PROCESS PARAMETERS OPTIMIZATION FOR BIOREMEDIATION OF CRUDE OIL IN CONTAMINATED COASTAL SEDIMENTS

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LIST OF ABREVIATIONS

$(NH_4)_2SO_4$	Ammonium Sulfate
ANOVA	Analysis of Variance
C.I.	confidence interval
$C_{12}H_{26}$	n-Dodecane
$C_{14}H_{30}$	n-Tetradecane
$C_{16}H_{34}$	n-Hexadecane
$C_{18}H_{38}$	n-Octadecane
$C_{20}H_{42}$	n-Eicosane
$C_{22}H_{46}$	n-Docosane
$C_{24}H_{50}$	n-Tetracosane
$C_{26}H_{54}$	n-Hexacosane
$C_{28}H_{58}$	n-Octacosane
$C_{30}H_{62}$	n-Triacontane
$C_{32}H_{66}$	n-Dotriacontane
$C_{34}H_{70}$	n-Tetratriacontane
$CaCl_2$	Calcium chloride
CCD	Central composite design
CCFD	Central composite face-centered design
CH_2Cl_2	Dichloromethane
CV	Coefficient of Variation
DOE	Design of Experiment

ESI	Environmental Sensitivity Index
$FeCl_3$	Ferric chloride
H_2SO_4	Sulfuric acid
HABs	Harmful algal blooms
HCl	Hydrochloric acid
HEM	hexane extractable material
HNO_3	Nitric acid
K_2HPO_4	Dipotassium hydrogen phosphate
KH_2PO_4	Potassium di hydrogen phosphate
$MgSO_4$	Manganese sulfate
Na_2HPO_4	Di potassium hydrogen phosphate
Na_2SO_4	Glauber's salt
NaOH	Sodium hydroxide
NH_4Cl	Ammonium chloride
NH_4NO_3	Ammonium nitrate
NOAA	National Oceanic and Atmospheric Administration
PRESS	Predicted Residual Sum of Squares
R^2_{Adj}	Adjusted R-Squared
R^2_{Pre}	Predicted R-Squared
RSM	Response surface methodology
SS	sum of squares
StD	Standard deviation
TPH	Total Petroleum Hydrocarbon
WCO	Weathered Crude Oil

LIST OF SYMBOLS

α	Alpha (axial distance from the center point which makes the design rotatable)	(-)
β_0	Constant coefficient	(-)
β_i	Coefficients for the linear effect	(-)
β_{ii}	Coefficients for the quadratic effect	(-)
β_{ij}	Coefficients for the cross-product effect	(-)
X_i, X_j	Variables corresponding to factors (independent variables)	mg/l
Y	Response calculated by model (dependent variables)	mg/l
mg	Milligram	mg
µg	Micro gram	µg
ppm	parts per million	
µm	Micrometer	
Mt	Million tons	

PROSES PENGOPTIMUMAN PARAMETER TERHADAP BIOREMEDIASI MINYAK MENTAH DALAM ENDAPAN PANTAI YANG TERCEMAR

ABSTRAK

Minyak mentah boleh mendatangkan pelbagai risiko apabila dilepaskan ke persekitaran. Bioremediasi kandungan hidrokarbon adalah proses yang sangat penting dalam pembersihan alam sekitar. Salah satu ruang pengetahuan yang belum terjawab sepenuhnya adalah kepekatan minyak mentah terdedah cuaca (WCO) di endapan pantai yang sesuai dibersihkan secara bioremediasi. Biorosotan tiga kepekatan minyak mentah kering sintetik diuji dalam penyelidikan ini pada skala makmal. Atenuasi semulajadi, biostimulasi dan bioaugmentasi ditentu dan dibandingkan pada 3, 30 dan 60 g minyak per kg endapan kering. Sampel diambil dari saliran di Butterworth dengan menggunakan mikroorganisma semulajadi yang telah disesuaikan. Hidrokarbon Petroleum Jumlah (TPHs) diukur secara gravimetrik pada hari 0, 7, 14, 28, 50, 70 dan 90. Penyingkiran tertinggi sebanyak 73.8% berjaya dicapai melalui bioaugmentasi pada kepekatan minyak 30 g/kg tanah kering, manakala 15.5% penyingkiran TPHs pada 90 hari dicatatkan oleh proses atenuasi semulajadi. Penyingkiran secara atenuasi semulajadi agak perlahan pada ketiga-tiga kepekatan minyak, walau bagaimanapun, penambahan nutrien meningkatkan kadar penyingkiran berbanding reaktor tak-distimulasi. Bioremediasi selama 70 hari menunjukkan pencapaian maksimum di mana tiada penurunan yang ketara diperhatikan sehingga hari ke 90. Pada bahagian kedua, TPHs dan n-alkana terpilih diukur pada 0, 6, 14, 25, 40 dan 60 hari selepas percampurann minyak mentah terdedah cuaca (WCO) bersama endapan pantai, meggunakan kaedah gravimetrik dan kromatografi gas. Eksperimen direka bentuk menggunakan Kaedah Tindakbalas Permukaan (RSM) dan Reka bentuk Tengah Komposit (CCD). Kepekatan awal minyak, mikroorganisma, nitrogen dan kepekatan fosforus digunakan sebagai pemboleh ubah tak berhubung (faktor). Penyingkiran TPHs dan n-alkana turut dikaji secara berasingan sebagai pemboleh ubah berhubung (tindakbalas). Model polinomial kuadratik turut dijana bagi setiap ujian. Kecukupan model disahkan menggunakan analisis varian (ANOVA) dan analisis baki. Kebarangkalian ($p<0.0001$) yang diperolehi menunjukkan terdapat perubahan yang

ketara terhadap model regresi. Pengoptimuman berangka berdasarkan fungsi kemahuan dijalankan pada kepekatan awal minyak 2, 16, dan 30 g/kg per endapan dan penyingkiran sebanyak 83.1, 78.1 dan 69.9 % berjaya dicapai berbanding masing-masing 77.1, 74.2 dan 69.9%. penyingkiran tanpa proses pengoptimuman. Untuk n-alkana, sebanyak masing-masing 97.1, 93,1 dan 90.2 % penyingkiran dicatatkan selepas pengoptimuman berbanding 95.15, 93.87 dan 89.05% penyingkiran tanpa proses pengoptimuman. Oleh itu, RSM boleh disyorkan sebagai kaedah yang boleh dipercayai untuk pengoptimuman parameter proses bioremediasi minyak mentah.

PROCESS PARAMETERS OPTIMIZATION FOR BIOREMEDIATION OF CRUDE OIL IN CONTAMINATED COASTAL SEDIMENTS

ABSTRACT

Crude oil causes a variety of risks when released into the environment. Biodegradation of hydrocarbon compounds is one of the most important processes for clean-up of the environment. One of the current knowledge gaps is the suitable concentration of weathered crude oil (WCO) in coastal sediment for selection of bioremediation as a clean-up option. In the first layout of this study, biodegradation of three different concentrations of synthetic weathered crude oil was investigated at laboratory scale. Natural attenuation, biostimulation and bioaugmentation were determined and compared at 3, 30, 60 g oil per kg dry sediment. Samples were collected from the Butterworth channel and acclimatized indigenous microorganisms were used. Total petroleum hydrocarbons (TPHs) were measured by gravimetric method on days 0, 7, 14, 28, 50, 70 and 90. Highest removal of 73.8 % was observed in bioaugmentation experiments for oil concentration of 30 g/kg dry soil while natural attenuation process removed 15.5 % of TPHs in 90 days. Removal by natural attenuation was slow; however, addition of nutrient increased its removal compared to non-stimulated reactors in all three oil concentrations. Bioremediation during 70 days reached the maximum value and no significant further decrease was observed up to 90 days. In the second part, TPHs and selected n-alkanes were measured at 0, 6, 14, 25, 40 and 60 days after introducing WCO to coastal sediment samples by gravimetric method and gas chromatography. Experiments were designed by response surface methodology (RSM) and central composite design (CCD). Initial oil concentration, microorganism, nitrogen and phosphorus concentrations were used as independent variables (factors). TPHs removal and n-alkanes removal were investigated separately as dependent variables (response). For each test a quadratic polynomial model was generated. The adequacies of models were confirmed by using analysis of variance (ANOVA) and residual analysis. The probability ($p<0.0001$) obtained showed significance of the regression models. Numerical optimization based on desirability function was carried out for initial oil concentration of 2, 16 and 30 g per kg sediment and for TPHs, 83.1, 78.1 and 69.9 %

removal were observed respectively, compared to 77.1, 74.2 and 69.9 % removal for un-optimized conditions. For n-alkanes 97.4, 93.1 and 90.2 % removal were observed respectively, compared to 95.15%, 93.87 and 89.05 % removal for un-optimized results. Thus, RSM can be recommended as a reliable tool for optimization of process parameters in crude oil bioremediation.

CHAPTER 1

INTRODUCTION

1.1 Marine pollution

Contamination of soils, sediments, groundwater, surface water, and air with toxic and hazardous chemicals is one of the major problems facing the industrialized world. Marine pollution has been defined as the introduction by man, indirectly or directly, of energy or substances into the marine environment, resulting in such effects as harmful to human health, hazards to living resources, difficulty to marine activities including fisheries, harm of quality for use of seawater (pollution affects the chemistry of water), and reduction of facilities (Clark, 2001). The pollutants, including toxic chemicals, can alter the conductivity, temperature, availability of oxygen in the water body and acidity of water. Marine life declines due to water pollution and start lethal killing of aquatic plants and fish in oceans, seas and rivers. The fauna and flora of rivers, sea and oceans is affected by water pollution harmfully. A marine system is a complex multimedia environment that can be divided into three main parts, namely water, air, and sediments. These bulk parts contain sub-sections such as suspended solids and biota in the water column, and solids and water in the sediment (Nazir et al., 2008).

1.2 Hydrocarbon in the marine environment

Annually, 1,300,000 tonnes of petroleum goes to the sea, around 53% of which comes from human activities such as oil extraction, transportation and consumption, and the remaining 47% is caused by natural seepage. Oil spills have a wide variety of impacts on the environment, in particular on marine ecosystems. Impacts can be either short or long term (Crain et al., 2009; Toledo et al., 2006; Mrayyan and Battikhi, 2005; AECIPE, 2002; Harris et al., 2001; Ziel et al., 1996).

Long-term effects usually require from 2 - 10 years for recovery (Kingston, 2002). Immediate effects are widespread and impact significantly on marine ecosystems. A variety of marine life is affected, including benthic invertebrate, mammals, birds, plankton and fish. Moreover, oil spills also cause negative impacts on mangroves, salt marshes, coral reefs as well as rocky and sedimentary shores. The economic effects of oil spills can be severe with marine culture, fishery, tourism and many other coastal activities being affected (Dao, 2007).

Seventy percent of the Earth's surface is covered by oceans and seas and more than half of the world's population lives within 60 km of the coast (Cook, 1984). The marine environment has complex relations between plant and animal species and their physical environment. The physical environment damage will often lead to harm for one or more species in a food chain, which may lead to damage for other species further up the chain. Whether an organism spends most of its time in open water, near coastal areas, or on the shoreline will determine the effects on oil spill are possible to have that organism (Clark, 2001).

Marine sediments, especially coastal industrial areas, are frequently polluted with petroleum hydrocarbons as the result of accidental oil spills, shipping activities, and urban and industrial runoff (Head and Swannell, 1999). The release of hydrocarbons into the environment, whether accidental or due to human activities, is the main cause of water and soil pollution and increases the risk of groundwater pollution. Many of these components are toxic, mutagenic and carcinogenic and clean-up can be very expensive (Kaiser and Pulsipher, 2006).

Marine oil spills have brought attention, generating intense pressure on the parties responsible for the spill, backed by legislation, for a prompt and effective response. Approximately, the total amount of oil released into the sea from catastrophic accidents is the same as that introduced by natural oil seeps. Furthermore, it is far less than that released from municipal sources and general transportation. The oil industry and consumers are working to minimize catastrophic releases and it is unlikely that oil spills can be completely prevented. There is thus a continuing need for environmentally-responsible cost effective tools for responding to oil spills (Prince, 1993).

The increasing number of marine oil spills requests for effective solutions for the environment. Remediation of these sites needs further development of technologies that emphasize the destruction of the pollutants rather than the conventional approach of disposal.

1.3 Problem statement

Coastal areas are directly subjected to anthropogenic impacts generally derived from industrial and urban activities. Petroleum causes a range of environmental risks when released into the environment. Hydrocarbons present in the marine environment can originate from natural sources such as forest fires, natural petroleum seeps and post-depositional transformations of biogenic originators, a large proportion can be attributed to human activities. Urban wastes, sewage disposal, industrial runoff, oil production and transportation are some of the most important sources of anthropogenic hydrocarbons (Ebuehi et al., 2005; Kennish, 2001). When they enter the marine environment, oil hydrocarbons tend to adsorb on to particulate material and deposit in the sediments, where they can accumulate to high concentrations and persist for many years, mostly under anoxic conditions.

Petroleum is one of the most world-wide spread contaminants and its components can produce harmful effects at different levels of biological organization. Also, petroleum introduction causes changes to the structure of benthic communities (Venturini et al., 2008). Sediments provide a temporally integrated indication of ecosystem condition and support a wide range of flora and fauna, which are important components of the aquatic food chain. Additionally, sediments are strongly influenced by anthropogenic activities, which can lead to the accumulation of trace metals and organic contaminants, and might represent an environmental risk due to toxicity (Barros et al., 2008). Hydrocarbons are highly toxic to plants and to living microorganisms and invertebrates, and constitute a potential risk to health, which increases as hydrocarbon resistance to degradation increases (Labud et al., 2007).

Coastal area of Malaysia is extremely sensitive to oil spill hazards. Therefore effective means for shoreline cleanup and a sacrificial area are the hard tasks in any contingency plan for oil spill response. In view of the fact that, coasts of Malaysia are important for fisheries, recreational and marine activities, tourism, and maintaining the biodiversity in the tropical area and there is no publish data in literature on application of bioremediation in Malaysia; this research focuses on oil bioremediation and effects of its factors to recommend a reasonable method for control, prevention and cleanup of oil spills pollution on Malaysian coastal areas.

Bioremediation techniques have become a major mechanism for removing oil residues on affected shorelines. Among the different techniques to enhance natural biodegradation by indigenous microorganisms, seeding and fertilizing the indigenous populations have attracted the most interest (Zhu et al., 2001). Hence, for successful bioremediation, basic environmental condition data is the most necessary factor. One of the current knowledge gaps is the suitable concentration of weathered crude oil in coastal sediment for selection of bioremediation as a clean up option. High oil concentrations are toxic and inhibitory and very low concentrations may be ineffectually utilized. Therefore, there is an optimum hydrocarbon concentration range for bioremediation applications (Zhu et al., 2001).

1.4 Research objectives

The overall aim of the present study is to investigate the effect of process parameters on bioremediation of weathered crude oil in coastal sediments. It specified in following detailed objectives:

(i) To investigate the feasibility of crude oil bioremediation and simulate oil spill bioremediation at laboratory scale.

(ii) To compare natural attenuation, biostimulation and bioaugmentation in bioreactors for cleanup of coastal sediment samples.

(iii) To identify the best possible duration and initial oil amount for weathered crude oil biodegradation.

(iv) To investigate optimize the important variables (initial oil amount, microorganism availability, and nitrogen and phosphorus concentrations) by using response surface methodology for bioremediation.

(v) To monitor biodegradation rate of crude oil (Total petroleum hydrocarbon and n-alkanes) during the experiments.

1.5 Scope of study

Oil release into the environment is a well-recognized problem in today's world. Oil spills affect many species of plants and animals in the environment, as well as humans. The search for effective and efficient methods of oil removal from contaminated sites has increased in recent years. One promising method that has been researched is the biological degradation of oil by bacteria. The scope of this study is bioremediation of spilled oil with consideration on the feasibility of bioremediation technology in the Malaysian environment for oil spill response. Even though several works have also been published on crude oil bioremediation, the literature is still inconclusive regarding optimization of oil amount, inoculums and nutrient concentration for weathered crude oil.

This study has two parts; in the first part petroleum hydrocarbons bioremediation was carried out in bioreactors at laboratory scale allowing evaluation of the effectiveness of natural attenuation, biostimulation and bioaugmentation. Comparison of bioaugmentation and biostimulation is not addressed well for crude oil in coastal sediments, on the other hand appropriate amount of oil, nutrient and bacteria strain is a big challenges for environmental engineers. Consequently optimization of additional parameters can fill the gap of information for bioremediation of weathered crude oil in coastal sediments. Total petroleum hydrocarbons were measured at 1, 7, 14, 28, 50, 70 and 90 days after introduction of nutrients and bacteria to monitor the bioremediation process. The study included bioremediation of weathered crude oil by the application of the central composite design and response surface methodology (RSM) to optimize the amount of oil, biomass and nutrient concentration. Response surface analysis was also used to evaluate the influence of oil, nutrient and bacteria concentration on the bioremediation of oil (the optimum operating conditions for the system) in reactors during 60 days.

1.6 Thesis organization

This section presents an overview of how each chapter contributes towards meeting the research objectives. The dissertation is organized as follows:

Chapter 1 Introduction; is a brief note on marine pollution and how hydrocarbons contaminate marine environment. It also presents the problem statements, objectives, scope of study and thesis organization. Chapter 2 Literature Review; reviews crude oil, petroleum hydrocarbon and hydrocarbon chemistry, fate and effect of oil in the marine environment and effects of oil on marine sensitive areas. The reviewer includes discussions of recent oil spills and disasters in the world and especially in the Malaysia, methods of oil spill clean up, bioremediation and its methods, factors affecting bioremediation, oil and hydrocarbon bioremediation studies, process modeling and optimization, design of experiments (DOE), Central Composite Design (CCD), and determination of optimal operating conditions for bioremediation of weathered oil. Chapter 3 Materials and Methods; explains sampling and analytical methods used for the parametric studies including chemical analyses for hydrocarbons and nutrients, design of the experiments, trials set up for each step. Chapter 4 Results and Discussion; presents results from parametric study, presentation and analysis of the bioremediation study. Conclusions and recommendations from this research are presented in end.

CHAPTER 2

LITERATURE REVIEW

2.1 Crude Oil and Petroleum Hydrocarbons

Oil is a nonrenewable resource that takes millions of years to create. Petroleum, the general name for all carbon byproducts like oil and natural gas is formed from organic materials. Oil was formed millions of years ago when tiny plants and animals died and sank to the bottom of a shallow body of water. Then, sand, clay and silt (lots of tiny pieces of rock) covered the thin layer of dead plants and animals. Over time these organic residues are changed by pressure and heat into petroleum, moving up, sometimes over widespread areas, either to reach the surface or be occasionally trapped in what are to become oil basins. The important point here is that only a small percentage of the oil produced in the rocks is trapped; most of it has found its way to the surface. Oil has been part of the natural environment for millions of years (Kingston, 2002). A variety of petroleum products are then derived from this natural resource. Because their compositions differ, each type of crude oil or petroleum product has unique characteristics or properties (Laws, 2000). Usually oils are explained with physical properties and chemical composition. Although very complex in structure, oils can be broken down into four basic classes: alkanes, naphthenes, aromatics and alkenes. Each class is distinguished on the basis of molecular composition (NOAA, 2008).

The term total petroleum hydrocarbon (TPH) describes a large family of several hundred chemical compounds that originally came from crude oil. Analysis of TPH in soil and groundwater samples measures the total concentration of all petroleum related hydrocarbons and the results explain in terms of the concentration of hydrocarbon compounds within various carbon ranges. Determination of the total amount of TPH is useful because there are so many different chemicals in crude oil and in other petroleum products that it is not practical to measure each one separately. Therefore the total petroleum hydrocarbon parameters indicate for hydrocarbon contamination, as it refers to the concentration of a complex mixture of compounds which do not display toxicological or fate and transport properties. Toxicological information of TPH compounds is available on only a very few.

2.2 Hydrocarbon Chemistry

Petroleum is a complex mixture of organic compounds and trace elements formed from a variety of organic materials that are chemically changed under different geological conditions over long periods of time. Crude oils contain primarily carbon and hydrogen (which form a wide range of hydrocarbons from light gases to heavy residues), but also contain smaller amounts of oxygen, sulfur, and nitrogen as well as metals such as vanadium, nickel and iron. The infinitely variable nature of these factors results in distinct chemical differences between oils (Wang et al., 1998). All crude oils and petroleum products, to some extent, have chemical compositions that differ from each other. This variability in chemical compositions results in unique chemical ‘fingerprints’ for each oil and provides a basis for identifying the source(s) of the spilled oil (Snape et al., 2005).

Alkanes (linear or branched), cycloalkanes, aromatic hydrocarbons, or more complicated chemicals like asphaltenes are the most commonly found hydrocarbons in petroleum. The alkanes (paraffins) are saturated hydrocarbons with straight and branched chains which contain only carbon and hydrogen and have the general formula C_nH_{2n+2}. They generally have from 5 to 40 carbon atoms per molecule; although in the mixture may be present small amounts of shorter or longer molecules. The alkanes from pentane (C_5H_{12}) to octane (C_8H_{18}) are classify as gasoline (petrol), the ones from nonane (C_9H_{20}) to hexadecane ($C_{16}H_{34}$) as diesel fuel and kerosene (primary component of many types of jet fuel), and the ones from hexadecane are defined as fuel oil and lubricating oil. Paraffin wax, at the heavier end of the range, is an alkane with approximately 25 carbon atoms, while asphalt has 35 and up, although these are usually cracked by contemporary refineries into more valuable products. The shortest molecules with four or fewer carbon atoms are found in a gaseous state at room temperature (petroleum gases).

The cycloalkanes or naphthenes are saturated hydrocarbons with one or more carbon rings to which hydrogen atoms are attached according to the formula C_nH_{2n}. Cycloalkanes have similar properties to alkanes, although they have higher boiling points. The aromatic hydrocarbons are unsaturated hydrocarbons, with one or more benzene rings and general formula C_nH_n. These hydrocarbons have a sweet aroma and some of them are carcinogenic (Krebs, 2006).

Oils typically are described in terms of their physical properties (density, pour point and so on) and chemical composition (such as percent composition of various petroleum hydrocarbons, asphaltenes, and sulfur).

2.3 Fate and effect of oil in the marine environment

The marine environment is subject to contamination by organic pollutants from a variety of sources. Human activities, economic development and industrial advancement have activated general pollution of the natural global environment (Ang et al., 2005). Environmental pollution of aquatic ecosystems caused by crude oil is still among the serious problems in the world. Sources of oil pollution in marine environments are shown in Table 2.1.

Oil hydrocarbon extraction can result in releases of both crude oil and refined products as a result of human activities associated with efforts to explore and produce oil. The main environmental concern with crude oil is that, if not handled carefully, it may cause significant hazards to human health and the earth ecology during all stages of production, processing and consumption. The major causes of environmental damage have been noted to be due to accidental spillages and sometimes discharge of petroleum or oily waste to water or land, through blow-outs from pipes and pumps, pipeline corrosion and spillages during transportation. Crude oil spills or discharges into water systems will often eventually end up on land. The best solution of course is to prevent spills or discharges, but this is not always possible (Urum et al., 2006).

Table 2.1 Sources of oil pollution in Marine Environments (Kennish, 2001; Laws, 2000)

Source	Oil Industry	Other	Total
Transportation	0.143		
Tanker accidents	0.110		
Dry docking	0.004		
Other shipping operations		0.229	
Other shipping accidents		0.018	
			0.504
Fixed installations			
Offshore oil production	0.045		
Coastal oil refineries	0.091		
Thermal loading	0.027		
			0.163
Other sources			
Industrial waste		0.181	
Municipal waste		0.635	
Urban runoff		0.109	
River runoff		0.036	
Atmospheric fallout		0.272	
Ocean dumping		0.018	
			1.251
Natural inputs		0.227	0.227
Biosynthesis			
Marine phytoplankton		23.582	
Atmospheric fallout		91-3.630	

Values in million metric tons per year

Oil spills can have very wide ranging impacts on the marine environment and human activities, reducing the scope for entertaining activities and tourism at sea or along the coast, harming fish farms and sea fisheries, and limiting the use of sea water for industrial processes. Oil spills also have many other serious impacts on marine ecosystems (Kingston, 2002).

In general, refined petroleum products are more toxic to organisms but less persistent in the environment. Crude oils and heavy fuel oils like bunker fuels are less toxic but more persistent. These compounds have physical impacts on wildlife (for example, coating feather, fur and skin of animals). Petroleum hydrocarbons integrated into sediments are not only accumulated within organisms and transported through the food chains but also show long-term toxicity. This long-term toxicity is from the persistence of low to medium molecular weight hydrocarbons (Barron et al., 1999; Miller and Mudge, 1997).

Crude oil and petroleum hydrocarbons are the most widespread soil pollutants. They are washed into the marine environment and adhere tightly to sediments. Since sediment resident invertebrates (fauna) are an important food source for many other species, the extent to which they accumulate, metabolize, and affect distribution and microbial degradation of contaminants in the sediment is critical not only for their own exposure but also for the circulation of contaminants along food chains (Kvenvolden and Cooper, 2003; Murthy et al., 2008). Commercial fish and crustacean species are important predators on fauna, and sediment processes and are therefore important for human health (Granberg and Selck, 2007). The degree of impact depends on many factors including: volume and type of oil spilt, seasons and weather conditions, physical characteristics of the affected area.

Small amounts of oil can persist for decades on some shorelines; 10- 20 and even 30 years old oil remains on coarse-sediment beaches have been known for some spills. For most spills, the bulk of the stranded oil is removed within months or a few years due to natural removal and weathering processes. Oil stranded on shorelines plays a critical role in biological and physical dynamics of the coastal environment (Owens et al., 2008).

Large amount of crude oil is released as seeps into the sea, however, these seeps most often occur in specific oil production areas, and release rates are relatively low and chronic. When crude oil enters the ocean from the seabed, the soluble compounds dissolve and the volatile compounds volatilize. This means that the toxicity associated with the low weight aromatics and the saturated hydrocarbons is lost early during their evaporation and dissolution (Ryder et al., 2004). But much of the oil rises to the surface, forming slicks. For most crude oils, about one-third is lost by evaporation (volatilization) in the first 24 hours. Low weight compounds can degrade by photooxidation and microbial processes over periods of weeks, and the residues form tar-balls. Where seeps occur close to shore, tar forms persistent deposits on the shoreline. In the coastal region, waters in areas dominated by seeps are possibly enriched in dissolved petroleum hydrocarbon, and then volatilization of petroleum hydrocarbon occurs (Kennish, 2001).

2.3.1 Oil weathering process

After oil is discharged into the environment, physical, chemical, and biological processes begin to transform the discharged oil. These processes are referred to as weathering and change the composition, behavior, exposure route, and toxicity of the discharged oil. Weathering processes include evaporation of volatiles, water-in-oil (emulsification), spreading and natural dispersion, dissolution into the water column, sedimentation, oxidation and biodegradation.

The weathering or fate of spilled oil depends on the oil properties and on environmental conditions. It is important to recognize the dynamic nature of spilled oil and the fact that the properties of spilled oil can change over time (Wang et al., 1998). During a response operation, it is important to monitor the continuous changes in the properties of the spilled oil, as response strategies may have to be modified.

Weathered oil is composed of relatively insoluble compounds and often combines into mats or tar balls. As a result, the potential for exposure to marine organisms through water column toxicity is lessened, as is the potential for birds or mammals to encounter the oil. Alternatively, certain species are known to tar balls and the potential for exposure of those species may increase as the oil weathers. Also, the loss of the lighter fractions through dissolution and/or evaporation during the weathering process can cause normally floating oil to sink, by contaminating subtidal sediment and contributing to water column toxicity.

2.4 Recent Oil Spills

Generally, oil spills are categorized by size (< 7 tonnes, 7-700 tonnes and > 700 tonnes) although the actual amount of oil spill is also recorded. About 10,000 incidents are recorded, most of which (84%) fall into the smallest category < 7 tonnes. Releasing large amount of oil is responsible for a high percentage of the oil spill. For example, in the ten-year period 1990-1999 there were 358 spills over 7 tonnes, totaling 1,138 thousand tonnes, but 830 thousand tonnes (73%) were released in just 10 incidents (just under 3%) (ITOPF, 2007). Table 2.2 shows recent oil spills and disasters in the world (Infoplease, 2009).

Table 2.2 Recent oil spills and disasters

Date	Area	Disasters
1990		
June 8	off Galveston, Tex.	Mega Borg released 5.1 million gallons of oil some 60 nautical miles south-southeast of Galveston
1991		
Jan. 23–27	southern Kuwait	During the Persian Gulf War, Iraq deliberately released 240–460 million gallons of crude oil into the Persian Gulf from tankers 10 mi off Kuwait.
Apr.11	Genoa, Italy	Haven spilled 42 million gallons of oil in Genoa port.
		May 28, Angola: ABT Summer exploded and leaked 15–78 million gallons of oil off the coast of Angola. It's not clear how much sank or burned.
1992		
Mar. 2	Fergana Valley, Uzbekistan	88 million gallons of oil spilled from an oil well.
1993		
Aug. 10	Tampa Bay, Fla.	Three ships collided, the barge Bouchard B155, the freighter Balsa 37, and the barge Ocean 255. The Bouchard spilled an estimated 336,000 gallons of No. 6 fuel oil into Tampa Bay.
1994		
Sept. 8	Russia	Dam built to contain oil burst and spilled oil into Kolva River tributary. U.S. Energy Department estimated spill at 2 million barrels. Russian state-owned oil company claimed spill was only 102,000 barrels.
1996		
Feb. 15	off Welsh coast	Supertanker Sea Empress ran aground at port of Milford Haven, Wales, spewed out 70,000 tons of crude oil, and created a 25 mile slick.
1999		
Dec. 12	French Atlantic coast	Maltese-registered tanker Erika broke apart and sank off Britanny, spilling 3 million gallons of heavy oil into the sea.
2000 Jan. 18	off Rio de Janeiro	Ruptured pipeline owned by government oil company, Petrobras, spewed 343,200 gallons of heavy oil into Guanabara Bay.
Nov. 28	Mississippi River south of New Orleans	Oil tanker Westchester lost power and ran aground near Port Sulphur, La., dumping 567,000 gallons of crude oil into lower Mississippi. Spill was largest in U.S. waters since Exxon Valdez disaster in March 1989.
2002		
Nov. 13	Spain	Prestige suffered a damaged hull and was drown to sea and sank. Much of the 20 million gallons of oil remains underwater.

2003		
July 28	Pakistan	The Tasman Spirit, a tanker, ran aground near the Karachi port, and eventually cracked into two pieces. One of its four oil tanks burst open, leaking 28,000 tons of crude oil into the sea.
2004		
Dec. 7	Unalaska, Aleutian Islands, Alaska	337,000 gallons of oil were released, most of which was driven onto the shoreline of Makushin and Skan Bays.
2005		
Aug.- Sept.	New Orleans, Louisiana	more than 7 million gallons of oil were spilled during Hurricane Katrina from various sources, including pipelines, storage tanks and industrial plants.
2006		
June 19	Calcasieu River, Louisiana	An estimated 71,000 barrels of waste oil were released from a tank at the CITGO Refinery on the Calcasieu River during a violent rain storm.
July 15	Beirut, Lebanon	The Israeli navy bombs the Jieh coast power station, and between three million and ten million gallons of oil leaks into the sea, affecting nearly 100 miles of coastline. A coastal blockade, a result of the war, greatly hampers outside clean-up efforts.
August 11th,	Guimaras island, The Philippines	A tanker carrying 530,000 gallons of oil sinks off the coast of the Philippines, putting the country's fishing and tourism industries at great risk. The ship sinks in deep water, making it virtually unrecoverable, and it continues to emit oil into the ocean as other nations are called in to assist in the massive clean-up effort.
2007		
Dec. 7	South Korea	Oil spill causes environmental disaster, destroying beaches, coating birds and oysters with oil, and driving away tourists with its stench. The Hebei Spirit collides with a steel wire connecting a tug boat and barge five miles off South Korea's west coast, spilling 2.8 million gallons of crude oil. Seven thousand people are trying to clean up 12 miles of oil-coated coast.
2008		
July 25	New Orleans, Louisiana	A 61-foot barge, carrying 419,000 gallons of heavy fuel, collides with a 600-foot tanker ship in the Mississippi River near New Orleans. Hundreds of thousands of gallons of fuel leak from the barge, causing a halt to all river traffic while cleanup efforts commence to limit the environmental fallout on local wildlife.

Several world regions where most oil spills accident occurred, both in terms of spill numbers and volumes, include the Northern European Atlantic and the Eastern Mediterranean; the Gulf of Mexico, the Caribbean and parts of the Southern Atlantic; the Persian Gulf and Arabian Sea; the South China Sea, the Gulf of Thailand and the Strait of Malacca, and around the Southern tip of Africa. Extremely large spills (≥ 100000 tonnes) also occurred mainly in these regions, with release of the Odyssey spill off Nova Scotia (Canada) in 1988 (Burgherr and Hirschberg, 2008).

2.4.1 Oil spill in Malaysia

Regarding the South East Asia Sea's resources, attention has been to hydrocarbons in general, and on oil in particular. Oil deposits can be found in most of these countries. It is estimated that these countries reserve about 7.0 billion barrels oil per day and produce 2.5 million barrels oil per day. Production of oil in the South East Asia has increased in the past few years and Malaysian production accounts for approximately one-half of the region's total (Clough, 2008). Some recent oil spills incidences in Malaysia include:

(a) **September 2, 2000 -** Oil spill from a sunken Chinese cargo ship at Tanjung Po anchorage point at the Sarawak River mouth. The ill-fated 5,000 ton Kingston registered vessel Double Brave was loaded with about 116 ton of diesel oil when it sank after a collision with a barge being towed by a tugboat. About 60 workers from the Marine Department, Department of Environment, and the Kuching Port Authority helped in the clean-up operation.

(b) May 28, 2001 – An oil tanker with some 67 ton of fuel, including diesel and 1,500 ton of bitumen, sunk after it was crashed from behind by a super tanker about 7.5 nautical miles off Pulau Undan, near Malacca. Officials said the crash caused MT Singapore Timor to take in water, and remained half-submerged in the sea floating southwards. Diesel and bitumen started to spill into the sea, and spreaded to about one nautical mile from the collision spot.

(c) June 13, 2001 -An Indonesian tanker laden with a toxic chemical capsized off Malaysia's southern Johor state, just across from Singapore. The 533 ton MV Endah Lestari was on its way to East Kalimantan in Indonesia with some 600 ton of the poisonous industrial chemical phenol, and 18 ton of diesel. Newspaper reports said the toxic spill had killed thousands of fish and cockles reared in 85 offshore cages, and Singapore authorities also warned its citizens to stay away from nearby waters. Officials said it would be tough to mop up the phenol, as it is soluble in water (ITOPF, 2007).

2.4.2 Penang region and pollution

The ocean plays an important role in energy cycling; it supplies energy, minerals and other natural resources, and habitat for sustaining living resources, and provides a medium for recreation, learning and explanation. Near shore ecosystems are supported by the ocean and the interrelationship between oceanic and land systems. This interrelationship can affect the income and growth potential of many economic regions, including natural resource harvesting (minerals and oil), commercial and entertaining fishing, tourism, real estate, manufacturing, and waste assimilation.

For communities and businesses around the country, clean water can mean the difference between economic decline and a clear, successful future. Oil and other chemical contaminants washed or discharged into the ocean may be suspended in the water column, at last settling in sediments and concentrating in marine organisms (NOAA, 2008). Once oil is released into the environment, a wide variety of physical, chemical, and biological processes begin to transform the spilled oil.

Seas are important for Malaysia because of transportation, revenue, source of food and defence. Penang Island has been the main port of Peninsular Malaysia for a long time. Now a days, it is the most important harbor in the northern part of Strait of Malacca. The presence of a large number of factories has made Penang Island and Prai Industrial Zone one of the most polluting sources for the marine environment. Industrial units in Prai Industrial Zone play an important role in aquatic pollution, additionally several oil spills occurred in this area including 2000 and 2001. A large percentage of oil pollution in Penang area occurs during deballasting, oil tank and cargo cleaning, and operation of fishing boats and small vessels. Oil spills also affect fisheries, mariculture, coastal tourism, and crops.

The coastal zone is extremely important, both economically and socially to the state of Penang, which at one time was a major magnetic pull for the tourism industry. The beaches could no more be the delight of Penang, especially with the extensive deterioration it has experienced for the last few years due to human activities. Penang relies on tourism industry that requires unpolluted, well maintained and unspoiled natural beaches where environmental quality is a basic resource.

Seberang Prai is a narrow hinterland of 760 square kilometers across a narrow channel whose smallest width is 4 km (2.5 miles). The body of water between Penang Island and Seberang Prai is the North Channel to the north of George Town and the South Channel to the south of George Town. Prai Industrial Area is part of Penang state and is located on the mainland with various industrial zones. The industrial zone in Prai is associated with heavy industries and wastewater discharge. Thousands of industrial plants including chemical, petrochemical, plastic manufacturing and electronics production and metal industries are active there and generate wastewater containing organic compounds, fertilizer and heavy metals.

The Butterworth ferry port is an important waterway for both passenger and cargo handling. Furthermore oil companies have coastal facilities on the mainland part of Penang province at Butterworth and Prai (Sgouridis, 2003).

2.5 Planning for oil spill on marine sensitive area

Marine shorelines and freshwater environments have an extensive variety of sensitivities to petroleum hydrocarbon and clean-up activities. Malaysian coastal sensitive area account for about 4675 km shoreline with coral reefs, bountiful fishing ground and fish farming, birds' breed and nesting areas, coastline wetlands, turtles' egg-laying regions, mudflats and mangrove swamps (Zaharudin et al., 2006).

In addition the Tourism Industry and the Port & Shipping Industry need to be safe enough and all this is possible just by an integrated oil bioremediation plan for the

Malaysian marine environment. This plan should be before shoreline cleaning starts, it needs to ensure that there is agreement about which shoreline areas are affected and need to be cleaned, the order in which this will occur, and what methods of cleaning will be employed. For each length of shoreline there will also need to be an agreed set of environmental standards, based on that location's sensitivity to both the oil pollution and cleaning methods, which will determine when cleaning operations begin and end.

Fig 2.1 Sight of Butterworth ferry and Penang Island

To classify shoreline types the National Oceanic and Atmospheric Administration (NOAA) and the American Petroleum Institute have developed the Environmental Sensitivity Index (ESI) for spill response. This classification has been used in oil spill contingency planning and spill response operations (Table 2.3) (Zhu et al., 2001). Where 1 is least sensitive and 10 is most sensitive to oil and clean up actions.

Table 2.3 Shoreline ESI ranking for habitats in marine shorelines (Zhu et al., 2001)

Environmental Sensitivity Index (ESI)	Shoreline Type
1	Exposed rocky shores sea walls and piers
2	Exposed wave-cut platforms
3	Fine-grained sand beaches
4	Coarse-grained sand beaches
5	Mixed sand and gravel beaches
6	Gravel beaches and riprap
7	Exposed tidal flats
8	Sheltered rocky shores
9	Sheltered tidal flats
10	Salt marshes and mangroves

Major factors considered in ranking habitat sensitivity include shoreline type (substrate, grain size, and tidal elevation), exposure to wave and tidal energy, biological productivity and sensitivity, and ease of cleanup (Runghen et al., 2003).

Marine environments have the potential to cause serious impact by oil spill in the sea. It is not always possible to prevent pollution incidents such as oil spills. Protection of the marine environment from pollution is a high priority for everyone.

In order to clean up pollution from marine oil spills successfully, an effective response strategy is a requirement. Bioremediation is the least impact remediation alternative that can speed the natural recovery of oil contaminated shorelines by raising the biodegradation rate of harmful crude oil (Wrenn et al., 2006).

2.6 Oil spill clean up

Oil recovery and shoreline cleanup methods of oil spill can be categorize as follows:

(a) Physical and mechanical methods Control or recovery is the primary line of cover against oil spills. Containment and recovery equipment include a variety of booms, barriers, and skimmers, as well as natural and synthetic sorbent materials. Mechanical containment is used to capture and store the spilled oil until it can be disposed of properly. Natural processes such as evaporation, oxidation, and biodegradation can start the cleanup process, but are generally too slow to provide adequate environmental recovery. Physical methods, such as wiping with sorbent materials, pressure washing, and raking and bulldozing can be used to assist these natural processes.

Physical collection of the oil is generally the first priority of responders, but this is neither very easy, nor very effective after a large spill. There is therefore a continuing search for alternative and additional responses. Amongst the most promising are those that aim to stimulate the natural process of oil biodegradation (Prince et al., 2003).

(b) Chemical methods Some chemical methods have been used in conjunction with mechanical means for containing and cleaning up oil pollution. Dispersants and gelling agents can be helpful to keep oil from reaching shorelines and other sensitive habitats in open seas. Some beaches are dedicated for tourism and oil pollution can be highly detrimental to them, so the use dispersant may perhaps be practical. The limiting factors