A Summary of the DOE/PERF Bioremediation Workshop

May 30, 2002

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Introduction

Bioremediation can be a cost-effective technology that is often used to treat oil spills in all types of environmental media including soils, groundwater, and surface water (both freshwater and marine). Bioremediation is also used to treat oily wastes. Once spilled, oil is subject to a wide range of physical, biological and chemical processes that serve to "weather" the oil, and attenuate it in the environment. One of these weathering processes, biodegradation, is unique because it is the primary process by which the oil is actually removed from the environment. Most of the other weathering processes transfer the oil from one medium to another (as in the case of volatilization where certain oil constituents evaporate into the air), or dilute it (such as from wave action, which may disperse oil throughout the water column in a marine environment).

Due to the obvious benefit of actually removing the oil from the environment and the fact that it can be cost effective compared to other treatment technologies, biodegradation has been, and currently remains, the subject of considerable research. Crude oil and refined oil products are frequently stored and transported on or over land, and as a result, oil spills that impact soil and groundwater tend to be quite common although usually smaller in volume than marine or freshwater spills. Methodologies for bioremediation of terrestrial oil spills are well developed. Research on bioremediation in surface soils is currently focused on optimizing biodegradation rates, developing appropriate treatment goals, and site restoration following remediation. Current research related to hydrocarbon-contaminated groundwater is primarily directed toward continuing to build a sound scientific basis for risk-based management strategies, methodologies for hydraulic control, and delivery of bioremediation amendments (oxygen, electron acceptors, and nutrients).

Research on biodegradation in marine environments has studied the use of surfactants, fertilizers, and exogenous (meaning not naturally-occurring) microbes, and has found that only the use of fertilizers consistently optimized biodegradation rates. A comprehensive review of biodegradation as applied to marine oil spills can be found in "Petroleum Spill Bioremediation in Marine Environments" by Roger C. Prince.[1] Research on oil spills in freshwater and marine wetland environments have indicated that oil should not be tilled into sediments, and that careful application of fertilizers is required to avoid toxicity issues.[2] Additional information on biodegradation in wetland environments can be found on the United States Environmental Protection Agency's (EPA's) web site, http://epa.gov/oilspill/docs/bioremed.pdf.

On May 30, 2002 the United States Department of Energy (DOE) and the Petroleum Environmental Research Forum (PERF) held a joint bioremediation workshop in Houston, Texas. The main objective of the workshop was to discuss the "state of the art" of bioremediation for hydrocarbon-impacted soil. In addition, key findings from bioremediation research on marine, freshwater, and wetland oil spills were presented.

Presentations at the workshop addressed bioremediation as practiced by the oil industry, toxicity assessment after bioremediation, and other technical issues. This workshop summary has been written in a "Question and Answer" format in order to provide the information in a concise manner for environmental professionals who are considering the use of bioremediation at sites where hydrocarbons have impacted soils. While some of the results presented below were the result of research on marine and freshwater oil spills, similar results have been observed in soils.

Frequently Asked Questions:

Bioremediation Basics

How does bioremediation work?

Bioremediation works when microscopic organisms (primarily bacteria) that live in soil "eat" chemicals, such as petroleum hydrocarbons. They use certain petroleum hydrocarbons as their food source, leave other chemicals behind, and ultimately generate water and carbon dioxide as their waste products.

Biodegradation is a natural process than can be harnessed or optimized to enhance the rate at which microbes biodegrade organic chemicals that have been released into the environment. The rate at which biodegradation will occur can be enhanced by providing an optimal living environment for the microbes. Most microbes that degrade petroleum hydrocarbons require oxygen, water, proper acidity (pH), and nutrients such as nitrogen and phosphorous. When the microbial environment is optimized by having the right amounts of water, air and nutrients and by maintaining proper acidity, biodegradation rates will be faster.

How are biodegradation rates enhanced?

In some environments, biodegradation can be very slow unless the environmental factors that affect biodegradation rates are modified. In order to determine which of the rate-limiting factors may require modification, an initial assessment should be performed. The rate-limiting factors that are typically assessed include oxygen, nutrients, salinity, temperature, pH, soil moisture content or water holding capacity, and the amount of soil organic carbon.

These rate-limiting factors may vary from medium to medium (i.e., soil, groundwater, fresh water, seawater, wastes, or wetlands) and they may vary from site to site depending upon climate, ecosystem, and human disturbance. For example, in groundwater, biodegradation is often limited by the amount of dissolved oxygen present rather than by nutrient (nitrogen and phosphorous) concentrations. In contrast, in seawater, nitrogen and phosphorous are usually rate-limiting rather than oxygen but, great care must be taken so that these nutrients are supplied at concentrations that do not result in toxicity to aquatic organisms. For most soils, nutrients are rate-limiting, but other factors such as moisture content and temperature may also limit biodegradation rates. If, however, the land has been previously used for farming, there may already be significant amounts of nitrogen and phosphorus present in the soil (the same may be true for marine or freshwater environments where there is run-off from farming). In wetlands, oxygen is often the rate-limiting factor for hydrocarbon biodegradation because it is an anaerobic environment.

Once the rate-limiting factors for biodegradation are assessed, it is important to develop a well-thought out plan for correcting them in a manner that will enhance microbial degradation rates without causing more harm to the environment.

How long does biodegradation take? Is it slow?

The time it takes to bioremediate a site and the rate at which biodegradation will occur depends on:

- the type and concentration/mass of petroleum hydrocarbons present,
- the depth of the impacted area,
- how well the environmental conditions are optimized, and
- the type of technology used

When conditions are optimized, biodegradation is an efficient and cost-effective remediation technique.

Selecting a Bioremediation Technology

How can I tell if bioremediation is the right technology to use at my site?

Determining whether bioremediation is the right technology to use at a site requires careful evaluation because it depends on several factors, including:

- the type and quantity of oil that has been spilled
- the amount of weathering that has occurred at an old spill site

- site conditions such as soil type, rainfall, temperature and space constraints
- regulatory considerations such as treatment goals.

These factors also affect the selection of a specific biotreatment technology. Information on each of these factors is provided in question and answer format in the sections below.

What bioremediation technologies are used at exploration and production (E&P) sites?

There are several biotreatment technologies that are used at E&P sites. In-situ technologies are those that are applied to soils in place; ex-situ technologies are those that are applied to excavated soils or other oily wastes (e.g. tank bottoms). The technologies used at E&P sites include:

- In-situ: bioremediation of oil spilled on soil in place

Other methods are used for treating oily production wastes:

- Land spreading: oily wastes are applied only once and tilled into the soil.[3]
- Land farming: oily wastes are repeatedly applied to the same tract of land.[4]
- Composting: bulking agents are added to oily soil or wastes to increase the air permeability and water holding capacity of the mixture. The mixture tends to be placed in piles or windrows, and sometimes air, water and nutrients have to be added.
- Biopiles: a modification of land treatment used when available space is limited, a small volume of soil is to be treated, or the hydrocarbons are volatile.[3]
- Land treatment units: constructed units for the ex-situ treatment of oily soil or wastes.

All of these methods, except land spreading, can be constructed with liners and allow for recycling or reuse of runoff and leachate water.[4] The need for liners and leachate collection depends upon the hydrogeology of the site and the need to protect groundwater.

Pilot studies have shown that composting is the technology that yields the fastest results (although at a higher cost). Land farming yields similar results, and biopiles have been found to be the least effective technology, as shown in **Figure 1**.[6] Therefore all of the technologies will yield

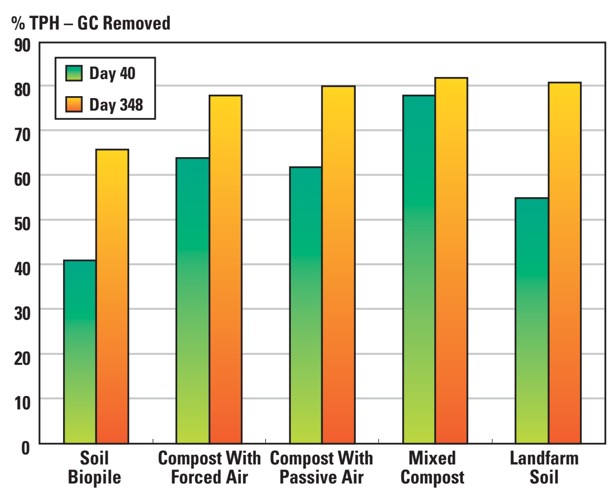


Figure1. Comparison of TPH Results

Source: Prince, Roger and Sara Mc Millen. Summary of PERF Bioremediation Projects. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002 similar results, but the length of time required can vary from six weeks to a year or more.

The Effect of Oil on Biotreatment

How can I determine whether bioremediation is suitable for meeting the treatment goals for my site?

Prior to selecting a specific bioremediation technology, the overall suitability of bioremediation for reducing the petroleum hydrocarbon concentrations to desired treatment goals must be determined for your site. There are two methods that can be used for this purpose, and they can be performed either independently or together. The first is chemical evaluation of the spilled oil coupled with soil analyses done for agricultural evaluations, and the second is a biotreatability study.

Because of the petroleum industry's experience with bioremediation, the relationship between the chemistry of an oil and its biodegradation potential is well understood. Chemical analyses can be used to determine the composition of the oil, which can then be used to estimate the biodegradation end-points that can be achieved (refer to Questions 34 and 35 for more information on end-points). These analyses usually cost less than $500.

Biotreatability studies are another way to determine if bioremediation will be able to meet the desired treatment levels. Biotreatability studies can also provide information on starting petroleum hydrocarbon concentrations, nutrient requirements, cost requirements, and other factors that can help optimize the treatment method.[3] Biotreatability studies can sometimes be costly and time-consuming, typically costing $5,000 and requiring 2 to 6 months of study time.

What oil concentrations can be treated efficiently with bioremediation?

Several factors determine the oil concentrations that can be treated efficiently. These factors include:

- the composition of the oil: greater fractions of heavy components (i.e., a lower API gravity) reduce the concentration of oil that can be biodegraded.
- the amount of oil added to the soil: if too much oil is added, soils can become oil-wet; this can inhibit biodegradation due to reduced moisture and can cause leaching of oil from soil.[8]
- the end-point or treatment goal that must be reached, e.g. 5% of a 10° API gravity crude oil in soil will never biodegrade to <0.1% total petroleum hydrocarbons (TPH).

A rule of thumb to ensure that oil concentrations are below soil saturation limits is <5% by weight for soil biotreatment methods, and <15% by weight for composting.[8] This oil loading rate can be adjusted based upon the oil type and soil type, and also the required regulatory treatment goals.[8]

What is weathering and how does it impact biodegradation?

Weathering is the physical, chemical and biological alteration of the original composition of a hydrocarbon mixture. Weathering is caused by at least three phenomena, which can occur alone or in concert with one another: microbial degradation (where microbes preferentially degrade certain components of the oil such as n-alkanes), volatilization (where components with the highest vapor pressures evaporate) and solubilization (where components with the highest solubilities, such as benzene, dissolve in water). **Figure 2** illustrates how the carbon range will change with time as biodegradation occurs.

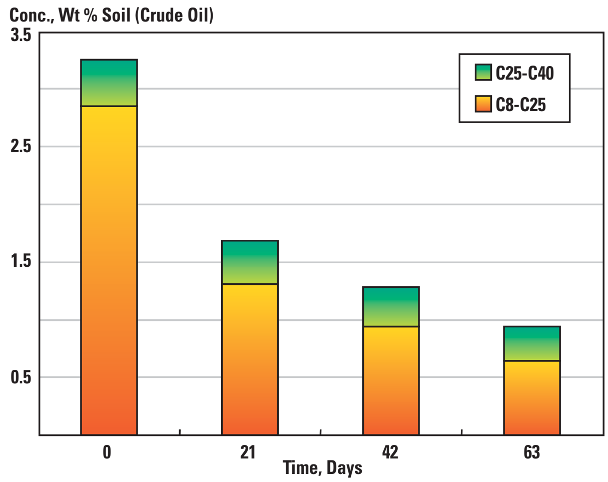
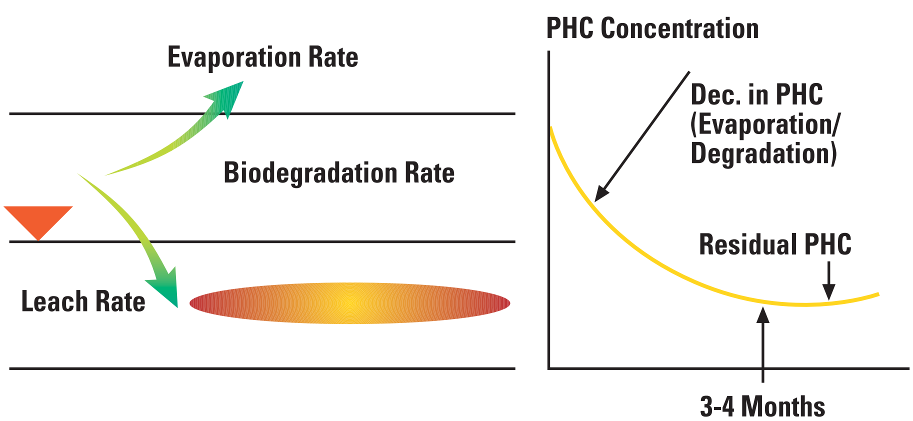


Figure 2. Changes in Carbon Range Due to Biodegradation

Source: Prince, Roger and Sara Mc Millen. Summary of PERF Bioremediation Projects. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002

What is natural attenuation?

Natural attenuation refers to the decrease in concentrations of chemicals in the environment due to natural phenomena such as microbial degradation, evaporation, and adsorption (where chemicals adsorb onto solids). **Figure 3** illustrates the effect of attenuating mechanisms on petroleum hydrocarbon concentrations.



• Residual PHC (NAPL)	
– Low Volatility (V.P. <E-5)	– Low Toxicity (C#/Class Versus Toxicity)
– High Sorption (Log K_{ow} >5)	– Sequestered (Non-Bioavailable)
– Low Leaching	– Recalcitrant (Non-Biodegradable)

Figure 3. Soil Bioremediation of Oily Waste "What We Know/Need to Know"

Source: Salanitro, Joe. Potential and Limits of Petroleum Hydrocarbon (PHC) Bioremediation in Soil – Shell Studies. Shell Global Solutions (US) Inc. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002.

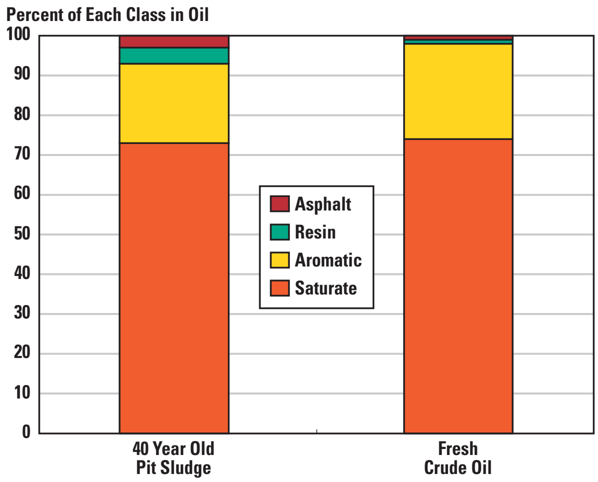
What is the effect of the age of the spill on biodegradation rates and end-points?

As the age of an oil spill increases, there will be more opportunity for the oil to weather and for its constituents to attenuate in the environment. In general, the more weathering that has occurred, the less biodegradation that can occur.[9] The composition of the oil can be evaluated by gas chromatography prior to conducting bioremediation. This analysis can be used to evaluate the amount of natural attenuation that has occurred at old spill sites and estimate the biodegradation potential of the oil. The mere age of a spill or of some wastes, such as pit sludges, does not indicate how much weathering may have occurred. **Figure 4** shows the composition of a 40+ year old pit sludge compared to the crude oil produced at the site. The composition of the sludge is very similar to the oil as produced, indicating that very little weathering and biodegradation had occurred even after 40+ years of storage in an open pit.

In another example, a site of an old crude spill was evaluated to determine if biodegradation could achieve an end-point of 1% TPH. **Figure 5** illustrates the results of a laboratory biotreatability test in which soil from the old crude oil spill site was brought into the laboratory and bioremediated for 42 weeks. Both oil and grease (O&G) and TPH were monitored over 42 weeks. Very little biodegradation was observed as shown by the lack of change in either the O&G or TPH measurements.

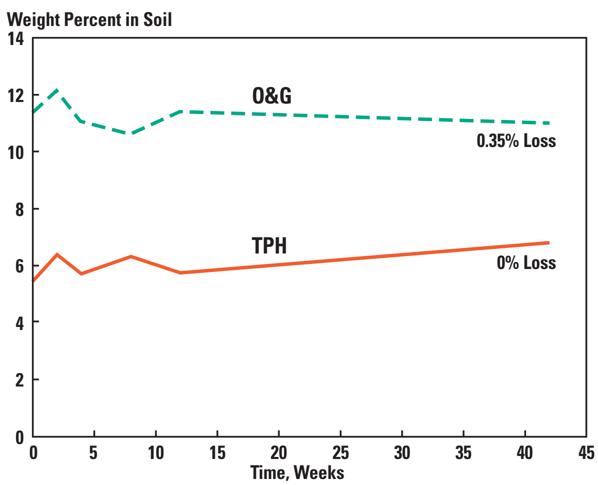
In another example (**Figure 6**), tank bottoms were composted for one year and the concentration of TPH dropped from 20% to 1.2%. Obviously, this waste was much more amenable to bioremediation than that of the old spill site described above.

Figure 4. Comparison of a 40 Year Old Pit Sludge With Fresh Crude Oil Produced at the Site



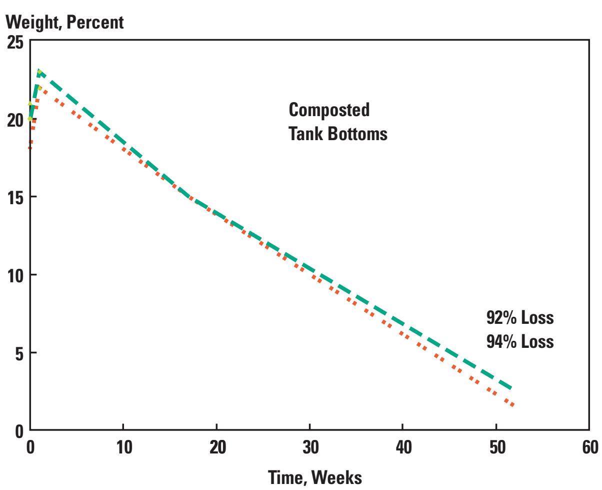
Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

Figure 5. Results From a Treatability Study for an Old Crude Oil Spill Site



Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

Figure 6. Biodegradation Results From Composting Tank Bottoms



Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

By comparing the initial composition of the old spill site with the tank bottoms, it is clear why such different results were obtained. The class composition data for the two cases are in **Figure 7**. The tank bottoms (crude oil produced at this site has an API gravity of 49.2°) consisted of oil containing over 70% saturates, while the oil extracted from the old spill site contained less than 20% saturates. The hydrocarbons extracted from the old spill site were also compared to the heavy crude oil (12.7° API) produced at this site. The spill site contained a higher percentage of resins and asphaltenes than did the heavy crude oil. This indicates that the oil had biodegraded since being spilled. The biodegradation potential for the crude oil as produced was low, and since biodegradation had already occurred at the old spill site, the potential for additional biodegradation was further reduced.

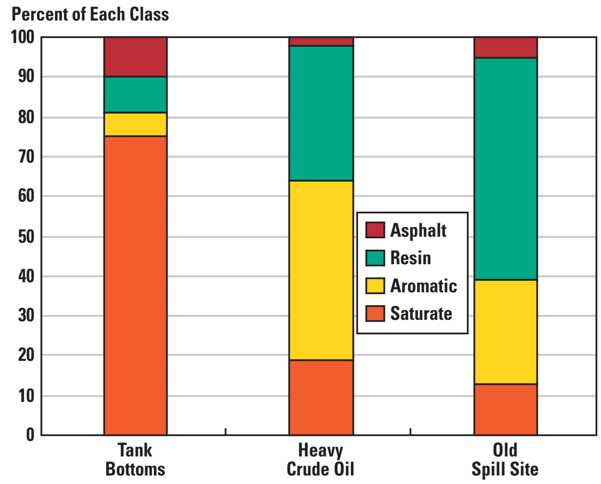


Figure 7. Class Composition Data for Tank Bottoms and Heavy Oil From an Old Spill Site

Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

Does biodegradation of oil leave behind other substances that are toxic to organisms that live in soil?

Potential toxicity that may result from biodegradation of oil in soil has been the subject of recent research.[7,8,9] There are several tests available for ecosystem function analysis: microbial response (e.g. most probable number); MicrotoxTM (solid and liquid phase); and for freshwater/marine sites: algal solid phase bioassay; daphnia survival; amphipod survival; gastropod survival; and fish bioassays.[1] **Figure 8** shows that after 17 weeks of biotreatment, diesel fuel in soil is non-toxic based on the results of the MicrotoxTM toxicity test.[8] These studies indicate that biodegradation results in a decrease, not an increase, in soil toxicity.

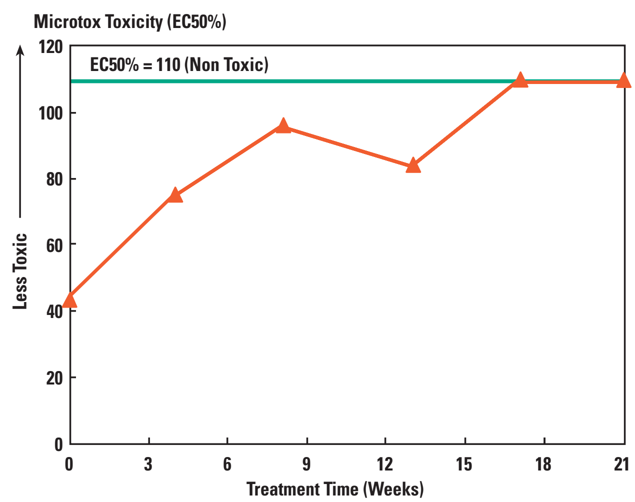
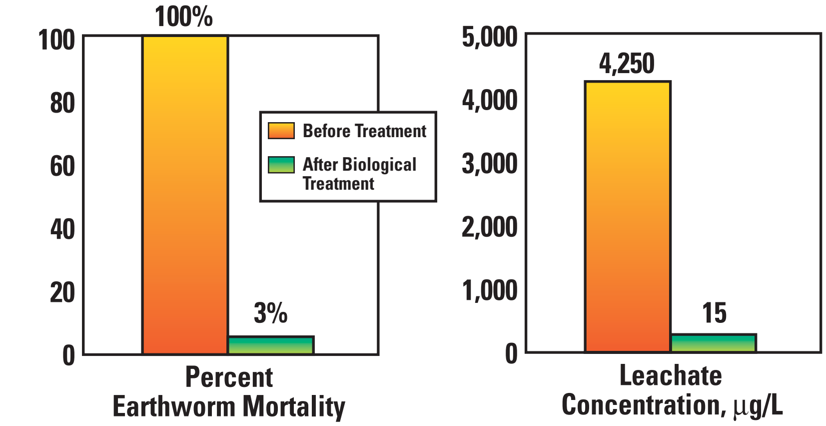


Figure 8. Micro-Tox™ Toxicity Profile for Diesel in Soil

Source: Nakles, Dave and Ray Loehr. Overview of Bioremediation Research of University of Texas and Gas Research institute. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002

Figure 9 also shows a significant decrease in earthworm mortality after biological treatment of a coal tar-impacted soil.

Studies performed in Prince William Sound, Svalbard and St. Lawrence all indicated that there was no evidence for any significant toxicity associated with bioremediation in marine or freshwater spills (**see Figure 10**).[9] Toxicity effects from fertilizer addition (toxicity to high nitrogen levels) have been observed, but when fertilizer amendments are applied carefully, they can stimulate oil biodegradation without incurring adverse environmental side effects.[9]



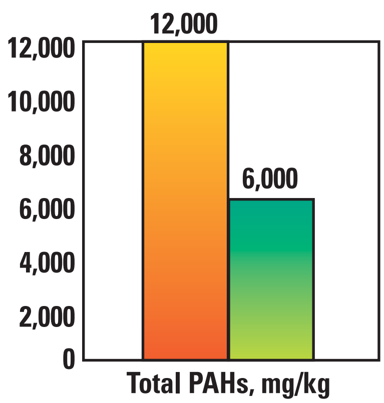


Figure 9. Treatment of Rapid-Release Fraction Yields Reduction in Toxicity and Mobility: Coal Tar Impacted Soil

Source: Nakles, Dave and Ray Loehr. Overview of Bioremediation Research of University of Texas and Gas Researchl institute. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002

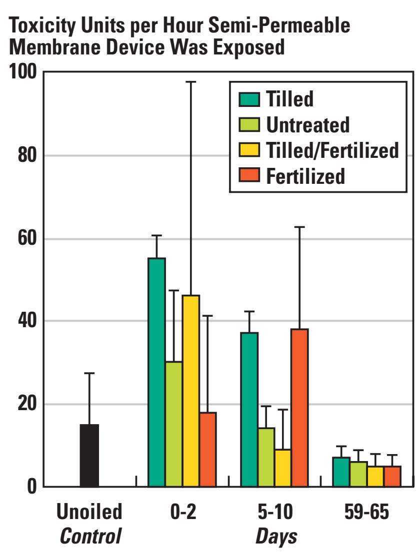


Figure 10. Svalbard Experiment

Acute Toxicity of Material Extracted into the Semipermeable Membrane Devices. Units are the Reciprocal of the EC50, Multiplied by 10,000 for Convenience and by the Reciprocal of the Exposure Time for Normalization. Error Bars Reflect the Standard Error of the Data.

Source: Prince, Roger. Bioremediation Effectiveness: Removing Hydrocarbons While Minimizing Environmental Impact. ExxonMobil Research and Engineering. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002

Can biodegradation be used for clay soils? Sandy soils?

Biodegradation will occur in all soil types but some may need additives or special care and equipment.[8]

Clay soils may need to be amended with bulking agents in order to improve oxygen transport. Sandy soils may need to be amended with organic matter to improve the soil water holding capacity. **Figure 11** illustrates that the sandy soil has a lower biodegradation rate than that of the clay and loam soils.

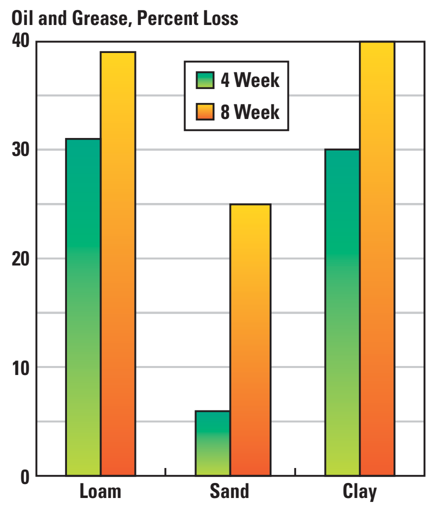


Figure 11. Biodegradation Rates

Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

A similar study was performed for refined products (jet fuel and heating oil), and the results also indicated that biodegradation rates are lower for sandy soils than for clay and loam soils. The reasons for this may be low water holding capacity, low total organic carbon content, and/or low surface area available for microbial growth in sandy soils. Biodegradation rates for the clay soils were similar or better than those for the loam; however in both studies, moisture content was maintained at optimum levels to improve soil tilth. If moisture content in clay soils is not optimized, tilth and thus aeration, will be impaired, leading to slower biodegradation rates in these soils.

What is the effect of brine on biodegradation?

Elevated concentrations of salt may be inhibitory or lethal to many classes of microorganisms because salts can disrupt the osmotic balance in the soil and interfere with enzyme activity.[3] As a result, soils with high electrical conductivity (EC) values will have retarded biodegradation rates.[4] If the soil salinity is extremely high, usually >40 mmhos, microbial activity will cease altogether.[4] However, the same basic methodology used for in-situ bioremediation of an oil spill works well to remediate a brine spill if the salt has a pathway out of the site. It has been observed that when combined spills of oil and brine are treated for oil remediation (and adequate drainage is provided to the site), the oil will be remediated long before all the salt is gone.

What are bulking agents and when should they be used?

Bulking agents are added to soil or wastes to improve permeability and water holding capacity, which in turn increases biodegradation rates. Bulking agents fall into two general categories: structural and organic.

Structural bulking agents improve the porosity and permeability of the soil by creating larger and more numerous pore spaces. Organic bulking agents initially act as structural bulking agents but also biodegrade themselves, producing degradation products that build soil structure on a long-term basis.

Organic bulking agents improve the water holding capacity which is especially important in sandy soils, but they can also increase fertilizer demand.[3] In composting applications, organic bulking agents allow for successful treatment of higher oil concentrations, increase the biodegradation rate and also increase the temperature.[8] Locally available bulking agents can be cost-effective, and can include palm husks, wood chips, saw dust, rice hulls, manure, straw, and hay. Of these, wood chips, palm husks, straw and hay can serve as structural bulking agents. The source of bulking agents should always be carefully checked to ensure that there is no potential for residual substances (like pesticides) to be present that could be toxic to the microbes.

How much bulking agent should be used?

The amount of bulking agent to be used in composting depends upon the original texture of the soil or waste. For sludges, a 1:1 to 1:3 ratio of sludge to structural bulking agent (by volume) has been reported in one study.[8] Typically 5 to 20% manure is added to enhance the water holding capacity of a compost or sandy soil.

For soil treatment, organic bulking agents should be blended into the soil until a porous soil structure is obtained with no visual evidence of oil. A rule of thumb for hay addition is 5 standard bales of hay per 1000 square feet of impacted soil.

How often should tilling be done?

Tilling the active biological zone should be performed regularly to overcome any oxygen deficiencies and to mix the soil with the nutrients and bulking agent.[5] Mixing also helps to optimize contact among the microorganisms, hydrocarbons, moisture and nutrients to enable maximum degradation rates.[5] Soil should be at or near the lower end of the recommended soil moisture range before tilling, because tilling of wet soil disrupts the soil structure and reduces oxygen infiltration.[3]

For landfarming operations, tilling is recommended at an interval of twice per month. When composting is used, tilling can be performed or a forced or passive aeration system can be used to supply oxygen. In general, the aeration method is selected by equipment availability and engineering considerations such as the compost pile configuration.[8]

What do I do if the soil is too rocky to till?

Rocky soils may not be ideal for bioremediation because of lower microbial populations, the inability to retain moisture, and difficulty in tilling. For rocky soils, one may need to consult with a bioremediation expert.

What are enzymes and why are they important?

Enzymes are protein catalysts that are responsible for driving almost all of the chemical reactions within a cell.[3] Enzymes act as efficient catalysts and alter the reaction mechanism of a cell in such a way as to lower the energy of activation for the overall reaction.[3] Enzymes are subject to inactivation (which may be lethal to the cell which contains that enzyme) by a variety of environmental conditions including: heat, adverse pH, salt, strong oxidizing or reducing agents; organic chemicals; and detergents or surfactants.[3] There are commercial "enzyme" products marketed for enhancing biodegradation, but there are little if any data that prove their cost-effectiveness in bioremediation projects.

What about surfactants?

The use of surfactants in soil to break up oil is not generally recommended because they can potentially interfere with cell membranes and enzymes, which in turn can slow biodegradation rates.[3] Surfactants have found use in deep vadose zone applications but precautions are necessary. Surfactants should be prescreened for toxicity to indigenous microbes. Dispersants, a type of surfactant, have been used successfully in breaking up marine oil spills.

The Effect of Soil Acidity on Biotreatment

What is the effect of pH on biodegradation?

pH provides a measure of the acidity of the environment, and is one of several environmental conditions that can serve to inactivate enzymes when levels are not optimal.[3] This has the effect of slowing microbial metabolism (the growth rate), which in turn has a detrimental effect on biodegradation rates.

The range in pH that is healthy for most bacteria is 6.5-8.5, and for yeasts and molds, 4.5-5.5.3 The optimal pH range for biodegradation is considered to be 6.0-8.5. Biodegradation processes may cause the soil pH to drop over time, and therefore frequent monitoring of pH is required.[8]

How do I control or adjust pH?

Lime or limestone can be added to correct low pH; sulfur, aluminum sulfate, and ferrous sulfate can be added to decrease high pH.[8] Sulfur is slow-acting since it requires conversion to sulfuric acid by soil microbes before it will counteract high pH. Lime and sulfur requirements are soil type dependent, with pounds required increasing as clay content increases.[8] Both acidifying and neutralizing amendments should be added to the soil gradually and thoroughly tilled in. Before adding pH amendments, local agricultural professionals (or in-house experts) should be consulted for guidance concerning appropriate amendment rates.

How do I measure soil pH?

Field test kits or portable pH electrode pens are available for the measurement of soil pH. Garden center pH test kits also work well and are inexpensive.

The Effect of Moisture & Temperature on Biotreatment

Is there a best time of year to initiate bioremediation?

Bioremediation can be initiated at any time of year, but consideration should be given to the potential effect of climate on the results. For example, rainy seasons may cause excessive runoff, while drought or seasonally dry conditions may result in the need for irrigation.[8] In addition, temperature can have an effect, since excessively cold or hot temperatures can slow biodegradation rates. For this reason, bioremediation projects are often initiated during the "growing season" for agricultural crops.

Can bioremediation be used in cold climates or dry, arid climates?

Yes, however, extremely low or high temperatures can have an adverse impact on biodegradation rates. In general, biodegradation will not occur below the freezing point of water.[8] A range of temperatures of 15 to 75 degrees Celsius (°C) can support one or more classifications of microorganisms.[3] Optimum temperatures for biodegradation are considered to be 65-115° Fahrenheit (F) or 18-45°C.[8] Temperatures above 100°F are usually only observed in compost piles where heat is generated and conserved.

Figure 12. Steam Rising From Compost During Tilling

Source: Sara Mc Millen, Ross Smart, Rene Bernier and Rob Hoffmann. "Biotreating E&P Wastes: Lessons Learned From 1992-2002." Presentation at the 2002 Integrated Petroleum Conference, Albuquerque, NM.

In cold climates, it may be advantageous to use composting in windrows or biopiles because these technologies generate and conserve heat during biodegradation and can extend the effective growing season into the winter months.[8] **Figure 12** illustrates steam coming off a compost pile during tilling even though there is snow on the ground and the temperature is below freezing. In dry, arid climates, temperature extremes and lack of moisture may retard biodegradation rates, unless moisture is frequently added.

Should water be added?

Whether or not water should be added depends upon the operating conditions at the site. The amount of water present is very important. Too little water inhibits biodegradation, but in wet climates too much water can be a problem as well. Research has shown that ideally, the water content of the soil should be 50 to 80% of its water holding capacity (the water holding capacity is the amount of water held in the soil pores under tension – it will not drain from the soil).[3,5] Water holding capacity depends on the soil matrix: it is typically higher for clays, and lower for sands as shown in **Figure 13**.[8] It can either be measured in the laboratory, or simply estimated from the soil type.

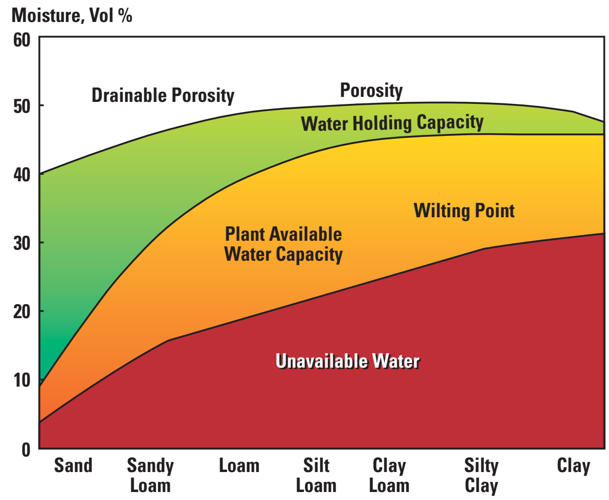


Figure 13. Relationship Between Soil Type and Water Holding Capacity

Source: Sara Mc Millen. Bioremediation Overview – ChevronTexaco. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002.

During dry conditions, water may have to be added to maintain the optimum soil moisture content, and during wet weather, drainage of the medium may have to be enhanced. Because it is so important to biodegradation, the moisture content should be monitored frequently.[5]

How do I measure moisture in the field?

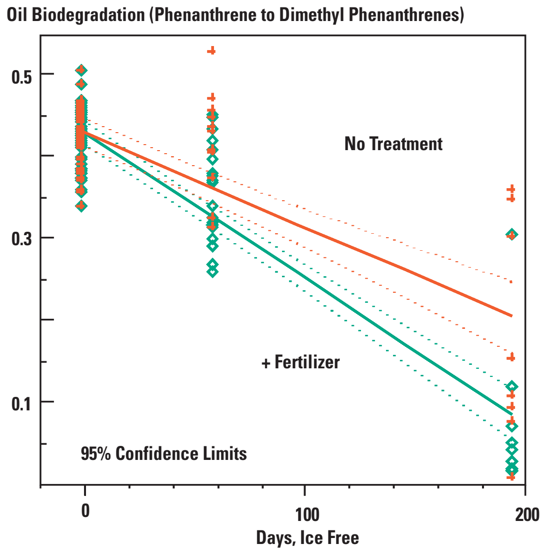
Field measurements of soil moisture content are typically made using a portable microwave or a drying oven and a reliable scale. The soil sample is removed from the ground, weighed and then dried and re-weighed at approximately five minute intervals until the weight of the sample is unchanged. The final weight is subtracted from the original weight and then divided by the original weight to obtain the percent by weight of water in the sample.

Alternatively, there are inexpensive moisture meters available for purchase at garden centers. These meters cannot be used to make precise moisture measurements, but they can be used to make "water" versus "don't water" decisions. If crops are grown in the vicinity of your biotreatment location, watering can be performed at the same frequency as crop irrigation.

Should nutrients be added, and what kind?

Most of the time nutrients have to be added to the impacted soil in order to enhance microbial growth. The two most important nutrients that need to be added are nitrogen and phosphorous. Nitrogen and phosphorous can be added simply by applying common fertilizers. An example of the effect of fertilizer addition on the rate of oil biodegradation along a shoreline after an intentional oil spill, is shown in **Figure 14**.

Figure 14. Svalbard Experiment — Bioremediation Treatment Doubled the Rate of Oil Biodegradation



Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

How much fertilizer should be added, and how often?

Nitrogen and phosphorous levels in the impacted soil should be measured to determine how much, if any, fertilizer is needed. Fertilizer should be added gradually to the impacted zone in the soil in order to avoid excessively high pH and high concentrations of nitrogen that might be toxic to soil microbes.[5] The water content of the soil must be sufficient for the nitrogen to dissolve and become available for use by the microbes, so watering may be needed after fertilizer addition.

It is important to note that nitrogen, which is applied in fertilizer as a soluble salt, will reside largely in the soil-water and will therefore increase the osmotic potential of the soil solution.[9] If too much fertilizer is added, the osmotic potential will increase to the point where it will have detrimental effects on the microbes. Also, if the soil is very sandy (and by its nature holds little moisture in the soil pores) or there has been a brine impact on the soil, less nitrogen should be added due to osmotic potential concerns.

In general, the exact amounts of fertilizer to be added and the frequency at which they need to be added depends upon field and operating conditions.[3] However, evidence from documented land treatment studies indicates that appropriate fertilizer dosages for a one-time application (to be repeated periodically) to loam-type soils are 500 pounds of nitrogen per acre, or 1100 pounds of urea per acre, and 250 pounds per acre of phosphorous.[8]

What kinds of fertilizers should be used?

Common agricultural fertilizers such as urea, ammonium nitrate, di-ammonium phosphate and manure can be used to maintain nitrogen and phosphorous levels, but consideration should be given to site-specific circumstances when selecting a fertilizer. For example, in slightly alkaline soils, organic nitrogen sources (such as manure) can cause accumulations of nitrite that are toxic to microbes. Research has shown that the use of specialty fertilizers such as oil-soluble and slow-release fertilizers yield similar biodegradation results and therefore are not necessary for most soils.[8] In fact, it is important that the fertilizers be water soluble so that they are available in the soil water for use by the microbes.[9] On the other hand, slow-release fertilizers should be used when treating marine or freshwater environments since aquatic organisms are very sensitive to nitrogen compounds in water.

What do those three numbers on the fertilizer bags mean?

The three numbers on the fertilizer bags represent the composition of the fertilizer with respect to nitrogen, phosphorous, and potassium. The first number is the percent by weight nitrogen. The second number is typically the percent by weight diphosphorous pentoxide (P_2O_5), and the third number is the percent by weight potash (K_2O).[3] Based on the information provided above regarding the amount of nutrients to add, the nitrogen to phosphorous ratios should be at least 2:1 depending on the type of fertilizer used. Potassium levels in the fertilizer are not as important as the nitrogen and phosphorous levels, but can be important to eventual revegetation following bioremediation.

What should I consider before using manure as a fertilizer?

Manure can be used as a fertilizer in bioremediation, but consideration should be given towards odor problems and the potential problem of nitrite accumulation as described above. Field experience has shown that chicken manure can cause odor problems, but horse and cow manure have been used without a significant odor problem.[5]

Another consideration in applying manure is that its nitrogen and phosphorous levels are not known. It is known however, that manure does supply trace nutrients and improves the soil structure, thereby stimulating microbial growth.[3] Manure is usually added as a bulking agent, not as the main source of fertilizer.

The Effect of Bacteria Biotreatment

Do "bugs" have to be added in order for bioremediation to work?

No, microbes (or "bugs") do not have to be added in order for bioremediation to work. Most soil already contains more than ten million naturally-occurring (indigenous) microbes per gram that have adapted to live in that environment. At least 10% of these microbes are capable of biodegrading petroleum hydrocarbons when the right conditions exist.[10] In fact, research has shown that indigenous populations of microbes will increase greatly within 24 to 48 hours of exposure to petroleum hydrocarbons.[11]

There are a lot of "bug products" sold commercially which may contain hydrocarbon-degrading microbes. However, these microbes are not adapted to a soil environment and usually die off quickly. Some of these products may also contain fertilizers and actually stimulate bioremediation when used. However, it is the indigenous microbes that are doing most of the work using the fertilizer in the product.

Regulatory Issues and Other Technical Considerations

When do I have to worry about groundwater contamination?

The circumstances under which you may have to worry about groundwater contamination are fairly complex, and depend to a significant degree on the hydrogeology of the area in which the biotreatment is to occur, the height of the soil column between the contaminated soil and any underlying aquifer, and the type of biotreatment taking place. For example, in areas where shallow groundwater is used heavily as a source of drinking water, protection of groundwater will be a concern. In these areas, liners and leachate collection systems may be used to ensure that no dissolved oil constituents are able to degrade the groundwater quality. Groundwater monitoring may be also performed to provide evidence that there is no groundwater quality degradation resulting from biotreatment. Groundwater protection is a greater concern during landfarming because the same soil receives multiple applications of oily waste.

Other areas may have shallow groundwater that is completely unusable as a drinking water source and some areas may only have groundwater present at depths greater than 500 feet. Regulatory authorities and in-house groundwater experts should be consulted in your area to determine the need for leachate collection systems or groundwater monitoring; in some cases there may be no need for a groundwater protection system based on site specific conditions.

A potential groundwater problem might exist if there is free-phase oil present in the soil or waste being treated, and the treatment area is unlined. Free-phase oil fully saturates the pore spaces of the soil and is mobile. If present in sufficient quantity, the oil can percolate downward to the water table where it may form a free-phase layer on top of the water table (if the oil is less dense than water), and its soluble constituents will dissolve and migrate in the direction of groundwater flow. A rule of thumb to prevent free-phase oil is to use an oil loading of no more than 5% by weight of oil in soil.

What are biodegradation "end-points?"

The term "end-point" is used to describe a criterion that will be used to measure whether biotreatment can be considered to be complete. End-point criteria are typically concentrations of specific components or chemicals that are measured in the impacted soil. Accordingly, end-points can be the bulk hydrocarbon content as measured by TPH or O&G or the concentration of specific petroleum hydrocarbons. Desired end-points should also be considered when selecting a treatment technology, since certain technologies may be capable of achieving the end-points in less time and with less money spent.

What kind of end-points are achievable using bioremediation?

The end-points that can be achieved by bioremediation are related to how much oil you start with, the composition of the oil, and the age of the spill. Research has shown that there is a linear relationship between API gravity of the oil, and the O&G (or TPH) end-point that can be achieved, as shown in **Figures 15 and 16**.[8]

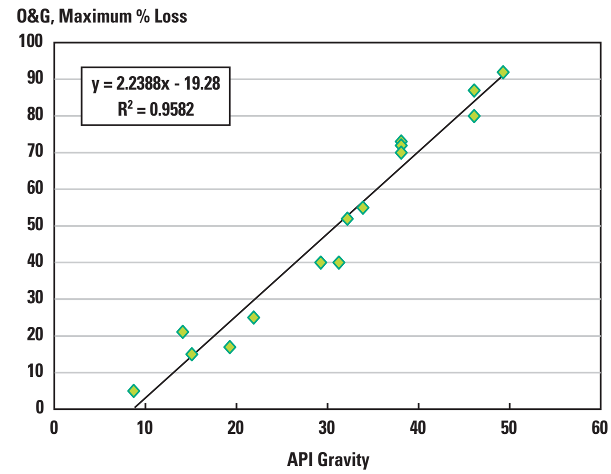


Figure 15. Correlation Between API Gravity and O&G End Points

Source: Sara Mc Millen. Bioremediation Overview – ChevronTexaco. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002.

In general, crude oils with API gravities greater than 40 degrees can expect to experience 70% or greater reductions in O&G (and 75% reductions in TPH).[8] Crude oils with API gravities less than 20 degrees can expect to experience 25% or less reductions in O&G (and 45% or less reductions in TPH).[8]

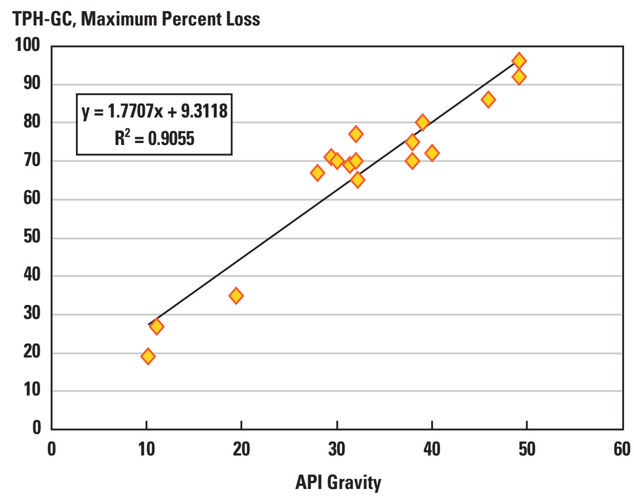


Figure 16. Correlation Between API Gravity and TPH End Points

Source: Sara Mc Millen. Bioremediation Overview – ChevronTexaco. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002.

To determine if bioremediation can achieve the treatment goal for your site, first determine the API gravity of the crude oil and use the graphs above to find the expected percent loss of hydrocarbons. Then multiply the current concentration of the oil (measured as oil and grease or TPH) by the expected percent loss to estimate the final expected end-point. The end-point concentration can then be compared to the treatment goal for the site to determine if biotreatment is a viable cleanup technology. As discussed earlier, weathering of old spill sites may further limit the biodegradation potential.

How do I know when I'm done with biotreatment?

If regulatory treatment goals have been established prior to initiating biotreatment, you will be done with biotreatment when there is regulatory acceptance that those goals have been achieved. Alternatively, a risk-based approach can be used to establish appropriate treatment goals in consultation with regulators. The risk-based approach takes into consideration who may be exposed to the oil constituents, and what the route(s) of exposure may be. (Note: Some regulatory treatment goals are risk-based, e.g., the new guidance from the Oklahoma Corporation Commission.)

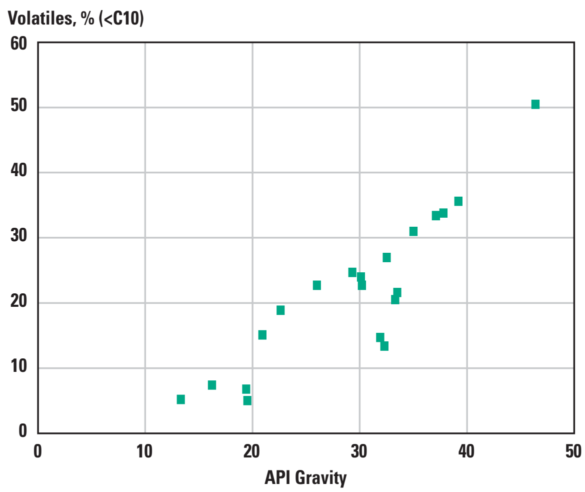
Are there regulatory considerations in selecting a bioremediation technology?

There may be regulatory constraints concerning whether bioremediation is permissible, and in which biotreatment methods are approved. One should always consult with all the local and state regulatory agencies prior to beginning biotreatment. For example, on some federal lands that are managed by the United States Bureau of Land Management (BLM), land treatment or land farming may not be allowed, while in other areas (like Colorado), the BLM allows land treatment.[15] Some agencies may require biotreated wastes and soils to reach certain TPH end-points before they can be removed and applied to soil or roads.[15]

When should I be concerned about volatile emissions?

Volatile emissions are a concern when the oil contains lighter aliphatics and aromatics (and hence has a high API gravity).[3] **Figure 17** shows volatilization losses measured for a broad range of crude oils by "topping" the oils to a constant weight at 40°C or 104°F. The weight of the oil before topping, minus the weight of the oil after topping, represents the portion of the oil that has the potential to volatilize.

Figure 17. Amount of Total Hydrocarbons That Can Be Volatized at 40°C For a Range of Crude Oils



Source: Sara Mc Millen. Society of Petroleum Engineers Distinguished Lecture, "Biotreatment at E&P Sites," 1994-95

Volatile emissions can be a health hazard if the emissions are inhaled. Care should be taken to prevent worker exposure to volatile hydrocarbons, and prevent exposure to residents if any live nearby. For example, volatile emissions can be prevented by using a covered biopile under negative pressure (meaning clean air is entering the pile) with an auxiliary air treatment system used to first treat the volatile emissions and then exhaust clean air to the atmosphere.

What is co-metabolism, and what does it mean if I am considering the use of bioremediation at a site?

Crude oil and refined products are complex mixtures of different types of hydrocarbons with varying biodegradabilities. In soil environments with complex microbial communities, co-metabolism is a major mechanism of the biodegradation process. Co-metabolism is the process by which a chemical will be metabolized (or eaten) by microbes because of the presence of a second chemical, which aids in the metabolization. In other words, certain chemicals are not easily biodegraded unless other chemicals are present that can act as co-metabolites. For example, research has shown that methyl tertiary butyl ether (MTBE) degradation gets a boost from addition of isopentane, as shown in **Figure 18.**[12]

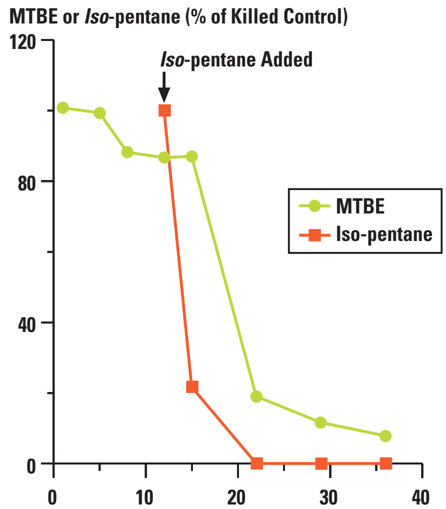


Figure 18. Co-Substrate and Target Both Degraded

Source: Stringfellow, William. Co-Metabolic Biodegradation: A Sophisticated Tool for Enhancing Bioremediation. Lawrance Berkeley National Laboratory. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002

The use of agents to aid in co-metabolism is not generally recommended for hydrocarbons, but they may be useful for recalcitrant, man-made chemicals like MTBE. A bioremediation specialist should be consulted when substances are present in soil or water that are not easily biodegraded (recalcitrant).

What are the best ways to monitor bioremediation? – hydrocarbons, nutrients, bacteria

Monitoring methods for bioremediation may include measuring concentrations of bulk or specific hydrocarbons, nutrients, pH, EC, moisture content, and the number and types of bacteria in the impacted soil or waste. The types and frequency of monitoring depend upon how much knowledge of the process is needed to achieve the goals for the site. For example, for a light API gravity crude oil spill onto a loam soil, one may only need to monitor using field test kits for nitrogen, phosphorous, pH, and TPH. For a field research project one may monitor population changes in the microbial community, loss of individual hydrocarbons, changes in toxicity to soil microbes or invertebrates, the concentrations and forms of nitrogen present, etc.

For field implementation the following analyses should be done:

- TPH or other hydrocarbon analysis requested by the local regulatory agency
- Field test kits for pH, nitrogen, and phosphorus
- Moisture meter

For a field research project the following additional analyses should be considered:

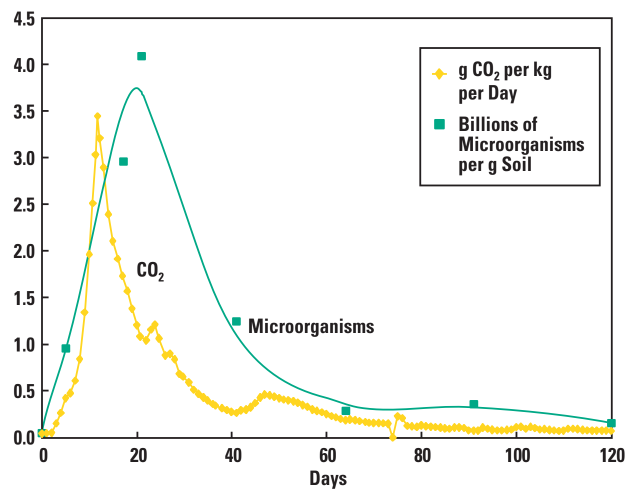
- Hydrocarbons: There are several methods available to measure hydrocarbons, but the choice of appropriate method depends on whether an understanding of bulk level of contamination is needed (e. g., O&G or TPH), or whether amounts of specific chemicals, e.g., BTEX and PAHs are needed.[3] If it is necessary to distinguish between physical loss (such as dilution in soil) versus biodegradative loss, it is a good idea to normalize to a conservative "marker" that is a component of the oil. These are typically referred to as biomarkers, which are molecular fossils that are found in oil and are highly resistant to biodegradation.[1]
- Nutrients: levels and forms of bioavailable N and P.
- pH.

For research projects in the laboratory or field, the following analyses may also be useful:

- Levels of dissolved oxygen and carbon dioxide (CO_2) in soil gas.
- Bacteria: evidence of microbial activity can be accomplished by the direct count, viable plate count, and most probable number methods. Microbial biomass and community structure can also be measured by phospholipid fatty acid analysis.[3]
- Temperature (temperature increases when bioremediation is occurring).[1]

Figure 19. Correlation Between CO_2 Production and Microbe Population – CO_2 Release Rate and Soil Microbe Numbers During Biodegradation of Crude Oil

Source: Prince, Roger and Sara Mc Millen. Summary of PERF Bioremediation Projects. Presentation at DOE/PERF Bioremediation Workshop. May 30, 2002



When collecting samples for the purpose of monitoring bioremediation, the samples should cover the entire depth of oil penetration and include statistical considerations.[1] Most monitoring of TPH, pH and nutrients can be performed using test kits in the field.[13]

Figure 19 illustrates how monitoring of CO_2 levels illustrates the correlation between increases in the microbial population and the onset of biodegradation as observed by the production of carbon dioxide.

How do I obtain a good representative soil sample?

Compositing of soil samples, where soils are collected from a variety of locations over a wide area and then mixed together to form one sample, is a good method for obtaining a sample that is representative of average soil conditions. If samples are desired that provide a measurement of variability, compositing should not be performed. Instead, discrete samples should be collected over the area.

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