

Biodegradation and Bioremediation of Oiled Beaches

A Primer for Planners and Managers

API TECHNICAL REPORT 1147
JANUARY 2014



AMERICAN PETROLEUM INSTITUTE

Special Notes

API publications necessarily address problems of a general nature. With respect to particular circumstances, local, state, and federal laws and regulations should be reviewed.

Neither API nor any of API's employees, subcontractors, consultants, committees, or other assignees make any warranty or representation, either express or implied, with respect to the accuracy, completeness, or usefulness of the information contained herein, or assume any liability or responsibility for any use, or the results of such use, of any information or process disclosed in this publication. Neither API nor any of API's employees, subcontractors, consultants, or other assignees represent that use of this publication would not infringe upon privately owned rights.

API publications may be used by anyone desiring to do so. Every effort has been made by the Institute to assure the accuracy and reliability of the data contained in them; however, the Institute makes no representation, warranty, or guarantee in connection with this publication and hereby expressly disclaims any liability or responsibility for loss or damage resulting from its use or for the violation of any authorities having jurisdiction with which this publication may conflict.

API publications are published to facilitate the broad availability of proven, sound engineering and operating practices. These publications are not intended to obviate the need for applying sound engineering judgment regarding when and where these publications should be utilized. The formulation and publication of API publications is not intended in any way to inhibit anyone from using any other practices.

Any manufacturer marking equipment or materials in conformance with the marking requirements of an API standard is solely responsible for complying with all the applicable requirements of that standard. API does not represent, warrant, or guarantee that such products do in fact conform to the applicable API standard.

All rights reserved. No part of this work may be reproduced, translated, stored in a retrieval system, or transmitted by any means, electronic, mechanical, photocopying, recording, or otherwise, without prior written permission from the publisher. Contact the Publisher, API Publishing Services, 1220 L Street, NW, Washington, DC 20005.

Copyright © 2014 American Petroleum Institute

Foreword

Nothing contained in any API publication is to be construed as granting any right, by implication or otherwise, for the manufacture, sale, or use of any method, apparatus, or product covered by letters patent. Neither should anything contained in the publication be construed as insuring anyone against liability for infringement of letters patent.

Suggested revisions are invited and should be submitted to the Director of Regulatory and Scientific Affairs, API, 1220 L Street, NW, Washington, DC 20005.

Contents

Illustrations	vi
Executive Summary	vii
1 Introduction	1
2 Beach Hydraulics	2
3 Oil Biodegradation	5
4 Measuring Environmental Factors of Biodegradation	9
4.1 Oxygen	11
4.2 Nutrients	11
4.3 Salinity, pH, and Temperature	11
4.4 Moisture	12
4.5 Microbial Communities	12
5 Monitored Natural Attenuation	13
6 Bioremediation	14
7 Techniques for Applying Bioremediation	15
7.1 Application of Nutrients in Solid Form onto a Beach Surface	16
7.2 Application of Amendment Solutions onto a Beach Surface	17
7.3 Application of Amendment Solution into the Beach Subsurface	17
7.4 Comparison between Methods	18
8 Acknowledgment	18
9 Reference	19
10 Appendix: Biodegradation Tables	28
10.1 Table 2 for Biodegradation of Saturates	28
10.2 Table 3: Decay Constant for the Biodegradation of Aromatics	36

Illustrations

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.	3
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.	3
Figure 3: Initial oil distribution following a spill.	4
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	4
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.	6
Figure 6: Pressure sensor in the beach (in the white pipe) (left inside) along with a multiport sampling well (right inside).	10
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. ...	10
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	10
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.	18
Figure 10: Injection wells for bioremediation of a beach in Prince William Sound, Alaska. The solution and pumps were placed landward of the beach.	18

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

A Primer for Planners and Managers

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).

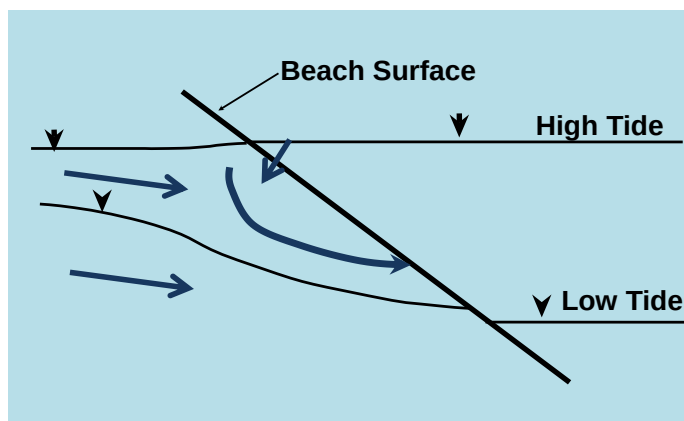


Figure 1: Water table movement due to tidal forces. The beach fills up with seawater near the high tide line and drains (mostly) near the low tide line. Influx of fresh groundwater (recharge) from the land side could replenish the beach with water, especially during low tides.

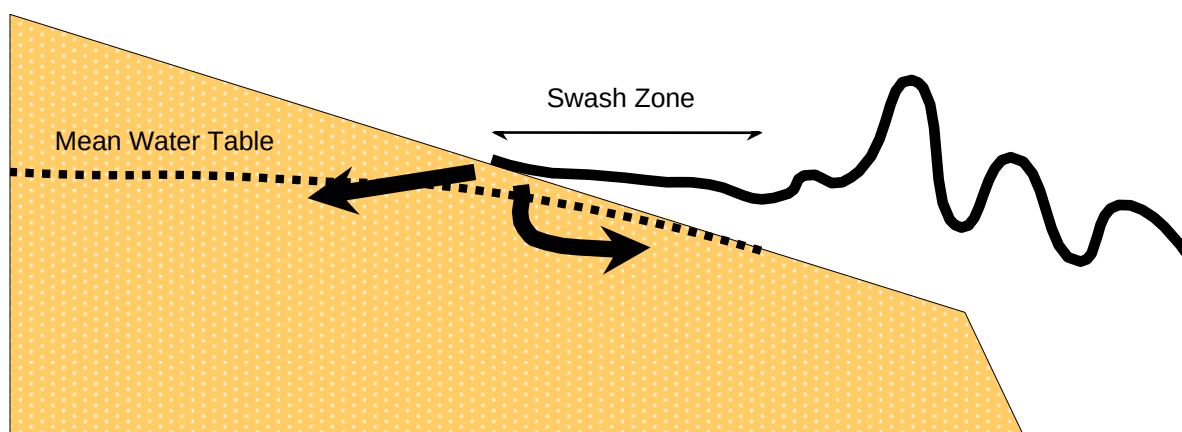


Figure 2: Waves running up a beach. The effect of waves on the beach water table is to create two zones with two different hydraulic gradients. The gradient is steep in the swash zone, and becomes mild landward of it.

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).

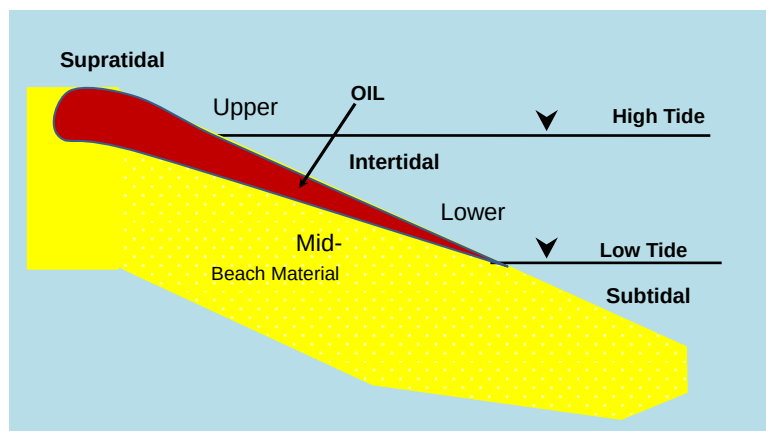


Figure 3: Initial oil distribution following a spill.

- 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).

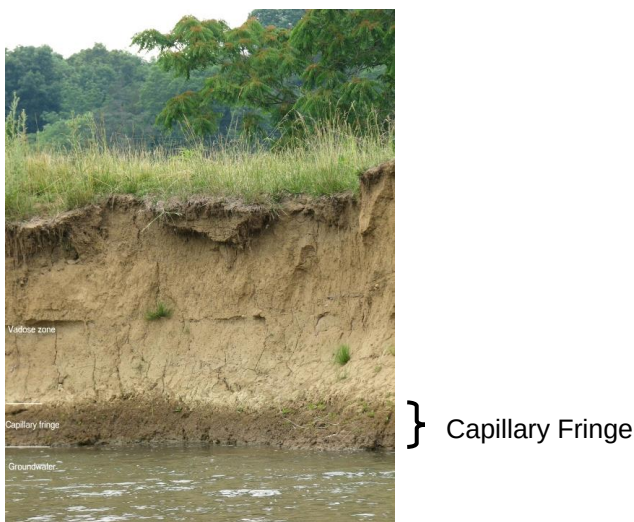


Figure 4: The capillary fringe (dark color) reflects the presence of high moisture content (more than 75% of the porosity). <http://wps.prenhall.com/wps/media/objects/5309/5437119/Figures/chapter06.htm>

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.

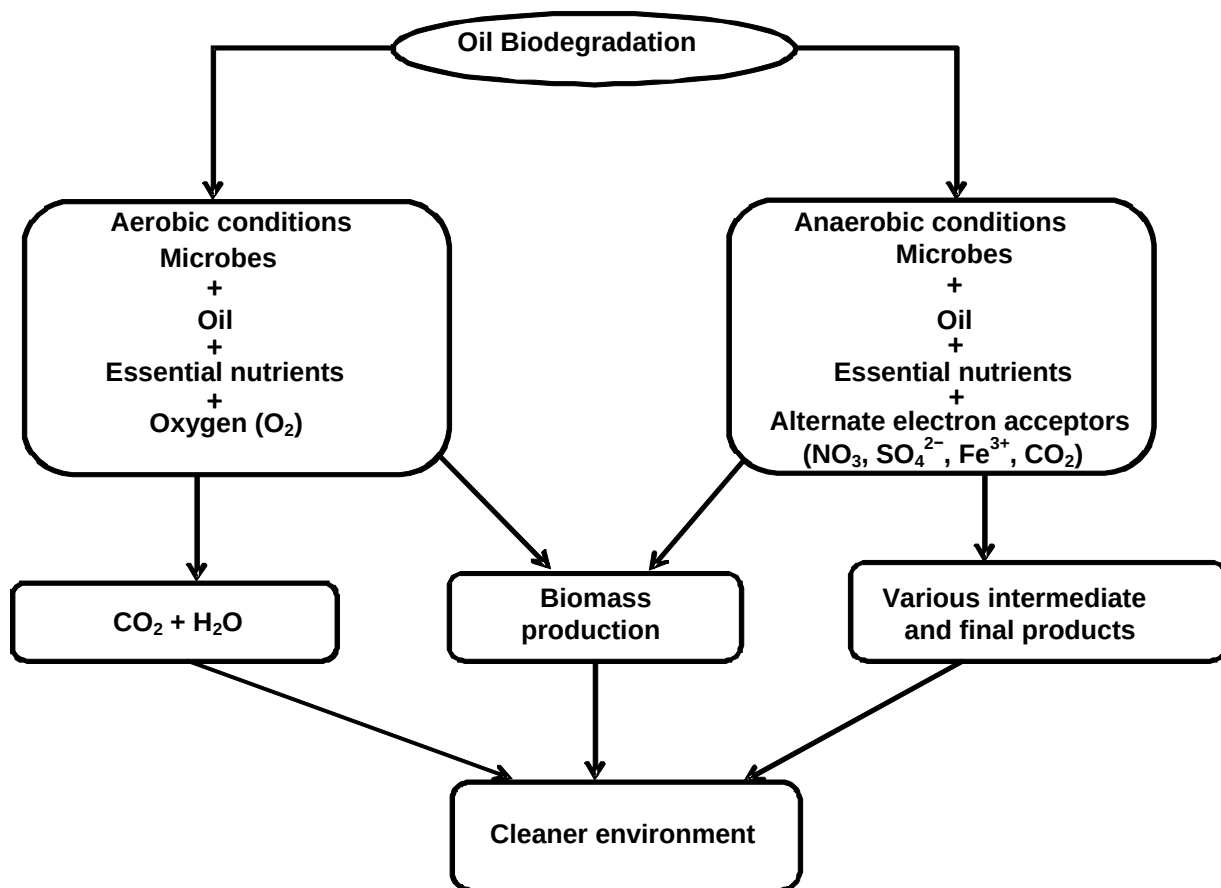


Figure 5: Potential aerobic and anaerobic oil biodegradation pathways. Shaded areas indicate aerobic oil biodegradation which is faster than anaerobic oil biodegradation. Aerobic oil biodegradation is the desired pathway in terms of practical bioremediation efforts of oiled beaches.

Due to the chemical complexity of crude oils, interspecies interactions often play a major role in 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 from 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, C30-17 α (H),21 β (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- $\alpha\beta\beta$ -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:

$$\frac{C}{C_0} = \exp(-k \cdot t) \quad (2)$$

Where C and C_0 are normalized concentrations of an oil component at times t and t_0 ; 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:

$$t = \frac{2.3}{k} \quad (3)$$

For example, for phytane under natural conditions, Table 1 gives $k=0.0125 \text{ day}^{-1}$, 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 $k=0.0212 \text{ day}^{-1}$ 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:

$$BI = \frac{BOD_5}{ThOD} \quad (4)$$

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 necessary 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.

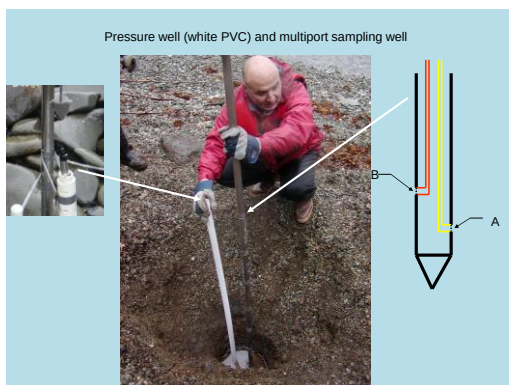


Figure 6: Pressure sensor in the beach (in the white pipe) (left inside) along with a multiport sampling well (right inside).

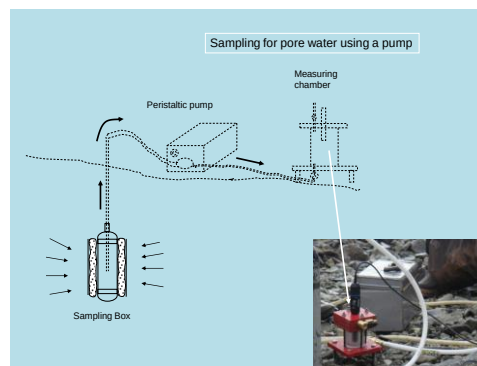


Figure 7: Pumping water by a peristaltic pump through a measurement chamber. Also shown (inset) is an optical probe (black vertical probe in the red measuring chamber) used to measure dissolved oxygen.

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.