

$$\text{Biodegradation (\%)} = \frac{(\text{initial TPH} - \text{final TPH}) / \text{TPH initial} \times 100\%}{1} \quad (1)$$

#### Data Analysis

Total bacterial and Total Petroleum Hydrocarbon (TPH) data were analyzed statistically using ANOVA with error degree ( $\alpha = 0.05$ ) using Statistical Package for the Social Science (SPSS ver. 2.1) while pH data was analyzed descriptively.

## RESULTS AND DISCUSSION

### Results

#### Bacterial Growth in Bio-slurry Reactors during Biodegradation of Hydrocarbons

The bacterial growth pattern during biodegradation lasted up to 14 days is illustrated in Fig. 2. The addition of a bacterial consortium had an impact on the number of initial bacteria in each treatment. The treatment with the addition of a bacterial consortium (code +) has a higher number of bacterial cells than the treatment without the addition of a bacterial consortium (code -). Pollutant concentration also affects the number of cells, the higher the concentration of pollutants, the higher

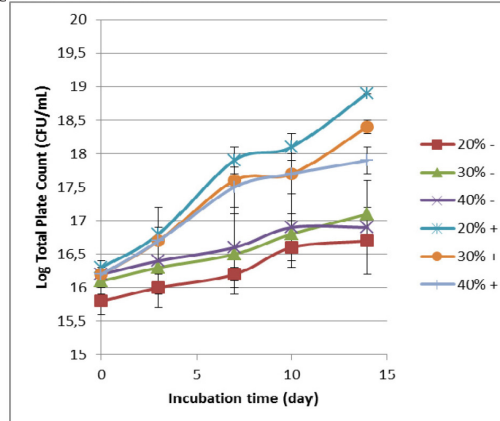


Fig. 2. Patterns of bacterial growth in various treatments during 14 days of incubation

the number of bacterial cells. This proves that in the oil sludge there is a bacterial indigenous. Growth occurs from 0 to 14<sup>th</sup> day and is relatively still in the logarithmic phase. From the bacterial growth pattern, it indicates that indigenous and exogenous bacteria are able to use hydrocarbons and molasses as nutrients. An increase in the number of cells until the end of the incubation time indicates that nutritional requirements (C, N, P, and K) and oxygen are still fulfilled until the end of the incubation time.

The results of the two-way ANOVA test showed that there was an effect of variations in pollutant concentration and incubation time on the total number of bacteria. Based on the Duncan test ( $\alpha = 0.05$ ), the number of bacteria on the 14<sup>th</sup> day between the treatment with the addition of a consortium and without the addition of a consortium was significantly different. The highest number of bacterial cells in H1420 + treatment was  $7.9 \times 10^{18}$  CFU/mL (log TPC = 18.9 CFU / mL). Although the pollutant concentration was high in H1440 + treatment, the number of bacteria on 14<sup>th</sup> day was lower than H1420 +. Bacterial growth in H1440 + slowed down from the 7<sup>th</sup> day of incubation while H1420 + increased significantly until the 14<sup>th</sup> day of incubation. Slowing growth shows that there is competition between bacteria in fighting for food and the presence of toxic compounds that allow bacterial cell death.

#### pH During Biodegradation in a Bio-slurry Reactor

Based on Fig. 3, it is known that there is a change in pH in the control and treatment reactors. The pH value in Fig. 4 shows that the longer the incubation time, the lower the pH value of the culture. On 0

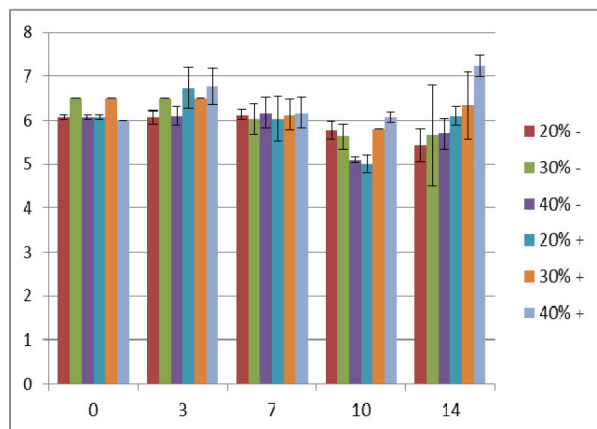


Fig. 3. The pH value of the culture in the bioslurry reactor during biodegradation

day to 7<sup>th</sup> day, the pH value of the reactor ranged from 6.00 to 6.77. However, at the 10<sup>th</sup> the pH value decreased, which ranged between 5.00-6.07, while on the 14<sup>th</sup> day the pH of the reactor increased, ranging from 5.43-7.23. At pollutant concentrations of 40% (-) and 20% (+) there was a lower pH decrease compared to other treatments, namely up to pH 5.0 on the 10<sup>th</sup> day. This can be due to the presence of more acidic microbial metabolites compared to other treatments.

#### Decrease of TPH and Percentage of Hydrocarbon Biodegradation

Based on the results of GC-MS analysis, the composition of hydrocarbons in Balongan oil sludge consists of 83.05% aliphatic hydrocarbons, 2.15% aromatic hydrocarbons, and 8.6% poly-aromatic hydrocarbons. In this study, pollutant concentrations of 20%, 30%, 40% (v/v) produced TPH values of 0.32 g/g, 0.48 g/g, and 0.64 g/g respectively. TPH values decrease along with the incubation time, this proves that hydrocarbons are

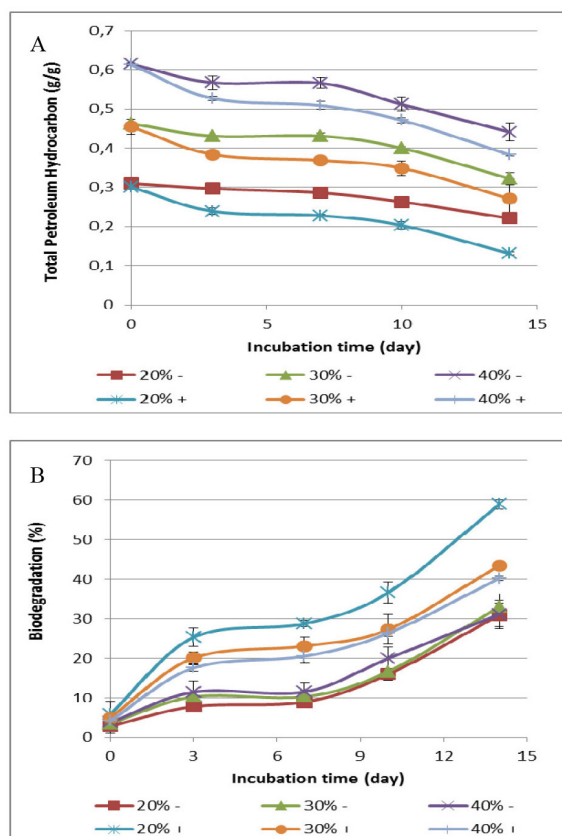


Fig. 4. A) TPH levels in various treatment for 14 days of incubation; B) Percentage of biodegradation of oil sludge hydrocarbons in various treatments for 14 days of incubation

used as nutrients for bacteria to grow. Duncan's test results ( $\alpha = 0.05$ ) showed that the H1420 + treatment had a TPH value that was significantly different from the other treatment groups. The treatment of H1420 + was able to reduce TPH levels by 0.189 g/g for 14 days of incubation. Decrease in residual levels are along with an increase in the percentage of biodegradation (Fig. 4). The highest percentage of hydrocarbon biodegradation in H1420 + treatment was 58.9%. The results showed that treatment with a pollutant concentration of 20% and the addition of a bacterial consortium of 5% in the bioreactor for 14 days of incubation gave the best results. This is reinforced by the highest total number of bacteria with the fastest generation time calculated at logarithmic phase (Table 2).

Based on the results of the GC-MS analysis, on the 14<sup>th</sup> day, the hydrocarbons that remain in the treatment are toxic and persistent mono-aromatic hydrocarbon groups in the environment.

## DISCUSSION

The ability of bacteria to grow in bio-slurry reactors is an important factor in the biodegradation of hydrocarbons. Hydrocarbonoclastic bacteria metabolize hydrocarbons enzymatically. The broken hydrocarbon bond begins with the oxygenase enzyme. Oxygen is used to metabolize hydrocarbon compounds by oxidizing the substrate with an enzyme oxygenase catalyst. Peripheral degradation pathway changes organic pollutants step by step into intermediates of the central intermediary metabolism, such as the Tricarboxylic acid cycle (TCA) Guangji *et al.* (2013). The occurrence of carbon metabolism in cells can trigger cell biomass biosynthesis which then leads to an increase in the number of cells.

Hydrocarbon biodegradation is a complex process that can be carried out jointly by the

bacterial community. None of the microbial species can degrade all components in a hydrocarbon group. Therefore, complete degradation is largely determined by the shared role of various microbial species (Soegiarto, 2005). The diversity and abundance of hydrocarbon degrading microorganisms has a linear relationship with the increase in degradation of hydrocarbon compounds (Hendriani *et al.*, 2011). However, in this case, there can be inhibitory interactions in growth caused by competition in obtaining nutrients. The interaction between bacteria starts from the use of shared nutrition and is influenced by environmental factors (Stubbenieck *et al.*, 2016). Besides the nutrition and the ability of bacteria to break down hydrocarbons, environmental factors also play an important role in the success of biodegradation, including oxygen, humidity, temperature, and pH. The level of acidity (pH) is one of the factors that influence the growth rate of bacteria, the ability of bacteria to build cells, transport through cell membranes and the balance of catalyst reactions (Cookson, 1995). The pH range in the control and treatment reactors is not much different from the optimum pH for bacterial growth, which is in the range of pH 6-8 (Charlena, 2010). The decrease in pH can be caused by organic acids produced by microbial consortia (Aliyanta *et al.*, 2011). The process of alkane degradation in petroleum forms alcohol, then becomes fatty acids. Fatty acids resulting from the degradation of alkanes are further oxidized to form acetic acid and propionic acid (Rosenberg, 1986). The acids produced by the metabolite can reduce the pH value of the culture. Increasing microbial activity in degrading hydrocarbons will also increase the amount of organic acids produced and the greater the decrease in pH produced (Aliyanta *et al.*, 2011).

Biodegradation of hydrocarbons using a bio-slurry reactor produces a high percentage of biodegradation. Study of hydrocarbon

**Table 2.** Relation of pollutant concentration to total bacterial count, generation time, TPH reduction, biodegradation percentage and time prediction of 100% hydrocarbon biodegradation

Concentration of pollutant	TPC <sup>day 14</sup> (CFU/mL)	Generation time (h)	$\Delta$ TPH (g/g)	Biodegradation (%)	Biodegradation rate (g/day)	Time prediction (day)
20% -	16.7	126	0.099	30.94	0.007	54
20% +	18.9	32	0.189	58.92	0.014	27
30% -	17.1	126	0.158	33.01	0.011	52
30% +	18.4	36	0.209	43.45	0.015	39
40% -	16.9	126	0.199	31.15	0.014	54
40% +	17.9	39	0.257	40.22	0.018	41

biodegradation of oily sludge waste using a *Bacillus*, *Pseudomonas*, and *Serratia* consortium to obtain 30% biodegradation for 15 days of incubation (Ramirez *et al.*, 2009). In addition, a similar study, using *Bacillus* and *Geomyces* consortiums, received 88% of degraded crude oil for 30 days (Maddela *et al.*, 2016). The reactor bio-slurry successfully gave a positive impact on biodegradation success. Intensive stirring and supplying oxygen through the aeration process in bio-slurry reactors can increase biodegradation of oil sludge. Agitation can homogenize slurry and increase microbial contact with the oil sludge substrate (Fava *et al.*, 2000).

In this research, bio-slurry has guaranteed the availability of oxygen and molasses has guaranteed the availability of dissolved nutrients. Ratio between pollutant and microbial concentrations is factor in the biodegradation of hydrocarbons. An increase in the concentration of oil sludge will automatically increase the microbial indigenous of oil sludge. In bio-slurry reactors with the same environmental conditions, the rate of biodegradation of indigenous bacteria is relatively same. Exogenous addition can change the rate of biodegradation due to the interaction between indigenous and exogenous bacteria. Addition of exogenous bacteria in 20% pollutant concentration can accelerate biodegradation up to two fold compared to treatment without exogenous addition. The low pollutant concentration with the addition of a number of microbes can accelerate the biodegradation time of hydrocarbons, while the high pollutant concentrations with the addition of a number of microbes require a longer time.

### CONCLUSION

Concentration of pollutant gives effect in biodegradation. Pollutant concentration of 20% and the addition of bacterial consortium of 5% in the bio-slurry reactor for 14 days of incubation give the best biodegradation up to 58.9%. The balance between pollutant concentrations and microbial concentrations in adequate environmental conditions and nutrients will reach optimal biodegradation. Exogenous bacteria (bacterial consortium) have proven useful in increasing the percentage of biodegradation.

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