

Effect of Organic Nutrient on The Biodegradation of Hydrocarbon Contaminated Marine Sediment in Malaysia

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Abstract—The effect of organic nutrient on the biodegradation of hydrocarbon contaminated marine sediment in Malaysia were investigated. Biodegradation was assessed in microcosm experiments containing 10%(w/w) of crude oil and either amended with inorganic nutrients (NP), or organic matter in the form of plant-based (*Elaeis guineensis*) or fish-based nutrient amendments (*Scomber australasicus*). Hydrocarbon mineralization occur in all treatments, with the highest biodegradation rates occur in *S. australasicus* supplemented sediments. The addition of *S. australasicus* have managed to reduce the oil concentration to 48% while the addition of *E. guineensis* and inorganic NP reduced the final oil concentration to 66% and 63% respectively. Bacterial analyses revealed the presence of *Pseudomonas aeruginosa* that are well known for its ability to degrade n-alkanes and polyaromatic hydrocarbon compounds in the spilled oil.

Keywords— Biodegradation, bioremediation, bio stimulation, crude oil, *Pseudomonas Aeruginosa*.

I. INTRODUCTION

Petroleum is operationally defined as any hydrocarbon mixture that can be recovered through a drill pipe. These hydrocarbon mixtures comprised of a various nature of molecules which their molecular weight can be as heavy as an asphaltenes (tens of thousands of g/mol) or as light as methane (16 g/mol) ("The Origin of Petroleum in the Marine Environment," n.d.). Paraffins, isoparaffins, cycloparaffins, aromatics, and olefins are the major categories of hydrocarbons found in the petroleum. Among these categories, polynuclear aromatic hydrocarbons (PAHs) have the highest molecular-weight aromatics and fused rings which made these compounds relatively incessant in the environment and most likely poisonous, carcinogenic, or mutagenic. For that reason, it is important for us to monitor the PAH compounds once crude oil spill occur (Yang et al., 2017).

Natural discharges of hydrocarbon fluids (crude oil and/or natural gas) occur frequently in nearly all of the petroleum basins worldwide (Abrams, 2005). Besides natural seepage, oil spill can also occur throughout the process of oil exploration, production and transportation. Statistics have shown that nearly 10% of the average yearly hydrocarbon input in the ocean around the world are caused by oil spilled from tank ships (García-Olivares et al., 2017). Based on the statistic from 1960 to 2010, the biggest contributor of major oil spills is the Asia regions with over 25 large-scale spills discharging more than 3.4 million tonnes of hydrocarbon into the environment. with, partly due to the aftermath of the Gulf War in 1991 (Li, Cai, Lin, Chen, & Zhang, 2016).

Oil spill have catastrophic impact to the world economic, environment and public health. On 2010, the disastrous Deepwater Horizon oil spill have caused more than \$5.5 billion of economic

losses to the fishing and tourism industries while threaten over 200,000 job opportunities (Division et al., 2010). During this tragic event, nearly 692 km of Gulf of Mexico's marsh shorelines were smothered in oil, 41% (309 km) were either moderately or heavily oiled and approximately 23,400 seabirds died due to the oil spill (Li et al., 2016). Marine sediments, particularly shorelines will suffer the greatest impact when oil spill accidents occur in the marine environment. (Samanta, Singh, & Jain, 2002). Unfortunately, the current cleanup technologies have limited consideration on the degradation process of spilled oil in the sediment.

The Straits of Malacca, without doubt is one of the world's busiest shipping routes due to its strategic location. However, the number of oil spill accidents in this area has been escalated in an alarming rate recently (Zakaria & Takada, 2007). There are several methods to counter these oil spill accidents which vary from physical, chemical and biological method. The oil pollution is usually recovered physically via containment boom and skimmer (Muttin, 2008). However, physical methods extremely time consuming, laborious and costly when employed on a large scale (Broje & Keller, 2007). Chemicals such as dispersants were also frequently applied onto the spilled oil slicks aiming to reduce the interfacial tension between the spilled oil and the sea water and thus increase the natural dispersion of the oil (Grote et al., 2017). Nevertheless, the dispersants effectiveness, toxicity, the influence to biodegradation and long-term concerns retract its applications (Li et al., 2016).

Intrinsic bioremediation is probably the most cost-effective and feasible option in removing oil spills that occur in an area where ex situ oil spills are too costly or causes additional disturbance to the ecosystem. This biological technology has been acknowledged as one of the least invasive cleanup methods and proven to be an efficient mechanism to combat oil spills in marine shorelines with low and medium nutrient content. (Zhu, Venosa, Suidan, & Lee, 2004). There are numerous environmental factors that will affect the effectiveness of bioremediation including the type of sediment, organic matter, water content, temperature, nutrients and sediment's pH value (A. Horel & Schiewer, 2009). There are numerous researches that has been done to investigate the effectiveness of inorganic nutrient in bioremediation. Hence, this paper will be focusing on the treatment of widely available and low-cost animal or plant based organic matter to stimulate the natural bioremediation process of hydrocarbon contaminated Malaysian shorelines.

II. METHODOLOGY

A. Marine sediment collection

The sediment was collected from sandy beaches in Port Dickson, Malaysia (2°27'17.3"N; 101°51'23.2"E). The site was selected because of the following considerations: 1) the site was known to be frequently impacted by oil spill accidents (Zakaria & Takada, 2007); and 2) the site contains bacteria with high ability to degrade oil (Hii, Law, Shazili, Abdul-Rashid, & Lee, 2009). The sediments were

collected randomly by disturb sampling, up to a depth of 30 cm. The sediment samples were stored in sealed bottles placed in a refrigerator (4 °C) according to the standard methods on sediment sample storage and handling (Epa, 2001), and were tested within one year. In the beginning of the experiment, nitrogen and organic carbon of the was determined and recorded (Table 2).

B. Crude oil

The crude oil sample was obtained from Bertam oil field, 160km of the shore of Kuantan in Peninsular Malaysia. The oil has an API value of 44.5 and specific gravity of 0.804.

C. Organic waste

The organic nutrient source that were used in this experiment are from empty fruit bunch (EFB) of palm oil mill and fish tissue waste. The EFB and fish tissue were collected from Kilang Minyak Sawit Meru Sdn. Bhd and Klang's Wet Market respectively. The fish tissue waste was lyophilized and pulverized beforehand. This experiment will also be using an inorganic nutrient source from NP fertilizer.

D. General experimental setup

The biodegradation rate of the hydrocarbon was evaluated in a 35-day experiment by using a 500ml microcosms. Each of the four-treatment setup (Table 1) were filled with 200 g dry weight (DW) of sand. The first treatment, which act as the control, only contained sand polluted with 10% (weight/weight) crude oil. The second and third treatments were also amended with 10% (w/w) of crude oil together with lyophilized and pulverized tissue of a fish (second treatment, *Scomber australasicus*, and EFB of palm oil mill (third treatment, *Elaeis guineensis*). The amount of the *S. australasicus* and *E. guineensis* added into the microcosm were equal to 10% of the amount contaminated sediment. Finally, inorganic fertilizers (NP) in the form of NH_4NO_3 as nitrogen(N) and KH_2PO_4 as phosphorous(P) were added to the fourth treatment together with 10% (w/w) of crude oil. Water was added into the microcosm to maintain its moisture content at 60%. To make sure that the microcosms have sufficient oxygen supply, microcosms was tilled three times a week. The microcosms were incubated at room temperature ($30 \pm 2^\circ\text{C}$). On day 1 and 35, total carbon and total nitrogen concentrations in the sediment samples were analyzed. Carbon and nitrogen were analyzed by using an elemental analyzer.

Table 1:Microcosms setup

Treatment Type	Materials
A	Sand + crude oil (Control)
B	Sand + crude oil + <i>S. australasicus</i>
C	Sand + crude oil + <i>E. guineensis</i>
D	Sand + crude oil + NP

E. Identification of hydrocarbon-degrading bacteria

The soil sample from Port Dickson beach was taken at the beginning of the experiment for bacterial identification. In this study, the focus was on identifying the indigenous hydrocarbon utilizing bacteria at Port Dickson beach. Hydrocarbon utilizing bacteria (HUB), particularly *Pseudomonas aeruginosa* in the soil samples were identified by using cetrimide agar. About 10g of soil sample was diluted with distilled water in a sterile tube. The soil suspension water is then poured and spread on the cetrimide agar plates. The plates were incubated at 37°C for 24 hours. The colonies of bacteria were then observed visually for any pigmentation.

F. Determination of total petroleum hydrocarbon

The moisture in the soil samples was remove by the addition of Anhydrous sodium sulphate (Na_2SO_4). To determine the hydrocarbon content of the soil samples, 10g of soil sample was suspended in 20ml of toluene (AnaLar grade) using a 250ml flask. The mixture was then shake at 200RPM for 60 minutes using an orbital shaker. A DR/4000 Spectrophotometer was used to measure the total petroleum hydrocarbon (TPH) of the liquid phase of the extract at 420nm. By comparing the result to the standard curve derived from fresh crude oil that has been diluted with toluene, we have managed to measure the exact TPH of the sediments.

$$\text{TPH reduction} = \frac{\text{Final hydrocarbon concentration}}{\text{Initial hydrocarbon concentration}}$$

III. RESULTS AND DISCUSSION

A. Identification of bacteria

A wide variety of prokaryotes, bacteria and eukaryotes, yeasts, and filamentous fungi are capable of utilizing hydrocarbons. Bacteria are the most active agents in petroleum degradation, and they are reported as primary degraders of spilled oil in environment. *Arthrobacter*, *Burkholderia*, *Mycobacterium*, *Pseudomonas*, *Sphingomonas*, and *Rhodococcus* were found to be involved for alkyl-aromatic degradation. Hii et al., (2009) reported that organic pollutants degrader such as *Pseudomonas pseudoalcaligenes* and *Pseudomonas aeruginosa* can be isolated from many different environments in Malaysia, including both contaminated and uncontaminated sites and even marine sediments. The inoculation of these two bacterial consortiums have managed to increase the biodegradation rate of the hydrocarbon contaminated sediment. Hence, one of the objective of this paper was to further prove that *Pseudomonas aeruginosa* is one of the major hydrocarbon degrader in oil-contaminated area.

In this paper, cetrimide agar was used to isolate *Pseudomonas aeruginosa* from the hydrocarbon contaminated marine sediment. After an incubation period of 24 hours at 37°C , small colonies of bacteria as shown in Figure 1 were observed which was an indication for bacterial growth. Cetrimide behave as a cationic detergent which hinder the growth of various microorganisms including *Pseudomonas* species other than *Pseudomonas aeruginosa*. The formation of yellow-green pigments on the cetrimide agar indicate presence of *Pseudomonas aeruginosa* in the soil sample from Port Dickson's shoreline as this bacteria produces a number of water soluble pigments, including the yellow-green or yellow-brown fluorescent pigment pyoverdine (fluorescein) ("CETRIMIDE SELECTIVE AGAR INTENDED USE," n.d.). The process of identifying the *Pseudomonas aeruginosa* was crucial as they have been proven to have the ability to degrade PAH compound that contain in the spilled crude oil. (Chettri, Mukherjee, Langpoklakpam, Chattopadhyay, & Singh, 2016; Gao, Zhang, Lai, & Xue, 2017; Thanjavarn et al., 2006).

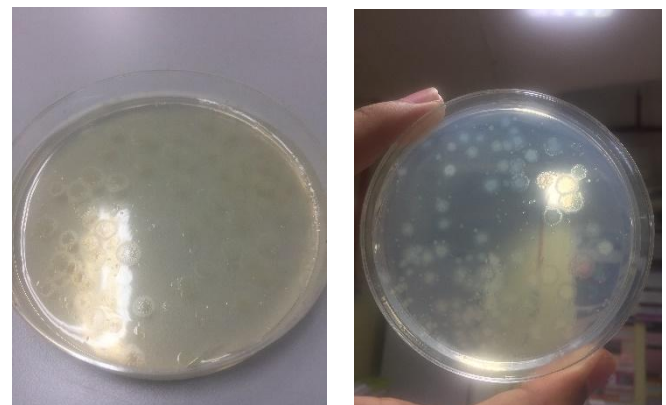


Figure 1: Catrimide agar with *Pseudomonas aeruginosa* colonies

Polynuclear aromatic hydrocarbon (PAH) derivatives and aliphatic derivatives have received a lot of attention due to their pervasive distribution in marine sediments since hydrocarbons from spilled petroleum can persist in the sedimentary environment for a substantial period of time that may be on the order of several years (Yang et al., 2017). Several PAH derivative-degrading members such as *Pseudomonas*, *Paenibacillus*, *Rhodococcus*, *Tsukamurella*, *Arthrobacter*, and *Sphingomonas* are commonly found in terrestrial habitats. *Pseudomonas*, as a biosurfactant producer (e.g., rhamnolipids and glycolipids), are the best-known bacteria capable of utilizing hydrocarbons as carbon and energy sources. Biosurfactants increase the surface area of oil available for the bacteria to metabolize (Thaniyavarn et al., 2006). They can also act as emulsifying agents, decreasing the surface tension and forming micelles, where, the microdroplets encapsulated in the hydrophobic microbial cell surface are taken inside and degraded (Speight & El-Gendy, n.d.).

B. Chemical and physical characteristics of the sediment and organic matter

Table 2: Physicochemical analysis of soil and organic matter

Parameter	Soil	Fish	Palm EFB
pH	7.53	6.84	5.87
Carbon (%)	3.16	39.77	19.16
Nitrogen (%)	3.30	11.09	1.84
Hydrogen (%)	2.61	9.57	5.06

This experiment was conducted in a laboratory at a temperature of 29.5°C. This temperature was maintained to simulate the average temperature at Port Dickson's beach which is between 28°C to 33.5°C (Hamzah, Saiful, Kipli, & Rahil, 2011). The optimum temperature for biodegradation to occur is normally between 25–35°C for anaerobic processes and 15–30°C for aerobic processes. The microbial biodegradation activity will even occur at sub-zero temperature, although the process will be at a minimum rate. Contaminants such as oil spill will diffuse through the pore liquid and soil organic matter more rapid at a higher temperature (Speight & El-Gendy, n.d.). During this experiment, the higher room temperature caused the viscosity of oil to reduce while the water solubility and volatilization of toxic short chain alkanes increased, stimulating the biodegradation activity.

The initial pH value of the soil from Port Dickson's beach is 7.53. The soil neutralization is usually preferable for the process of biodegradation as most of soil microorganisms are reported to thrive best in the pH range of 6–8. Based on Table 2, the addition of fish-based organic fertilizer caused the pH value of the sediment drop to 6.84. This drop in pH value is still acceptable as the optimum hydrocarbon biodegradation occurs around pH 6.5 to 8.0 (Speight & El-Gendy, n.d.). Meanwhile, the addition of plant-based organic fertilizer has caused further drop in pH value to approximately 5.87. It should be noted that during the soil bioremediation process, a recorded decrease in pH would occur due to fertilizer application or the production of aliphatic acids during alkane biodegradation.

As for polynuclear aromatic hydrocarbons (PAH) degrading bacteria such as *Pseudomonas aeruginosa*, the optimum pH condition is around pH 7.0 and 30°C (Speight & El-Gendy, n.d.). The ability of the microbial population to degrade hydrocarbons is reduced significantly under extreme pH environment and it is observed that nitrogen fixating bacteria are strongly hindered by high acidity. Hence, the physical condition of the microcosm in this experiment is very conducive for the growth of *Pseudomonas aeruginosa*. Due to the sensitivity of aromatic degraders to the pH, PAH compound in the spilled oil became persistence in the environment as the result of lower biodegradation of the organic contaminants. Yu, Li, Tang, & Wu, (2011) observed that the biodegradation rate constants for various polycyclic aromatic

hydrocarbons (PAHs) doubled when pH changed from 7.0 or 9.0 to the optimal pH value of 8.0.

In this paper, only carbon and nitrogen were analyzed as these nutrients are essential for the growth of oil degrading bacteria and greatly influence the oil biodegradation rate. Based on the physicochemical analysis results in Table 2, it can be said that the initial nutrient availability at Port Dickson's beach is relatively low. Organic carbon and nitrogen only make up 3.16 % and 3.30 % respectively of the total sand's elements. Two types of organic nutrient amendment were used in this experiment, fish-based, and plant-based which are *Scomber australasicus* and Palm EFB respectively. These fertilizers were used to enhance the nutrient availability inside the soil for a greater biostimulation potential. From the Table 2, the total organic carbon which available inside the fish-based organic fertilizer is roughly two times higher compared to the plant-based organic fertilizer.

Carbon is needed in greater quantities compared to other elements as it is the most basic element of living forms and vital for synthesizing their cell components. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells, while phosphorous and sulfur contribute with 70% of the remainder. Next, the physicochemical analysis also indicates that the total nitrogen which contain inside the fish-based organic fertilizer was nearly six times higher compared to the plant-based nutrient amendment. Nitrogen is essential for the synthesis of protein as well as of DNA and RNA by the oil degrading bacteria (R. Vasanthakumari, n.d.). From these result, it can be concluded that the fish-based fertilizer has higher nutrient content and greater potential to stimulate the biodegradation process inside the oil-contaminated soil.

C. Nutrient utilization

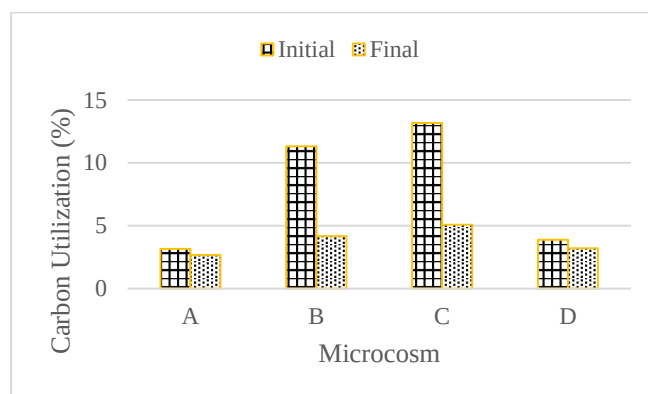


Figure 2: Percentage of carbon utilization by microorganisms

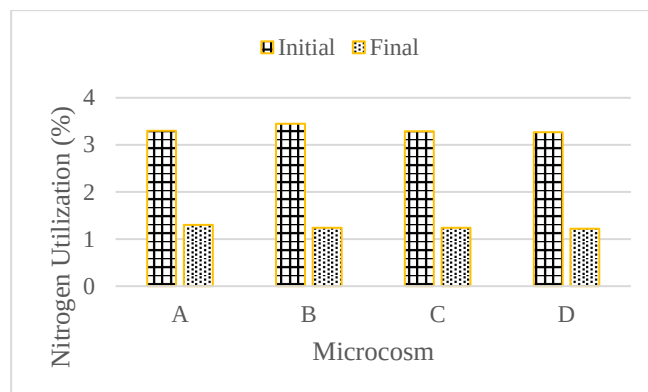


Figure 3: Percentage of nitrogen utilization by microorganisms

The soil sample from microcosms A, B, C and D was taken at the beginning and at the end of the experiment for nutrient analysis and the result of these analysis was presented in Figure 2 and Figure 3. From the result, the initial nutrient which contained inside the

microcosms B, C and D have significantly increased due to the addition of organic and inorganic fertilizers. Microcosm B have the highest carbon and nitrogen utilization which are 63% and 64% respectively. Agota Horel, Mortazavi, & Sobecky, (2014) reported that the increase in bacterial population leads to swift assimilation of available nutrients by soil microbes, thus increasing the rate of nutrient reserves depletion. Hence, it can be said that the high nutrient depletion rate in microcosm B was caused by the rapid microorganism hydrocarbon mineralization. Microcosm C have the second highest carbon and nitrogen utilization at 61% and 62% respectively followed by microcosm D. The organic and inorganic nutrient amendment have also managed to stimulate the indigenous microorganism activity in these microcosms. As for microcosm A, the lack of nutrient amendment results in a lower nutrient depletion rate which may retard the microorganism's hydrocarbon mineralization activity.

Petroleum is a very complex mixture which contains various compounds that may not be readily degraded. Weathered petroleum may even pose a bigger threat as they were enriched with poorly degradable components (Agota Horel et al., 2014). Due to the nature of crude oil which only consist of carbon and hydrogen, its contamination in soil results in an imbalance in the carbon-nitrogen ratio at the spill site. The input of large quantities of organic carbon sources such as crude oil will cause rapid depletion of available inorganic nutrients, which consequently limits the biodegradation process. Nitrogen deficiency will also occur in the oil-soaked soil, which therefore reduced the utilization of carbon sources and retards the growth of indigenous microorganisms. Although hydrocarbons are an excellent source of carbon and energy for microbes, they are incomplete foods due to the lack of significant concentrations of other nutrients such as phosphorus and nitrogen which was crucial for microbial growth (Speight & El-Gendy, n.d.).

As stated previously, the soil in Port Dickson's beach ecosystem contain relatively low nutrient concentration. Hence, the release of hydrocarbons into this ecosystem will eventually results in an excessively high carbon-nitrogen or carbon-phosphorus ratios, which inhibit microbial growth. Hence, the addition of nutrient can play a vital role during the remediation process of weathered crude oil where the more readily degradable components are either partially oxidized or even completely removed during weathering. To ensure a successful bioremediation process, it is very essential that the addition of sufficient and controlled amounts of nutrients is done periodically. Lepo & Cripe, (1999) reported that the addition of nitrogen and phosphorous nutrients also stimulates the alkane-degrading microbes and results in a substantial depletion of the n-alkane fraction. Apart from vaporization of oil, this approach will further help reducing the shorter alkane chain in the spilled hydrocarbon.

D. Biodegradation of crude oil

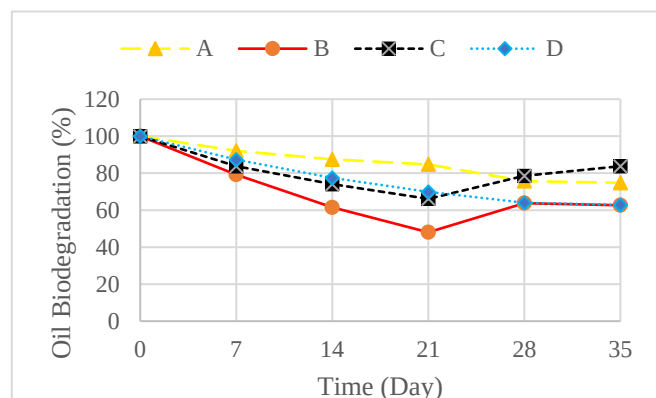


Figure 4: Biodegradation percentage of petroleum hydrocarbon in the contaminated soil

From Figure 4, it was observed that the highest total petroleum hydrocarbon (TPH) reduction occurs at the first seven days. The total hydrocarbon in microcosm A, B, C and D reduced to 87%, 79%, 83% and 85% respectively during this period. The high-rate of hydrocarbon reduction in the soil samples demonstrates that the native soil microbes were capable of degrading hydrocarbons to a large extent. This high biodegradation rate is also due to vaporization of the crude oil (Agota Horel et al., 2014). Under normal condition, indigenous biodegrading microorganisms would need an adaptation period of 4–10 days and sometimes takes weeks, during which no destruction of pollutants would be markedly evident. However, fast acclimation periods, as shown by a lack of lag phases was observed in sample B, C and D. This fast biodegradation response was the result of organic and inorganic nutrients amendment which led to a large decrease in the hydrocarbon concentration and deletion of the lag (adaptation)period (N, 2018). On the other hand, we can observe lag phase in microcosm A due to the lack of nutrient amendment to boost the biodegradation rate.

The highest TPH concentration reduction was observed in microcosm B with the final concentration of 48% at day 21. Based on Figure 2 and Figure 3, the elemental analysis on microcosm B shows that the addition of fish-based organic fertilizer has significantly increase the nutrient concentration inside the soil. Microcosm B also have the highest carbon and nitrogen utilization rate which are at 63% and 64% respectively. The high nutrient availability helps to boost the indigenous oil degrading bacteria community which result in higher oil biodegradation rate (Agota Horel et al., 2014). It is also observed that the addition of fish-based organic fertilizer caused the microcosm B to give off a very strong odor that persists for three to four weeks. This foul odor is assumed to be caused by the rapid decay of the fish-based fertilizer as it contain a very high nitrogen and is a source of several trace element (Mary Choate, 2007).



Figure 6: Fine suspended solid in microcosm B



Figure 5: Fine sedimentation in microcosm C

Microcosm C which are amended with plant-based organic fertilizer yield 66% hydrocarbon reduction at day 21, the second highest biodegradation rate behind microcosm B. The addition of plant-based fertilizer has also increased the nutrient concentration in the soil to as stated in Figure 2 and Figure 3. However, lower nutrient utilization rate was observed in Microcosm C compared to Microcosm B. Microcosm C have the second highest carbon and nitrogen utilization rate which are 61% and 62% respectively. One of the major findings in this experiment is that the decaying process of the fish-based and plant-based fertilizer in microcosm B and microcosm C respectively, start to generate some fine suspended particles at day 28 and day 35, as shown in Figure 5 and Figure 6. These suspended particles have increased the turbidity of the liquid extract, which caused some disturbance to the UV-Vis

spectrophotometer readings as the UV-Vis light is being absorbed or deflect by the suspended particles (Goodner, 2008; Thomas & Burgess, n.d.; Van Eerdenbrugh, Alonzo, & Taylor, 2011). Hence, these factors have caused the result in Figure 4 show a significant increase in the hydrocarbon concentration for both microcosm B and microcosm C.

As for microcosm D which are amended with inorganic NP fertilizer, the final hydrocarbon concentration is 63% at day 35. Hence, it is proven that the addition of organic and inorganic fertilizer had managed to increase the hydrocarbon biodegradation process and yield much higher rate as compared to the control microcosm A. Although the addition of inorganic NP fertilizer have increased the hydrocarbon biodegradation rate, this approach may not be a viable option due to their toxicity and excessive use of inorganic nutrient might bring negative impacts to the coastal ecosystems such as eutrophication (Rabalais, Turner, Diaz, & Justic, 2009). It was also reported by (Agota Horel et al., 2014) that the addition of inorganic NP fertilizer caused a very high pH drop in the microcosm, which eventually can further compromise the already disturbed environment in real oil spill accident. Extremes in pH (observed in some soils) could retard the ability of the microbial population to degrade hydrocarbons. For example, nitrogen fixating bacteria are strongly hindered by high acidity.

From this result in Figure 4, it was proven that the use of organic fertilizer has significantly better efficiency in stimulating the process of microbial biodegradation. Microcosm B have the highest hydrocarbon degradation rate followed by Microcosm C, Microcosm D and Microcosm A. Although the addition of organic and inorganic fertilizer can greatly improve the bioremediation process of contaminated soil, acute toxicity from fertilizer application can occur if nutrients at high concentrations are immediately solubilized, forming a highly toxic condition to microorganisms. Thus, the adjustment of C/N/P ratio is very important for the achievement of successful bioremediation process. It was frequently reported that the carbon-nitrogen-phosphorus ratio of 100:10:1 has been successfully optimize the biostimulation process of various contaminated soil (Speight & El-Gendy, n.d.). However, this would change in highly contaminated soil and/or with soil type that is characterized by depleted concentrations of nitrogen and phosphorous.

IV. CONCLUSION

In the present study, the additions of organic matter in a form of fish-based and plant-based fertilizers have managed to enhance the biodegradation process of the hydrocarbon contaminated marine sediment. The microcosms with the addition organic matter results in higher hydrocarbon removal compared to inorganic nutrient. Hence, it is proven that the organic nutrient amendment is as effective as or more effective than the inorganic nutrient amendment. The addition of organic matter also managed to improve the nutrient availability in the contaminated soil significantly. This paper also managed to prove that the microorganisms in the contaminated soil metabolize the organic fertilizer's nutrient at a higher rate compared to the inorganic fertilizer. Although the addition of inorganic nutrient in a form of NP is the most common approach to stimulate the hydrocarbon biodegradation, several studies have shown that this method might pose several threats to the contaminated sediment due to its toxicity.

This paper has also managed to identify the major species of bacteria which contribute to the biodegradation process of the hydrocarbon contaminated sediment. Through the use of cetrimide agar, *Pseudomonas Aeruginosa* bacteria was successfully isolated from the Port Dickson's marine sediment. *Pseudomonas Aeruginosa* is a well-known for its ability to metabolize and biodegrade hydrocarbon as well as its ability to produce biosurfactant. Biosurfactants increase the surface area of oil available for the

bacteria to metabolize by becoming an emulsifying agent, decreasing the surface tension and forming micelles, where, the microdroplets encapsulated in the hydrophobic microbial cell surface are taken inside and degraded. The cost of hydrocarbon spillage cleanup will also significantly reduce with the use of organic fertilizer to stimulate the biodegradation process. This is because the organic matter used in this study was obtained from the waste generated by food processing factory. These wastes are relatively cheap and abundant in our country.

For future reference, there are several areas of this experiment that can be further develop. To get more detailed and accurate reading, the qualitative method of determining the hydrocarbon concentration can be change. The use of gas chromatography with Flame-Ionization Detection (GC-FID) and gas chromatography with Mass Spectrometry (GC-MS) will provide a better insight on the spilled hydrocarbon behavior and characteristics. Hence, it is possible to determine type of bonds and substance that have been remove through the process of biodegradation. Next, to minimize the disturbance in the UV-Vis spectrophotometer reading, the extracted microcosms sample need to undergo centrifuging process. This process will eliminate the fine suspended solid generated by the decaying process of the organic matter. Future research can also focus more in identifying different type of bacteria that are able to degrade the spilled hydrocarbon in Port Dickson's beach. The result from this identification process will allow us to have a better understanding on the effect of the organic nutrient amendment to various indigenous microorganism in the whole ecosystem.

ACKNOWLEDGMENT

Thank you to my supervisor, Azzah Nazihah Che Abdul Rahim and Universiti Teknologi Mara.

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