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# Fates of spilt oil and factors affecting the bio-degradation of oil: a review

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**ABSTRACT**

**FATES OF SPILT OIL AND FACTORS AFFECTING
THE BIO-DEGRADATION OF OIL: A REVIEW**

**by**
**Amogh-Ranganath Chakravarthy**

The usage of crude oil and its products is increasing; in turn, the number of spills is also going up. The inland spills are the more recurrent spills in the U.S today. This review article deals with the weathering of oils, which commences just after oil spills into the environment with various processes such as evaporation, dissolution, photo-oxidation, dispersion, emulsification and bio-degradation along with other possible fates. Bio-degradation is one of the most significant processes of weathering which depends on oil physical and chemical characteristics, environmental characteristics, pH, salinity and oil concentration. There is a range of oil concentration for bio-degradation to occur; otherwise inhibition of microbial activity is observed. The microbial activity is dependent on the type of the microbes present. The interfacial area of oil and water is also an important factor which varies significantly with variation in water saturation. The degree of bio-degradation varies between saturates, aromatics, resins and asphaltenes.

**FATES OF SPILT OIL AND FACTORS AFFECTING**
**THE BIO-DEGRADATION OF OIL: A REVIEW**

**by**
**Amogh-Ranganath Chakravarthy**

**A Thesis**
**Submitted to the Faculty of**
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My master's thesis would not have been possible without the invigoration and succor from my parents, Mr. Ranganath K. Chakravarthy and Mrs. Shashikala Krishnaswamy, which was one of the arduous tasks in my life to endure and bring to fruition. Accordingly, I would like to dedicate this thesis to my parents.

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# CHAPTER 1

# INTRODUCTION

## 1.1 Objective

The objective of this thesis is to review the different weathering processes such as evaporation, dissolution, photo-oxidation, dispersion, emulsion and to focus on oil bio-degradation. The review on biodegradation considers several factors affecting the bio-degradation such as the chemical and physical properties of oil, oil concentration, inhibition of microbial-activity due to too low or too high substrate concentration and other environmental factors.

The goal of this review is to provide insight about the possible fates of the oil after being spilt. The thesis provides information about the factors affecting oil bio-degradation, one of the chief weathering processes which could be the response action for cleanup. It also helps in identifying the suitable cleanup strategy based on the degree of bio-degradation happened.

## 1.2 Background Information

The Fossil fuel products are gaining importance with the broadening of their uses in almost all of the realms of life. The Petroleum products are being used widely all over the world. Thus, the need for the transportation of petroleum and its products, have increased continuously. The increased demand for the petroleum and its products necessitates the transport of crude oil and refined products all over the world. In this regard, crude oil

transport has been drastically increased over the last half of the previous century which in turn, has resulted in an increase in the number of oil spill accidents. These accidents have resulted in major adverse ecological and economic impacts. The accidents have been happening in both the marine and freshwater environments. Since marine oil spills usually occur on a large scale, they have received special attention in research as well as in clean-up processes. Inland oil spills occur more often in freshwater habitats than marine spills, yet freshwater spills have received less attention. The oil released in a river or other freshwater habitat has more impact on the sensitive environments like marshes and wetlands and also impacts people living near the banks. Some of the important freshwater habitats are lakes, streams, rivers, ponds, wetlands, swamps and marshes.

Inland oil spills are increasing in number in the US. One of the major inland oil spills that occurred recently was the Kalamazoo river spill in the state of Michigan. The Kalamazoo River spill occurred by the rupture of a pipe for a stretch of six feet, which caused a major inland oil spill, by releasing 819,000 gallons of Cold Lake crude oil into the river (Dollhopf & Durno, 2011). Another inland oil spill worth mentioning is the more recent spill in the lower Mississippi River where, a vessel carrying oil released 31,500 gallons of light crude oil into the river due to its collision with a tow vessel (Flower, 2014). The large volume of spills in the U.S. occurred at sea in the early 90's (Owens, Taylor, Marty, & Little, 1993; Yoshioka & Carpenter, 2002). But, the large volume became inland after the late 90's. Typically, pipeline spills occur more frequently and also release larger amounts of oil than vessel spills (Yoshioka & Carpenter, 2002). According to recent study, in the US 88% of oil spills above 10,000 gallons are inland and the remaining are coastal (Brody, Bianca, & Krysa, 2012). The selection of suitable

clean up technologies is dependent on the properties of the oil, degree of weathering and

environmental conditions (Miller & Mudge, 1997).

# CHAPTER 2

# WEATHERING OF OILS

Oil spilt in the environment gets altered physically, chemically and biologically with time. The several processes responsible for the weathering of oils decide the fate of the oil with time. Also, weathering processes vary with time. However, it is important to know about these processes to come up with a response plan for the spill based on the correct assessment of the fate of the oil.
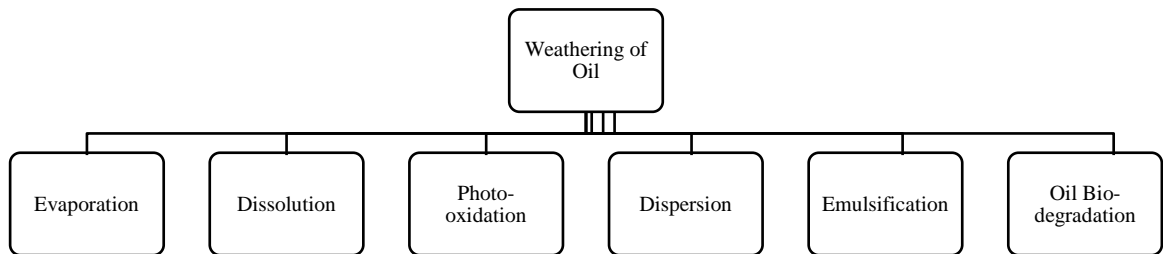


**Figure 2.1** Flowchart of different weathering processes.

## 2.1 Weathering Processes

Weathering includes bio-degradation along with many other physical and chemical processes. The oil forms a thin film on the surface of the water in a spilt environment which is known as oil slick, and then starts spreading due to gravity, and interfacial tension and is resisted initially by inertia and later by viscosity (Wang, Shen, & Zheng, 2005; Zhu, Venosa, Suidan, & Lee, 2001). Spreading results in an increase in the oil interfacial area with the atmosphere and with the water, which facilitates processes such as evaporation, dissolution and bio-degradation.

Evaporation is the process in which most of the low molecular weight compounds in oil like alkanes less than $<C_{15}$ and volatile aromatics, such as benzene and toluene are removed within the first ten days of the oil spill (Zhu et al., 2001). This tends to reduce the toxicity of oil to the spilt environment (Anderson & Hess, 2012). The rate of evaporation depends on the chemical and physical properties of the oil, wind energy, water current and water temperature (Zhu et al., 2001). The slow evaporation of a few compounds makes the oil to spread more (Ryerson et al., 2011). Evaporation dominates at the initial stages and bio-degradation at the later stages of oil spills (Yim et al., 2011). The low molecular weight aromatics are more soluble in water and loss of oil through dissolution is small for surface spills, but it is important for its influence on bio-degradation and its impact on the biota in the impacted environment. Some components that undergo evaporation tend to undergo dissolution into the water column from an oil slick at the surface (Wang et al., 2005).

Photo-oxidation is the process of oxidation of high molecular weight aromatics and polar compounds into simpler compounds, with the help of sunlight. Increase in solubility, results in formation of new lower molecular weight compounds and new polar compounds, which cause an increase in bio-degradation. Dispersion is the mixing of the oil droplets in the water column and tends to increase oil bio-degradation by increasing the contact area between oil components and micro-organisms, and also by increasing dissolution of oil. Emulsification is another important weathering process in which water forms droplets within the oil forming an emulsion (sometimes called "mousse" because it resembles a gel). Emulsification makes physical recovery of oil difficult. It also reduces the rate of bio-degradation of oil as it makes the oil surface area more resistant to bio-degradation (A. D. Venosa & Zhu, 2003). Other factors affecting bio-degradation are sinking, sedimentation, tar ball formation and adsorption to the suspended particle (Zhu et al., 2001).

## 2.2 Kinetics of Weathering Processes

### 2.2.1 Evaporation

The major processes which take at the initial stages of the spill are evaporation and dispersion. In most of the light crude oils evaporation is found to be a major weathering loss as the loss may be around 50 to 60%. The difference in thickness of the film causes the difference in evaporative losses. The evaporative losses are reduced with the increase in oil thickness (Brandvik & Faksness, 2009). During the study of evaporative losses before and after the formation of oil slick, it was found evaporative losses decreased five

folds after the formation of oil slick (Mervin F Fingas, 1995). At the Heibei Spirit spill, it was observed that after four days of the spill, 22-30% of oil had evaporated, 0.7-10% of oil had dispersed depending on the oil type as three types of Middle East crude oils were mixed and 60-76% of oil remained. During the study period, the indices for photo-degradation remained unchanged, so it has not been considered as a major weathering process. Most oils within three after the spill are found to be in moderate weathering stage and weathering loss during this stage are chiefly due to combined effects of evaporation and dissolution. Average weathering loss at Heibei Spirit spill at this stage was found to be 37.7%. This study concludes that evaporation was the dominant with continuous sequential changes in composition over time with an estimated half-life constant to be 2.6 months (Yim et al., 2011). At the Deepwater Horizon spill, approximately after one and a half months, the analysis of airborne data was conducted and it showed the evaporation of hydrocarbons at the rate of 258000 kg/day. On the same day around 33% of the surfacing hydrocarbon was found to have dissolved and around 14% was found to have evaporated. The evaporation of volatile hydrocarbons does not include the slow evaporation of the semi volatile compounds (Ryerson et al., 2011). Oil film thickness is used in determining the evaporation rate by many models. The evaporation of pure compounds with time will be linear. If several compounds are evaporating simultaneously the evaporation rate will be a logarithmic or square root equation. It has been found in light oils, if approximately 7 components are evaporating simultaneously then the evaporation rate equation is a logarithmic equation. In case of a heavy oil if approximately 5 compounds are found evaporating simultaneously, the evaporation rate equation is found to be a square root equation (Merv F Fingas, 1997).

The important method of determining the evaporation pseudo component concept in which the oil has been divided into a number of fractions based on the boiling temperatures. The vapor pressure of each component is calculated average boiling point and oil temperature. It is assumed that the evaporation rate of each component is proportional to the partial pressure of each component. The actual rate of evaporation depends on a mass transfer coefficient, which is related to temperature and wind speed (Reed et al., 1999).

The mass transfer rate is given by

$$\frac{dm_i}{dt} = \frac{K_2 P_i f_i A M_i}{RT} \tag{2.1}$$

Where $K_2$ is given by the equation (3), $P_i$ is the vapor pressure (atm), $f_i$ is the fraction of spill which is constituent I, $A$ is area of the spill, $M_i$ is molecular weight of the compound I, $R$ is the universal gas constant (8.314 Joules/Mole-K) and $T$ is the temperature (K) (Reed et al., 1999; Sebastiao & Guedes Soares, 1995)

The evaporation rate of oil was determined during a study with the following the equation

$$N = \frac{k_m A P}{RT} \tag{2.2}$$

Where

$$k_m = 0.0292 U^{0.78} X^{-0.11} Sc^{-0.67} \tag{2.3}$$

Here $N$ is the evaporative molar flux (mol/s), $A$ is the area, $P$ is the vapor pressure of the bulk liquid $R$ is the gas constant (8.314 Joules/Mole-K), $T$ is the temperature, $k_m$ is the mass transfer co-efficient, $U$ is the wind speed, $X$ is the pool diameter and $Sc$ is the Schmidt number. Using this expression evaporation rate variation with area keeping the mass of oil constant was conducted. This showed a poor correlation between area and evaporation rate. In another set of experiments, evaporation rate variation with volume was carried out in which the area and thickness were kept as constant. A linear increase in the evaporation rate found with the increase in the volume. In another set of experiments with wind and no wind conditions, evaporation rate was identical for the hydrocarbon compounds having 12 and more number of carbon atoms, while in the with the wind, the compounds less than 12 carbon atoms had a higher evaporation rate than in the no wind conditions and this increased exponentially with the decrease in the number of carbon atoms (Mervin F Fingas, 1999).

The rate of evaporation either follows a logarithmic or a square root equation. The percent and weight evaporated are given by

(a) For oils following a logarithmic equation

$$\text{Percentage evaporated} = [0.0165\,(\%D) + 0.045(T - 15)]\ln(t) \qquad (2.4)$$

(b) For oils following a square root equation

$$\text{Percentage evaporated} = [0.0254(\%D) + 0.01(T - 15)]\sqrt{t} \qquad (2.5)$$

(Mervin F Fingas, 1999)

## 2.2.2 Natural Dispersion

Natural dispersion rate is determined using the oil film thickness. Shear spreading of oil is the process caused by natural dispersion and subsequent resurfacing of oil droplets. So relating spreading to dispersion would let us know about both dispersion and spreading. The dispersion rate is dependent on the rate of mass entrained by the breaking wave. The expression for the droplet size distribution of the oil mass entrained by each breaking wave is

$$Q_{d \leq D} = CD^p \tag{2.6}$$

Where $Q_{d \leq D}$ is the entrained oil mass per unit area in droplets up to a certain diameter D. The exponent p was found to be 1.7 observed power law distribution of droplet size determined. The proportionality factor C depended on height of breaking wave H and oil type where $C = aH^q$. Here a is the dispersion co-efficient and from the wave fume experiments q was found to be 1.14. As the dispersion rate depends on the mass entrained by each breaking wave, the dispersion rate can be obtained by multiplying $F_w$ which is fraction of sea surface hit by breaking waves per unit time obtained by the white cap coverage divided by mean wave period (Reed et al., 1999).

The fraction of oil at the sea surface dispersed into the water column is calculated by the loss of fraction of oil at the sea surface which is given by

$$D = D_a D_b \tag{2.7}$$

Where $D_a$ is the fraction of sea surface dispersed per hour

10

$$D_a = 0.11(W + 1)^2 \qquad (2.8)$$

And $D_b$ is the fraction of dispersed oil not returning to the surface

$$D_b = (1 + 50\mu^{\frac{1}{2}}\delta S_t)^{-1} \qquad (2.9)$$

Where $W$ is the wind speed (m/s), $\mu$ is the viscosity (cP), $\delta$ is the slick thickness (cm) and $S_t$ is oil water interfacial tension (dyne cm$^{-1}$) (Sebastiao & Guedes Soares, 1995).

### 2.2.3 Oil biodegradation

The biodegradation rate for a particular compound or an analyte can be found using this equation.

$$\left(\frac{dA}{dt}\right)_t - \frac{A}{H}\left(\frac{dH}{dt}\right)_t = -kA \qquad (2.10)$$

Where A is the concentration of the Analyte, H is the concentration of hopane, k is the first order rate constant which is calculated by the method described by Kvålseth (1985) ("API Technical Report," January 2014; A. D. Venosa et al., 1996).

### 2.3 Further Changes Occurring in the Spilt Oil

The dispersed oil droplets or dissolved oil constituents might have further changes in their fate. They may either form oil-suspended particulate material aggregate (OSA) by aggregating directly or incorporation in or adsorption on the sediments (Gong et al., 2014; Lee, 2002; Sterling Jr, Bonner, Ernest, Page, & Autenrieth, 2005). Weathering processes are responsible for the changes in physical and chemical properties. That is,

after evaporation, volatile components are lost and oil becomes viscous making it less soluble and it concentrates, thereby restricting the spread of the oil (Mendelssohn et al., 2012; Owens et al., 1993). The loss of Polycyclic Aromatic Hydrocarbons (PAH) and Benzene Toluene Ethylbenzene and Xylenes (BTEX) was observed with a very high weathering rate within the three days of the spill (Lee, Stoffyn-Egli, & Owens, 2001). Apart from the processes discussed above affecting bio-degradation, bio-degradation itself is an important weathering process. Although, bio-degradation is one of the important processes, 15.6.% of oil loss is by natural depletion processes in 60 days and it has been observed that the maximum amount of oil lost, is through the natural depletion processes during the early days of the spill (Abu & Dike, 2008).

# CHAPTER 3

## OIL BIODEGRADATION

Oil bio-degradation is a process which involves the interactions between oil and micro-organisms under environmental conditions. Oil bio-degradation depends on the properties of the oil, the environment in which the spill has occurred, and the behavior of oil in the spilled environment. The spills that happen normally are either different types of crude oil or any petroleum products which are constituted by mixture of thousands of compounds which have a variety of physical properties.

The natural bio-degradation of the oil is important because it is an economical process and also a process to be preferred in sensitive eco-systems when there is ample time for cleanup (Abu & Dike, 2008).
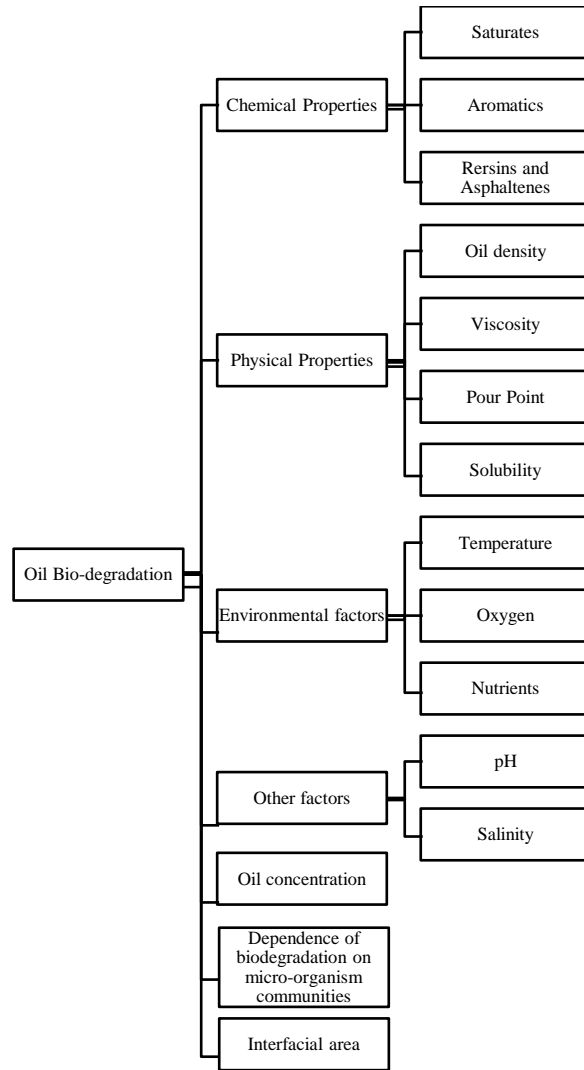
**Figure 3.1** Flowchart of different factors affecting oil bio-degradation.

### 3.1 Crude Oil and its Chemical Properties

Crude oil is a mixture of hydrocarbon and non-hydrocarbon compounds in different patterns of combinations. The hydrocarbon compounds consist of elements of hydrogen and carbon in the ratio of 2:1 and non-hydrocarbon compounds are made up of elements like nitrogen, sulfur and oxygen which contributes generally to less than 3% (v/v) of oil (Okoh, 2006). Refined petroleum products such as fuel oil or gasoline have higher percentage of toxic constituents than the crude oil (Owens et al., 1993). Major classes of petroleum compounds of the crude oil are saturates, aromatics and resins and asphaltenes. Saturates are the aliphatic compounds or especially alkanes, which are either normal or branched or cyclic alkanes.(Zhu et al., 2001). Different kinds of saturates which constitute to most of the crude oil composition are straight chained, branched and cyclic structures (Mendelssohn et al., 2012). The degradation of large saturate compounds (e.g., alkane of $C_{44}$) has been observed, but the compounds from $C_{10}$ to $C_{26}$ are the most commonly observed degradable compounds (A. D. Venosa & Zhu, 2003). The chief method of weathering by which most of n-alkanes are lost is through evaporation. (Miller & Mudge, 1997). Aromatics are the class of compounds which contain either monocyclic or polycyclic compounds which are of most concern, due to the possibility of their transformation into carcinogens. Mono-aromatic compounds have a toxic effect on cell membranes of micro-organisms, however they are also bio-degradable at low concentrations. Resins are the class of compounds that contain nitrogen, sulfur and oxygen. Asphaltenes are compounds that are constituted by high molecular weight compounds, weakly identified hydrocarbons, along with some of the NSO (Nitrogen Sulfur Oxygen) compounds, and metals iron and nickel (Zhu et al., 2001). Resins and

asphaltenes which are most resistant to bio-degradation can be used in road asphalt and tar for roofing(Mendelssohn et al., 2012). However, bio-degradation of asphaltenes has also been observed through a process of co-metabolism and low molecular weight resins degradation at low concentrations (Leahy & Colwell, 1990; A. D. Venosa & Zhu, 2003). The larger the proportion of asphaltenes and resins the more are the polarities of the crude oil (Gong et al., 2014; Guyomarch, Le Floch, & Merlin, 2002).

Crude oil composition depends on the area where it is formed, and is also based on the different areas having different boiling temperatures. Crude oils may be mainly categorized using boiling fraction from $20^0$C to $205^0$C and fraction above $205^0$C. The naphtha boiling fraction, a low boiling fraction has its boiling fraction composition by weight of around 20% to 30% of oil which consists of saturates, aromatics, resins and asphaltenes. The other boiling fraction (>$205^0$C) is around 70% and 80% by weight of the oil. Refined oil products such as gasoline, kerosene, jet fuel, diesel fuels, wax, asphalt may have unsaturated compounds in them, the only in which these products differ from the crude oil. These unsaturated compounds are being formed during the catalytic processes (Zhu et al., 2001).

### 3.2 Physical Properties of the Crude Oil

The physical properties of oil are also important to assess the fate of the oil after the spill. Some of the important physical properties are density, pour point and solubility in water. Oil density is expressed as either specific gravity or API (American Petroleum Institute) gravity, an important physical property needed to know the composition of the oil by

which the fate of the oil can be predicted. Pour point is the temperature at which the oil stops flowing. Pour point of crude oils ranges from $-57^0$C to $32^0$C which is also an important property to be considered for oil spill response strategies. Solubility of oil in water is low and is about 30mg/L, which mainly depends on the quantity of compounds within the oil and also the temperature of the boiling fraction. This property is important in predicting the behavior of the oil, oil toxicity and bio-degradation process (Zhu et al., 2001).

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# CHAPTER 4

## ENVIRONMENTAL FACTORS IMPACTING BIO-DEGRADATION

Oil bio-degradation rates can be affected by the different weathering processes, properties of the oil, and characteristics of the spill environment. As discussed above, there are several weathering processes like evaporation, dissolution, photo-oxidation and many others. The physical and chemical properties of the oil has also been considered and explained in detail. There are several other environmental factors such as temperature, nutrients and oxygen.

### 4.1 Temperature

Temperature is one of the important factors for predicting the rate of bio-degradation. It influences the characteristics of the oil and also the micro-organisms. At the same time, the micro-organisms present in the environment affects the rate of the bio-degradation. If most of the bacteria are thermophiles, then high temperature is favorable for bio-degradation.
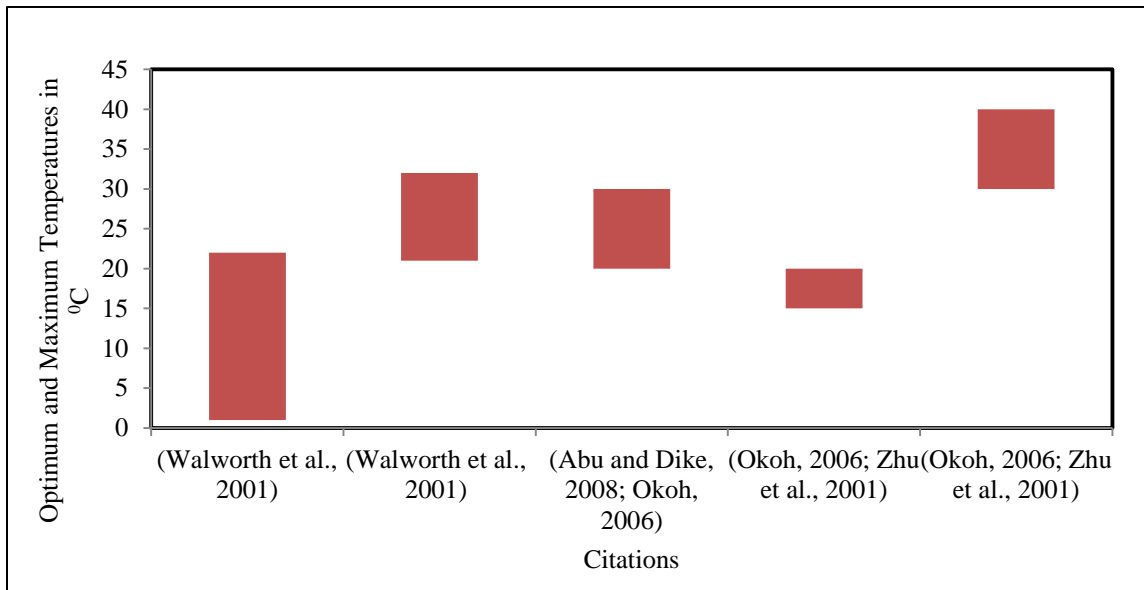
**Figure 4.1** Optimum and Maximum Temperatures for oil bio-degradation from different articles.

In general, a decrease in temperature results in the reduction of rates of bio-degradation (Okoh, 2006). The bio-degradation rate was observed to increase from $1^0$C to $21^0$C whereas, the maximum bio-degradation rates were observed between $21^0$C and $31^0$C (Walworth, Braddock, & Woolard, 2001). At low temperature, the viscosity of the oil is high while the toxic low molecular weight volatile compounds are not easily degraded (Abu & Dike, 2008; Atlas, 1981; Okoh, 2006). At high temperatures, the oil viscosity decreases, the volatility of the short chained compounds increases and the rate of microbial metabolism increases. These factors improve the removal of oil (Haigh, 1995). Diesel contaminated soil was studied to observe the degradation rates of diesel oil at different temperatures. During the degradation one soil sample was kept at room temperature and the other was kept at hot air oven with forced ventilation. In the hot air oven the faster degradation rates were observed than the one kept at the room temperature

(Van Gestel, Mergaert, Swings, Coosemans, & Ryckeboer, 2003). The bio-degradation of the oil with different temperatures was studied. The soil contaminated with oil was kept open, the other was covered with straw and the other contained in a greenhouse covered with straw. The treatment with straw that contained in a greenhouse had the highest bio-degradation rate when compared to the one covered with straw or the one kept open (Chaîneau, Yepremian, Vidalie, Ducreux, & Ballerini, 2003). The temperature even affects the chemical composition of the oil (Yang et al., 2009). Temperature appears to be very important in freshwater environments (Abu & Dike, 2008; Okoh, 2006). The highest rates of biodegradation are evident at temperatures $15^0$C to $20^0$C for bio-degradation in sea water, $20^0$C to $30^0$C in freshwater environments and $30^0$C to $40^0$C for bio-degradation in soils (Okoh, 2006; Zhu et al., 2001).

## 4.2 Oxygen

The presence of oxygen is another favorable factor for the bio-degradation of the spilled oil. The pathway of the degradation for saturates and aromatics have been the breakdown with the help of oxygen (Zhu et al., 2001). Biodegradation with drastic reduced rates is observed in subsurface sediments, freshwater wetlands, mudflats and salt marshes. Increased aeration results in the increase of the microbial population which implies the increased rates of bio-degradation (Abu & Dike, 2008). Testing the BOD value in the affected environment is one of the ways to find out the oxygen in the environment (Zhu et al., 2001). For instance, if the BOD is too high (>500mg/L) it implies that, there are many biologically oxidizable compounds and drastic depletion of oxygen takes place

affecting the living organisms in the environment (Abu & Dike, 2008). In an affected mangrove swamp, oxygen gets depleted with the increasing concentration of PAHs (Li, Zhou, Wong, & Tam, 2009; Mendelssohn et al., 2012). Flooding in freshwater environments do not only contribute to mixing energy but also contribute in creating anaerobic environments, which in turn have an effect especially on the rate of biodegradation of aromatic components (Mendelssohn et al., 2012). Anaerobic degradation has been found helpful in degrading benzene, toluene and other low weight molecular compounds. Some PAHs and alkanes can be degraded in sediments under sulfate reducing conditions (Zhu et al., 2001).

## 4.3 Nutrients

Oil spills provide a lot of carbon for the micro-organisms from the substrate itself, whereas nutrients such as nitrogen and phosphorous increase the rates of bio-degradation (Okoh, 2006; Zhu et al., 2001). Bio-degradation studies of oil in arctic and sub-arctic soils showed that the bio-degradation rates were at their peaks, when the concentration of nitrogen was in between 50mg/kg and 100mg/kg (Walworth et al., 2001). Another study involving the bio-degradation of hexadecane as the substrate, one of the chief constituent in oils, showed that the rates of bio-degradation were maximum around 36mg/kg/day to 40mg/kg/day, when the nutrients concentrations were 50mg/kg to 200mg/kg (Børresen & Rike, 2007). Enhancement of bio-degradation has been conducted by the addition of straw, manure, compost etc., which helps in upgrading soil texture, oxygen transfer and supplying energy to the micro-organisms (Haritash & Kaushik, 2009). In marshes, the microbial diversity gets more prolific with the increase in the amount of carbon available

for it to degrade. The nutrients gets depleted, if the spill happens repeatedly in a particular area with vegetation, where shoot growing capacity also decreases with time (Mendelssohn et al., 2012). The observed limiting concentration of nitrate for oil biodegradation is 10mg/L (Du et al., 1999). The surplus amount of nitrogen levels especially when the C/N ratio is less than 20, may lead to nitrite toxicity (Yang et al., 2009). The nutrients in freshwater environments during oil spills are highly variable which may be oligotrophic to eutrophic. A river which is oligotrophic in its upstream reach may become eutrophic in its downstream locations due to the addition of agricultural run-off and industrial effluents within its drainage area downstream (Okoh, 2006; Zhu et al., 2001). It has also been observed that nutrient concentrations vary in a freshwater habitat seasonally (Zhu et al., 2001).

## 4.4 Other Factors

Other factors that impact bio-degradation are oil properties, environmental factors and situational factors. Examples of situational factors are pH and salinity. These factors are related to the environment in which the spill has happened.

### 4.4.1 pH

Sea water has neutral or slightly alkaline pH, whereas organic soils have acidic pH and mineral soils have slightly alkaline pH (Zhu et al., 2001). It has been observed that at slightly alkaline conditions optimum bio-degradation rates occur. According to Abu and Dike (2008) at a pH of 4.26 maximum bio-degradation rates were observed. But, there is

no steady order of variability of pH with the change in environments (Abu & Dike, 2008; Zhu et al., 2001).

## 4.4.2 Salinity

Salinity is also an important factor which affects the bio-degradation rate. It has been observed that oil has reduced rates of hydro-carbon biodegradation in salt water ponds with an increase in salinity (Zhu et al., 2001).

**Table 4.1** Different Optimum and Maximum Salinities.

| Author | Description | Salinity |
|---|---|---|
| (Díaz et al., 2002) | Bio-degradation in Mangrove sediments with MPD-M consortium | 20-70 g/L |
| (Minai-Tehrani et al., 2009) | Bio-degradation of oil in oil affected soils | 10g/kg |
| (Zhao et al., 2009) | Bio-degradation of Phenanthrene with varying Sea Salt Defined Media Yeast extracts (SSDMY) concentration | Degradation occurred at 50g/L,100g/L,150g/Land no degradation at 0g/L and 200g/L |
| (Al-Mailem et al., 2010) | Oil Bio-degradation with Halophilic bacteria | 0-262.98g/L |
| (Ulrich et al., 2009) | Bio-degradation of hydrocarbons in soil and groundwater at $10^0$C | 0-10g/L |
| (Kumar et al., 2007) | Maximum bio-degradation of hydrocarbons with bacterial strain Bacillus Licheniformis | 116g/L |
| (Díaz et al., 2000) | Degradation of $nC_{17}$, $nC_{18}$ and naphthalene with consortium MPD-M and MPD-7 | 40g/L |
| (Nicholson and Fathepure, 2005) | Optimum of bio-degradation of benzene at Oklahoma soils | 146g/L |
| (Mille et al., 1991) | Bio-degradation of ashtart crude oil with a mixed bacterial community | 23.37g/L |

The effect of salinity on the changes of the rates of bio-degradation was tested with a bacterial consortium of MPD-M (meta-Phenylene Diamine) isolated from sediments related to Columbian Mangrove roots. It was found that concentrations above 20g/L of salinity increases biodegradation significantly until 70g/L. No significant changes were observed 70g/L to 180g/L (Díaz, Boyd, Grigson, & Burgess, 2002). The oil affected soils can receive salinity of up to 10g/kg of soil, where degradation rates are significant and if the same rises above 10g/kg high reduction in rates of bio-degradation are observed (Minai-Tehrani, Minoui, & Herfatmanesh, 2009). Phenanthrene one of the important PAH was tested for bio-degradation under varying salinities, with the help of Sea Salt Defined Media added Yeast extract (SSDMY), from concentrations ranging from 0.1% to 20% salinity, where bio-degradation was observed only at 5%, 10% and 15% but not at .1% and 20% (Zhao, Wang, Mao, & Li, 2009). The halophilic bacteria namely Haloferax, Halobacterium and Halococcus were tested for bio-degradation under varying salinities and it was observed that bio-degradation increased up to a concentration of 263 g/L and at a concentration of 234g/L all strains had higher biodegradation rates (Al-Mailem, Sorkhoh, Al-Awadhi, Eliyas, & Radwan, 2010). Bio-degradation of hydrocarbons were tested in both soil and groundwater at a temperature of $10^0$C, which showed significant bio-degradation rates at 0 to 10 g/L salinity, after which highly reduced rates of bio-degradation was observed (Ulrich et al., 2009). The bacterial strain of *bacillus licheniformis* isolated from oil contaminated soils, was able to degrade hydrocarbons in a large range of salinity variations, with a maximum bio-degradation rate of hydrocarbon being observed at 116g/L (Kumar, León, Materano, & Ilzins, 2007). The degradation of naphthalenes and nC17 and nC18 were carried out with the help of the bacterial consortia

of MPD-M and MPD-7, isolated from Cormant Field in the North Sea. In most of the cases, the results demonstrated that the maximum bio-degradation was at 40g/L, but the same decreased with the increase in salinity (Díaz, Grigson, Peppiatt, & Burgess, 2000). In the soil samples taken from the great salt plains, Oklahoma it was observed that the bio-degradation of benzene was tested along the salinity range of 0 to 234 g/L. The optimum bio-degradation rates was observed at 146.1g/L and it decreased with higher salinities (Nicholson & Fathepure, 2005). Degradation studies of Ashtart crude oil by a mixed bacterial community was conducted and it was found that the bio-degradation rates increased with the increase in salinity from 0 to 24 g/L and further decreased with the increase in the salinity (Mille, Almallah, Bianchi, Van Wambeke, & Bertrand, 1991).

### 4.5 Oil Concentration

Oil concentration is also an important factor in determining the rate of bio-degradation. The longer the time it takes, the more the oil it has (Fayad & Overton, 1995; Pritchard, Mueller, Rogers, Kremer, & Glaser, 1992). But, if the bio-availability of the compounds are maximized by adding surfactants or by photo-oxidation, then even with greater mass of oil, faster bio-degradation rates can be achieved (Pritchard et al., 1992). According to a study by A. Venosa and Holder (2007), two different undiluted and diluted oil concentrations were taken. It has been observed that the rates are faster in the diluted samples than the undiluted and the residual concentration is lower in the diluted samples. In a study, the assessment of different factors on the microbial population was done. The decrease of hydrocarbon concentration was observed, due to which bio-degrading micro-organisms mortality decreased. Concentrations higher than 600mg/kg of hydrocarbon had

reduced rates of bio-degradation are observed (Chaîneau et al., 2003). Bio-degradation of oil was studied using different initial oil concentrations. The more the concentration of oil, the lesser it bio-degrades for the same time when compared with other lesser concentrations. This means that, greater concentrations get reduced to lesser concentrations and it takes more time than to be reduced for lesser concentrations (M. A. Zahed, H. A. Aziz, M. H. Isa, & L. Mohajeri, 2010; Zahed et al., 2011). Soil was treated with diesel with different concentrations of 5000, 10000 and 20000mg/kg of soil and bio-degradation of diesel was observed in all the three different soils. It was found that the soil with the highest percentage of bio-degradation was the soil with 5000mg/kg concentration (Walecka-Hutchison & Walworth, 2006). The effect of different crude oil concentrations such as 2.5%, 5%, 7.5% and 10% (w/v) on a mixed bacterial consortium was studied. The degradation rate was 70% at 2.5%, 67% at 5%, 63% at 7.5% and 52% at 10% with approximately equal concentrations of microbes. Thus, it can be said that the crude oil bio-degradation rate is inversely proportional to the concentration of oil (Rahman, Thahira-Rahman, Lakshmanaperumalsamy, & Banat, 2002). Fuel oil in microcosms along with the soil were treated and the logarithmic decrease of hydrocarbon concentration was observed with an initial hydrocarbon concentration of around 2190µg/g (ChaIneau, Morel, & Oudot, 1995).

The inhibition of bio-degradation of oil depends on the composition of the oil. At too high concentrations of PAHs bio-degradation of oil is inhibited (Balba, Al-Awadhi, & Al-Daher, 1998; A. D. Venosa & Zhu, 2003; M. Zahed, H. Aziz, M. Isa, & L. Mohajeri, 2010). The effect of oil concentration on bio-degradation was studied with sandy sediment with three different oil concentrations 14, 21 and 28g/kg. There were no

significant differences in degradation observed with time for the concentrations of 14 and 21 g/kg whereas, significant reduced degradation was observed at 28 g/kg due to partial inhibition of microbial activity at this concentration (Del'Arco & De Franca, 2001). The crude oil or hydrocarbon concentration affects the rate of bio-degradation such that, above a threshold value of the concentration inhibition of the microbial activity is observed (Leahy & Colwell, 1990). If the depth of oil penetration increases along with the increase in oil concentration, then bio-degradation capacity is negatively affected (A. D. Venosa, 2004). The inhibition of the activity of Spartina alterniflora and Spartina patens was observed at a concentration greater than 8 liters / m2 of the application of oil (Lin & Mendelssohn, 1998). In recently contaminated soil, bio-degradation was not observed at a concentration higher than 6.8% (w/w), whereas in weathered soil the threshold was found as 0.5% (w/w) above which no bio-degradation was observed (Trindade, Sobral, Rizzo, Leite, & Soriano, 2005). The maximum concentration has been found to be 80g of oil per kg of dry sand during lab scale experiments. However, the optimum range is not common for all the oils which also depends chiefly on the composition of the oil (Zhu et al., 2001). Hydrocarbon degrading bacteria gets enhanced just after contamination, but inhibitory effects are observed at high Total Petroleum Hydrocarbons (TPH) concentrations (Margesin, Hämmerle, & Tscherko, 2007). The maximum degradation rate achieved during the 24 hours and 48 hours of degradation was dependent much on the initial concentration of the oil especially for the volatile hydrocarbons. The initial concentration is linearly related to the mass of the oil degraded (Van Hamme & Ward, 2001).

## 4.6 Dependence of Oil Bio-degradation on Micro-organism Communities

The bio-degradation rate study of PAH was studied with respect to the fungi *Cunninghamella Echinulata* and was found that the bio-degradation of PAH follows a first order reaction. That is, the rate of change in contaminant concentration is directly proportional to the concentration of the contaminant. The article also states that the kinetics of the bio-degradation also depends on the biomass involved, the type of contaminant and the contaminant concentration (Haritash & Kaushik, 2009).

## 4.7 Interfacial Area

In this study, the limitation for the growth of bacteria has been studied and interfacial surface area of oil and water has been found as an important factor. If the interfacial surface area becomes restricting, then the increase in growth of bacteria would be linear rather than exponential (Ron & Rosenberg, 2002). The smaller the oil droplet size the larger is the interfacial area obtainable by the micro-organisms, thereby boosting the rate of bio-degradation (Lessard & DeMarco, 2000). The variation of interfacial area in $cm^2/cm^3$ has been shown along with the variation of water saturation.

**Table 4.2** Variation of Interfacial Area with Water Saturation.

| Citations | Variation range of water saturation | Interfacial area ($cm^2/cm^3$) |
|---|---|---|
| Bradford and Leij (1997) | .2 to .75<br><br>.75 to .8 | 110 to 10<br><br>10 to 50 |
| Schaefer, DiCarlo, and Blunt (2000) | 0 to 1 | 200 to 0 |
| Dalla, Hilpert, and Miller (2002) | .2 to .9 | 50 to <20 |
| K. Culligan, Wildenschild, Christensen, Gray, and Rivers (2006)<br><br>K. A. Culligan et al. (2004)<br><br>Reeves and Celia (1996) | .1 to .3<br><br>.3 to .9 | 2 to 3.5<br><br>4 to .7 |

# CHAPTER 5

# MICROBIOLOGICAL STUDY OF BIO-DEGRADATION

Micro-organisms which are able to degrade petroleum hydrocarbons and other related compounds are widespread in all the different habitats. More than 200 species of bacteria, yeasts and fungi are capable of degrading hydrocarbons with more than 40 carbon atoms, where yeasts and fungi have a major role in the freshwater habitat (Leahy & Colwell, 1990; Zhu et al., 2001). Fungi and yeasts have a subtle role in bio-degradation. One of the positive aspects about the fungi is that its hyphae can penetrate through the anoxic sediments and hydrophobic conditions, and also it can degrade several hydrocarbons partially. Yeast can degrade most of the straight chained alkanes (Ijah, 1998; Mendelssohn et al., 2012). The distribution of the bacteria in a particular freshwater environment depends on the previous exposure of the environment to the oil spill. That is, if the oil has spilled recently, then more hydrocarbon degrading bacteria can be found and vice versa (Zhu et al., 2001). For instance, the hydrocarbon degraders are found around 0.1% in a pristine environment whereas, 100% of hydrocarbon degraders can be found in the oil polluted environments (A. D. Venosa & Zhu, 2003). To understand the bio-degradation, it is essential to know about the degradation of the components.

## 5.1 Degree of Degradability from a Micro-Biological Perspective

Oil can be classified majorly into saturates, aromatics, resins and asphaltenes. The extent to which the various chemical compounds can be degraded by micro-organisms is based on the chemical structure of the compounds. If the structure is more complex, the longer the time or shorter the level up to which degradation occurs.

### 5.1.1 Saturates

Saturates is one of the major class of petroleum compounds in which normal alkanes degrade more easily especially from $C_{10}$ to $C_{26}$ compounds. But branched alkanes show some resistance to bio-degradation due to methyl branching. Cycloalkanes are more resistant, where hopanes and steranes tend to persist for a long time in the environment (Zhu et al., 2001).

### 5.1.2 Aromatics

The aromatics are more resistant to bio-degradation but few of the low molecular weight aromatics are more bio-degradable than saturates. Mono-aromatic hydrocarbons are toxic to some of the micro-organisms, but bio-degradable only at low concentrations. Other PAHs with two to four rings are more bio-degradable when the structure is simpler. Other PAHs with five or more rings are bio-degraded by the method of co-metabolism (Zhu et al., 2001). PAHs are the compounds that have distinct structure and bonding, which increases the solubility in turn, increasing the toxicity to the biota in the affected environment (Mendelssohn et al., 2012). The increase in the number of benzene fused

rings in the PAH composition decreases the solubility and volatility (Juhasz & Naidu, 2000). Photo-oxidation has been observed as an important process which improves the bio-availability and bio-degradability of PAHs (Maki, Sasaki, & Harayama, 2001; Okoh, 2006). Evaporation of several volatile hydrocarbons can be prevented if oil infiltrates into the soil (Leahy & Colwell, 1990). Hydrocarbons in the spilt oil, floating in the affected environment could get reduced continuously with the help of evaporation and dissolution. The velocity in an affected environment like a river, helps in transporting the hydrocarbon rich plume continuously, which help in continuous dissolution and allows the bio-degradation of the available and degradable compounds (Reddy et al., 2012). According to recent studies, it has been found that several marine gamma-proteo-bacteria secrete bio-surfactant molecules improving the solubility of many hydrocarbon compounds, which in turn improves the bio-degradability of hydrocarbons in a marine environment (Gutierrez et al., 2013). Diol and trans-diols are the product of degradation of PAHs. The important way of degradation of PAHs and aromatics involves oxygenases enzymes (Abu & Dike, 2008; Atlas, 1981). Phenanthrene, one of the persistent three ring PAH has been observed to bio-degrade from 42% to 78% in different sediments, with the help of the bacteria isolated from mangrove sediments (Haritash & Kaushik, 2009; Tam, Guo, Yau, & Wong, 2002). Degradation of naphthalene is as focused on, as it is toxic (Lemkau et al., 2010; Page et al., 2002). Degradation of PAHs is also possible along with the FE (III) reduction with the help of freshwater aquifers derived sediments (Haritash & Kaushik, 2009).

### 5.1.3 Resins and Asphaltenes

Resins and asphaltenes are the most resistant compounds to degradation as they have a very complex structure. Some of the asphaltenes can be bio-degraded by co-metabolism, which is the process of degradation of a secondary compound during degradation of a primary compound. The degradation rate for different compounds is normally observed in the way described above, but the degradation rate for the same oil components may alter differently for different oils (Zhu et al., 2001).

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# CHAPTER 6

## CONCLUSION

The dependency on oils and transportation of oils in all the walks of life makes it essential to be prepared to provide suitable clean up response during an oil spill. This thesis deals with the weathering process of oil among which, bio-degradation is the chief one. This paper also examines the factors affecting bio-degradation. To be prepared and taking appropriate action during a spill is one of the crucial aspects, as the spill affects the biota of the eco-system and sometimes even abiotic vital components like water or soil. The first and foremost attention must be given in analyzing the type of oil spilt, the amount weathered and all other environmental factors to select the satisfactory cleanup strategy. There are many variables that are interrelated, associated with the degree of biodegradation during oil spills. Thus, it is necessary to study all the different factors potentially impacting the spill in order to choose a suitable response plan.

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