Bioremediation of Hydrocarbon Contaminated Soils using Locally P.Aeruginosa with Organic and Inorganic Fertilizer

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***Abstract*— The discharge of oily wastewater to environment caused serious damages to human, animal and environment and bioremediation are an attractive alternative to chemical method for removal of hydrocarbon from oily wastewater. *Pseudomonas Aeruginosa* were utilized in degradation of different hydrocarbon (kerosene and diesel oil) contaminated soils amended with inorganic (NPK and Urea) and organic (Shrimp waste and Egg Shell Powder) fertilizers and in some of their combination. The incubation period ranged from 3-16 days. Results showed that bacterial population count increased as the microbes' utilized hydrocarbon for carbon and energy, residual hydrocarbon decreased was stimulated by the fertilizer and percentage degradation increased. *Pseudomonas Aeruginosa* degraded kerosene and diesel better in the presence of NPK fertilizers and urea fertilizer. More than 90% of the hydrocarbons were degraded with each incubation period. Bacterial population is keep increasing for first 15 days but after 15th day it begins to drop because of the secretion of toxic secondary metabolites. Furthermore, the usage of *Pseudomonas Aeruginosa* to degrade hydrocarbon has been proven as it can degrade kerosene and diesel.**

Keywords*— Biodegradation; Bioremediation; Microbes; Hydrocarbon; Pseudomonas sp.; Fertilizer; Contaminated Soil

I. INTRODUCTION

The world, in which we live as it is today, is the world in which everything we do as regards human growth, biological, physical, economic, industrial and infrastructural growth, science and technological growth etc. revolves around energy. Therefore, the amount of oil used is getting increasing with the industrial development. For the reason that, the producing of crude oil is getting higher due to high demand. Sometimes due to technical and management reasons a lot of oil is brought into the water, which formed great environmental pollution. Oil contaminated wastewater has been identified as one of the most concerned pollution sources. Source of oily wastewater is very broad and it is mainly generated from crude oil production, oil processing, petrochemical, metallurgical, mechanical industries and maritime transport. According to statistics, every year at least 500 to 1000 million of oil is discharged to water through a variety of ways, which not only cause water pollution, but also proves to be a waste of oil resources [6].

Besides that, crude oil spills also produce waters containing oil. According to the U.S Department of Energy, 1.3 million gallons (4.9 million liters) of petroleum are spilled into U.S. waters from vessels and pipelines in a typical year. A major oil spill could easily double that amount. Environmental pollution with petroleum and petrochemical products has attracted much attention in recent decades. Hydrocarbon contamination of the air, soil, and freshwater especially by polycyclic aromatic hydrocarbons (PAHs) attracts public attention because many PAHs are toxic, mutagenic, and carcinogenic. Organic toxic waste that can be found in oil can cause environmental harms for marine life, plant, animal and human being. Therefore, hazards on the environment and human health of oil pollutants have caused great concerns.

Bioremediation plays a great role in solving some of these problems. Bioremediation is the application of biological treatment to clean up hazardous chemicals. Bioremediation of oily wastewater is treatment technology that use of microorganisms or their enzymes to reduce the concentration or toxicity of hydrocarbon into less toxic forms [9]. Bioremediation method therefore come in handy and have correctly received favorable publicity as promising environmentally friendly technique for the remediation of hydrocarbon contaminated ecosystem.

Several studies have reported on the catabolic abilities of microorganisms such as fungi, bacteria, and algae to degrade petroleum hydrocarbon [12]. A number of gram positive and negative microbes have been reported to be capable of utilizing a wide variety of hydrocarbons as carbon and energy. The microorganisms include bacteria of *genera Klebstella, Protes Bacillus, Escherichia, Pseudomonas, Streptomyces, Norcardia, Xanthomonas* and others.

Usually these natural microbes can be found in marine sediments. The marine condition in Malaysia constitutes an excessive supply of undiscovered resources for the revelation of bioactive compounds. Malaysia is an oceanic nation with one of a kind fortunes that has plenty of resources such as mangroves, mudflat, and etc. to determine the natural microbes. Unfortunately there is less research does on the discovery of biological diversity in these part, especially marine bacteria. Besides that, lack of essential nutrients such as nitrogen and phosphorus is one of the major factors affecting biodegradation of hydrocarbon in soil and also limit the microbial growth. Thus, a study has been carried out to study the effect of organic fertilizer and inorganic fertilizer addition to the growth rate of locally isolated *Pseudomonas Aeruginosa*. Other than that, is to investigate the effectiveness of inorganic nutrients (NPK and urea) and organic nutrient (shrimp waste and egg shell powder) to stimulate the natural bioremediation process of hydrocarbon contaminated soil.

II. METHODOLOGY

A. Marine sediment collection

The sediment was collected from sandy beaches in Port Dickson, Malaysia (2°30'59.99"N; 101°47'59.99"E). The site was selected because of it has a port and two refineries. The sediments were

collected randomly by disturb sampling up to a depth of 30cm. The sediment samples were stored in a sealed bottles placed in a refrigerator (4ºC) according to the standard methods on sediment sample storage and handling, and were tested within one year. In the beginning of the experiment, nitrogen and carbon of the sediment was determined and recorded (Table 1). The soil samples was dried using oven and sieved through screens with 300 µm diameter openings to remove stones, wood particles and other debris. The soil samples was sterilized using autoclave (121ºC, 15 minutes).

B. Hydrocarbons

Diesel and kerosene were purchased from the gasoline filling station and local market, respectively.

C. Organic waste and Inorganic waste

The organic nutrient source that were used in this experiment are shrimp waste and egg shell powder (ESP). The ESP was collected from homes and the shrimp waste from GL Marine Sdn.Bhd., Klang. The shrimp waste are dried in an oven for two days at a temperature 80ºC. Then, are manually and mechanically crushed using Waring 1L blenders and sieved to 2mm. Egg shells were thoroughly washed with soap and water and air dried. Egg shells is crushed into pieces initially and grinded to fine powder using Waring 1L blenders. The inorganic waste used in this experiment were NPK and urea. The NPK and urea fertilizers were purchased from the local markets.

D. Hydrocarbon Utilizing Bacteria (HUB) Count

The microbial growth is determined by cell count method. The bacterial count is taken for every 4 days. The method used to count the hydrocarbon-utilizing bacteria (HUB) was serial dilution plating technique by direct cell count. 6 sterile tube was labeled from 1 to 6 and then 9 ml of distilled water was added in each tube. Then 1 ml of the stock solution was pipetted into test tube 1. This bacterial suspension was mixed thoroughly using vortexer before proceeding to the next step. Then 1 ml of the diluted bacterial suspension from the first test tube was pipetted into the second tube. The step is repeated until serially diluted the original bacterial suspension into test tube 6. Then the samples is plated onto cetrimide agar and was incubated at 37ºC for 24 hours before the cell counting. The cell count was determined by using the digital colony counter. The colony forming unit (CFU) was determined as below;

Plate count x Dilution Factor = Cells (CFU)

E. General experiment setup

The biodegradation rate of hydrocarbon was evaluated in a 16-day experiment by using a 100 ml microcosms. The composition of the mineral salts medium used contain 0.29 g KCl, 10g NaCl, 0.42g $MgSO_4.7H_2O$, 0.83g KH_2PO_4, 0.42g $NaNO_3$ and de-ionized water. The mineral salts was autoclaved at 121ºC for 15 minutes. The experiment have twelve (12 samples) and six (6) samples for each hydrocarbon for kerosene and diesel.

The six (6) composite experiments were performed as follows:

1. Biodegradation of kerosene contaminated soil using *Pseudomonas sp*. amended with inorganic fertilizer (NPK and urea) and organic waste (shrimp waste and ESP). It consist of six test as:
    I. Sample A: 0.4ml stock solution of *Pseudomonas sp.*, 1ml of kerosene, 10g of soil, 1g of shrimp waste and 50ml mineral salt.
    II. Sample B: 0.4 ml stock solution *of Pseudomonas sp.*, 1ml of kerosene, 10g of soil, 1g of ESP and 50ml mineral salt.
    III. Sample C: 0.4 ml stock solution of *Pseudomonas sp*., 1ml of kerosene, 10g of soil, 1g of NPK fertilizer and 50ml of mineral salt.
    IV. Sample D: 0.4 ml stock solution of *Pseudomonas sp.*, 1ml of kerosene, 10g of soil, 1g of Urea fertilizer and 50ml of mineral salt.
    V. Sample E: 0.4 ml stock solution of *Pseudomonas sp*., 1ml of kerosene, 10g of soil, 0.5g each of NPK and Urea and 50ml of mineral salt.
    VI. Sample F (Control): 0.4 ml stock solution of *Pseudomonas sp*., 1ml of kerosene, 10g of soil and 50ml of mineral salt.
2. Biodegradation of diesel oil contaminated soil using *Pseudomonas sp*. amended with inorganic fertilizer (NPK and Urea) and organic waste (Shrimp waste and ESP). It consist of six (6) test as;
    I. Sample A: 0.4 ml stock solution of *Pseudomonas sp.*, 1ml of diesel oil, 10g of soil, 1g of shrimp waste and 50ml of mineral salt.
    II. Sample B: 0.4 ml of stock solution of *Pseudomonas sp*., 1ml of diesel oil, 10g of soil, 1g of ESP and 50ml of mineral salt.
    III. Sample C: 0.4 ml of stock solution of *Pseudomonas sp*., 1ml of diesel oil, 10g of soil, 1g of NPK fertilizer and 50ml of mineral salt.
    IV. Sample D: 0.4 ml of stock solution of *Pseudomonas sp*., 1ml of diesel oil, 10g of soil, 1g of Urea and 50ml of mineral salt.
    V. Sample E: 0.4 ml of stock solution of *Pseudomonas sp*., 1ml of diesel oil, 10g of soil, 0.5g each of NPK and Urea and 50ml of mineral salt.
    VI. Sample F (Control): 0.4ml of stock solution of *Pseudomonas sp.*, 1ml of diesel oil, 10g of soil and 50ml of mineral salt.

The moisture was adjusted to 60% water holding capacity and incubated at room temperature (28ºC). In all for each sample analysis, the total nitrogen and carbon was determined on day 1 using elemental analyzer. The residual hydrocarbon is determined using spectrophotometric analysis.

F. 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 samples 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 37ºC for 24 hours. The colonies of bacteria were then observed visually for any pigmentation.

G. Total Petroleum Hydrocarbon (TPH) Determination

The moisture in the soil samples was removed by the addition of anhydrous sodium sulphate (Na_2SO_4). To determine the hydrocarbon content of the soil samples, 5g of soil sample was suspended in 10ml of cyclohexane using 100 ml Erlenmeyer flask. The mixture was then shake at 200RPM for 60 minutes using an orbital shaker. A U-Visible Lambda 750 spectrophotometer was used to measure the total petroleum hydrocarbon (TPH) of the liquid phase of the extract at 255 nm for diesel and 272 nm for kerosene. By comparing the result to the standard curve derived from fresh kerosene and diesel oil that has been diluted with cyclohexane, the exact TPH of the sediment was measured. The initial concentration can be calculated by the following equation:

$$C_o = (m_o/V_w) \times 10^3$$

Where C_o (mg/L) is the oil concentration, m_o (mg) is the corresponding mass of oil in the standard sample and the V_w (mL) is the mineral salt solution volume. The TPH was calculated from the measured residual oil content by equation:

$$TPH = (C_o - C_i)/C_o$$

Where C_o and C_i are the initial and residual hydrocarbon concentration after each incubation period.

III. RESULTS AND DISCUSSION

A. Identification of bacteria

There are few bacterial species have been recognized as having the capacity for oil degradation. The commonly used microorganisms in bioremediation are bacteria, fungi, cyanobacteria and algae. Bacteria are the most active agents in petroleum degradation, and they work as primary degraders of spilled oil in environment [11]. Contamination of water hydrocarbon waste stimulates indigenous microbial populations, which are capable utilizing the hydrocarbon substrates as their sole carbon and energy sources thereby degrading the contaminants. They have been studied in many researches, *Penicillium, Yarrowia, Geotrichum, Bacillus, Acinobacter, Serratia* and *Pseudomonas Aeruginosa* are sample of these microoganisms, among which bacteria are more applied in oily wastewater treatment [2] and could degrade the hydrocarbon [11]. 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 study, cetrimide agar was used to isolate and identify *Pseudomonas Aeruginosa* from the hydrocarbon contaminated marine sediment. After an incubation period of 24 hours at 37^{o}C, the pigmentation of bacteria as shown in Figure 1 were observed which indicate the growth of microorganisms. Cetrimide agar is a selective agent and also constituents to enhance pigment production by inhibits the growth of many microorganisms whilst allowing *Pseudomonas Aeruginosa* to develop typical colonies. It exhibits inhibitory actions on a wide variety of microorganisms including *Pseudomonas* species other than *Pseudomonas Aeruginosa*. The formation of yellow-green pigments (pyocyanin) on the cetrimide agar indicate the presence of *Pseudomonas Aeruginosa* in the soil sample from Port Dickson. *Pseudomonas Aeruginosa* is the only species of *Pseudomonas* or gram- negative rod known how to excrete pyocyanin [3]. Therefore, these media important in the identification of *Pseudomonas Aeruginosa*. The identification of *Pseudomonas Aeruginosa* from the soil samples is very important because it has been studied that *Pseudomonas Aeruginosa* can be used to degrade hydrocarbon.

Based on the number of published reports, the most important hydrocarbon-degrading bacteria in both marine and soil environments are *Achromobacter, Acinetobacter, Alcaligenes, Arthrobacter, Bacillus, Flavobacterium, Nocardia* and *Pseudomonas spp* [8]. In the marine environment, bacteria are generally considered to represent the predominant hydrocarbon-degrading element of the microbial community. The microorganisms will degrade the hydrocarbon as a sole carbon and energy sources, as a means of reducing PAH toxicity and as co-metabolic substrates [4].

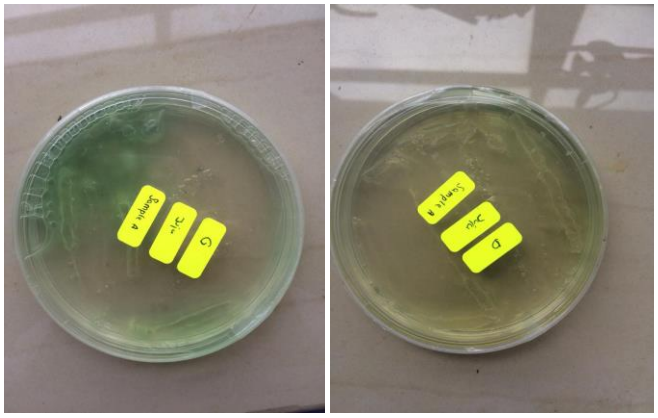

Figure 1: Cetrimide agar with *Pseudomonas Aeruginosa* colonies

B. Physicochemical analysis of the soil samples and organic waste

Table 1: Physicochemical analysis of soil and organic waste

Parameter	Soil	Shrimp Waste	Egg Shell Powder
pH	7.60	7.08	6.71
Carbon (%)	1.99	21.17	10.34
Nitrogen (%)	0	6.36	3.12
Hydrogen (%)	1.68	1.72	22.17

The physicochemical properties of soil and organic wastes used for the bioremediation studies are shown in Table 1. This experiment was conducted in a laboratory at room temperature which is 28^{o}C. The temperature of the experiment was maintained at room temperature because among the others physical factors, temperature plays an important role in biodegradation of hydrocarbon. This is because temperature directly affecting the chemistry of the pollutants as well as affecting the physiology and diversity of the microbial flora [11]. Besides that, based on the studies found that at low temperatures, the viscosity of the oil increased, while the volatility of the toxic low molecular weight hydrocarbons were reduced, delaying the onset of biodegradation [11]. Although hydrocarbon biodegradation can occur over a wide range of temperatures, the rate of biodegradation generally decreases with the decreasing temperatures. According to Nilanja Dass, the highest degradation rates that generally occur in the range 30-40^{o}C in soil environments, 20-30^{o}C in some freshwater environments and 15-20^{o}C in marine environment [11]. Other than that, the optimum temperatures for biodegradation to occur is normally between 25-35^{o}C for anaerobic processes and 15-30^{o}C for aerobic processes [11]. During this experiment, the temperature was maintained at room temperature for the optimum biodegradation rates. Furthermore, the optimum temperature for *Pseudomonas Aeruginosa* for biodegradation is at 30^{o}C.

The initial pH value of the soil from Port Dickson's beach is 7.60. The pH of the soil is neutral and it is favor for the bacteria to degrade the hydrocarbon. Most heterotrophic bacteria and fungi favor a pH near neutrality, with fungi being more tolerant of acidic condition [8]. The optimum pH for *Pseudomonas Aeruginosa* for biodegradation is 7. Based on Table 1, the addition of shrimp waste organic fertilizer caused the slight decrease in the pH which is 7.08. Even though, there is decreasing in the pH but it still acceptable because the optimum hydrocarbon biodegradation occurs around pH 6.5 to 8. Meanwhile, the addition of egg shell powder organic fertilizer has caused further dropped in pH value which is 6.71.

In this paper, only carbon and nitrogen were analyzed as these nutrients are essential for the growth of oil degrading bacteria.

According to Table 1, the carbon and nitrogen value of soil is quite low which is 2% and 0%; this is a low carbon and nitrogen content for effective biodegradation of oil in the soil, hence the need for addition for organic wastes as a source of nutrients (N and P). Shrimp waste had the highest N content among the other organic wastes used which is egg shell powder. Shrimp waste is rich in nitrogen because of the higher content in proteins. Based on the studies, dried shrimp waste contain about 52% of protein [5]. This indirectly contribute to the higher value of nitrogen content. Besides that, the nitrogen content of egg shell powder also rich sources of essential nutrients. Based on Table 1, the nitrogen content of egg shell powder is 3.1%.

Carbon is usually needed in higher amounts and can be provided by pollutants because it is important for synthesizing their cell components. According to Kalantary, nitrogen and phosphorus as macronutrients are 14% and 3% of dry weight of a typical microbial cell respectively [7]. From these result, it can be concluded that the shrimp waste fertilizer has higher nutrient content and greater potential to stimulate the biodegradation process inside the oil-contaminated soil.

C. Nutrient Utilization

The soil sample from sample A, B, C, D, E and F for diesel and kerosene was taken at the beginning of the experiment for nutrient analysis and the result of these analysis was presented in Figure 2. From the result, the initial nutrient which contained inside samples A, B, C, D and E for diesel and kerosene have increased due to the addition of organic and inorganic fertilizers. Samples D and E for both kerosene and diesel have highest nitrogen content while samples A and B for kerosene and diesel have highest carbon content. Samples D and E have highest nitrogen content because NPK and urea have 15% and 46% of nitrogen in fertilizer respectively. This caused the significant increase of nitrogen content in samples D and E for both kerosene and diesel. This addition of nutrients can stimulate the biodegradation rates of bacteria.

From the figure 2, addition of organic nutrients in samples A and B also increase the carbon and nitrogen content in the samples. This is because egg shell powder and shrimp waste is high in protein thus indirectly contribute to the high contain of nitrogen. The addition of nutrient enhance the microbial growth of the microorganisms and stimulate the biodegradation of hydrocarbon in soil. From the figure, it can be seen that samples that amended with inorganic nutrients have high carbon and nitrogen content than the organic nutrients. As for the sample F for both the kerosene and diesel, the carbon and nitrogen content is the lowest among all because of the lack of nutrients which may retard the microorganism's hydrocarbon mineralization activity.

Addition of nutrients play a vital role during the remediation process which can stimulate the microorganisms to degrade hydrocarbon. To ensure a successful bioremediation process, it is very essential that the addition of sufficient amounts of nutrients and also ensure that the nutrients is suitable for the microorganisms. Addition of nutrients will increase the population of microorganisms thus increasing the degradation rate of the hydrocarbon.

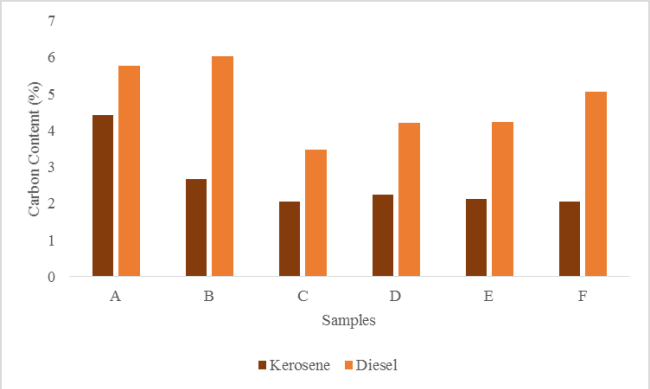

Figure 2(a): Percentage of carbon content in kerosene and diesel oil for micoorganisms.

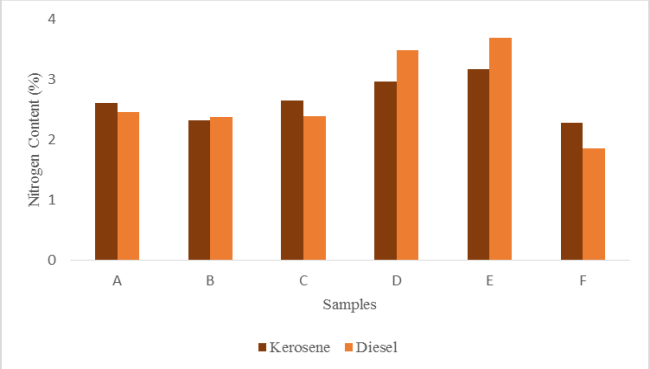

Figure 2(b): Percentage of nitrogen content in kerosene and diesel oil for microorganisms.

D. Microbial Growth

Figure 3 shows the growth profile of the hydrocarbon degrading bacterial population. An initial hydrocarbon-degrading bacterial population is 7.3 x 10^6 CFU/mL at the start of the biostimulation. As seen from Figure 4, the bacterial population is increasing for both in kerosene and diesel when amended with organic and inorganic fertilizers. The increasing in bacterial population, it also indicates that increasing in the degradation rates of hydrocarbon. The counts of hydrocarbon utilizing bacteria amended with organic and inorganic wastes were higher compared to those unamended. This might be because of the presence of appreciable quantities of nitrogen and phosphorus in the fertilizers.

The reason for increased biodegradation rates of hydrocarbon in amended soils compared to the control because of the addition of organic and inorganic wastes. This is because the organic and inorganic wastes helps to loosen the compactness of the soil making sufficient aeration available for the indigenous bacteria, thereby enhancing their metabolic activities in the contaminated soils [1].

Based on figure 3, after day 14 the bacterial population is decreasing. This is because there is insufficient nutrients for the microorganisms and also due to the secretion of secondary metabolites by the microbes which may be toxic to microbes themselves.

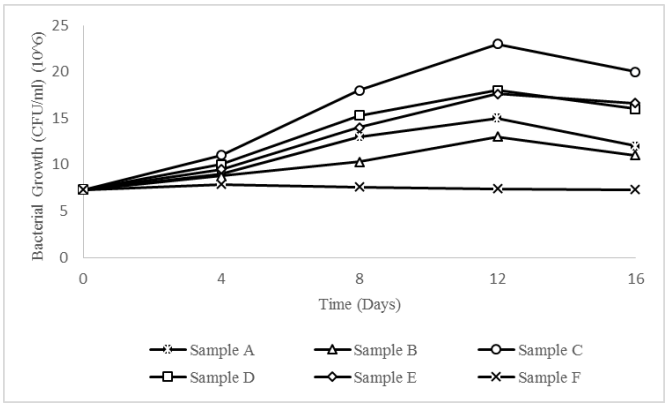

Figure 3(a): Bacterial growth in kerosene oil amended with organic and inorganic fertilizers.

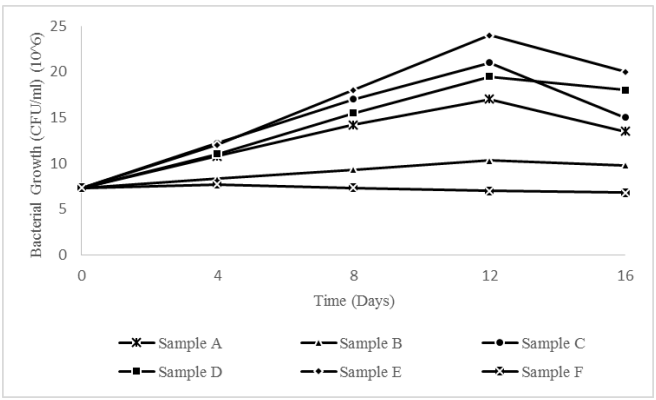

Figure 3(b): Bacterial growth in diesel oil amended with organic and inorganic fertilizers.

E. Biodegradation of Hydrocarbon

The percentage of oil biodegradation in the soil contaminated with kerosene and diesel is shown in Figure 3 and the microbial growth is shown in Figure 4, respectively. From Figure 3, it was observed that the highest total petroleum hydrocarbon (TPH) reduction occurs at the first seven days. The total hydrocarbon in microcosms A,B,C,D,E and F reduced to 95%, 97%, 98%, 97%, 98%, 98% for kerosene and 90% and 95% for diesel. It can be seen that first three days the biodegradation percentage for both kerosene and diesel is about 90-97%. The high rate of biodegradation shows that the *Pseudomonas Aeruginosa* is capable of degrading hydrocarbons. Addition of organic waste and inorganic wastes does increase the degradation rate of hydrocarbon. It can be seen that, first seven days there is rapid degradation occur but after seven days it slowly continue until day 15. Besides that, addition of organic and inorganic nutrients helps to reduce the lag phase of the microorganisms and supply nutrients to the microbial population present in the contaminated soil. The result shows that increase in the rate of biodegradation of hydrocarbon,as the concentration of oil reduced [1].

Sample C and D shows more population bacterial count and less residual kerosene was observed than in samples A and B. It shows that NPK and urea is better source of food than shrimp waste and egg shell powder. Based on previous studies, it shows that *pseudomonas aeruginosa* degraded kerosene better in the presence of NPK fertilizer [10]. More population count, higher percentage degradation and less residual hydrocarbon (kerosene) were observed in sample C (NPK fertilizers) and followed by sample D (Urea). In sample A which using shrimp waste as an organic fertilizer shows that slowly degrade the hydrocarbon concentration compare to the NPK and urea fertilizer. Sample A using shrimp waste shows increase in bacterial population but it slowly degrade the hydrocarbon. This is because it does not have enough nutrients to degrade the hydrocarbon. Sample B using ESP as an organic nutrients shows the rapid increase in first seven days compared to the sample A using shrimp waste as an organic nutrients and after the seven days it slowly continue to degrade the hydrocarbon. Microbial growth in sample B which used ESP as organic nutrients shows that slowly increasing in microbial growth and slowly degrade the concentration of hydrocarbon.

As for diesel, it can be seen that the degradation rate is almost the same for all samples. The first seven days the percentage of biodegradation is around 90-95%. After that, it slowly degrades the hydrocarbon until day 16. The highest microbial growth shown in sample E which is the combination of NPK and Urea and also has 95% of oil biodegradation. Based on the graph, it can be seen that addition of shrimp waste has no significant difference in degradation of diesel. Addition of ESP shows the percentage of biodegradation is 95% for the first seven days. Combination of NPK and urea has higher biodegradation rate than organic waste (shrimp waste and ESP) and inorganic waste (NPK and urea). Based on this experiment, addition of organic and inorganic nutrients or the combination (NPK and urea fertilizer) was effective. Moreover, the usage of mineral salt solution also help the microorganisms to degrade hydrocarbon. This is because mineral salt aided the microorganism's ability to degrade hydrocarbon. *Pseudomonas Aeruginosa* can be reffered to as halophiles which is the microorganisms that required salt for growth [10]. But in all, type of microbe, hydrocarbon used and fertilizer amendment determined the bacterial population count and the percentage of hydrocarbon degraded [10].

From the figure, it can be seen that after day 12 the percentage biodegradation is decreasing. This is because after day 14 the bacterial population is decreasing thus give impact to the degradation process of the hydrocarbon. Besides that, sample A in kerosene and samples B and E for diesel show significant decrease in percentage compare to others. This is because shrimp waste in sample A, egg shell powder in sample B and combination of urea and NPK in sample E start to generate some fine suspended particles after day 12. These suspended particles have increased the turbidity of liquid which caused some disturbance to the UV-Vis spectrophotometer readings. Hence, this factors have caused the biodegradation percentage for sample A, B and E rapidly decreasing at day 15.

Even though, there is no difference in the biodegradation of hydrocarbons when stimulated with inorganic fertilizers (NPK and urea) and organic waste (shrimp waste and ESP), the use of fertilizer had led to better degradation of hydrocarbon, it is better to use organic waste which are cost effective and more environmental friendly.

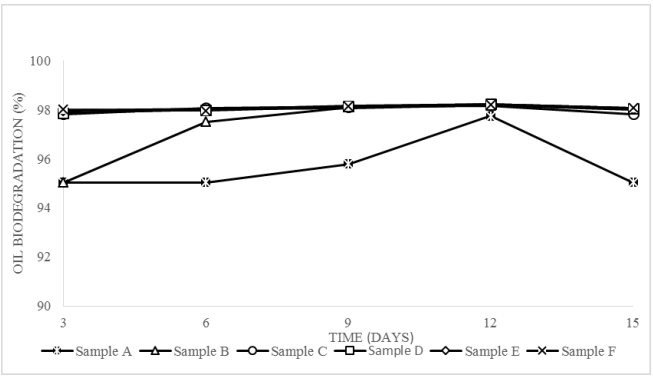

Figure 4(a): Biodegradation percentage of kerosene oil using *Pseudomonas Aeruginosa* amended with organic and inorganic fertilizers.

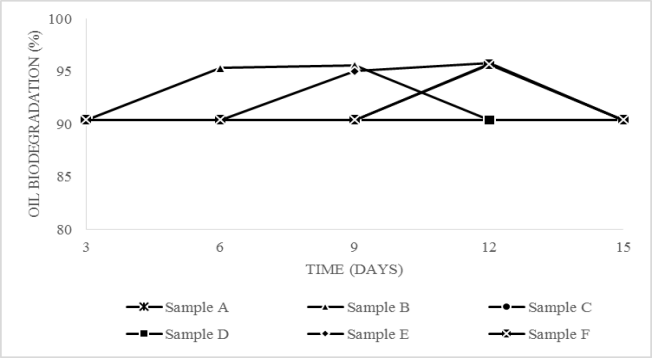

Figure 4(b): Biodegradation percentage of diesel oil using *Pseudomonas Aeruginosa* amended with organic and inorganic fertilizers.

IV CONCLUSION

The addition of organic and inorganic wastes which is shrimp waste and ESP for organic and NPK fertilizer and urea as inorganic wastes have managed to enhance the biodegradation process of the hydrocarbon contaminated marine sediments. From the studies, it can be seen that addition of inorganic wastes (NPK and urea) is higher hydrocarbon removal compared to organic both in kerosene and diesel. Even though, the addition of inorganic nutrients have higher hydrocarbon removal but it is advisable to use organic wastes as it is more cost effective and environmental friendly.

This paper also has managed to identify that *Pseudomonas Aeruginosa* can degrade hydrocarbon. The *Pseudomonas Aeruginosa* was identified by using cetrimide agar that was isolated from the Port Dickson's marine sediment. Besides that, there are several studies and has been proven that *Pseudomonas Aeruginosa* is well-known microorganisms that can degrade polycylic aromatic hydrocarbons (PAH). Biostimulation method can be used for the treatment of oily wastewater as it is environmental friendly and cost effective. This is because the organic wastes used in this study was obtained from the waste-generated by food processing factory. These wastes are relatively cheap and can be find easily.

For future references, there are some development can be done for this research. To get more detail and accurate reading of hydrocarbon concentration the use of gas-chromatography-mass spectrometry (GC-MS) combined with U-Visible spectrophotometry can be done. This is because GC-MS will provide a better insight on the spilled of hydrocarbon behavior and characteristics. Besides that, GC-MS provide the information which type of bonds and substance that have been degrade by microorganisms. Next, for the bacterial growth, it is better to do cell count method and dry-weight method for more accurate results. Furthernore, future research can identify the different type of bacteria in Malaysia marine environment for the bioremediation process.

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References

[1] Abioye, O. P., et al. "Biodegradation of Used Motor Oil in Soil Using Organic Waste Amendments." Biotechnology Research International, vol. 2012, 2012, pp. 1–8., doi:10.1155/2012/587041.

[2] Azhdarpoor, A., Mortazavi, B., & Moussavi, G. (2014). Oily wastewaters treatment using Pseudomonas sp. isolated from the compost fertilizer. Journal of environmental health science & engineering, 12, 77. doi:10.1186/2052-336X-12-77

[3] Cetrimide Agar Base (n.d.), Retrieved from http://www.himedialabs.com/TD/M024.pdf

[4] Doyle, E., Muckian, L., Hickey, A. M., & Clipson, N. (2008). Microbial PAH Degradation. Advances in Applied Microbiology, 27-66. Doi: 10.1016/s0065-2164(08)00602-3

[5] F.Z Razouk R Kajji A, Mamouni & Daoui, Khalid. (2016). Characterization of haracterization of Organic Waste Used as U Bio-fertilizers: Case ase of of Potato Peelings, Almond lmond Hulls and Shrimp Shells. International International Journal Journal of of Agriculture Agriculture Innovations Innovations and Research. 4. 2319-1473.

[6] Hui, L., Yan, W., Juan, W., & Zhongming, L. (2015). A Review: Recent Advances in Oily Wastewater Treatment. *Recent Innovations in Chemical Engineering (Formerly Recent Patents on Chemical Engineering), 7*(1), 17-24. Doi: 10.2174/2211334707666140415222545

[7] Kalantary, Roshanak Rezaei, et al. "Effectiveness of Biostimulation through Nutrient Content on the Bioremediation of Phenanthrene Contaminated Soil." Journal of Environmental Health Science and Engineering, vol. 12, no. 1, 2014, doi: 10.1186/s40201-014-0143-1.

[8] Leahy, J. G., & Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. Microbiological reviews, 54(3), 305-15.

[9] Marina Bujang, and Noor Azlina Ibrahim, and Aweng Eh Rak, (2013) *Biodegradation of oily wastewater by pure culture of bacillus cereus.* ARPN Journal of Agricultural and Biological Sciences, 8 (2). pp. 1-8. ISSN 1990-6145

[10] Nduka JK, Umeh LN, Okerulu IO, Umedum LN, Okoye HN (2012) Utilization of Different Microbes in Bioremediation of Hydrocarbon Contaminated Soils Stimulated With Inorganic and Organic Fertilizers. J Pet Environ Biotechnol 3:116. doi:10.4172/2157-7463.1000116

[11] Nilanjana Das, and Preethy Chandran (2011). "Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview." Biotechnology Research International, vol. 2011, 2011, pp. 1–13., doi:10.4061/2011/941810

[12] Obi, L. U., Atagana, H. I., & Adeleke, R. A. (2016). Isolation and characterisation of crude oil sludge degrading bacteria. *SpringerPlus*, *5*(1), 1946. http://doi.org/10.1186/s40064-016-3617-z