Biodegradation and Bioremediation of Oiled Beaches

A Primer for Planners and Managers

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Executive Summary

Physical/mechanical removal is the primary response action for addressing the bulk oil deposited on shorelines from an offshore release. However, the biodegradation of oil (i.e., the natural breakdown by microorganisms) would still need to be considered for three non-exclusive reasons:

1. There are land owners who refuse to allow the usage of mechanical means to remove the oil,

2. Some patches of oil may sometimes be overlooked by physical removal approaches, especially when dealing with long impacted shorelines, and

3. Any protocol to physically remove oil has end-points beyond which additional physical action would not result in a net environmental benefit (as determined through a net environmental benefits assessment, NEBA).

Therefore, it is worthwhile to have a basic understanding of oil biodegradation and to be aware of the challenges and advantages of accelerating this natural process through bioremediation. Unlike physical removal, bioremediation is a minimally-disruptive response technique. Furthermore, it mimics or enhances natural oil biodegradation and can continually operate over time rather than as a finite event.

This document is a primer on oil biodegradation and bioremediation. It is intended to provide planners and managers responding to oil spills with sufficient information to decide whether or not natural biodegradation is occurring sufficiently and whether bioremediation should be used as a response technology. Specifically, this document would allow responders to communicate effectively with bioremediation experts, concerned citizens, and public officials. This document is not intended to be an extensive literature review, but rather a simple document that sets general bounds for actions. The salient points of the document are reported below:

1. Under optimal biological conditions (pH, temperature, availability of nutrients, oxygen, and microorganisms), in combination with a dynamic tide, a large portion of the oil should naturally attenuate within 3 to 9 months. Tables at the end of this document summarize the attenuation rates of various hydrocarbons.

2. A conceptual model of the beach should be adopted, and it should account for the transport of water within three locations of a shoreline. They are (see Figure 3): the lower intertidal zone, the mid-intertidal zone, and the upper intertidal zone. The supratidal zone (i.e., landward of the high tide) should be considered if it contains oil. The sizes of these zones depend on the type of shoreline (e.g., sand beach, gravel beach, wetland, etc.).

3. Monitoring natural biodegradation and natural hydraulic flushing, the two main processes in the monitored natural attenuation (MNA) of oil, may be used as an alternative or follow up to the active cleanup of contaminated beaches. MNA should be accompanied by a conceptual model that adequately interprets field measurements.

4. In a typical offshore oil spill scenario, oil deposition is highest in the landward portion of shorelines (upper intertidal and supra-tidal zones) and decreases going seaward (lower and mid-intertidal zones). The deposition is directly related to the drop of the water table during low tides, where the magnitude of the drop increases going landward.

5. Biodegradation in combination with natural hydraulic flushing causes oil in the upper intertidal zone to disappear faster than from the seaward portion. Therefore, a decrease in the oil concentration with time in the upper intertidal zone might not necessarily reflect the fate of oil in the lower intertidal zone. Biodegradation of oil in the supratidal zone of beaches could occur at relatively high rate provided the pore moisture is larger than 20% of the porosity and the pore water salinity is not too large (i.e., less

than 60 g/L). Therefore, biodegradation of oil in the supra-tidal zone should not be neglected even if waves do not reach that zone.

6. To differentiate between oil disappearance due to biodegradation from that due to hydraulic flushing(also known as washout), the concentrations of oil components would need to be normalized to the concentration of biomarkers (oil compounds that biodegrade at very slow rates). The ratios of oil components to biomarkers would be tracked over time. Commonly adopted biomarkers for crude oils and bunker fuels include hopane and stigmastane. For diesel, C3-phenanthrenes and C3-chrysene have been used, but terpanes are probably a better choice.

7. Gas Chromatography (GC) is the preferred method for the analysis of oil components. Besides measuring oil concentrations, other parameters such as oxygen, nutrients should also be measured. Standard methods and field instruments exist for measuring oxygen and nutrients. Microbial populations can be also estimated using the most probable number (MPN) method and molecular techniques.

8. Oil biodegradation is faster in the presence of oxygen (aerobic biodegradation) than in the absence of oxygen (anaerobic biodegradation). While anaerobic biodegradation occurs, it has not been shown to contribute as significantly as aerobic biodegradation to the reduction in oil. Therefore, conditions supporting aerobic biodegradation are desired over anaerobic biodegradation due to the accelerated rates of oil removal.

9. For optimal oil biodegradation, the oxygen concentration in the pore water should be larger than 2.0 mg/L. Similarly, nitrogen should be upwards of 2 to 5 mg/L and phosphorous should be above 0.2 to 1.0 mg/L. When oil, oxygen, and nutrients are at sufficient levels, indigenous microorganisms that biodegrade oil tend to proliferate in response to an oil spill, often increasing several orders of magnitude in population numbers, provided the oil concentration is not too high to inhibit growth.

10. There are two principle bioremediation approaches for enhancing natural biodegradation: biostimulation (addition of nutrients) and bioaugmentation (addition of microorganisms). Biostimulation seems to be more effective than bioaugmentation. However, bioaugmentation could still be considered in situations where the indigenous microbial population numbers are too small following the oil release.

11. There are several types of biostimulants available commercially:

    - Slow release fertilizers (SRF) have been used successfully. They should be used at locations of relatively high pore-water mixing or flushing (e.g., in the intertidal zones). Otherwise, the resulting pore water nutrient concentration might be too small to significantly affect biodegradation rates. However, the SRF might be washed out due to the action of waves. SRF have been successfully used at gravel beaches following the Exxon Valdez oil spill in Alaska.

    - Common agriculture fertilizers have been successful at bioremediating oil polluted shorelines. They are inexpensive, but may require frequent application.

    - Oleophilic fertilizers are nutrients that attach to oil. They are commonly combined with a surfactant that helps remove oil from surfaces thereby enhancing the availability of the oil and nutrients to microorganisms. These fertilizers are recommended at high energy shorelines (e.g., influenced by significant wave action) or at shorelines where the oil is at or just below the surface. However, they can be expensive. Oleophilic fertilizers have been successfully used at gravel beaches following the Exxon Valdez oil spill in Alaska.

12. When applying these amendment solutions, the height of the zone of high moisture above the water table, known as the capillary fringe, needs to be taken into consideration. If the applied volume is too large then ponding and subsequently runoff would occur, causing the loss of nutrients from the beach

even if they were leached from slow release fertilizers at the surface. For example, a beach with a capillary fringe of 1.0 m would not imbibe the applied volume if the water table is shallower than 1.0 m. Therefore, monitoring water table fluctuations should account for the capillary fringe; otherwise, applying an amendment solution onto a beach surface may result in the solution running off into the sea.

13. The high biological oxygen demand (BOD) associated with the addition of nutrients can cause a rapid depletion of oxygen which could lead to a subsequent decrease of the biodegradation rate after an application. Therefore, to maintain optimal conditions, ongoing monitoring of the oil, oxygen, and nutrient levels should be conducted to facilitate any potential follow up applications. Subsequent monitoring of microbial populations may also be conducted but are considered a secondary line of evidence that the biodegradation is proceeding or the bioremediation effort is effective.

Biodegradation and Bioremediation of Oiled Beaches
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1 Introduction

Oil is made up of thousands of chemicals that can be placed into four groups: saturates, aromatics, resins, and asphaltenes (referred to as SARA). “Light oil” generally contains more saturates and aromatics, while “heavy oil” contains more resins and asphaltenes. However, for most crude oils, the saturates and aromatics make up a larger portion of the entire composition on a mass basis. The physical, chemical, and biological properties of these components dictate their overall environmental fate in an oil spill situation.

Understanding the conceptual model of the environmental fate of oil under natural conditions provides a basis for decision makers to develop response plans for shorelines impacted by an offshore release. While physical/mechanical removal is the primary response action for addressing the bulk oil deposited on shorelines, natural attenuation (the combination of hydraulic flushing and biodegradation) and the applicability of bioremediation of the oil would still need to be considered for three non-exclusive reasons:

1. There are land owners who refuse to allow the usage of mechanical means to remove the oil,

2. Some patches of oil are likely to be missed by physical removal approaches, especially when dealing with large distances of impacted shorelines, and

3. Any protocol to physically remove oil has end-points beyond which additional physical action would not result in a net environmental benefit (as determined through a Net Environmental Benefits Analysis, NEBA).

Therefore, the goal of this manuscript is to provide guidelines for understanding the natural attenuation processes and subsequently, whether applying bioremediation would provide any additional benefits or challenges to cleaning up oiled shorelines.

For the purposes of this document, a “beach” is defined as the soil or sediment matrix of a “shoreline” that may or may not contain vegetation rooted in the matrix. Within a beach, fluids can move in and out within the pore spaces between sediment particles (known as the “pore water” space) and is measured as the “permeability” of the sediment. In general, larger sized sediments have higher permeability than finer-grained materials. When oil moves into the sediment matrix, it is “deposited”, and when it is moved out by water, this is known as “hydraulic flushing”. The ease with which oil moves in or out is called the “oil hydraulic conductivity”. Similarly, water also moves in and out of the pore spaces and is termed the “water hydraulic conductivity”. While permeability is a function of the sediment matrix, the hydraulic conductivity depends on the pore space geometry, the fluid, and the content of that fluid in the pore space. Thus, oil and water do not behave the same way in the pore space.

Oil present in a shoreline is amenable to processes of “biodegradation”, which refers to the natural breakdown by microorganisms into more elementary compounds. Biodegradation occurs provided the necessary conditions to promote it are met, and it can occur either in aerobic conditions (in the presence of oxygen) or anaerobic conditions (in the absence of oxygen). Typically, substantial biodegradation requires the presence of certain mineral conditions for the microorganisms to flourish. A key piece of information for decision-making is the rate of biodegradation (i.e., how fast oil components break down). For instance, it would be important for a manager or planner to know whether a component will deplete to 10% of its original mass in one month or in 10 years.

To accelerate the rate of natural biodegradation, decision makers can consider “bioremediation” as a standalone or supplemental response action. Two major categories of bioremediation are “biostimulation”,

which refers to the addition of nutrients or oxygen to stimulate indigenous oil-degrading microorganisms, and “bioaugmentation”, which refers to the addition of microorganisms with desirable catabolic traits.

2 Beach Hydraulics

When an oil spill reaches a shoreline, it tends to penetrate into a beach (e.g., enter into the exposed sediment matrix) as the tide recedes from high to low tide. Vegetation and small sediment size, such as in a wetland, tend to prevent the initial penetration of oil into the sediment matrix. However, over time if the vegetation begins to weaken and fall over, die off, or get removed through physical processes, the oil could come into direct contact with the sediment and begin to penetrate. In addition, the movement of water laden with dissolved compounds such as nutrients and oxygen has a direct influence on the biodegradation of oil present in the same beach. Therefore, the biodegradation of oil is influenced by four major factors:

1) Beach permeability, determined by the size of the sediment particles (coarse sediments such as gravel) tends to be more permeable than finer sediments such as clays.

   Beaches can be made up of a combination of clay (<4 micrometer), silt (4 to 60 micrometer), sand (60 micrometer to 2 mm), or larger aggregate materials. For example, beaches in the Gulf of Mexico are often publicized for their fine-grained, “playground” sand, or even “sugar grain” textures. Alternatively, beaches in northern latitudes are usually made up of granule (2 to 4 mm) and pebbles (4 to 64 mm), interspersed between cobbles (64 to 256 mm) and boulders (>256 mm) (Hayes and Michel, 1999; Owens et al., 2008). Beach permeability generally increases with the average size of the sediments, thus for a given tidal range, oil and water would penetrate deeper into a gravel beach than into a sandy or clayey beach. Furthermore, beaches can range from highly heterogeneous in permeability to relatively homogenous. The heterogeneity could result from (apparent) random permeability zones or from large zones each with a different permeability. Examples of the latter include beaches in Prince William Sound, where the permeability is higher in the top 0.10 to 0.30 m, and decreases sharply with depth (Li and Boufadel, 2010; Xia and Boufadel, 2011).

2) Tides, waves, and the landward aquifer.

   The tidal range (vertical distance between the high and low tide levels) plays an important role in beach hydraulics. As the tide rises, the water table rises relatively fast in response. However, as the tide drops, the water table tends to lag behind (Philip, 1973), and could result in the formation of a seepage face (Naba et al., 2002 and citations therein). In other words, the beach fills much faster than it drains. When averaged over a tidal period, the mean water table within the beach is higher than the mean sea level, causing a persistent seaward hydraulic gradient in beaches. In terms of exchange flux, most of the seawater that enters the beach occurs near the high tide line. Conversely, most of the pore water leaves the beach near the low tide line (Boufadel, 2000). These concepts are illustrated in Figure 1.

   In addition to the tides, waves create two seaward hydraulic gradients in a beach (Riedl et al., 1972). A steep hydraulic gradient exists in the swash zone (region between run-down and up-rush) while a mild hydraulic gradient exists as one moves landward (Figure 2). Consequently, the pore water that is landward of the swash zone moves slowly seaward until it reaches the swash zone where it moves rapidly. Boufadel et al. (2007) conducted tracer studies and found that the superposition of waves and tides reduced the residence time of a tracer plume by about one third in comparison to the tide-only case. The effect of buoyancy (due to the presence of freshwater in a saltwater environment) on beach hydraulics is typically negligible in comparison with tide and waves (Li and Boufadel, 2010).

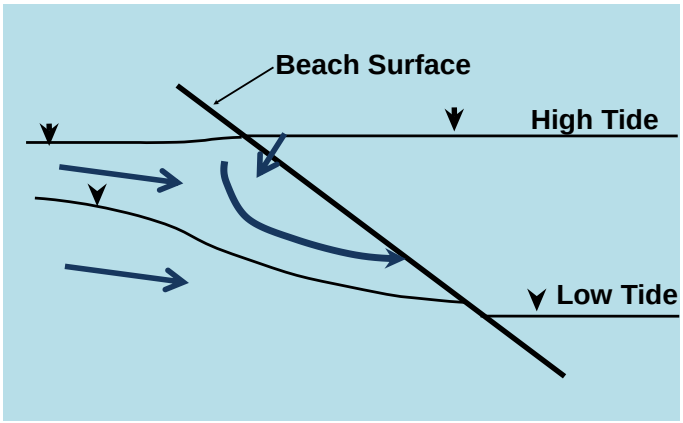

Figure 1: Water table movement due to tidal forces. The beach fills up with seawater near the high tide line and drains (mostly) near the low tide line. Influx of fresh groundwater (recharge) from the land side could replenish the beach with water, especially during low tides.

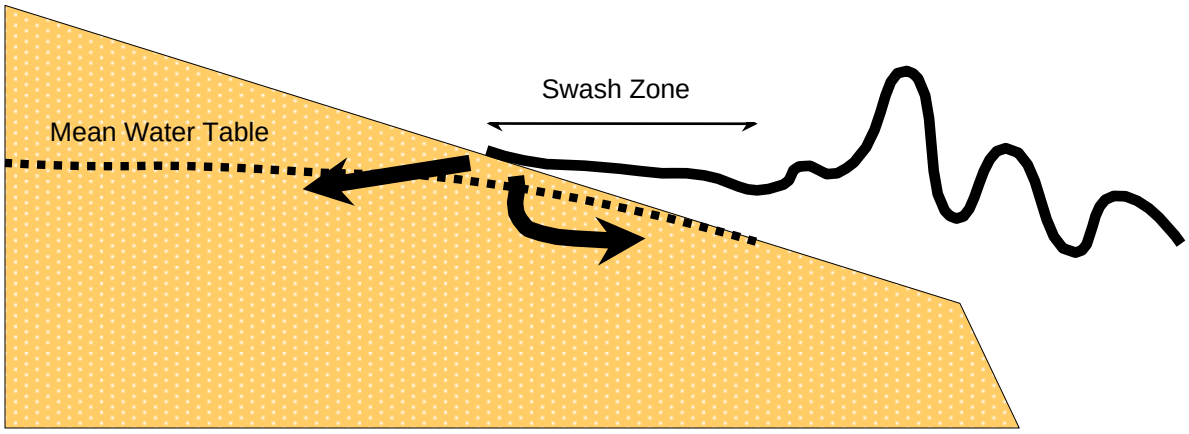

Figure 2: Waves running up a beach. The effect of waves on the beach water table is to create two zones with two different hydraulic gradients. The gradient is steep in the swash zone, and becomes mild landward of it.

For beaches with a considerable transition into a landward feature, fresh (non-saline) inland groundwater can flow from the landward side and mix with the saline seawater at the beach. However, low permeability (e.g., rock, peat, clay) formations landward of the beach can create hydraulic barriers with the regional aquifer thus reducing the recharge of the beach water table with fresh groundwater. Therefore, in such beaches, hydraulic flushing from the landward portion of the beach is generally limited. This influence could be inferred through measurements of salinity in the pore water within beaches compared to values found in open seawater.

Figure 3 provides an illustration of the initial distribution of oil in a shoreline. In general, the deposition of oil is highest (on a mass of oil per mass of sediments basis) in the upper intertidal zone due to the large drop in the water table associated with that location. The mass of oil within the sediments decreases when one moves seaward (from upper to mid- to lower intertidal zones), which is due to the smaller water table drop as one moves seaward. Oil could also reach the supratidal zone (i.e., the zone landward of the intertidal zone) due to the action of waves resulting from storms (e.g., storm surge). Once deposited onto the supratidal zone, the oil could penetrate there as well (Hayes and Michel, 1999; Owens et al., 2008) and/or get covered by sediments from subsequent storm waves (OSAT2, 2011). However, the concentration of oil deposited into the supratidal zone tends to be lower

than that of the oil deposited into the intertidal zones. Oil can also bind to sediments forming the so-called oil-mineral-aggregates (OMA), which was observed to accelerate oil biodegradation (Owens and Lee, 2003).

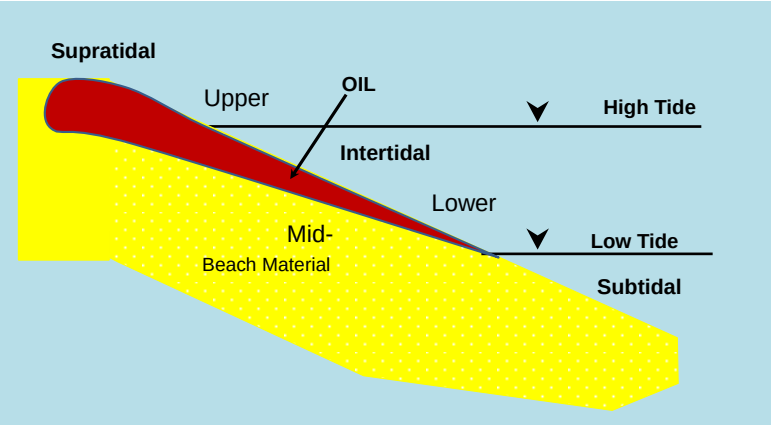

Figure 3: Initial oil distribution following a spill.

3) Depth of the high moisture zone (capillary fringe) within the beach during low tides.

Another important factor in beaches is the height of the capillary fringe, the zone of considerable water moisture above the water table where 75% or more of the porosity is water-filled (Bear, 1972; Boufadel, 1998). In this zone (Figure 4), capillary forces cause water to rise into the matrix (also known as capillary rise) where the height is also determined by the particle sizes. In beaches made up of coarse-grained material, the capillary rise is small (a few centimeters). In beaches with fine textured particles, the capillary fringe could be up to 2.0 m high (Van Genuchten, 1980).

Figure 4: The capillary fringe (dark color) reflects the presence of high moisture content (more than 75% of the porosity).http://wps.prenhall.com/wps/media/objects/5309/5437119/Figures/chapter06.htm

Monitoring only the water table without accounting for the capillary fringe could result in an overestimation of the oil holding capacity of the beach since beaches with large capillary fringes would not allow oil to penetrate deeply into them. Similarly, when the water table drops, little water is released so these beaches also do not allow oxygen from the atmosphere to penetrate deeply into them either.

4) Oil characteristics - fluidity (viscosity), density (oil being lighter than water tends to float), composition of SARA, and biodegradability all influence how oil initially, and over time, interacts with the soil/sediment, water, microorganisms, and nutrients in the beach.

   Light oil, which generally has high fluidity (i.e., low viscosity), would penetrate deeper in beach sediment than heavier oil or an emulsion of the oil, both of which typically have high viscosity. In addition, oil tends to float on top of the capillary fringe, whose elevation changes with the tidal water table. Thus, the depth of oil penetration also depends on the range of depths of the water table between high and low tides. During rising tides, a portion of the oil floats back to the open water while the remaining portion gets entrapped within the beach due to attraction forces (adhesion and capillarity) between the sediments and the oil. Thus, the net effect of tide is to cause the oil to be deposited in the intertidal zones.

   Under favorable conditions, the saturates and aromatics are amenable to biodegradation while the resins and asphaltenes biodegrade extremely slowly. Biodegradation of oil is covered in more detail in Section 3.

Although the initial oil deposition tends to be skewed to give more oil mass in the landward portion of a shoreline, the landward zones get flushed more vigorously by natural processes, and the flushing tends to replenish the nutrients and oxygen more rapidly in comparison with the mid- to lower intertidal zones. Therefore, oil in the upper intertidal zone tends to deplete faster than the oil in seaward zones due to natural hydraulic flushing and biodegradation. This attenuation which depends on the zone of a shoreline was noted in gravel beaches of Prince William Sound that contained lingering oil from the *Exxon Valdez* oil spill (Short et al., 2004; 2007).

In terms of developing a plan for applying a bioremediation solution or water onto the beach, the available pore space should be considered; otherwise, applying any water or solution may result in it running off into the sea (called “washout”). For example, a beach with a capillary fringe of 1.0 m would not imbibe a bioremediation solution if the water table is shallower than 1.0 m since the majority of the pore space would already be water- or oil-filled and unable to be easily replaced by the solution. Furthermore, when averaged over a tidal cycle, the net effect of the waves is to accelerate the washout of chemicals applied onto the beach (Wrenn et al., 1997a; Wrenn et al. 1997b). Given these complexities, a strong understanding of the conceptual model of an impacted shoreline is needed to better design, apply, and monitor bioremediation solutions.

3 Oil Biodegradation

Figure 5 illustrates the potential oil biodegradation pathways. Oil biodegradation can occur either in aerobic or anaerobic conditions. In aerobic environments, microorganisms respire oxygen while in anaerobic environments, microorganisms use other terminal electron acceptors (such as nitrate, sulfate, ferric iron, etc.). Under both conditions, the microorganisms extract energy from oil to build biomass. These processes depend on the availability of essential nutrients and other environmental factors (discussed further in Section 4).

The components of oil biodegrade at different rates. In general, the rates are fastest for normal alkanes, followed by branched alkanes, single-ring aromatics next, then polycyclic aromatic hydrocarbons (PAH) in increasing number of rings, and the cyclic alkanes. The isoprenoids, such as pristane and phytane, have been reported recalcitrant over durations of months (Lee and Levy, 1989; Bragg et al., 1994; Venosa et al., 1996). Finally, the biomarkers such as hopane and stigmastane are considered non-biodegradable.

If electron acceptors, nutrients, microorganisms with selected catabolic traits, and other environmental factors are optimal, the alkanes and aromatics can biodegrade considerably within 1 to 9 months (Haines and Alexander, 1974; Venosa et al, 1996; Boufadel et al., 1999; Du et al., 1999; Venosa et al., 2010).

However, as oil biodegrades, it becomes “weathered” where the composition shifts to contain an increasing number of degradation byproducts. Furthermore, the increased presence of alkylated groups on a compound biodegrade less rapidly than less alkylated ones. With this in mind, monitoring only the U.S. EPA’s 16 listed priority PAH pollutants may in some cases prove insufficient to fully characterize an oil spill.

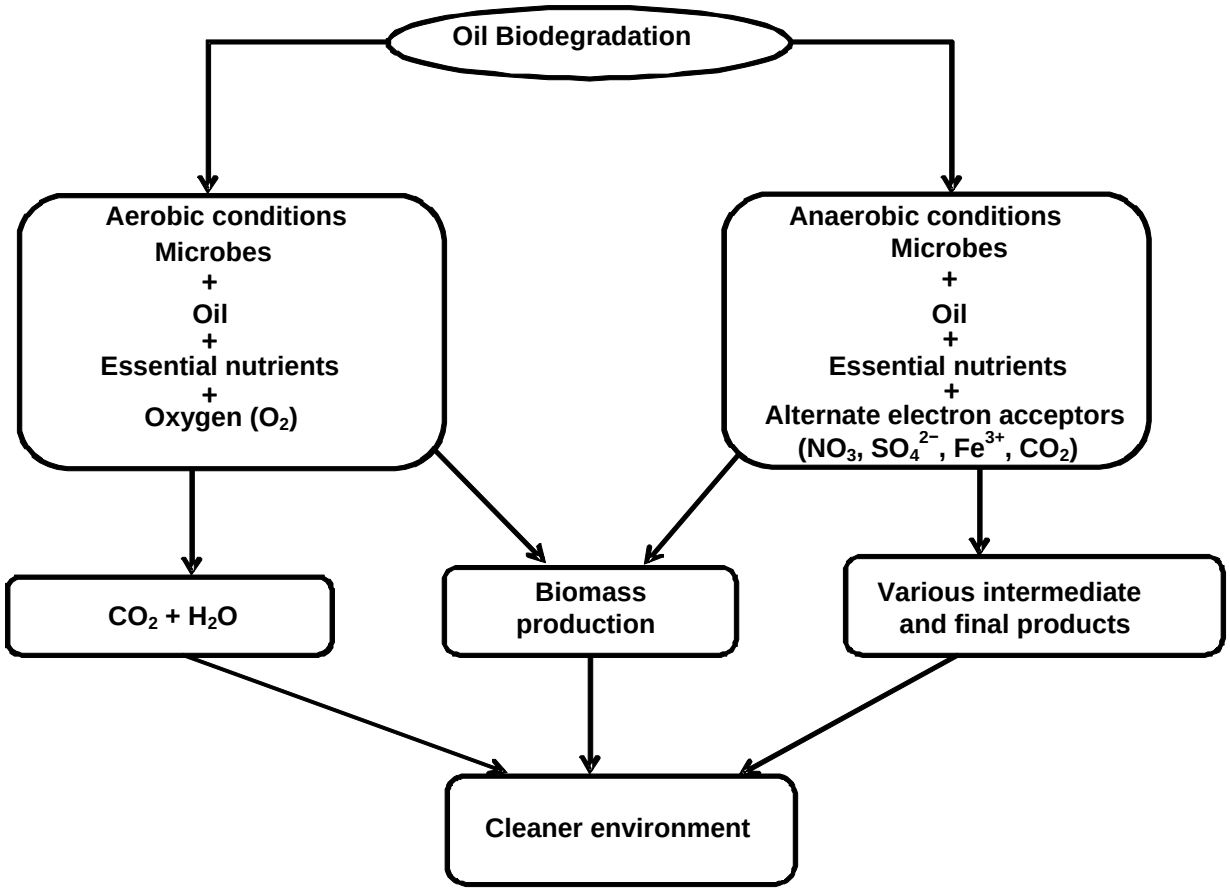

Figure 5: Potential aerobic and anaerobic oil biodegradation pathways. Shaded areas indicate aerobic oil biodegradation which is faster than anaerobic oil biodegradation. Aerobic oil biodegradation is the desired pathway in terms of practical bioremediation efforts of oiled beaches.

Due to the chemical complexity of crude oils, interspecies interactions often play a major role in in their biodegradation (McGenity et al., 2012). The complete oxidation of hydrocarbons to CO_2 and H_2O does not always occur. Aerobic hydrocarbon biodegradation can lead to numerous oxyhydrocarbons (Aeppli et al., 2012), and many organisms can be involved in the release of oxyhydrocarbons from the incomplete oxidation of measurable amount of PAHs (McGenity et al., 2012). It has been demonstrated that mixed cultures drive faster aerobic hydrocarbon biodegradation in comparison with a single species isolated form a consortia (see McGenity et al., 2012 for a review). Thus, multiple species interactions need to be considered during aerobic oil biodegradation processes.

Oil biodegradation involves the mass transfer of the oil components to a water interface where the different microorganisms reside. Therefore, oil biodegradation tends to increase with the interfacial area between the oil and water. Without solubilizing or mixing the oil components into dispersion in water, the water-bound microorganisms will have difficulty accessing the bulk of the oil for consumption. Heavier oils tend to be more viscous which further limits the bioavailability to microorganisms. However, some microorganisms produce extracellular biosurfactants-sometimes called pseudo-solubilization factors (Nicol

et al., 1994; Desai and Banat, 1997). These biosurfactants promote the emulsification of hydrophobic hydrocarbons to facilitate their transport into the hydrophilic intracellular space of microorganisms (Desai and Banat, 1997; Southam et al., 2001). Taken together, the composition of the oil, degree of weathering, and dissolution or emulsification greatly affect oil biodegradation potential.

In order to assess oil biodegradation, the concentrations of specific oil components, or a more all-inclusive parameter called total petroleum hydrocarbons (TPH), are measured as a function of time. The primary advantages of TPH measurements are that many of the methods are relatively simple, do not require specialized equipment or expertise, and depending on the method that is selected, may provide a measure of the total oil mass in the sample. Disadvantages of using TPH include the results between methods may not be comparable, they are severely affected by spatial heterogeneity, and are subject to interference by biogenic (i.e., naturally-occurring organic) compounds (Zhu et al., 2004) unless the extraction procedure applied (e.g. silica gel column) effectively removes biogenic compounds from the oil-contaminated samples (Wang et al., 2012).

For measuring the concentrations of specific compounds, gas chromatography (GC) is the preferred method. GC can be coupled to either flame-ionization detection (FID) or mass spectrometry (MS). GC-FID is typically used to measure the concentration of normal and branched alkanes (Douglas et al., 1994; Prince et al., 1994) whereas GC-MS is used for measuring the concentrations of aromatics and biomarker compounds, such as the hopanes, steranes, and cholestanes (Douglas et al., 1994; Venosa et al., 1996; Wang and Fingas, 1997, 1998; Short et al., 2004).

Biomarkers are compounds that biodegrade very slowly within the time frame of interest and have been used to evaluate the effectiveness of biodegradation. Specifically, the main metric for evaluating effectiveness is the percent biodegradation through time as given in the equation below (Bragg et al., 1994; Venosa et al., 1996). In this equation, the concentration of the compound of interest, A, has been normalized to the concentration of a biomarker, H.

$$\text{percent biodegradation} = \left[1 - \frac{\left(\frac{A}{H}\right)_t}{\left(\frac{A}{H}\right)_o}\right] \times 100 \quad (1)$$

Typically, C30-17α(H),21β(H)-hopane has been used (Bragg et al., 1994; Prince et al., 1994; Venosa et al., 1996), but more recent studies have demonstrated that this compound can eventually be biodegraded in the laboratory and in the field (Wang et al., 2001; Frontera-Suau et al., 2002; Watson et al., 2002; Atlas and Bragg, 2007). Some steranes (e.g., C29-αββ-stigmastane) may be more resistant to biodegradation than hopane (Wang et al., 2001; Frontera-Suau et al., 2002; Watson et al., 2002). Atlas and Bragg (2007) argued for usage of stigmastane instead of hopane. Alkylated PAHs, such as C2-chrysene and C3-chrysene have been also used as the recalcitrant compound (Lee et al., 1997).

If only GC-FID is available for the analysis of oil components, the ratio of C17/pristane and C18/phytane (Lee and Levy, 1987; 1989) can be used when applying Eq. 1, particularly in the early stage of the biodegradation processes. However, the approach is not as rigorous as using biomarkers. In this approach, the ratios of heptadecane (C17) to pristane (tetramethylpentadecane; C19) and octadecane (C18) to phytane (tetramethyl hexadecane; C20) can be used to determine whether concentration changes observed in oil-contaminated environments are due to biodegradation or physical transport. This is based on differences between the biodegradation rates of normal alkanes (e.g., heptadecane and octadecane) and branched alkanes (e.g., pristane and phytane). In general, normal alkanes are degraded more quickly than branched alkanes. So, C17/pristane and C18/phytane would tend to decrease as biodegradation

proceeds. Because pristane and phytane are both biodegradable as well, this method is generally only useful in the early biodegradation stages, and is less rigorous than normalizing by biomarkers.

It is important to note that the normalization discussed herein could provide incorrect results if the oil is subjected to considerable evaporation and photooxidation during biodegradation, as these processes could greatly affect the concentration of low biodegradability compounds, such as the biomarkers, and pristane and phytane.

Numerous models (e.g. BIOWIN in EPI Suite software) can be used to estimate biodegradation (Aronson et al., 2006). In order to represent the overall attenuation rate of oil as a function of time, it is common to use a simple decay model such as:

$$\frac{C}{C_o} = \exp(-k \cdot t) \tag{2}$$

Where C and C_o are normalized concentrations of an oil component at times t and t_o; the term "k" is known as the decay constant (1/day) and is specific to the oil component being evaluated. Values of "k" are reported in alphabetical order in the Appendix in Table 1 for different alkanes and in Table 2 for different aromatics. With a given value of "k", one could estimate the time it takes for the decay of 90% of the initial concentration (i.e., 10% of the initial concentration is remaining) according to the following formula:

$$t = \frac{2.3}{k} \tag{3}$$

For example, for phytane under natural conditions, Table 1 gives k=0.0125 day^{-1}, which results in t=184 days. Thus, it takes around 6 months for phytane to decrease to 10% of the initial value under natural attenuation. In comparison, Table 1 also gives a decay constant of k=0.0212day^{-1} for the biodegradation of phytane with nitrogen supplied at 6.4 mg/L. Using this value, the biostimulation approach decreases the time to 108 days or approximately 3.5 months. While the values in Tables 1 and 2 were obtained under specific conditions, and should therefore not be used indiscriminately, they should provide general guidelines for estimating biodegradation times.

In some cases, a biodegradability index (BI) could be calculated to assess the biodegradation potential. This is shown in the equation below where BOD_5 is the biochemical oxygen demand of the oil at 5 days and ThOD is the theoretical chemical oxygen demand, representing the amount of oil that oxidizes by both biological and chemical reactions:

$$BI = \frac{BOD_5}{ThOD} \tag{4}$$

A large BI (>0.5) indicates that the oil is highly biodegradable while a small BI (<0.05) represents an oil whose potential for biodegradation is small. When determining this index, the oxygen demand values should be based on the oil in the environment, not based on fresh oil. Furthermore, if possible, the oil in the environment should be separated from the biogenic material in the sediments in order to prevent erroneous estimations of the BI that are based on the biodegradation of both oil and biogenic material. In this case, laboratory procedures developed for "cleaning up" a sample of the biogenic material should be employed prior to analysis. However, these can introduce significant errors; thus, it is best to use this index to answer qualitatively questions such as whether the spilled oil is biodegradable or not? Both BOD and COD (the chemical oxygen demand component of ThOD) are measured exogenously using material collected from an impacted shoreline and conducted in a laboratory.

4 Measuring Environmental Factors of Biodegradation

Whether to assess natural biodegradation or the effectiveness of bioremediation, several field measurements are recommended. Oil concentrations are the primary measurements, but it is advisable to measure several additional environmental factors that influence oil biodegradation. These factors are also affected by oil biodegradation and can thus be used to help predict the future extent of the biodegradation. These additional measurements include:

- Oxygen concentration
- Nutrients concentrations such as nitrate, ammonium, and phosphate.
- Salinity, pH, and temperature
- Moisture content
- Microbial community

These factors, discussed in further detail below, are measured in samples taken from the water table, pore water in the sediment, and the sediment. Furthermore, these measurements would need to be conducted, when possible, at a minimum of four locations: the lower intertidal zone, the middle intertidal zone, the upper intertidal zone, and the supratidal zone of the beach (as indicated in Figure 3). For oil located in the supratidal zone, an additional location would be landward of that zone. Also, when possible, additional measurements at un-oiled sites with similar geomorphic properties to the oiled sites should be taken to serve as control. Similarly, samples taken before an oil impacts a beach are also desirable in order to provide baseline information. However, this is not always possible depending on the spill scenario.

For measuring the variations in the water table with time, as discussed earlier in Section 2, pressure transducers can be placed into slotted or perforated pipes installed into the beach down to depths below the low tide at that location. These measurements could also be used to assess the moisture in the beach and to predict the horizontal fluxes of pore water. Furthermore, the sensors used to measure water table changes could also provide the depth-averaged water temperature and salinity (see for example, Li and Boufadel, 2010).

Other parameters can also be measured through the collection of water samples from the wells. Specifically, multiport sampling wells which consist of a stainless steel well casing with screen-covered holes drilled at several locations (Figure 6) could be installed with care to dig holes as large as nccessary to allow depth-discrete sampling of the water. At each of these holes, separate stainless steel tubes are welded on the inside of the well casing (labeled A, B in Figure 6), allowing one to take samples from each depth using a syringe attached to Luer fittings connected to the top of each tube (Boufadel et al., 2010). Alternatively, using a peristaltic pump, water could be pumped from the ground and through a sealed flow cell (Figure 7). Inside of the flow cell, electrochemical or optical oxygen probes can be installed. The sample container is filled from the bottom upwards to prevent the sample from being exposed and compromised by atmospheric oxygen (Boufadel et al., 2010). Colorimetric test kits are also available (e.g. http://www.chemetrics.com/) for rapid measurements of dissolved oxygen (1-12 ppm) using the Indigo Carmine method.

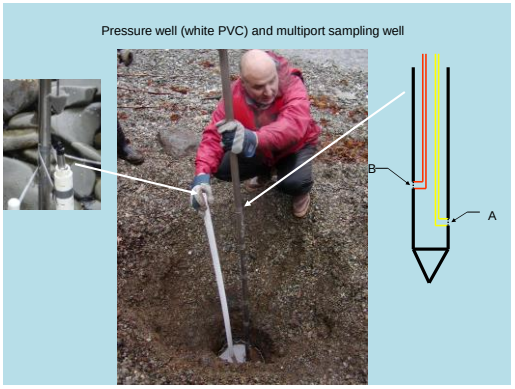

Figure 6: Pressure sensor in the beach (in the white pipe) (left inside) along with a multiport sampling well (right inside).

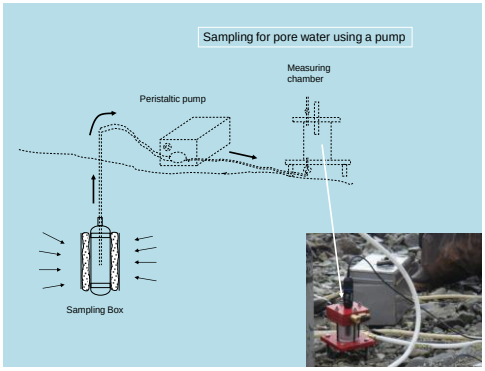

Figure 7: Pumping water by a peristaltic pump through a measurement chamber. Also shown (inset) is an optical probe (black vertical probe in the red measuring chamber) used to measure dissolved oxygen.

Other systems, such as dialysis cells, commonly called diffusion samplers or “peepers” (Laforce et al., 2000), could be used to sample the pore water. These cells use permeable membrane enclosures filled prior to installation with deionized water. They are then installed into wet sediments, left in place for a sufficient time (typically a week), and then removed for analysis. The basic principle is that the water contained in the cells equilibrates with the pore water in the surrounding sediments; thus reflecting the concentration of constituents from those sediments at the corresponding depths. These can be used to quantify the pore water concentrations of dissolved oxygen, nutrients, salinity, and pH.

For sediment sampling, coring should be conducted when possible to obtain an intact core that allows one to map the vertical distribution of oil (Figure 8). Otherwise, one would need to excavate a pit (Figure 6), and take samples from the walls of the pit to avoid vertical mixing of sediments. Sediment samples would be used to obtain the grain size distribution and the hydraulic permeability of the sediments (as discussed in Section 2), microbial community, chemical oxygen demand, and TKN. Sediment samples could be also used to characterize the capillary fringe by conducting a capillary-retention experiment (Bear, 1972; Boufadel et al., 1998).

Figure 8: Core sample in a transparent sleeve obtained from the supra-tidal zone (i.e., landward of the high water mark). The discoloration at approximately one third from the top represents oil deposition**.**

4.1 Oxygen

Although anaerobic biodegradation of petroleum hydrocarbons has been reported over the past 20 years (e.g., Ackersberg et al., 1991; Coates et al., 1997; Caldwell et al., 1998; So and Young, 2001), the rates are relatively slow compared to those commonly observed in the presence of oxygen under comparable experimental conditions (e.g., Bauer and Capone, 1988; Prince et al., 1994; Berthi-Conti and Bruns, 1999). In addition, hydrocarbons from marine oil spills have persisted for decades in anoxic sediments (e.g., Corredor et al., 1990; Wang et al., 2001; Reddy, 2004; Peacock et al., 2005). In carefully-controlled laboratory studies, the dissolved oxygen (DO) concentration needed for the biodegradation of hydrocarbons in open water has been suggested to be as low as 0.1 mg/L (Michaelsen et al., 1992; Berthe-Conti and Bruns, 1999, 2001). However, results from field studies suggest that a concentration greater than 1.5 mg/L in the pore water is needed for the efficient biodegradation of oil (Borden et al., 1989; Chiang et al., 1989; Li and Boufadel, 2010).

These observations also suggest that particular attention needs to be paid to the concentration of DO within the sediment pore water, which could be measured using various methods. The Winkler method can be very accurate for extremely low DO concentrations (APHA, 2005) but requires considerable analytical skill and will most likely be difficult to conduct in the field. In general, measuring dissolved oxygen in the field is challenging because care must be taken to prevent contamination of the sample by atmospheric oxygen, and the sample must be analyzed as quickly as possible to preclude concentration changes due to biological activity (APHA, 2005). Electrochemical probes are commercially available and can be placed into the beach, but require good mixing or rapid flow past a membrane surface contained within the probe (Snoeyink and Jenkins, 1980). Similarly, optical probes (Thermo Scientific, 2008) can be used on stagnant water, and are thus more versatile. While both of these probes are relatively easy to use in the field, their accuracy depends upon proper calibration and maintenance. Details on their usage on oil spills can be found in Boufadel et al. (2010). However, as mentioned earlier, colorimetric test kits are also available (e.g.http://www.chemetrics.com/) for rapid measurements of dissolved oxygen (1-12 ppm) using the Indigo Carmine method.

4.2 Nutrients

Nutrients such nitrogen and phosphorus compounds are needed for microbial activity. Nitrogen and concentrations can be measured using colorimetric test kits (e.g. http://www.chemetrics.com/) using the Cadmium or Zinc Reduction Methods. Phosphorus can be measured using the Vanadomolybdophosphoric Acid Method or The Stannous Chloride Method. Evidently, water both nitrogen and phosphorous compounds can be measured in an analytical laboratory using standard ion chromatography methods (Michalski, 2006) from samples collected at a site.

From a theoretical point of view, the conversion of 1.0 g of hydrocarbon to cell material requires 0.150 g of nitrogen and 0.030 g of phosphorus (Rosenberg and Ron, 1996). However, maintaining these ratios remains a challenge in the field due to the potential for washout of nutrients to sea (see Section 2.0 Beach Hydraulics).A more practical approach has been to maintain the concentration of the limiting nutrients measured in the pore water (Bragg et al., 1994; Venosa et al., 1996; Boufadel et al., 1999). Several studies suggest that nitrogen concentrations between 2 and 10 mg/L are required to support maximal rates of oil biodegradation (Venosa et al., 1996; Boufadel et al., 1999; Du et al., 1999). A similar concentration limit has not been established for phosphorus, but N:P ratios of about 10:1 on a mass basis typically give good results (Atlas and Bartha, 1973; Smith et al., 1998; Oh et al., 2003; Garcia-Blanco, 2004; Zahed et al., 2010).

4.3 Salinity, pH, and Temperature

Under natural conditions, neither salinity or pH are expected to play significant roles in the biodegradation of oil in a beach environment. Indigenous microorganisms are acclimated to the water environments in

which they evolved. This is no different for oil degrading microorganisms in a saline environment. However, salinity could be used to infer the magnitude of fresh groundwater recharge and the washout of applied water soluble chemicals to sea. Similarly, seawater has a pH around 8.0 whereas hydrocarbon biodegradation is practically unaffected at pH conditions between 6 and 9 (Atlas and Bartha, 1992). The optimal pH range for biodegradation is reported to be slightly alkaline water with a pH between 7 and 8.5 (Zhu et al., 2001; Saadoun and Al-Ghzawi, 2005).

Microbes are also well adapted to different temperature environments, including extremely low temperatures (Delille et al., 2009; McFarlinet al., 2011). Oil biodegradation has been shown to occur by psychrophilic (cold-loving), mesophilic, and thermophilic (heat-loving) organisms. However, low temperatures might make the alkanes more “waxy”, decreasing their biodegradation rate relative to the aromatics (Campo et al., 2013). Low temperatures could also reduce the flux of nutrients from slow-release fertilizers due to a reduction in permeability (Lee et al., 1993). However, there have been successful demonstrations of biodegradation in the Arctic where temperatures were quite low (Prince et al., 2003).

In order to measure salinity (as conductivity), pH, and temperature, standard field probes are commercially available including multi-parameter instruments. Furthermore, these instruments can be deployed to collect information semi-continuously (e.g. datalogged) or at discrete times by a field team.

4.4 Moisture

Moisture content is a measure of the water-filled vs. air-filled pore spaces in a soil matrix. Since microorganisms and dissolved nutrients reside together within the aqueous environments of a beach, moisture content within the sediments is an important factor influencing the natural biodegradation of oil. Depending on the exchange of water and oxygen diffusion limitations in a sediment profile, a high moisture content could inhibit aerobic hydrocarbon biodegradation. Also, a low moisture content due to evaporation could cause nutrients concentration and salinity to damage soil microbes due to osmosis (Walworth et al., 1997).

Maximum biodegradation has been shown to occur at moistures varying between 30% and 80% of the pore space (Vaughan and Malcolm, 1985; Cole, 1994; Atlas and Bartha, 1993; Liu et al., 2001) depending on soil texture and type (Kooirevaar et al. 1983; Walworth et al. 1997). Fallgren et al. (2010) found that the optimum value is between 30% to 50% of the pore space. In laboratory experiments, Horel and Schiewer (2009) noted that a moisture saturation of 8% provided essentially the same biodegradation as that of 50% moisture saturation (100% is equal to the porosity). Tibbett et al. (2011) investigated the potential of moisture content and nutrient amendments to enhance the biodegradation of crude oil in sediments from a semi-arid island. They found that the greatest levels of CO_2 respiration resulted when the percent saturation was between 50% and 70%, suggesting increased biodegradation activities within this range of moisture.

Thus, it can be concluded that oil biodegradation occurs at a broad range of soil moisture, down to 20% of the porosity, which occurs typically in the supratidal zone of beaches.

4.5 Microbial Communities

Contamination of sediments with crude oil or refined petroleum products usually results in an increase in the relative proportion of hydrocarbon degraders in the microbial community (Atlas, 1995). Therefore, it may be useful to look for increased abundance of known or suspected hydrocarbon degraders to infer the initiation and progress of biodegradation following a release. This increase in hydrocarbon degraders is sometimes accompanied by a decrease in the phylogenetic diversity of the microbial population, especially in laboratory microcosms (Roling et al., 2002; Evans et al., 2004). However, this reduced microbial diversity has not been consistently observed in field studies (MacNaughton et al., 1999; Roling et al., 2004). Recently, a study conducted during the Deepwater Horizon oil spill observed dramatic reductions in

the diversity of eukaryotic microorganisms (Bik et al., 2012). These investigators reported eukaryotic microbial communities that were dominated by fungi (including hydrocarbon degraders) in oil-contaminated sediments, whereas unoiled sediments and sediments collected before oil contamination consisted of a diverse assemblage of nematodes and other metazoans.

Sediment samples properly collected for microbial analysis could be used to measure the microbial population through various techniques including:

1) Growth-dependent characterization using the most-probable-number (MPN) methods to estimate the numbers of heterotrophic bacteria, alkane degraders, and PAH degraders (Wrenn and Venosa, 1996).

2) Growth-independent characterization methods such as the measurement of lipid phosphate to estimate the total size of the microbial population (Findlay et al., 1992; Boufadel et al., 1999) and analysis of the community metagenome and metatranscriptome (Hazen et al., 2010; Simon and Daniel, 2011; Bomar et al., 2011; Valentine et al., 2012).

The advantage of the MPN methods is that they assign a concentration value for the surveyed microorganisms. The disadvantages include using the time needed for microbial growth in the lab (around 21 days for aromatic degraders), and the fact that microorganisms that grow in the lab represent a small fraction (5% to 50%) of the total population in the field (Herbert, 1990; Wrenn and Venosa, 1996; Harris et al., 1998; Rittman and McCarty, 2001). Conversely, the molecular methods can provide a complete map of the microbial population and activity, providing insights into the effectiveness of bioremediation of oil contaminated sediments. Specifically, polymerase chain reaction (PCR) amplification of gene fragments encoding specific enzymes that catalyze key steps of oil biodegradation and subsequent hybridization with gene probes can be used to test the suitability of bioremediation (Meyer et al., 1999). In addition, the combination of phospholipid fatty acid analysis(PLFA) of the lipid profiles of microorganisms and the nucleic acid-based denaturing gradient gel electrophoresis (DGGE) for fingerprinting the 16S ribosomal deoxyribonucleic acid (rDNA) carried by all bacteria, is useful for identifying the composition of microbial communities responsible of oil degradation and assessing the end point of oil removal (MacNaughton et al., 1999). More recently, Adetutu et al. (2013) combined PCR-DGGE with chemical approaches of hydrocarbon extraction and analysis to assess the potential and predict the end-point of hydrocarbon biodegradation. The main disadvantages of molecular methods include high cost and the lack of a direct relation between them and oil biodegradation rates. However, both of these problems are vanishing due to increased use.

Although microbial growth could help infer about the progress of biodegradation, real time measurements of O_2 and nutrients as discussed above are more useful in terms of rapid adjustment as necessary for stimulating oil biodegradation in response to a spill.

5 Monitored Natural Attenuation

Monitored Natural Attenuation (MNA) may be used as an alternative or follow up to active remediation for contaminated shorelines. MNA relies on a combination of physical, chemical, and biological processes to achieve site-specific cleanup goals without human intervention (EPA, 1999; Brauner et al., 2004). The processes that contribute to natural attenuation include biodegradation, biological stabilization, chemical oxidation or reduction, sorption to soil or sediments, volatilization, dispersion, advection by waves to the open water, or dilution. EPA guidance (EPA, 1999) suggests that destructive processes (e.g., biodegradation) are preferable to processes that merely reduce the concentration of contaminants (e.g., dispersion) or transfer them to another physical compartment (e.g., advection or volatilization).

MNA could be selected based on a risk-based corrective action (RBCA) process (ASTM, 1995) or implemented after completion of active remediation. The potential advantages of MNA include generation of a smaller volume of waste and being less intrusive than active remediation. Because MNA allows contaminants to be degraded in place, it may be less likely to mobilize them or introduce new exposure

pathways. In addition, MNA is not limited by equipment availability, down time, scale of implementation, or access to the contaminated area. However, as MNA relies on natural processes, the time required to meet site-specific cleanup goals may be longer than that would be achievable by active remediation. The slow rate of cleanup requires extensive long-term monitoring to demonstrate that contaminant migration is not occurring and progress is being made toward achieving the cleanup goals. In particular, the long-term monitoring program should ensure that toxic byproducts are not formed by the natural degradation processes and that the hydrological and geochemical conditions that enable those processes have not changed. Institutional controls (e.g., site access and use restrictions) may be required to ensure that people are not exposed to the contaminants before acceptable concentrations are achieved.

The decision to implement MNA should be supported by evidence that contaminant mass is being reduced by natural processes under *in situ* conditions, and that hydrogeological or geochemical data are consistent with the hypothesized mechanism for mass reduction (Brauner et al., 2004). If either of these lines of evidence is inconclusive, field or microcosm experiments may be performed to demonstrate that a competent microbial community exists in the contaminated sediments. Selection of MNA requires extensive site characterization and development of a conceptual site model, which should include definition of the source, identification of potential exposure pathways for human and ecological receptors, and consideration of all potential current and future receptors (Brauner et al., 2004). In addition, as beach hydraulics preclude one from treating the beach as a homogeneous unit, one could benefit from using a groundwater model for pore water flow to interpret the physical, geochemical, and biological measurements. Physical and biological heterogeneity of the contaminated shoreline will tend to increase the complexity of the conceptual site model and increase the information requirements for site characterization.

6 Bioremediation

Bioremediation has been defined as the “use of biological methods, usually involving microorganisms, to break down or neutralize contaminants in soil, water, and wastes” (http://www.usgs.gov/science/science.php?term=113). For an oil spill, this response action can be accomplished through the application of bioaugmentation and/or biostimulation agents (Zhu et al., 2001). In order for any bioremediation product to be used in the field, it would need to be included on the EPA National Oil and Hazardous Substances Pollution Contingency Plan (NCP). Bioremediation agents are listed on the NCP schedule (April 2013), which can be found at: http://www.epa.gov/oem/docs/oil/ncp/schedule.pdf.

Bioaugmentation relies on providing microorganisms that have been selected for their ability to rapidly degrade contaminants of concern. Although addition of exogenous hydrocarbon degraders is sometimes effective in laboratory experiments conducted in closed batch systems such as microcosms (Aldrett et al., 1997; Hozumi et al., 2000; McKew et al., 2007), this has never been shown to be effective in the field. In general, these exogenous microorganisms simply cannot outperform indigenous microorganisms or persist if they are introduced into a relatively foreign environment (Lee and Levy, 1987; Venosa et al., 1992, 1996; Lee et al., 1997; MacNaughton et al., 1996). In addition, there are concerns of safety and ecological damage due to the introduction of alien microorganisms into an ecosystem (Leahy and Colwell, 1990). Similarly, there are also public relations concerns when bioaugmentation is perceived by the public (often incorrectly so) as the release of “genetically engineered” organisms into the environment. A less controversial approach that has been done in the field by Venosa et al. (1996) is to cultivate microorganisms on hydrocarbons in the lab and return them to the same beach they were obtained. Although Venosa et al. (1996) reported no noticeable differences in oil biodegradation rates between oiled plots subjected to bioaugmentation and oiled plots subjected to biostimulation, there would be situations where such a form of bioaugmentation could be conducted. Examples include situations where microbial counts of hydrocarbon degraders within the beach are low and/or the washout of applied solutions from the contaminated zone is too rapid to allow sufficient microbial growth to support prolonged oil biodegradation.

Biostimulation involves addition of chemicals, such as mineral nutrients or oxygen that stimulate the growth of naturally occurring hydrocarbon degraders. Several different nutrient formulations have been used in field-scale bioremediation studies (Zhu et al., 2004). Biostimulation has been demonstrated to be successful in many controlled field studies (Zhu et al., 2001) and was used extensively in response to the *Exxon Valdez* oil spill (Prince and Bragg, 1997; Prince et al., 1994; Bragg et al., 1994).

- The most commonly available biostimulants are water-soluble nutrients found in common fertilizers. This is due to their low cost and wide availability (e.g., Lee and Levy, 1987; Venosa et al., 1996).

- Slow-release fertilizers (SRF) are nutrient products available normally in solid forms (granules) that consist of either relatively insoluble nutrients, water-soluble nutrients coated with hydrophobic materials such as vegetable oil and paraffin or sulfur compounds (Lee et al. 1993; Zhu et al., 2004). These fertilizers can be spread on the beach surface using common fertilizer spreaders, and then either watered or placed such that the rising tide submerges and slowly dissolves them into the seawater. The main advantage of the SRF is that they can be applied less frequently than water-soluble nutrients because the latter tend to washout quickly when the contaminated area is submerged by tides. However, if the SRF is placed in slow moving waters (e.g., supratidal zone in the absence of waves) and/or if the mass of SRF applied per surface area of a beach is small, then the resulting pore water concentrations would be too small to cause an increase in the biodegradation rate.

- Oleophilic fertilizers are designed to stick to oil-contaminated sediments and therefore, introduce, and make available, the nutrients right at the oil-water interface where hydrocarbon-degrading bacteria tend to grow (Rosenberg et al., 1992; Pritchard et al., 1992; Prince, 1993). However, no oleophilic fertilizer is currently on the EPA NCP schedule.

Several studies have been conducted on the effectiveness of each of these different biostimulants independently and to each other. Croft et al. (1995) compared the efficiency of a well-known oleophilic fertilizer to a slow-release inorganic fertilizer and found in their study that the oleophilic fertilizer was more effective at stimulating oil degradation than the slow-release product. Likewise, Sveum and Ramstad (1995) found that organic products such as fish meal and stick water (a fish meal byproduct) were also more effective than a slow-release fertilizer tested in their study. Regardless of the bioremediation agent, most of the successful field-scale applications have been conducted on shorelines with well-drained sediments. Bioremediation on shorelines with water-saturated sediments during low tides has been less successful (Venosa et al., 2002; Garcia-Blanco et al., 2007).

In terms of benefits and disadvantages, nutrient addition to oil contaminated wetland sediments has been shown to stimulate plant growth (Lin et al., 1999; Venosa et al., 2002). This tends to stabilize the wetland and reduce the extent of erosional loss from storms subsequent to an oil spill. On the other hand, although the nutrients added are only to meet the needs of microbial consumption, there is a remote risk for eutrophication with their application in closed embayment, which could lead to a concomitant stimulation of algal growth. This potential should be included in the evaluation process for response alternatives in closed embayments. Small enclosed bays with limited exchange with a large body of water would be more susceptible to eutrophication than an open, well-mixed coastline. However, no evidence for bioremediation-induced eutrophication has been observed, during the USEPA's nutrient test on the Valdez oil spill (Prince et al., 1994).

7 Techniques for Applying Bioremediation

Prior to applying bioremediation to oil-impacted beaches, mechanical techniques or hydraulic flushing should be used to actively remove as much bulk oil from the surface as possible. Surface washing agents could be used on rocky shorelines to cause the oil to dislodge from the rocks and subsequently collected. However, in most cases, all of the surface oil cannot be completely removed. Furthermore, in situations

where these techniques are not recommended based on a net environmental benefit analysis (NEBA), bioremediation may be an alternative to consider. Similarly, oil from an offshore release can also wash up onshore in the form of “balls” or “patties” that may or may not be encapsulated in a protective layer of dry oil or asphaltene (i.e., tar). If these oil remnants are in the form of “tarballs”, their physical removal can be accomplished using various manual or mechanical approaches (Owens, 2013). However, if they are not encapsulated, and water is able to penetrate into them, one could consider bioremediation as well.

For oil on or trapped in beach sediments, three options for delivering bioremediation solutions can be considered:1) application of a nutrient in solid form onto the beach surface (biostimulation only), 2) application of a liquid solution (biostimulation or bioaugmentation) onto the beach surface, or 3) subsurface delivery of an amendment (biostimulation or bioaugmentation). The required water to make up the solution of nutrients (and/or microorganisms) is typically obtained from the water body associated with the beach. Due to the dilution upon mixing with the existing pore water, the concentration of the applied solution should be high enough to ensure that the resulting concentration is larger than needed. Primarily, this will be based on measurements of the existing concentrations of nutrients (or population numbers for microorganisms), estimates of the moisture content with depth, and the contribution of the applied solution to attain target concentrations (e.g.,10 mg/L nitrogen,1 mg/L phosphorus). Given the variability, particularly with depth, a dilution safety factor of 10 to 100 should be adopted. This means the solution concentrations in the tank prior to applying onto the beach should be 100 to 1000 mg/L for nitrogen and 10 to 100 mg/L for phosphorus. For bioaugmentation solutions, the recommendations from commercial suppliers should be followed. The nitrogen compounds that are commonly used (nitrate, nitrite, and ammonia) are highly soluble in water (freshwater or saltwater). However, phosphorus compounds have typically with low solubility, especially in saltwater, which would limit the maximum concentration in the mixing tank. Details on the application of each method are discussed in subsections 7.1 to 7.3.

7.1 Application of Nutrients in Solid Form onto a Beach Surface

Nutrients in solid granular or prilled (pelletized) form could be spread onto the beach surface during low tide and, one could rely on the tide and/or waves to dissolve them into the water column, delivering them to the oiled zone. Slow release fertilizers (SRF) have been used extensively with a great success on the beaches of Prince William Sound to bioremediate the Exxon Valdez oil spill (Prince et al., 1994). However, for primarily addressing oil on the surface of the beach, the use of solid fertilizers is not recommended due to the high dilution that occurs at the beach surface compared to that in the pore water, and could lead to significant washout and issues such as eutrophication in the receiving water body.

The goal is to spread the solid nutrients as evenly as possible throughout the impacted zone. The main advantage of the approach is that it precludes the use of major hydraulic systems (tanks, generators, pumps) for mixing and pumping solutions, particularly at locations where access to the beach by major machinery is difficult. The disadvantages include:

1) For common fertilizers (i.e., not SRF), nitrogen compounds in inorganic fertilizers have high solubility and tend to generate solutions that are heavier than seawater (e.g. densities greater than 1.0mg/L). This density difference causes rapid sinking and an uneven distribution of the solution due to the formation of high water density regions, labeled “fingers”, in the sediment profile (Schincariol and Schwartz, 1994).

2) For common fertilizers, phosphate compounds have low solubility (Slomp and Van Cappellen, 2004) so they tend to stay behind on the surface until their concentration drops below the solubility limit. Thus, the 10:1 ratio of nitrogen to phosphorus desired for optimal biodegradation can be difficult to attain throughout the beach profile and results in inefficient usage of the nutrients.

3) For beaches subjected to a high wave activity, the SRF might not produce sufficiently high pore water concentration to enhance oil biodegradation.

7.2 Application of Amendment Solutions onto a Beach Surface

Solutions of common, water-soluble inorganic fertilizers and oleophilic fertilizers in liquid form, with or without microorganism, could be sprayed onto the beach surface during low tide. For addressing surface oil, oleophilic fertilizers are particularly recommended as dilution by the tide does not affect them greatly if they are in contact with the oil when the tide rises. A successful application of an oleophilic fertilizer took place on gravel beaches following the *Exxon Valdez* oil spill in Prince William Sound. The treated area appeared to be visibly cleaner than the untreated area (Pritchard et al., 1992). A similar study conducted in Brittany France showed that the surfactant in another oleophilic fertilizer accelerated oil biodegradation in comparison with the same oleophilic fertilizer without the surfactant (Le Floch et al., 1997 and 1999). These results are in agreement with laboratory studies which showed that chemically dispersing the oil can accelerate the biodegradation rate provided the toxicity limit of microorganisms is not reached (Venosa and Holder, 2012).

In general, if a sufficient volume of a bioremediation solution is applied onto the surface of a beach, the solution would begin propagating downward through “fingers” of high moisture content surrounded by volumes of low moisture content. This process takes place above the capillary fringe (Steenhuis et al., 1996). This transport of the solution proceeds until the solution reaches a high moisture zone (i.e., the capillary fringe) at which time the applied solution spreads laterally, and occupies the whole pore space. The solution volume applied should be large enough to insure inundation of the pore space. Therefore, if addressing subsurface oil, it is best to apply volumes of solution large enough to ensure that the downward flux is greater than the hydraulic conductivity of the sediments.

In order to deliver a bioremediation solution onto a beach, different approaches are available depending on the situation. For small areas, manual or semi-portable pump sprayers can be used. The advantage of this approach includes a large degree of control of the location and volume of solution applied. The disadvantage is the labor involved which limits the area that this can be used. For beaches with a large intertidal zone, measured based on the beach slope and the tidal range (vertical distance between the high and low tides), a water jet powerful enough to deliver the solution would be needed. For example, a beach with a 10% slope and a tidal range of 2.0 m results in an intertidal zone larger than 20 m. Since the lower intertidal zone tends to be saturated with water due to the formation of a seepage face, bioremediation solutions should not be applied onto that portion of the beach due to excessive washout. Therefore, the water jet would need to reach the high tide line and seaward approximately 15 m onto the upper and mid-intertidal zones. Thus, the nozzle would need to be selected carefully to ensure that the splash velocity is not too large to cause significant beach erosion as the solution impinges onto the beach surface. Li et al. (2008) proposed an alternative method that relies on releasing the bioremediation solution near the high tide line through a manifold that could be buried within the sediments, placed on the beach surface, or suspended in the air. The main advantage of this method is a more complete saturation of the pores in the intertidal zone while a disadvantage is that it requires infrastructure to be deployed.

In terms of the timing of when to apply a bioremediation solution, it is best to apply it after the high tide (i.e., when the tide is falling), which would ensure maximum soak time of the solution into the beach, and maximum contact with the oil prior to the returning tide. In addition, one needs to allow sufficient time following when the tide begins to fall to allow the capillary fringe above the water table to drop as well. If a beach has a capillary fringe of 0.20 m and the water table is 0.20 m below the surface, the moisture at the beach surface would be nearly 100% of the porosity, and thus the beach cannot imbibe more liquid. In such a case, one would need to wait until the water table drops below 0.20 m from the surface to apply the bioremediation solution.

7.3 Application of Amendment Solution into the Beach Subsurface

In gravel beaches, application of amendment solutions at the beach surface could result in its washout to sea due to the high permeability in the upper beach layer (Li and Boufadel, 2010). For this reason, one recommendation is to inject the amendments (including oxygen) under pressure deep into the beach. The

injection pressure should be kept lower than the blowout pressure (preferably one third of the blowout pressure), which could be estimated based on sediment properties, or more accurately by conducting a blowout test (Boufadel and Bobo, 2011). If the blowout pressure is exceeded, the applied solutions would short-circuit and upwell to the surface in the borehole annulus rather than travelling laterally from the injection well. Logistically, injecting bioremediation solutions can be very demanding, as one needs to shelter the wellheads in the intertidal zone, and the distance between wells might not be large. For larger areas this would require a large number wells.

After the *Exxon Valdez* oil spill, Boufadel and Bobo. (2011) conducted tracer studies of injected solutions in a gravel beach in Prince William Sound and found that the diameter of influence around an injection well was approximately 4.0 meters. Therefore, for bioremediating this beach, it would be recommended to place wells spaced at 4.0 m along the beach. Results from a follow up bioremediation study at the site indicated a variable degree of success. At some locations, up to 80% of the oil biodegraded within a month while other locations of the shorelines exhibited no biodegradation at all. Figures 9 and 10 provide basic information about the operation.

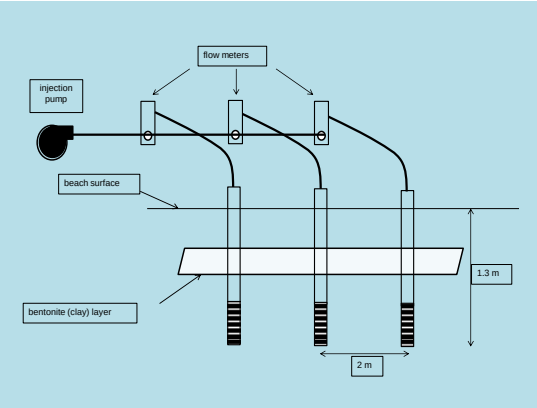

Figure 9: Setup for injection of solutions deep into the beach. Note the presence of the bentonite layer that prevents short-circuiting in the vicinity of the injection points.

Figure 10: Injection wells for bioremediation of a beach in Prince William Sound, Alaska. The solution and pumps were placed landward of the beach.

7.4 Comparison between Methods

The application of the slow release fertilizer (SRF) is the most widely used, and its success was demonstrated in dealing with the Exxon Valdez oil spill in the early 1990s. However, increased knowledge of oil biodegradation and beach hydraulics suggests that other techniques could be adopted with a great success if engineered correctly. A comparison of the three techniques reported above is presented in Table 1.

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Table1: Comparison of Various Biostimulation Methods

Techniques for applying bioremediation	Mode of delivery/ Recommendations	Major advantages	Major disadvantages
Nutrients in solid form onto beach surface	Applied granular or prilled form spread onto beach surface	No need to use major hydraulic systems Less frequent application than amendment solutions	Potential washout and eutrophication of adjacent water body
Amendment solutions onto a beach surface	Applied a large volume of solutions spread onto beach surface after the high tide	Dissolved fertilizers are commonly available.	Potential beach erosion if the splash velocity of water is large. Frequent application is needed.
	Applied oleophilic fertilizers spread onto the oiled beach material during low tide	No need to use major hydraulic systems Dissolution and release of nutrients directly into the oil at high tide	Oleophic fertilizers are not recommended unless or until approved by EPA NCP schedule
Amendment solutions into a beach subsurface	Inject solutions under pressure deep into the beach	Deliver the solutions/O_2 directly in the subsurface contaminated areas	Logistically demanding (installation of wells)

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10 Appendix: Biodegradation Tables

10.1 Table 2 for Biodegradation of Saturates

Table 2: Decay Constant for the Biodegradation of Saturates

One could use the equation $t = \frac{2.3}{k}$ to obtain the time it takes a compound to biodegrade to 10% of its initial value.

The expression "inoculum" below means that microorganisms were added to the system.

In the column of "Measurements": C= Hydrocarbon. O=Oxygen, X= biomass, N=Nutrients

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Heptane (C_7H_{16})	k=0.1351 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)
Octane (C_8H_{18})	k=0.1102 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)
Decane ($C_{10}H_{22}$)	k=0.05 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)
Decane ($C_{10}H_{22}$)	k=0.118 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.118 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.044 day^{-1} N=0.8 mg/L (Natural attenuation)				
Undecane ($C_{11}H_{24}$)	k=0.0658 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)
Undecane ($C_{11}H_{24}$)	k=0.089 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.085 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.036 day^{-1} N=0.8 mg/L (Natural attenuation)				
Dodecane ($C_{12}H_{26}$)	k=0.0991 day^{-1}	99% pure chemical	Experimental Solution	C,O [(1)]	Haines (1974)

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Dodecane ($C_{12}H_{26}$)	k=0.075 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.07 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.033 day^{-1} N=0.8 mg/L (Natural attenuation)				
Tridecane($C_{13}H_{28}$)	k=0.0724 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0648 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0312 day^{-1} N=0.8 mg/L (Natural attenuation)				
Tetradecane($C_{14}H_{30}$)	k=0.0708 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa(1996)
	k=0.0621 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0309 day^{-1} N=0.8 mg/L (Natural attenuation)				
Pentadecane($C_{15}H_{32}$)	k=0.0701 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0598 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0305 day^{-1} N=0.8 mg/L (Natural attenuation)				
Hexadecane ($C_{16}H_{34}$)	k=0.8481 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Hexadecane ($C_{16}H_{34}$)	k=0.0648 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0541 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0282 day^{-1} N=0.8 mg/L (Natural attenuation)				
Heptadecane ($C_{17}H_{36}$)	k=0.0641 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0528 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0272 day^{-1} N=0.8 mg/L (Natural attenuation)				
Heptadecane ($C_{17}H_{36}$)	μ_m=1.57 day^{-1} Ks=36500mg/L K_d=0.013 day^{-1} Y_x= 0.18 mg/L K_N=0.28 mg/L	Chemical	Sediment	C, O, and N	Boufadel (1999)
Heptadecane ($C_{17}H_{36}$)	k=0.0301 day^{-1} (N=5.0 mg/L)	Chemical	Sediment	C,O, and N[(1)]	Boufadel (1999)
	k=0.0295 day^{-1} (N=2.5 mg/L)				
	k=0.0198 day^{-1} (N=1.0 mg/L)				
	k=0.0131 day^{-1} (N=0.5 mg/L)				
	k=0.0044 day^{-1} (N=0.1 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Pr Pristane ($C_{19}H_{40}$)	k=0.0222 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0195 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0135 day^{-1} N=0.8 mg/L (Natural attenuation)				
Octadecane ($C_{18}H_{38}$)	k=0.0756 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)
Octadecane ($C_{18}H_{38}$)	k=0.0644 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0528 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0521 day^{-1} N=0.8 mg/L (Natural attenuation)				
Ph Phytane ($C_{20}H_{42}$)	k=0.0212 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0188 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0125 day^{-1} N=0.8 mg/L (Natural attenuation)				
Nonadecane ($C_{19}H_{40}$)	k=0.0654 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0514 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0278 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Eicosane ($C_{20}H_{42}$)	k=0.0853 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)
Eicosane ($C_{20}H_{42}$)	k=0.0614 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0481 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0261 day^{-1} N=0.8 mg/L (Natural attenuation)				
Heneicosane ($C_{21}H_{44}$)	k=0.0604 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0461 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0248 day^{-1} N=0.8 mg/L (Natural attenuation)				
Docosane ($C_{22}H_{46}$)	k=0.0807 day^{-1}	99% pure chemical	Solution	C,O [(1)]	Haines (1974)
Docosane ($C_{22}H_{46}$)	k=0.0584 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0457 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0258 day^{-1} N=0.8 mg/L (Natural attenuation)				
Tricosane ($C_{23}H_{48}$)	k=0.058 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0424 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0254 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Tetracosane ($C_{24}H_{50}$)	k=0.0887 day^{-1}	99% pure chemical	Solution	C,O [1]	Haines (1974)
Tetracosane ($C_{24}H_{50}$)	k=0.0553 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0414 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0264 day^{-1} N=0.8 mg/L (Natural attenuation)				
Pentacosane ($C_{25}H_{52}$)	k=0.057 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa(1996)
	k=0.039 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0244 day^{-1} N=0.8 mg/L (Natural attenuation)				
Hexacosane ($C_{26}H_{54}$)	k=0.0537 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0384 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.025 day^{-1} N=0.8 mg/L (Natural attenuation)				
Heptacosane ($C_{27}H_{56}$)	k=0.0566 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.039 day^{-1} N= 3.5 mg/L (Inoculum)				
	k=0.0254 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Octacosane ($C_{28}H_{58}$)	k=0.0513 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.038 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0247 day^{-1} N=0.8 mg/L (Natural attenuation)				
Nonacosane ($C_{29}H_{60}$)	k=0.0546 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.035 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0234 day^{-1} N=0.8 mg/L (Natural attenuation)				
Triacontane ($C_{30}H_{62}$)	k=0.0506 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0376 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.024 day^{-1} N=0.8 mg/L (Natural attenuation)				
Hentriacontane ($C_{31}H_{64}$)	k=0.0539 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0343 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0227 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Dotriacontane ($C_{32}H_{66}$)	k=0.0931 day^{-1}	99% pure chemical	Solution	C,O [1]	Haines (1974)
Dotriacontane($C_{32}H_{66}$)	k=0.0443 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa(1996)
	k=0.0346 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0227 day^{-1} N=0.8 mg/L (Natural attenuation)				
Tritriacontane ($C_{33}H_{68}$)	k=0.035 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.028 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.02 day^{-1} N=0.8 mg/L (Natural attenuation)				
Tetratriacontane ($C_{34}H_{70}$)	k=0.0286 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.0203 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0153 day^{-1} N=0.8 mg/L (Natural attenuation)				
Pentatriacontane ($C_{35}H_{72}$)	k=0.0289 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X, and N	Venosa (1996)
	k=0.016 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.012 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Hexatriacontane ($C_{36}H_{74}$)	k=0.0924 day^{-1}	99% pure chemical	Solution	C,O (1)	Haines (1974)
Tetracontane($C_{40}H_{82}$)	k=0.0312 day^{-1}	99% pure chemical	Solution	C,O (1)	Haines (1974)
Tetratetracontane($C_{44}H_{90}$)	k=0.0384day^{-1}	99% pure chemical	Solution	C,O (1)	Haines (1974)

(1): Oxygen was measured by Haines (1974). The amount of C was estimated based on the stoichiometry. No oxygen was assumed to be consumed by microorganisms.

10.2 Table 3: Decay Constant for the Biodegradation of Aromatics

Table 3: Decay Constant for the Biodegradation of Aromatics

One could use the equation $t = \frac{2.3}{k}$ to obtain the time it takes a compound to biodegrade to 10% of its initial value.

The expression “inoculum” below means that microorganisms were added to the system.

In the column of “Measurements”: C= Hydrocarbon. O=Oxygen, X= biomass, N=Nutrients

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
TPAH (Total Polycyclic Aromatic Hydrocarbons)	KN114A: k=0.0138 day^{-1} (Natural attenuation, N=0.8 mg/L) k=0.0284 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM006B: k= 0.0043 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0099 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0072 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0170 day^{-1} (Nutrient, N:5~10 mg/L)				
Nap Naphthalene ($C_{10}H_8$)	k=0.293 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa(1996)
	k=0.216 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.189 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_1-nap C_1-Naphthalenes (C_1-$C_{10}H_7$)	SM 006B: k=0.0064 day^{-1} (Natural attenuation, N=0.8 mg/L) k=0.0082 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	EL107: k=0.0071 day^{-1} (Natural attenuation, N=0.8 mg/L) k=0.0095 day^{-1} (Nutrient, N:5~10 mg/L)				
C_1-nap C_1-Naphthalenes (C_1-$C_{10}H_7$)	k=0.11 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0883 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0775 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_2-nap C_2-Naphthalenes (C_2-$C_{10}H_7$)	SM 006B: k=0.0197 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0097 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	EL107: k= 0.0525 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.1234 day^{-1} (Nutrient, N:5~10 mg/L)				
C_2-nap C_2-Naphthalenes (C_2-$C_{10}H_7$)	k=0.0487 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.036 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0324 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_3-nap C_3-Naphthalenes (C_3-$C_{10}H_7$)	KN114A: k=0.0282 day^{-1} (Natural attenuation, N=0.8 mg/L) k=0.0342 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k=0.0135 day^{-1} (Natural attenuation, N=0.8 mg/L) k=0.0159 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k=0.0206 day^{-1} (Natural attenuation, N=0.8 mg/L) k=0.0593 day^{-1} (Nutrient, N:5~10 mg/L)				
C_3-nap C_3-Naphthalenes (C_3-$C_{10}H_7$)	k=0.0324 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0216 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0198 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_4-nap C_4-Naphthalenes (C_4-$C_{10}H_7$)	KN114A: k=0.0353 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0556 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0067 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0156 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0099 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0259 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_4-nap C_4-Naphthalenes (C_4-$C_{10}H_7$)	k=0.0233 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0198 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0162 day^{-1} N=0.8 mg/L (Natural attenuation)				
Phe Phenanthrene ($C_{14}H_{10}$)	EL107: k= 0.0671 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.1596 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
Phe Phenanthrene ($C_{14}H_{10}$)	k=0.0468 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Vebosa (1996)
	k=0.0303 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0282 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_1-Phe C_1-Phenanthrenes (C_1-$C_{14}H_9$)	EL107: k= 0.0324 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0455 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
C_1-Phe C_1-Phenanthrenes (C_1-$C_{14}H_9$)	k=0.0299 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0239 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0204 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_2-Phe C_2-Phenanthrenes(C_2-$C_{14}H_9$)	KN114A: k=0.026 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0405 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0062 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0111 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0116 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0179 day^{-1} (Nutrient, N:5~10 mg/L)				
C_2-Phe C_2-Phenanthrenes (C_2-$C_{14}H_9$)	k=0.0202 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0149 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0129 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_3-Phe C_3-Phenanthrenes (C_3-$C_{14}H_9$)	KN114A: k= 0.0158 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0319 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.003 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0104 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0053 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0131 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_3-Phe C_3-Phenanthrenes (C_3-$C_{14}H_9$)	k=0.0152 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.00965 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0085 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_4-Phe C_4-Phenanthrenes (C_4-$C_{14}H_9$)	KN114A: k= 0.0076 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.024 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0014 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0079 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0026 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.01 day^{-1} (Nutrient, N:5~10 mg/L)				
Flu Fluorene ($C_{13}H_{10}$)	EL107: k= 0.0518 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0903 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
Flu Fluorene ($C_{13}H_{10}$)	k=0.0484 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0411 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0317 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_1-Flu C_1-Fluorenes (C_1-$C_{13}H_9$)	KN114A: k= 0.0345 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0583 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0195 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0234 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0211 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0368 day^{-1} (Nutrient, N:5~10 mg/L)				
C_1-Flu C_1-Fluorenes (C_1-$C_{13}H_9$)	k=0.029 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0257 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0226 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_2-Flu C_2-Fluorenes (C_2-$C_{13}H_9$)	KN114A: k= 0.0319 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0497 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.006 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0130 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0091 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0178 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_2-Flu C_2-Fluorenes (C_2-$C_{13}H_9$)	k=0.0215 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0154 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0142 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_3-Flu C_3-Fluorenes (C_3-$C_{13}H_9$)	KN114A: k= 0.0190 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0376 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0034 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0115 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0055 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.015 day^{-1} (Nutrient, N:5~10 mg/L)				
C_3-Flu C_3-Fluorenes (C_3-$C_{13}H_9$)	k=0.0176 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0129 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0115 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Dbt Dibenzothiophene ($C_{12}H_8S$)	SM 006B: k= 0.0302 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0280 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	EL107: k= 0.0478 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0768 day^{-1} (Nutrient, N:5~10 mg/L)				
Dbt Dibenzothiophene ($C_{12}H_8S$)	k=0.0429 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0344 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0282 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_1-Dbt C_1-Dibenzothiophenes (C_1-$C_{12}H_7S$)	KN114A: k= 0.0339 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0545 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0086 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0144 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0154 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0249 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_1-Dbt C_1-Dibenzothiophenes (C_1-$C_{12}H_7S$)	k=0.0233 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0191 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0171 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_2-Dbt C_2-Dibenzothiophenes (C_2-$C_{12}H_7S$)	KN114A: k= 0.0226 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0401 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0042 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0117 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.007 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0155 day^{-1} (Nutrient, N:5~10 mg/L)				
C_2-Dbt C_2-Dibenzothiophenes (C_2-$C_{12}H_7S$)	k=0.0187 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0138 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0121 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_3-Dbt C_3-Dibenzothiophenes (C_3-$C_{12}H_7S$)	KN114A: k= 0.0128 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0308 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0021 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0097 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0036 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.012 day^{-1} (Nutrient, N:5~10 mg/L)				
C_3-Dbt C_3-Dibenzothiophenes (C_3-$C_{12}H_7S$)	k=0.0129 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa(1996)
	k=0.0116 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.00929 day^{-1} N=0.8 mg/L (Natural attenuation)				
Nbt Naphthobenzothiophene ($C_{16}H_{10}S$)	KN114A: k= 0.0136 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0303 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0025 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0095 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0061 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0123 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Nbt Naphthobenzothiophene ($C_{16}H_{10}S$)	k=0.0199 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa(1996)
	k=0.0211 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0191 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_1-Nbt C_1-Naphthobenzothiophenes (C_1-$C_{16}H_9S$)	KN114A: k= 0.0064 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0214 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0017 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0087 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0031 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0108 day^{-1} (Nutrient, N:5~10 mg/L)				
C_1-Nbt C_1-Naphthobenzothiophenes (C_1-$C_{16}H_9S$)	k=0.0149 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.0088 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0088 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_2-Nbt C_2-Naphthobenzothiophenes (C_2-$C_{16}H_9S$)	KN114A: k= 0.002 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0134 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0007 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.006 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0012 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0076 day^{-1} (Nutrient, N:5~10 mg/L)				
C_2-Nbt C_2-Naphthobenzothiophenes (C_2-$C_{16}H_9S$)	k=0.0208 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.00857 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.00659 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_3-Nbt C_3-Naphthobenzothiophenes (C_3-$C_{16}H_9S$)	KN114A: k= 0.00035 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0046 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.00073 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0035 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0012 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0035 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_3-Nbt C_3-Naphthobenzothiophenes (C_3-$C_{16}H_9S$)	k=0.00909 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.00606 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.00437 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_1-Pyr C_1-Pyrenes (C_1-$C_{16}H_9$)	KN114A: k= 0.0109 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0181 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0032 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0078 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0043 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0091 day^{-1} (Nutrient, N:5~10 mg/L)				
C_1-Pyr C_1-Pyrenes (C_1-$C_{16}H_9$)	k=0.00967 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.00776 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.00634 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_2-Pyr C_2-Pyrenes (C_2-$C_{16}H_9$)	KN114A: k= 0.0088 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0136 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0025 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0065 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0035 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0074 day^{-1} (Nutrient, N:5~10 mg/L)				
C_2-Pyr C_2-Pyrenes (C_2-$C_{16}H_9$)	k=0.0044 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa(1996)
	k=0.00275 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.00274 day^{-1} N=0.8 mg/L (Natural attenuation)				
Cry Chrysene ($C_{18}H_{12}$)	KN114A: k= 0.0009 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0065 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0012 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0041 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0033 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.005 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
Cry Chrysene ($C_{18}H_{12}$)	k=0.00553 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa(1996)
	k=0.00639 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.00192 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_1-Cry C_1- Chrysenes (C_1-$C_{18}H_{11}$)	KN114A: k= 0.0081 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0153 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.001 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0051 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0025 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0063 day^{-1} (Nutrient, N:5~10 mg/L)				
C_1-Cry C_1-Chrysenes (C_1-$C_{18}H_{11}$)	k=0.00637 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.00528 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.0443 day^{-1} N=0.8 mg/L (Natural attenuation)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_2-Cry C_2-Chrysenes (C_2-$C_{18}H_{11}$)	KN114A: k= 0.005 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0118 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.001 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0048 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0016 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0057 day^{-1} (Nutrient, N:5~10 mg/L)				
C_2-Cry C_2-Chrysenes (C_2-$C_{18}H_{11}$)	k=0.00582 day^{-1} N= 6.4 mg/L (Nutrient)	Oil	Sediment	C, X and N	Venosa (1996)
	k=0.00501 day^{-1} N=3.5 mg/L (Inoculum)				
	k=0.00471 day^{-1} N=0.8 mg/L (Natural attenuation)				
C_3-Cry C_3- Chrysenes (C_3-$C_{18}H_{11}$)	KN114A: k= 0.0036 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0065 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0005 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0026 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0005 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0032 day^{-1} (Nutrient, N:5~10 mg/L)				

Compound By increasing MW	Rate	Hydrocarbon state	Matrix	Measurements	Reference
C_4-Cry C_4-Chrysenes (C_4-$C_{18}H_{11}$)	KN114A: k= 0.0013 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0021 day^{-1} (Nutrient, N:5~10 mg/L)	Oil	Sediment	C, N	Venosa (2010)
	SM 006B: k= 0.0005 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.0015 day^{-1} (Nutrient, N:5~10 mg/L)				
	EL107: k= 0.0001 day^{-1} (Natural attenuation, N=0.8 mg/L) k= 0.00078 day^{-1} (Nutrient, N:5~10 mg/L)				

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