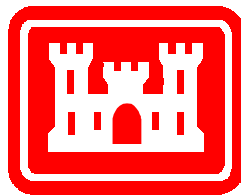


PUBLIC WORKS TECHNICAL BULLETIN 420-49-27  
31 MARCH 1999

**BIODEGRADATION OF POL-CONTAMINATED  
WASHRACK SLUDGE**



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DEPARTMENT OF THE ARMY  
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Public Works Technical Bulletin  
No. 420-49-27

31 March 1999

FACILITIES ENGINEERING  
Operation and Maintenance

BIODEGRADATION OF POL-CONTAMINATED WASHRACK SLUDGE

1. Purpose. This Public Works Technical Bulletin (PWTB) provides information on use of biodegradation for treatment of petroleum, oil, and lubricant (POL) contaminated waste. Biodegradation is defined as the use of applied or naturally-occurring micro-organisms with or without additional nutrients or other amendments for the biological breakdown and utilization of carbon-based materials.
2. Applicability. This PWTB applies to all Department of the Army installations responsible for construction, operation and maintenance of oil water separators and interceptors or disposal of POL (petroleum hydrocarbon) contaminated waste soil, grit, and sludge.
3. Reference. AR 420-49, Utility Services, 28 April 1997.
4. Discussion.
  - a. AR 420-49 contains policy and criteria for the operation, maintenance, repair, and construction of facilities and systems, for efficient and economical solid (nonhazardous) waste management including source reduction, re-use, recycling, composting, collection, transport, storage, and treatment of solid waste. Chapter 3 gives general guidance on all aspects of solid waste management, including composting (in section 3-3i).
  - b. U.S. Army installations are increasingly being asked to comply with more stringent regulations for disposal of POL and petroleum hydrocarbon-contaminated soils, grit, and sludge. Among the methods for treatment and disposal is the use of biodegradation.
  - c. This PWTB will review findings and provide supporting documentation for use of bioaugmentation/ biostimulation as appropriate. A background review of the subject of petroleum hydrocarbon biodegradation will be presented. Definition of terms, discussion of Total Petroleum Hydrocarbon (TPH) and a site specific application information is provided.
  - d. Appendix A gives a detailed explanation of this technology and an example of biodegradation as it has been implemented at Fort Hood, TX. Appendix B lists references cited throughout this PWTB. Appendix C includes an expanded discussion of TPH. Appendix D gives a review of literature related to recent bioremediation efforts.

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5. Points of Contact. Questions and/or comments regarding this subject that cannot be resolved at the installation level, should be directed to the U.S. Army Corps of Engineers, ATTN: CEMP-RI, 441 G Street, NW, Washington, DC 20314-1000; or the U.S. Army Engineer Research Center, Construction Engineering Research Laboratory at 1(800) USA-CERL, for Richard Scholze (e-mail: r-scholze@cecer.army.mil).

FOR THE DIRECTOR:

/S/  
FRANK J. SCHMID, P.E.  
Director of Engineering

## APPENDIX A BIODEGRADATION OF POL-CONTAMINATED WASHRACK SLUDGE

### 1 Bioremediation.

a. Bioremediation has gained considerable recognition in recent years as an innovative remedial technology to help reduce concentrations of petroleum hydrocarbons in soil. A major concern raised when comparing bioremediation to other remedial techniques is the time required to achieve the treatment target level. Significant strides have been made in optimizing systems to maximize the degradation rate. Supplements used to optimize bioremediation systems include specialized bacterial cultures that are claimed to increase the rate of contaminant loss. Many of these enhancements require significant effort and money to implement. Therefore, most bioremediation users choose to carefully determine the advantages to such supplements relative to their cost before including them in full-scale remedial designs.

b. Although many successes have been reported using bioaugmentation, those who fund and implement remediation technologies must be aware of the overall benefit of such an approach. The results of these case studies suggest that some conventional applications may not require bioaugmentation.

### 2. Bioremediation Activities

a. Army installations generate petroleum-contaminated soil from a variety of military operations. Examples include fuel spills, LUST cleanups, and waste sludge from oil water separators (OWS). Often, installations will store hydrocarbon-contaminated materials until sufficient quantities are generated to justify contracting for soil processing and/or disposal. Bioremediation has the capability to transform contaminated soil into a useful recyclable material at relatively low processing costs.

b. Fort Hood has successfully demonstrated the use of bioremediation for treatment of POL-contaminated sludges and soil. Fort Hood constructed a permanent bioremediation site with the capacity to handle 1600 cu yd of soil in treatment, with another 250 cu yd in a staging area. Pilot projects provided an estimated treatment time of 6 months, which allows Fort Hood an annual treatment capability of 3200 cu yd.

c. The pilot project remediation level goals for hydrocarbon treatment of contaminated soil were below 1500 ppm, which allows the soil to be reused as intermediate cover in the installation sanitary landfill. The post is also able to add the savings from not having to purchase outside soil as top cover for the landfill, which was estimated at \$10/cu yd.

### 3. Site Design

a. Fort Hood staff designed the bioremediation facility in-house. Construction was performed under contract.

b. An 8-ft chain-link security fence was constructed around the entire bioremediation treatment site to provide a buffer zone ensuring site security. A single access point is secured by lock and key. Signs deter entry by unauthorized personnel.

c. Staging Area: An area is marked and set aside on the concrete bioremediation pad away from all in-treatment materials. The area is open across the front, allowing for placement of contaminated soil and grit-trap material awaiting laboratory analysis, and for drainage of excess moisture.

d. Remediation Pad: The remediation pad is constructed of reinforced concrete 6 in. thick with a surface treatment area of 130 by 80 ft. All seams and joints are sealed so that no contaminants can leak into the ground under the concrete. Under the concrete pad is a sand base and then a 80-mil impermeable plastic liner. Also, in the sand base area between the concrete and the liner, leach field pipes were installed as a detection system for contaminant leaks in the concrete treatment surface.

e. Drainage System: The entire remediation pad area is sloped at a 0.5 percent grade to a concrete settlement area at the west end of the pad. This settlement area works as a sand or grit interceptor. The settlement area enables easy access of front-end loaders to remove these materials for further treatment. The water from the settlement system drains into an impoundment after any residue that accumulates in the area has been separated.

f. Impoundment and Irrigation System: The bioremediation site uses water piped from an already established Central Vehicle Wash Facility. A separate pump station located at the impoundment serves the bioremediation site. The water becomes an irrigation source as it is pumped by two irrigation pumps through a line system to the remediation pad, where it is applied by reciprocating sprinklers.

g. Grit Collection Chamber: The grit collection chamber (Figure A-1) is the receptacle that holds the semi-solid slurry gathered by the suction trucks used to empty over 100 installation oil water separators (maintenance site interceptors) on the post. The chamber is also designed to facilitate entry of equipment used for soil removal and cleaning. Soil removed from the chamber is classified as interceptor grit material and is placed in the staging area as newly arrived contaminated soil. The grit collection chamber ramp that is connected to the grit collection chamber is also used as an equipment wash pad and collects any contaminated soil washed from vehicles and equipment. This minimizes cross-contamination of materials treated on the pad.



Figure A1. Grit Collection Chamber

h. Operations Building: The building provides office space for site workers to do documentation or laboratory on-site soil testing, and for storage of products used in the remediation process.

#### 4. Operating Procedures.

a. The following is derived from the work of Klinger (1997). Seeding with facultative micro-organisms is used to optimize degradation of petroleum-contaminated soil. Materials are worked in using agricultural plowing and tilling techniques, to both thoroughly mix additives and to condition the soil. The bioremediation of collected grit material from the interceptors takes place in two stages.

b. Contaminated soil is transported to the staging area at the bioremediation treatment site. It is placed in a grid-identified location and documentation is started. The staging area is divided into grids to accurately identify the location of each batch of contaminated soil received and to keep all nonpetroleum storage tank (NPST) soil separate from petroleum storage tank (PST) soil, which requires special reporting. After receipt on the staging area, soil samples are obtained for initial testing, which is conducted by an external analytical laboratory to determine extent and type of contamination. If the soil is classified nonhazardous (per 40CFR261), it is contained in the staging area until transfer to the nearby remediation pad. If determined to be hazardous, the soil is transferred from the site immediately. No soil is allowed to remain in the staging area for more than 90 days.

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c. After transfer of the contaminated soil to the treatment pad, it is spread to a depth of 1 to 1.5 ft and treated. Then a second layer of soil of equal depth is placed atop the first and similarly treated. The depth of each layer is a function of the capability of the equipment used.

d. The pH of the contaminated soil may need to be adjusted to ensure optimal degradation. Agricultural lime is typically used to raise pH levels. The initial amount to be added is based on the development of a limiting curve. A soil slurry in distilled water is mixed with incremental additions of lime, and the resulting pH values are recorded. Adequate time between intervals of lime addition must be allowed for pH stabilization. Lime is added as necessary according to field monitoring of pH drop during bioremediation. Care must be taken to avoid excess lime, since pH levels above 9.0 will hinder microbial growth.

e. Nutrients to sustain a healthy microbial metabolism are an important additive. At Fort Hood, a standard agricultural fertilizer containing a nitrogen/phosphorous mixture with a ratio of three to one (3:1) is generally used. Fertilizers are applied in a dry form and are worked in by plowing then tilling. The nutrient requirement is based on stoichiometric calculations (calculation of the quantities of elements involved in a chemical reaction), treatability studies, or the need to control a specific microbial response. Soil samples should be analyzed at intervals to maintain optimum process parameters, and adjustments will need to be made.

f. Current practice at Fort Hood is to use a commercial mix of specific micro-organisms. They believe the use of facultative micro-organisms provides flexibility for the micro-organisms to modify their metabolism as required to live in the presence of free oxygen or an anaerobic environment.

g. After all additives have been applied, the contaminated soil is plowed then tilled (Figures A-2 and A-3). The plowing/tilling operations mix the soil to increase homogeneity, distribute the added chemicals and microbes, and increase oxygen transfer by promoting atmospheric diffusion. Mixing operations will also optimize degradation by redistribution of nutrients, contaminants, and micro-organisms. Mixing operations are performed directly after the soils are placed on the remediation pad. Micro-organisms and nutrients are added using a plow and power take off driven roto-tiller at the Fort Hood site. Frequency of mixing varies and is usually done only at the onset of the treatment and/or when sampling tests indicate it could be helpful. At Fort Hood, it was found to be beneficial to till every 2 weeks during the last month of the 6-month remediation process.

h. Throughout the degradation process, watering, if required, is performed after tilling. The soil will need the addition of approximately 1.5 in. of water per week during months of little or no rainfall. Moisture is determined by a standard moisture content test. Water is pulled from the impoundment by the use of an irrigation pump system and supplied by using common reciprocating sprinklers.



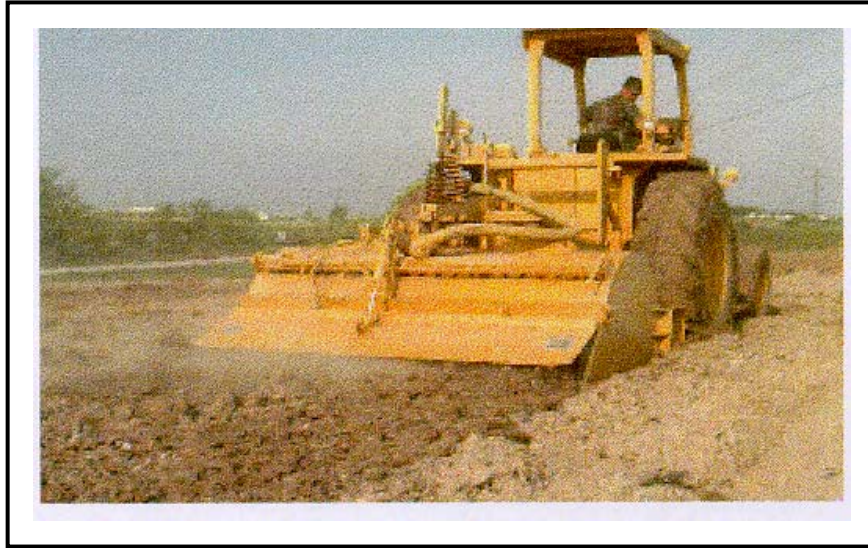


Figure A2. Tilling Operations.



Figure A3. Plowed/Tilled Soil.

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i. Contaminated soil should be sampled immediately on its arrival and placement at the staging area. Sampling will continue at the remediation pad after the initial mixing of additives, near the middle of the treatment, and at the end of the degradation cycle. Samples are collected from evenly spaced grid areas of each soil group on the remediation pad through use of a hand auger at a 1-ft depth. Samples are analyzed by an external analytical laboratory for hydrocarbon content (Figure A-4). Leachate samples must be monitored to document the loss by water transport versus true degradation. The following tests are to be run at established intervals during the remediation process unless initial testing results indicate specific tests are not needed:

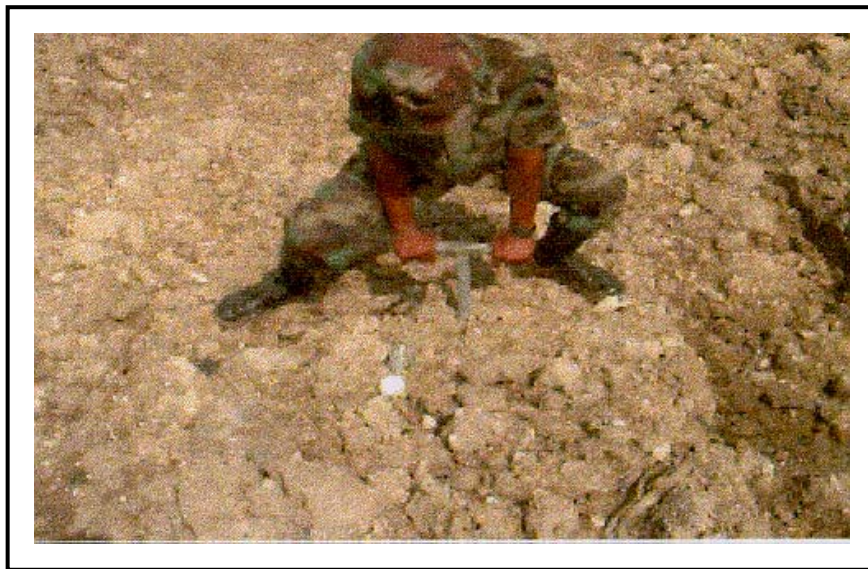


Figure A4. Extracting Sample Material.

(1) BTEX (Benzene, Toluene, Ethylbenzene, and Xylene) test to determine pollution levels for aromatics.

(2) TPH test to determine pollution levels in ppm for all hydrocarbons.

(3) TKN (Total Kjeldahl Nitrogen) test to determine levels in ppm of nitrogen.

(4) TP (Total Phosphorous) test to determine levels in ppm of phosphorous.

(5) pH test to determine acidity or alkalinity of soil.

(6) Bench test to determine potential for bacterial toxic shock.

5. Bioremediation of Interceptor Grit Materials. The bioremediation of interceptor grit materials is accomplished in two stages. The process at Fort Hood involves materials recovered from over 100 oil-sand-interceptors using vacuum trucks that clean out all grit material on a 14-

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day cycle and deposit the grit in the collection chamber at the bioremediation site. Subsequently the materials are dried and treated on the remediation pad. Remediation procedures are divided into two stages.

a. Stage One. The treatment process starts before any of the interceptor cleanouts when 2 lb of BIOPetro® (a commercial microbial additive) are placed in the empty vacuum truck container. This is enough for a full load of semi-solid slurry (approximately 10 cu yd). At the same time, the operator also puts into the container 2 lb of a selected nitrogen/phosphorous mix fertilizer. Because of the vacuum action and continuous agitation of the truck during/between pickups, the slurry is well mixed with the additives. When the truck is full, the operator drops his load into the grit collection chamber (Figure A-5).

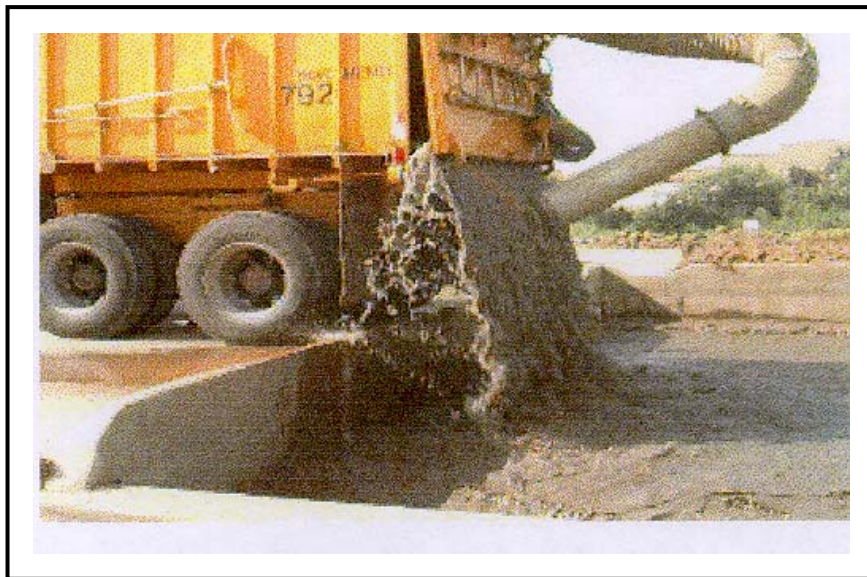


Figure A5. Vacuum Truck Dumping into the Grit Interceptor

b. Stage Two. When the grit collection chamber is full, its contents are retrieved by use of a front-end loader and placed on the bioremediation pad to dry out and be tested for hydrocarbon levels and type. If not found hazardous, the materials will be transferred to the staging area and follow the same treatment cycle as established for petroleum-contaminated soils.

c. Inspection. The bioremediation treatment site requires a daily inspection to determine if malfunctions have occurred in any of the mechanical devices used in the degradation process. After any storms, the site should always be monitored and checks made for run-on and runoff problems. The impoundment pond should be checked for high water levels.

d. Disposition of Treated Soil. Generally, treated soil is removed from the remediation pad only when the following conditions are met: (1) the contaminated soil has undergone degradation for a period of not less than 6 months, and (2) the soil meets the sanitary landfill reuse standards of less than 1500 ppm TPH and/or the topsoil cover standard of less than 500 ppm. In either event, the soil will be properly disposed with final disposition (location) of soils documented for reference and closure reports.

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e. Documentation. The documentation needed on soil being treated is extensive. From the moment the soil enters the bioremediation treatment site to its final destination as a recyclable cover material, full documentation is essential. The following information is to be collected and kept on file:

- Location from which the contaminated soil was generated.
- Location by grid of material being treated on the bioremediation pad.
- Test results throughout the degradation process from TPH drops.
- Dates and quantities of additives, by type, introduced into the soil during the process.
- Type and quantities of micro-organisms added.
- Plowing, tilling, and watering cycles.
- Final disposition site locations and identified quantities delivered to each.

f. Costs. Cost information calculated by the installation from pilot project studies indicate treatment using the commercially available micro-organisms was \$41/cu yd treated. This is broken out as \$35/lb for the micro-organisms using 1 lb/ton of soil. Support cost (equipment and manpower hours used for tilling, etc.) were \$5/cu yd treated and the nutrients added cost \$1/cu yd treated. The construction cost for the bioremediation facility was approximately \$300,000. Equipment is assigned zero value as it is on loan from the Directorate of Public Works.

## 6. Pilot Project Summaries.

a. The installation performed several pilot projects to evaluate the performance and cost effectiveness of the use of facultative micro-organisms for cleanup of petroleum-contaminated soils and interceptor grit. Pilot project A followed bioremediation of 900 cu yd of petroleum-contaminated soil generated from three different sources/dates and collected together.

b. The first source was a JP8 spill on November 11. Five hundred cu yd of soil containing an estimated 6,200 gal of JP8 were removed and stored. The second source resulted from a spill on 2 January. A tank farm valve left open resulted in a spill of 2,000 gal of fuel and removal of 300 cu yd of soil. The third source was the collection of regularly scheduled cleanup operations around the Fort Hood Recycle Used Product Storage site resulting in the collection of 100 cu yd of petroleum-contaminated soil.

c. Assuming a fuel weight of 6.24 lb/gal and a soil weight of 3,375 lb/cu yd, the concentration of fuel was approximately 16,800 ppm immediately after the spills. The contaminated soil concentrations ranged from 965 to 7260 ppm of TPH, indicating that a significant fraction had been volatilized or degraded before initiation of the treatment process.

d. Reduction of hydrocarbons in contaminated soil to a level allowing reuse as intermediate cover for the sanitary landfill (<1500 ppm) was accomplished in 6 months. The total cost determined by base personnel was \$41/cu yd treated. The primary cost was \$35/lb for the micro-

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organisms. Support costs (equipment and manpower hours used for tilling, etc.) were \$5/cu yd treated and the nutrients added cost \$1/cu yd treated.

e. The \$41/cu yd treatment cost compared very favorably with costs of contaminated soil treatment/disposal when thermal burning was used. These costs have ranged from \$40 to \$250/cu yd and have averaged \$150/cu yd (Klinger 1997).

f. A bioremediation cell was constructed and filled with the hydrocarbon-contaminated soil. The completed cell was 25 ft wide, 350 ft long, and 3 ft deep, adequately holding the 900 cu yd of contaminated soil.

g. Initial hydrocarbon contamination level of the soil was analytically determined on 9 January 1996. Samples were collected at four equally spaced locations at depths of <1 ft and analyzed externally for extent and type of contaminants present. The soil was also classified non-hazardous per 40CFR261.

h. Treatment of the contaminated soil was started on 12 January 1996. The process used microbial seeding by sowing facultative micro-organisms into the soil, adding nutrients, tilling, and adding 1.5 in. of water. BioPetro, a product of BioEnviroTech, Inc (BET) of Tomball, TX was used. Microbe application was 1 lb for each cu yd of contaminated soil (900 lb). Nutrients were locally procured fertilizer rated as 24-8-8 with an application rate of 0.5 lb to the cu yd of contaminated soil (450 lbs.). After all additives had been applied, the contaminated soil was tilled. The tilling operations mixed the soil to increase homogeneity, distribute the chemicals and microbes, and increase oxygen transfer. Tilling also optimized degradation through redistribution of nutrients, contaminants, and micro-organisms. Frequency of tillage was weather-dependent and was performed only when sampling indicated that more additives needed to be mixed into the soil.

i. Moisture is essential to the bioremediation process. Throughout the degradation process, watering, if required, was performed after tilling. The soil needed approximately 1.5 in. of water per week during most of the remediation process because of very little or no rainfall. Water was applied by using a water truck from the start of the project until 21 February 1996. A water line was then run to the site allowing for the use of common reciprocating sprinklers.

j. Samples were collected at 4- to 6-week intervals and analyzed to record the degradation of the contaminants. After the initial sampling, later samples were taken adjacent to the originally designated sample locations. A grid was later developed to simplify this task. Figure A-6 shows the sampling results obtained from this pilot project. As Figure A-6 shows, initial levels of 965 to 7260 ppm of TPH were reduced to between 76 and 1170 ppm over 6 months.

k. Considerable variation in performance by sample area was noted during testing. Hot spots developed in some areas, presumably because of the difference in the levels of hydrocarbon contamination at the various sites where the soil was collected, and the different delay times before soil was transferred into the bioremediation cell. This problem was successfully addressed by additional tilling. In area four, an investigation of a steady increase in hydrocarbon ppm levels revealed that a previously unnoticed slope in the bioremediation cell design caused contaminants to flow in the leachate to that area. This variation was corrected by

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introducing additional microbes and nutrients in the area, tilling again, and watering intensively to accelerate degradation.

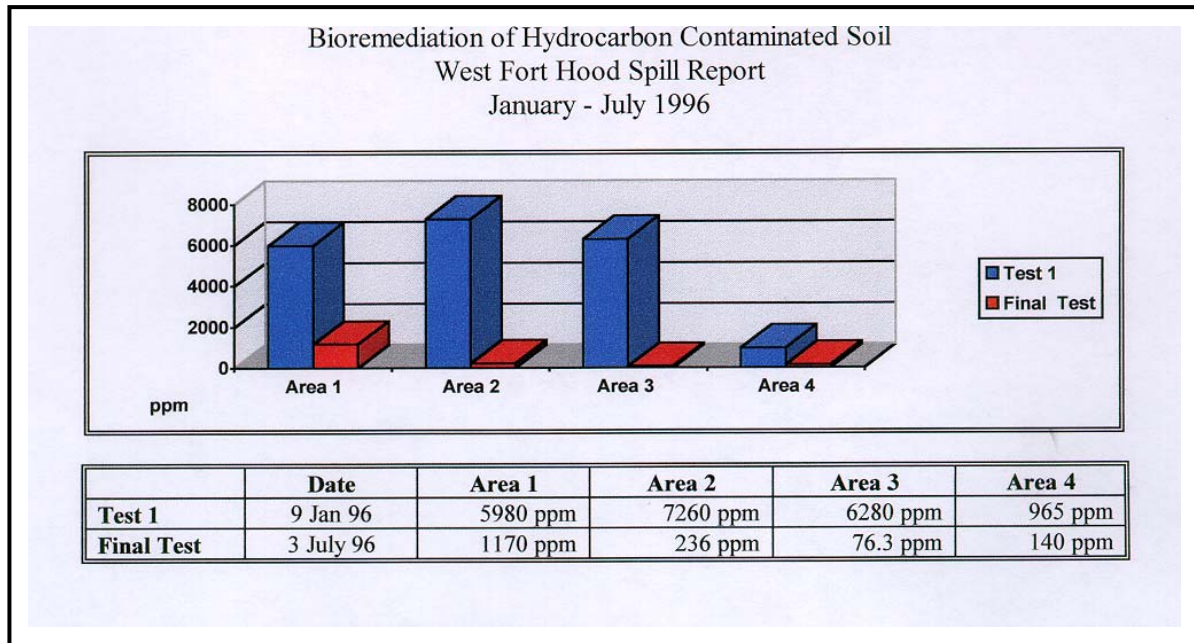


Figure A6. Reduction in Levels of TPH over 6 months at Site 1.

l. Degraded soils were removed from the remediation cell after the soils were treated for 6 months and met the reuse requirements for final disposal to the sanitary landfill.

m. Site 2 had a bioremediation treatment of 300 cu yd of soil that had been contaminated by an estimated 1000 gal of JP8.

n. Bioremediation of soil to a TPH level suitable for disposal in the sanitary landfill was completed in under 6 months at a cost of \$41/cu yd. This included \$35/cu yd for the micro-organisms, \$1/cu yd for nutrients, and \$5/cu yd as the cost of equipment and manpower as at Site 1. Two hundred gal of free-floating liquid was removed. The cell was constructed including containment with a 2-ft depth of soil. On 3 May, the cell was completed and filled with contaminated soil. The initial soil sample collection was taken 13 May 1996 at a 1-ft depth. Initial values of the six samples ranged from 10,100 ppm TPH to 13,100 ppm.

o. A similar procedure was followed as at Site 1. Facultative microbes were added at a rate of 1 lb/cu yd, and 24-8-8 fertilizer was added at a rate of 0.5 lb/cu yd. The soil was tilled and watered. Tilling occurred during the project and watering was at a rate of 1.5 in./week because of little or no rainfall.

p. Figure A-7 shows initial and final sample results of tests conducted on the soil treated in this bioremediation cell. Final results ranged from 195 to 1170, except for Area 4. Results from

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the 18 October sample had met closure requirements except for Area 4 where a spike had occurred. This was traced to an unauthorized dumping in the treatment area of an estimated 1 yd of soil excavated from a hose rupture spill of 20 gal of JP8. Four additional samples were collected on 14 November and the soil had met required reuse level of TPH. The remediated spoil was removed and reused in the sanitary landfill.

q. A third pilot site was for a site spill where an estimated 1200-gal spill saturated 200 cu yd of nearby soil. Using the same approach, a reduced overall cost to \$37/cu yd was developed. This was due to a reduction in the price of micro-organisms (quantity discount), a nutrient cost increase of \$1/cu yd and an increase of \$5 to \$10/cu yd for labor and equipment support. Elapsed treatment time remained at 6 months.

r. On 10 October 1996, the 200 cu yd of JP8-contaminated soil were placed on a drying pad. Spreading was in two layers of 1.5 ft, one on top of the other to form a single row 30 ft wide by 70 ft long by 3 ft deep.

s. Treatment began 10 October. The process used was identical to the previous two. Microbes (200 lb), 24-8-8 fertilizer (100 lb), till and add 1.5 in. of water per week. Throughout the degradation process, watering was performed when required, always after tilling and/or when moisture content in the soil dropped. Water application was by using hoses and common household reciprocating sprinklers.

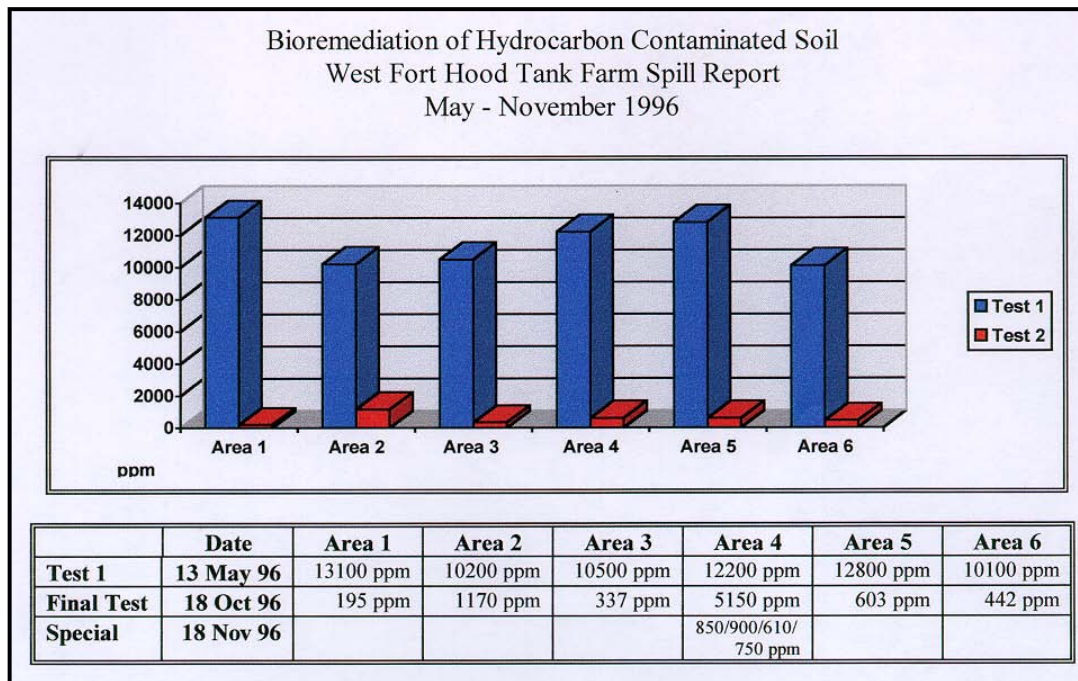


Figure A7. Initial and Final Sample Results of Tests at Site 2.

t. Sample collection began on 18 October 1996 at eight equally spaced locations in the soil to determine initial contamination level of the soil. Samples were collected at a 1-ft depth. Sample dates were 18 October, 11 December, and 14 April 1997. The following graph and chart

(Figure A-8) show initial and final sample results of tests conducted in this bioremediation cell. Initial concentrations varied from 660 to 3030 ppm of TPH. Final results varied from 220 to 1090 ppm of TPH.

u. Migration of contaminants in test areas 7 and 8 caused an elevated concentration. This was presumed to be cross contamination from another batch of contaminated soil during tilling. The treated soil was removed for use in the sanitary landfill.

v. Another pilot study was conducted presenting results of using bioremediation to treat hydrocarbon-contaminated grit material from maintenance facility wash rack interceptors. (i.e., motor pool oil water separators). Fort Hood has more than 100 oil-sand interceptors at maintenance facility washracks that require periodic cleaning. The scheduled cleaning is done by vacuum trucks that clean out all grit material on a 14-day cycle and deposit the grit in a large interceptor at the 4th Infantry Division Central Vehicle Wash Facility. Before development of the bioremediation process, the hydrocarbon levels in the 4ID interceptor were extremely high. Every 6 months when the interceptor was emptied, costly thermal burning was required.

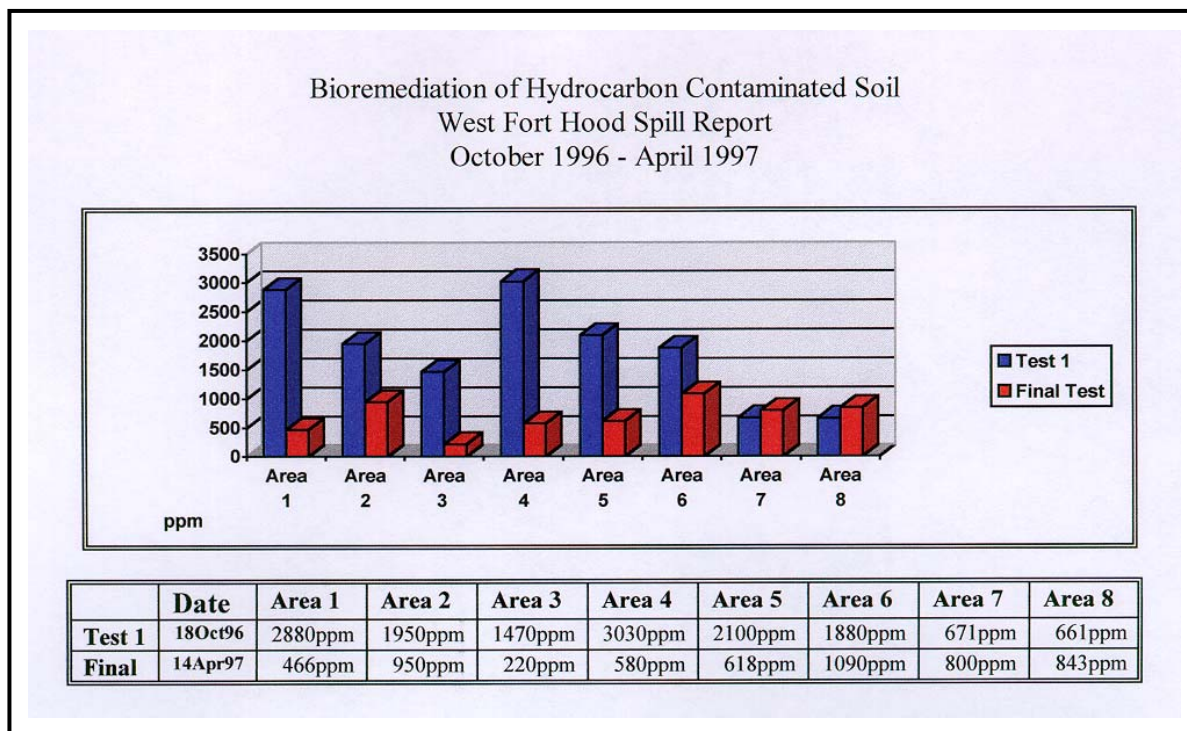


Figure A8. Initial and Final Sample Results of Tests at Site 3.

w. Initial samples were collected in March 1996 before start up of the bioremediation process. These samples identified a high level of hydrocarbon contamination. The chamber was then emptied, cleaned, and returned to service as a collection container for grit materials whose bioremediation has already started. In October 1996, after 5 months use, the content of the grit collection chamber was again sampled. Figure A-9 shows the reductions. Initial results ranged



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from 10,300 ppm of TPH to 31,100. Seven months later, results indicate a substantial decline to 1810 to 11000 ppm.

x. Equipment List. Some of equipment recommended is shown in Figures A-10, A-11 and A-12:

- 1 each Rubber Tire Tractor, w/ Bucket Loader Attachment.
- 1 each Roto-Tiller, 6 ft, TPH, PTO-driven.
- 1 each Seeder/Spreader, 800 lb capacity, TPH, PTO-driven.
- 1 each Irrigation System.
- 1 each Box Blade, TPH, 6 ft.
- 6 each pH recorders for measuring and recording cell pH conditions.
- TPH = Three Point Hitch.

\* PTO = Power take Off.

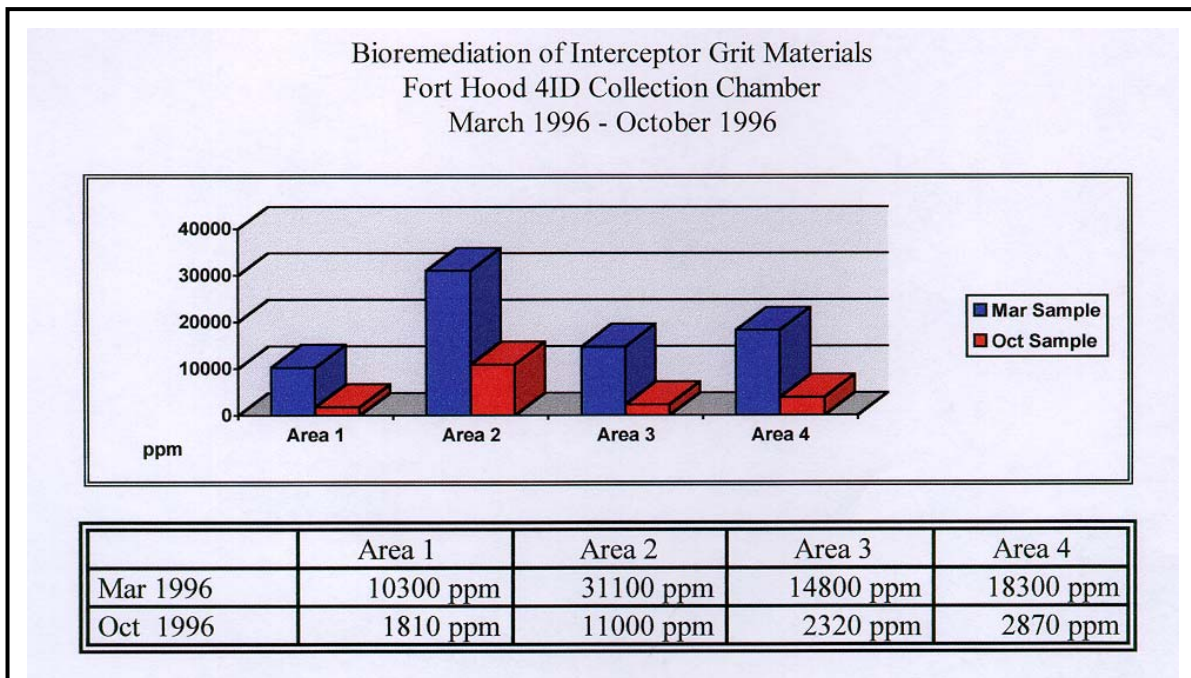


Figure A9. Results of Tests on the Content of the Grit Collection Chamber.



Figure A10. Grader.



Figure A11. Front End Loader.

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Figure A12. Earth Mover.

7. Summary. Fort Hood has been successful with the use of bioremediation for treatment of hydrocarbon-contaminated soil to levels that allow it to be used for intermediate landfill cover at the sanitary landfill located on the installation. They use a process that includes addition of facultative micro-organisms with addition of nutrients and moisture with appropriate tilling, and have achieved satisfactory results within 6 months. However, there have not been appropriate controls conducted that may show that similar results may have happened without the use of the proprietary microbe addition. The scientific literature, moreover, generally has found that inocula are of limited use for treating hydrocarbon-contaminated soil. They may speed up the initial rate, but indigenous micro-organisms usually present already include hydrocarbon degraders. Nevertheless, the Fort Hood approach has been successful. A unique approach is the collection of interceptor grit in a vacuum truck that has been dosed with microbes and nutrients, which, during the course of collection, become thoroughly mixed before being drained into the holding tank and then moved into a soil treatment facility. Costs are estimated at \$37 to \$41/cu yd for micro-organisms, nutrients, and labor and equipment operating costs. (The proprietary micro-organisms represent the dominant cost.) Capital costs are not available, although the bioremediation facility was constructed for \$300,000. Equipment is on loan from the Directorate of Public Works.

APPENDIX B  
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## APPENDIX C Total Petroleum Hydrocarbons Discussion

1. The chemical composition of petroleum products is complex and may change over time following release into the environment. The significant number of petroleum hydrocarbon impacted sites at Army installations across the United States results from a wide range of past practices. Most site cleanup or disposal investigations involving petroleum hydrocarbons are regulated by individual states that may have differing requirements in methodologies, action levels, and cleanup criteria.
2. Total Petroleum Hydrocarbons (TPH) is a parameter used to indicate the level of contamination present in the environment. The use of TPH concentrations to establish target cleanup levels for soil or water is a common approach implemented by U.S. regulatory agencies. Approximately 75 percent of the states use TPH-based cleanup criteria. Because these values have become such key remediation criteria, it is essential that everyone using TPH data understand the various analytical methods. Minor variations may be found between states.
3. TPH is sometimes referred to as mineral oil, hydrocarbon oil, extractable hydrocarbon, and oil and grease. Many analytical techniques are available to measure TPH concentrations in the environment. No single method measures the entire range of petroleum-derived hydrocarbons. Because the techniques vary in the way hydrocarbons are extracted, cleaned up, and detected, they each measure slightly different subsets of the petroleum-derived hydrocarbons in a sample. The definition of TPH depends on the analytical method used because the TPH measurement is the total concentration of the hydrocarbons extracted and measured by a particular method. The same sample analyzed by different TPH methods may produce different TPH values. For this reason, it is important to know exactly how each determination is made. Interpretation of the results depends on understanding the capabilities and limitations of the selected method. If used indiscriminately, TPH data can be misleading and could lead to an inaccurate assessment of risk.
4. TPH data do not provide ideal information for investigating sites and establishing target cleanup criteria for several reasons. For example, use of the term "TPH" suggests that the analytical method measures the combined concentrations of all petroleum-derived hydrocarbons, thereby giving an accurate indication of site contamination. In fact, this is not always the case. Furthermore, target cleanup levels based on TPH concentrations implicitly assume that: (1) the TPH result is an accurate measurement of petroleum-derived hydrocarbon concentration, and (2) the TPH result indicates the level of risk associated with the contamination. These assumptions are not correct. Many factors can affect the TPH result including the nonspecificity of some of the methods used and the complex nature of petroleum hydrocarbons and their interaction with the environment over time. There are many TPH measurement techniques. Since petroleum and petroleum-derived products are complex, some TPH methods are more appropriate than others for measuring specific contaminants.

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5. TPH concentration data cannot be used to quantitatively estimate human health risk. Under various circumstances, the same concentration of TPH may represent very different compositions and have very different risks to human health and the environment. For example, suppose two sites both have TPH measurements of 500 ppm. The constituents at one site may include carcinogenic compounds while the constituents at the other site may include no carcinogens. Moreover, the risk at a specific site will change with time as contaminants evaporate, dissolve, biodegrade, and become sequestered.

6. Although the utility of TPH data for risk assessment is limited, it is still an inexpensive tool that can be used to: (1) determine if there is a problem, (2) assess the severity of contamination, and (3) follow the progress of a remediation effort. A collection of reports has been developed by the Total Petroleum Hydrocarbon Criteria Working Group (TPHCWG), which details the subject in its complexity. The TPHCWG is a working group convened in 1993 to address the large disparity among cleanup requirements used at states with sites contaminated with hydrocarbon materials such as fuels, lubricating oils, and crude oils. These requirements usually focus on TPH with widely ranging numerical standards. The Group is guided by a steering committee consisting of representatives from industry, government, and academia with many sources of support (including DoD). The Group's goal is to develop scientifically defensible information for establishing soil cleanup levels that protect human health at petroleum-contaminated sites.

## APPENDIX D Literature Review

1. Berkey et al. (1991) described the undertaking to evaluate bioremediation products for the Exxon Valdez oil spill. Relative to the bioaugmentation approach, Roy (1992) describes the site evaluation process and some successful applications of bioaugmentation. However, no biostimulation controls were included. Compeau et al. (1991) compared two different commercially available cultures to uninoculated and sterilized inocula for petroleum degradation in soil. Compared to indigenous micro-organisms, neither of the cultures led to an increase in petroleum hydrocarbon degradation even though the microbial density was higher.
2. Leavitt and Brown (1994) present case studies examining biostimulation versus bioaugmentation. The first case was a 2-month pilot study in 1991 at the U.S. Environmental Protection Agency (USEPA) Test and Evaluation facility in Cincinnati, OH. The objective was to demonstrate the biodegradation of crude oil sludges generated during storage. The project used two 64-L slurry reactors operated in batch mode. The first (R1) was augmented with nutrients and the pH adjusted to 7.0. The second (R2) was bioaugmented with a naturally isolated, petroleum-degrading bacteria culture. The consortium had demonstrated significant TPH removal during bench-scale fermentation experiments. Fertilization and pH adjustment were conducted similarly to R1. Microbial density of hydrocarbon degraders and organic compounds were monitored regularly throughout the study. Contaminants were analyzed in the mixed liquor and headspace of each reactor over a 2-month period. Through the combined mechanisms of volatilization and biodegradation, 100 percent of the compounds having eight or fewer carbon atoms were removed from both reactors. R1 and R2 demonstrated 98 and 97 percent removal of compounds containing 9 to 12 carbon atoms. R1 demonstrated 60, 53, and 30 percent removal of compounds containing 13 to 14 carbon atoms, 15 to 18 carbon atoms, and greater than 18 carbon atoms, respectively. R2 illustrated reduced performance, with only 14 percent removal of compounds containing 12 to 14 carbon atoms and no reduction of higher molecular weight compounds. The poor performance in R2 prompted the conclusion that bioaugmentation did not benefit this bioreactor system. Leavitt and Brown presented another study designed to demonstrate accelerated biodegradation of weathered crude oil in drilling mud using pilot-scale soil volume and equipment. The demonstration was intended to compare the extent of biodegradation with conventional treatment, with additional bulking agents, and with bioaugmentation. Three plots were constructed at the site, each containing approximately 500 cu yd of waste soil. Plot 1 received fertilizer, mixing, and irrigation as needed. Plot 2 received the same treatment, but initially was mixed with 7 percent straw by volume. Plot 3 was augmented with a proprietary culture and nutrient blend and treated as recommended by the vendor. Mixing and irrigation in Plot 3 were the same as for plots 1 and 2. The inoculation rate was 1 gal for every 2 cu yd. Additional nutrients were also added to Plot 3. The demonstration was maintained for 6 months.



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a. Oil and grease, as well as total recoverable petroleum hydrocarbon (TRPH) analyses were conducted on samples after slurring. TRPH was also reported for samples collected just before slurring. Considering oil and grease results, Plot 3 was the best performer, with a loss of 86 percent at the end of the study. Plot 2 exhibited an 82 percent loss in oil and grease, whereas Plot 1 exhibited only a 32 percent loss. TRPH analyses in slurried samples proved Plot 2 to be the best performer (55 percent loss), followed by Plot 1 (45 percent) and then Plot 3 (27 percent). TRPH results in samples that were not slurried had a 62 percent loss in Plot 3, 59 percent in Plot 2, and 59 percent in Plot 1. When all analyses were averaged for each plot, Plot 2 showed the highest percent loss at 65 percent. Plot 3 averaged a 58 percent loss, and Plot 1 a 45 percent loss.

b. The recommendations drawn from this study were applied to a full-scale system. Considering the scale, nutrients were a significant factor. Nutrient costs for Plots 1 and 2 were \$0.35/lb. At the time of the study, the cost for bacteria was \$1700 per drum and \$3.00/lb for the proprietary nutrient blend. Following the vendor's recommendations would have resulted in more than a \$1 million cost for bacteria alone. Considering the cost and marginal benefit in reduction, bioaugmentation was not recommended for the full-scale treatment. The bulking agent was recommended because it did render the soils more workable and was relatively inexpensive.

3. Reynolds et al. (1994) discuss the application of field-expedient bioreactors and landfarming in Alaskan climates. They found that landfarming can be used to treat the less-contaminated soil, which often comprises the bulk of contaminated soil volume. Highly contaminated soils can be readily contained and treated on site using recirculating leachbeds. In field evaluations, the spatial average of total petroleum hydrocarbon concentration in a diesel fuel-contaminated soil decreased from 6,200 mg/kg dry soil to 280 mg/kg in approximately 7 weeks. At another site, a recirculating leachbed was used to decrease TPH concentration in diesel fuel-contaminated soil from between 300 mg/kg and 47,000 mg/kg to between 240 and 570 mg/kg in a 5-week period.

4. Landfarming is a frequently chosen treatment for contaminated soil because of containment, relatively low cost, and high potential for success. Lining and leachate recovery systems, often used to assure that soluble fractions or high concentrations do not leach, essentially double the construction cost. A possibility for maximizing cost effectiveness of landfarms is using centrally located, lined landfarms to treat multiple batches of contaminated soil where the volume of contaminated soil is large. The landfarm site occupied about 1 acre. Nutrient amendments were 270 lb of nitrogen, 34 lb of phosphorous, and 26 lb of potassium. Nutrients were added 1 month apart and were periodically tilled to approximately an 8-in. depth. About 1200 cu yd of soil were treated (Reynolds et al. 1994).

5. Prince et al. (1994) discuss a study on application of fertilizers to stimulate oil biodegradation by indigenous micro-organisms following the Exxon Valdez spill. They found that fertilizer application at the study site was generally successful in delivering nutrients, that it

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stimulated microbial hydrocarbon degradation activity, and that it increased the degradation rate of the spilled oil severalfold. They also found no adverse ecological effects following fertilizer application. They found that bioremediation was a useful part of an overall plan for cleanup. Bioremediation was found to be successful in stimulating the biodegradation of the more bioavailable components of the oil, and was thus able to accelerate ecological recovery by diminishing the duration of oil impact. For example, at one site they extrapolate data to conclude that it would have taken 500 days for the unfertilized site to achieve the same depletion achieved on a fertilized site in 100 days. They further conclude that fertilizer application was a safe and successful approach for stimulating natural biodegradation in areas with nutrient limitations.

6. Reisinger (1995) presents background information on bioremediation. Bioremediation processes transform xenobiotics (e.g., pesticides or carcinogens) introduced into the environment to a less toxic or harmless form, or mineralize them to an inorganic form (e.g., carbon dioxide and water). Bioremediation can be carried out aerobically or anaerobically (with or without oxygen) by “bugs” normally found in the soil, or by specially selected or engineered organisms. The technique can be applied either in place or at a treatment center.

a. As a class, hydrocarbons have a wide range of physicochemical characteristics. Their molecular weights range from very low to very high, as do their boiling points. They can be very fluid or very viscous, very volatile or relatively nonvolatile, and highly soluble or rather insoluble. This combination of physicochemical characteristics causes the behavior of individual hydrocarbons and mixtures to vary greatly.

b. The driving force behind hydrocarbon bioremediation is that micro-organisms can use hydrocarbons as sources of energy and food to develop and maintain cell mass. These organisms produce enzymes, used in their metabolic pathways, to extract energy from the degradation of hydrocarbon molecules. As such they are “heterotrophic.”

c. The organisms residing in the soil that are responsible for bioremediation include bacteria, fungi, and protozoans. Of these, bacteria appear to play the dominant role in hydrocarbon degradation. Just as a great variety of organisms exist in the soil, many organisms can, to a greater or lesser extent, utilize hydrocarbons to satisfy their cell growth and energy needs. Of the total consortium of organisms in the soil, a dominant organism or group of organisms exists that, through natural selection, has developed because they can thrive in the unique physical and chemical setting in which they reside. Although the dominant organism or organisms generally predominates in terms of numbers and biomass, other organisms exist within the consortium. As long as the physical and chemical regime does not change, the population dynamics do not change. However, when the environment changes — for example through the introduction of hydrocarbon contamination — the population changes in response. Those organisms that are best adapted to the new environment assume the position of dominance. Their dominance is a function of their capacity to use hydrocarbons as a primary

source of carbon and energy in their metabolism and of their ability to better compete in the new environmental setting.

d. The metabolic pathways that hydrocarbon-degrading heterotrophs use can be either aerobic (using oxygen as the primary electron acceptor) or anaerobic (using an alternative electron acceptor such as nitrate or sulfate). Although hydrocarbons can be degraded via both pathways, the aerobic pathway is generally considered to proceed most rapidly and most efficiently because aerobic reactions require less free energy for initiation and yield more energy per reaction. Borden et al. (1995) have suggested that the aerobic process dominates in the presence of oxygen. However, when oxygen in the system is depleted, denitrification processes dominate.

e. Indigenous organisms with the ability to biodegrade hydrocarbons are present in most subsurface systems. Thus, many workers believe that it is unnecessary to introduce nonindigenous organisms to address hydrocarbons in the environment biologically. Although specially adapted bacteria are sometimes introduced, they are difficult to distribute and they cannot compete with the indigenous population. As a result, they soon become a minor element of the consortium.

f. For a microbiological consortium to successfully degrade a given xenobiotic, a number of conditions must be met. The compound(s) must be biodegradable; that is they must be compatible with the enzymes produced in the metabolic pathways of the organisms present. They must also be of a form that produces adequate energy and carbon for the organisms to use. The environmental setting also must be hospitable for the organisms. The most significant environmental factors include pH, temperature, moisture content, terminal electron acceptor content, and macronutrient content and form (i.e., nitrogen and phosphorous). If these factors are not satisfactory, biodegradation will occur at a rate that is less than optimal, or it will not occur at all. One of the primary tasks facing the bioremediation practitioner is to optimize the environmental setting.

g. Hydrocarbon bioremediation can be carried out either ex situ or in situ. The primary advantage of in situ bioremediation is that it is carried out without removing the hydrocarbon-impacted soil or groundwater. The ex situ approach to hydrocarbon bioremediation is carried out aboveground by physically extracting the impacted medium. Soils are treated aboveground via landfarming, biopiling, and composting. The primary advantage to these ex situ approaches is the degree of control that can be exerted over the processes being used to manipulate the system. The primary disadvantage is the expense and disruption associated with removal, treatment, and disposal or replacement of the impacted medium.

7. Venosa et al. (1991) describe USEPA screening of commercial inocula for efficacy in stimulating oil biodegradation in closed laboratory systems. The USEPA issued a public solicitation for testing commercial microbial products for enhancing degradation of weathered

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Alaska crude oil. After submission of 40 proposals, 10 were selected for laboratory testing. The laboratory testing consisted of electrolytic respirometers set up to measure oxygen uptake over time and shake flasks to measure oil degradation and microbial growth. The conclusions after laboratory testing and following evidence supplied through microbiology, respirometry, and oil chemistry, were that only two products should be continued to the next stage for field testing. It appeared from all the available evidence that the indigenous Alaskan micro-organisms were primarily responsible for the biodegradation in the closed flasks and respirometer vessels and that any enhancement provided by the two products selected for further testing might have been due simply to metabolites, nutrients, or co-substrates present in the products.

8. Pritchard (1992) indicates that there have been several attempts at inoculation of oil-contaminated soil but with little success. He indicates the work of Jobson et al. (1974) showed that inoculation had no more effect than simple nutrient addition. Tilling or mechanical re-working of the soil is also often needed to get oxygen to the microbes. Mueller et al. (1992) state that sustained stimulatory effect of inoculation was no greater than that observed with the addition of inorganic nutrients alone.

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