



## BIODEGRADATION OF CRUDE OIL IN DIFFERENT TYPES OF MARINE SEDIMENT

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### ABSTRACT

An active oil-oxidizing bacterium, named Nap C was isolated from the sediment sample of Port Dickson coastal area for this study. Nap C is a gram negative, rod shape marine bacterium. It forms spore when the condition is not favorable. Three different types of treated marine sediment; sand, silt and clay were used in this study. The degradation of Malaysian Tapis A crude oil in the different types of marine sediment were assessed. Silt type of marine sediment was found to sustain highest biodegradation compared to clay type and sand type. 86.67% of the Malaysian Tapis A crude oil was degraded in silt type of marine sediment within 10 days of incubation. Where as there were only 60% and 73% of the Malaysian Tapis A crude oil was degraded in sand and clay type of marine sediment respectively. Microbial biomass estimation in the sediment was estimated by indirect phospholipid enumeration technique.

**Keywords:** Oil bacteria, biodegradation, sediment, petroleum hydrocarbons.

### Introduction

Rapid development of the economy in this region, especially maritime and petroleum-base industry had led to the increase of petroleum hydrocarbons flux into the marine environment. Oil level in the Straits of Malacca had shown an increasing trend over the past decade. In 1990, the range of the hydrocarbon level in the sediment samples of Port Dickson was only 21.73 – 74.50 mg/kg sediment (Law et al., 1990). However, in 1998, the level of hydrocarbon has increased to 171.53 mg/kg at the same locations (Sue, 1999). According to DOEs' (Department of Environment Malaysia) environmental quality report 1997, Oil and grease are the major contaminant in Malaysian waters. 37% of the DOEs' monitoring stations were found to have exceeded the proposed Marine Interim Standard for oil and grease (0 mg/l).

Sediment is an important matrix in environmental safety as well as for many economical activities such as aquaculture. However, less attention is given to its condition compared to water supply, water quality and water management (Boyd, 1995). Most soil contained large number of indigenous microorganisms that are able to degrade



hydrocarbons. Zobell (1973) reported that 70 genera (28 bacteria, 30 filamentous fungi and 12 yeast) of microorganisms were able to degrade one or more type of hydrocarbons. Bacteria appear to be the most important microbe for breaking down hydrocarbons in the marine environment. Activities of the active oil-oxidizing microbes are mainly affected by the characteristics of the sediment such as, pH, nutrient availability,  $E_h$ , temperature, moisture, porosity and the texture of the sediment. Aerobic bacteria in oxygenated sediment were capable to break down petroleum hydrocarbons than that of the anaerobic bacteria in oxygen free sediment (Delaune, 1980). Hence, biodegradation of hydrocarbons in sediment is a very complex and heterogeneous process. Therefore, this study aims to evaluate the types of sediment on biodegradation of hydrocarbons in the sediment.

## **Materials and methods**

### *Oil-oxidizing bacterium*

Nap C, an oil-oxidizing bacterium, was isolated by naphthalene-enriched medium from the sediment sample of Port Dickson coastal environment. Correlations between the logarithm cell population and optical density at 600nm as well as the logarithm cell population and phospholipid content were established. The correlation coefficients were  $Y = 5.788x + 6.9834$ ;  $r = 0.9371$  and  $Y = 7.147x$ ;  $r = 0.9407$  respectively.

### *Culture media*

Double strength synthetic seawater medium of Law and Button (1977) with pH 8 and salinity 30 g/liter was used in this study. The liquid medium was sterilized by autoclaving at 121°C, 15 p.s.i for 15 minutes. Vitamin B<sub>1</sub>, B<sub>12</sub> and d-Biotin which had been sterilized by Millipore filtration technique was added into the liquid medium by using sterilized syringe after autoclave. Solid medium used in this study was prepared by adding 55.1 g of marine agar 2216 (Difco) into 1 liter of distilled water. The solid medium was sterilized by autoclaving.

### *Sediments*

Marine sediment sample (sand, silt and clay) was collected from Port Dickson coastal water by using a box corer. The sediment was washed, air-dried and sieved (Endecott) to obtain a homogeneous substrate. Sieved sediment was burnt in muffle furnace (Heraeus) at 600°C for 2 hours to remove the organic fraction of the sediment. pH of the treated sediment were measured by calibrated pH (Hanna, HI 9622). Total nitrogen and total phosphorous content in the sediment were determined by methods described by McGill and Figueiredo (1993) and Halloran (1993) respectively.

### *Incubation*

100 g of treated sediment (sand, silt and clay) sample was weight into 500ml low form sample bottle. 150 mg of Malaysian Tapis A crude oil was added and mixed thoroughly with the sediment to give 1500 mg crude oil /kg sediment. Approximately 75 ml of liquid medium was added to the sediment in order to maintain the optimum moisture for the activities of the bacteria. An initial of  $9.50 \times 10^5$  cells/ gram sample was inoculated into the sample. Samples were incubated for 10 days. At an interval of 48 hours, the concentration of crude oil and cell biomass were determined.

### *Biomass Determination*

Indirect microbial biomass determination by using phospholipid analysis (Findlay et al., 1989) was employed to assess microbial biomass in the sediment. Briefly, lipid was recovered from specimen by chloroform-methanol extraction. After the extraction, lipid contented chloroform was filtered through Whatman 2V filter paper and recovered in a round bottom flask. Solvent was removed in vacuum. Phosphate was liberated from lipids recovered by potassium persulfate digestion. Phosphate released by digestion was determined by the methods of Van and Mannaerts (1987). The absorbance at 610 nm was then read by using spectrophotometer (Unicam 5625 UV/VIS spectrophotometer). Concentration of phosphate was calculated by using the linear regression line from a standard curve prepared by digesting glycerol phosphate. Recovery test of the method was conducted by using glycerol phosphate. Cell population of Nap C was determined by the standard curve of Nap C versus glycerol phosphate produced.

### *Hydrocarbons analysis*

Gravimetric method (Kimura et al., 1990) was used to measure the content of crude oil in the sediment. Residue oil in 10 grams sediment sample was extracted with 200 ml dichloromethane (J.T. Baker) by using SOHLEX for 8 hours. Residue was then transferred into round bottom flasks and concentrated by rotary evaporator (Ika-Heizbed HB-250). Initial weight of the round bottom flasks were weighted after it has been cleaned and dried. After removing dichloromethane, the round bottom flasks were dried at 50°C in oven for an hour and then put into desiccator for another hour before weighing with a 3 decimal points balance (Sartorius BASIC).

### *Biodegradation rate*

Biodegradation rate of hydrocarbon,  $v_c$  (mg/kg/day) in the sediment is estimated by the following equation.

$$v_c = (O_i - O_f) / t \text{ mg/kg/day}$$

Where  $O_i$  is the concentration of the hydrocarbon at the beginning of the experiment, mg/kg;  $O_f$  is the concentration of the hydrocarbon at the end of the experiment, mg/kg; and  $t$  is the incubation time, day.

## Results and discussion

Many studies had been conducted on the effects of nutrient, pH, salinity and water activity on the biodegradation of petroleum hydrocarbons (Huesemann, 1994; Law and Teo, 1997). Among the factors, nutrient and pH are at critical. According to Huesemann (1994), biodegradation will only be stimulated when the weight ratio of carbon, nitrogen and phosphorous is 100:1:0.2 and above. Nitrogen and phosphorous are always the limiting factors in the natural environment. Frankenberger (1991) reported that, the optimum pH for biodegradation was between pH 6.0 to 8.0. N : P in this study; total nitrogen and total phosphorous content in the treated sediment were  $721 \pm 14 \mu\text{g-at N/g}$  and  $45.15 \pm 0.01 \mu\text{g-at P/g}$  respectively. The ratio of N : P in the treated sediment was 16:1. These additional nutrients from the sediment were an advantage to the degradation. pH of the treated sediment was constant ( $\text{pH } 8.0 \pm 0.005$ ) through out the experiment. The constant pH was due to the calcareous content in the marine sediment. Consistent pH of the sediment had minimized the effects of pH on the experiment.

Preliminary study indicated that, the optimum conditions for the activities of Nap C was at pH 8.0, temperature of  $30^\circ\text{C}$  and salinity of 30 g / Liter. There is strong correlations between Nap C and concentration of phospholipid ( $Y = 1.4034 \times 10^7 X$ ;  $r = 0.9407$ ). This correlation was utilized to estimate the microbial biomass in the sediment.

The effects of different type of sediment on the extent and kinetic of petroleum hydrocarbons degradation was not fully understood. Theng and Orchard (1995) reported that, chemical bonding between particles of different type of sediment will influent the interaction between sediment and microbe. This mean, the interaction between different type of sediment and microbes will have effects on the activities and survival of the bacteria in the sediment. However, Burns (1995) reported that, soil texture and its water content are the primary influences on the activities of inoculated bacteria.

Biodegradation of petroleum hydrocarbons have been conducted on different type of sediment. Figure 1 and 2 reveal that, silt type of sediment (0.005mm – 0.05 mm) sustained highest oil degradation compared to sand and clay types of sediment. The results showed that, 86.67% of crude oil was degraded in silt type of sediment after 10 days of incubation, where as, percentage of petroleum hydrocarbons degraded in sand type (larger than 0.05 mm) and clay type (less than 0.005 mm) of sediment were only 60% and 73% respectively. The maximum degradation rate of crude oil in silt, sand and clay types of sediment by Nap C were 130 mg/Kg/day; 90 mg/Kg/day and 110 mg/Kg/day accordingly.

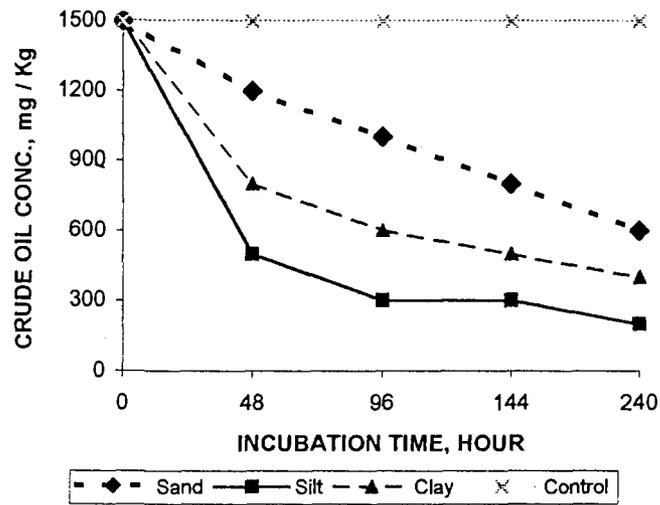
Higher degradation of petroleum hydrocarbons was observed in silt type of sediment (130 mg/Kg/day). This was most probably due to the texture of silt type sediment. Generally, silt has well sorted texture compare to the rest. This well sorted texture enables optimum nutrient transportation and aeration in the sediment. Beside, larger surface area per volume of silt type sediment also enhance the association of the microbe on the sediment particles. Clay type of sediment tend to have compact-pattern of sediment texture. This compact pattern of sediment texture will decrease the quality of the nutrient transportation and sediment aeration. Reduction of the quality of sediment aeration and transportation probably caused lower degradation. Although sandy sediment tend to have loose-pattern of sediment texture, high surface evaporation rate of this sediment will probably decreased the oil degradation. Beside, big particle size of the

sandy sediment will also have least surface area per volume for the association of the microbe on the particles.

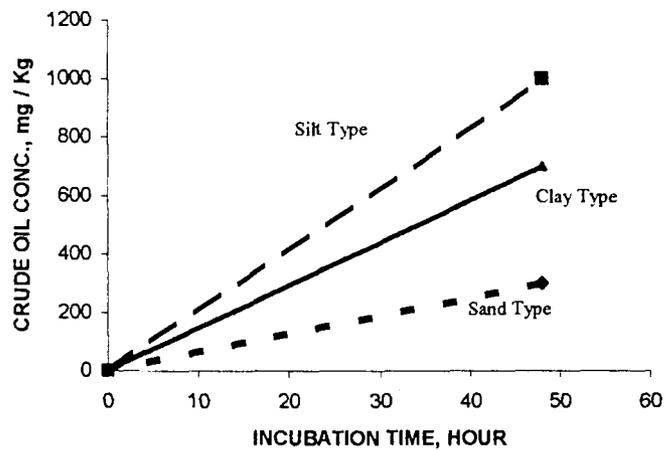
Similar study was also conducted *in-situ* by Huesemann (1994). According to Huesemann, highly sorptive surface of clay which content organic matter fractions limit the bioavailability of petroleum hydrocarbons to the active oil degrading bacteria. Biodegradation kinetic of clay type of sediment is not limited by the number of hydrocarbon degrading microbe but rather by mass transportation phenomena. Diversion between Huesemann (1994) and present study was due to the organic content and the bonding between particles in the sediment. In this study, all the sediment were burnt at 600°C for 2 hours to remove the organic matter. As a result, chemical bonding between the sediment particles and organic matter have been eroded. Quantity of water, aeration and nutrients transport have been increased and hence enhanced the activities of the bacteria in the sediment.

Figure 3 shows the degradation of crude oil by Nap C in different type of sediments. It clearly revealed that, the maximum growth of the cells (Log phase) occurred during the maximum degradation of the crude oil. Disappearance of the hydrocarbons were due to the process of assimilation by the strain Nap C. Microbe was believed to utilize hydrocarbons as carbon sources for reproduction. Experiment indicated that, every single gram of the hydrocarbon utilized by the cells during optimum condition (silt type sediment, 30°C, 30 g/Liter and pH 8.0),  $5.05 \times 10^{-3}$  grams of Nap C will be produced.

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**FIGURE 1. Degradation of Crude Oil by Nap C in Different Types of Sediment.** The experiment was conducted at pH 8.0, 30°C and at salinity of 30 g/liter. The initial concentration of crude oil was 1500 mg/kg.



**FIGURE 2. Maximum Degradation Rate of Malaysian Tapis A Crude Oil in Different Types of Sediment.**

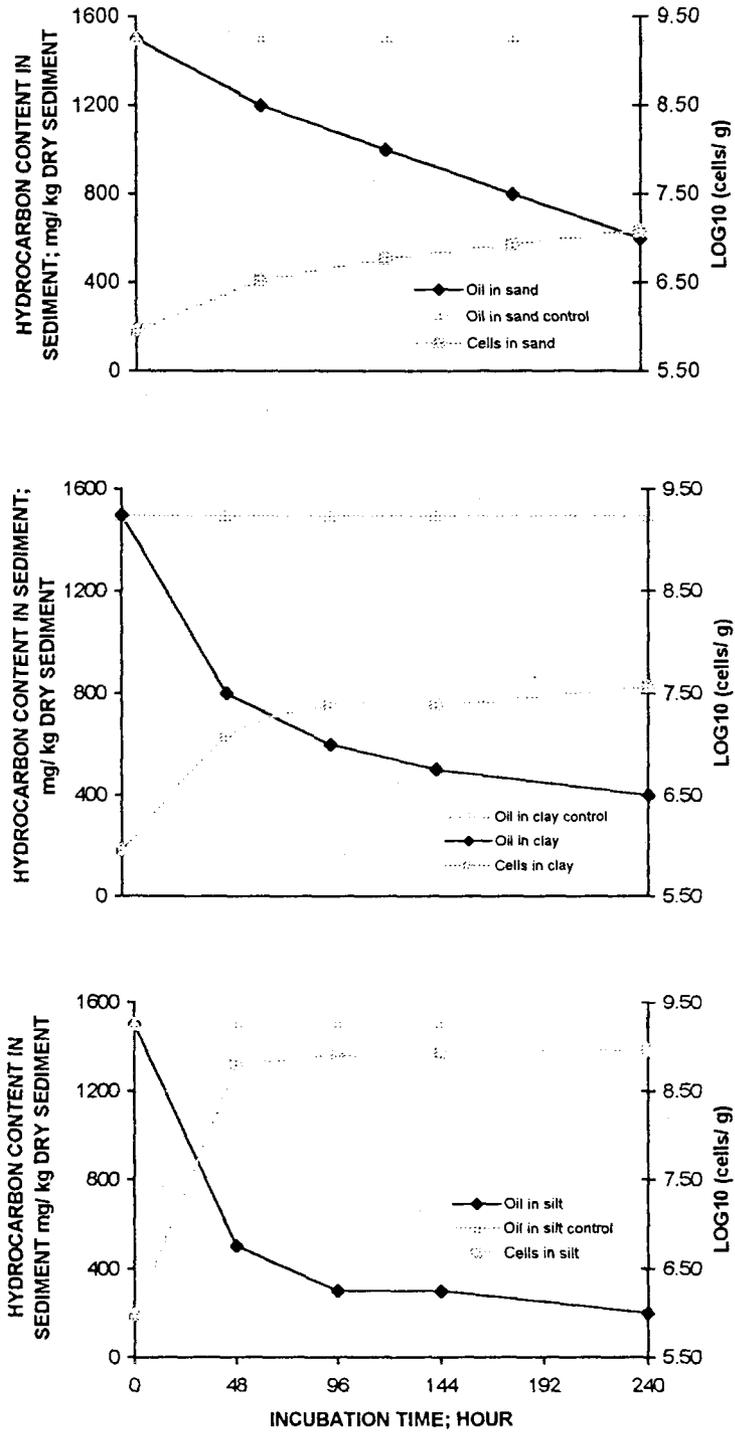


FIGURE 3. Degradation of Malaysian Tapis A Crude Oil by Nap C in Different Types of Marine Sediment. Condition of incubation was the same as Fig. 1.

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