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Standard Guide for
Consideration of Bioremediation as an Oil Spill Response Method on Land[1]

This standard is issued under the fixed designation F 1693; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ε) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 The goal of this guide is to provide recommendations for the use of biodegradation enhancing agents for remediating oil spills in terrestrial environments.

1.2 This is a general guide only, assuming the bioremediation agent to be safe, effective, available, and applied in accordance with both manufacturers' recommendations and relevant environmental regulations. As referred to in this guide, oil includes crude and refined petroleum products.

1.3 This guide addresses the application of bioremediation agents alone or in conjunction with other technologies, following spills on surface terrestrial environments.

1.4 This guide does not consider the ecological effects of bioremediation agents.

1.5 This guide applies to all terrestrial environments. Specifically, it addresses various technological applications used in these environments.

1.6 In making bioremediation-use decisions, appropriate government authorities must be consulted as required by law.

1.7 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.* In addition, it is the responsibility of the user to ensure that such activity takes place under the control and direction of a qualified person with full knowledge of any potential or appropriate safety and health protocols.

2. Referenced Documents

2.1 *ASTM Standards:*

F 1481 Guide for Ecological Considerations for the Use of Bioremediation in Oil Spill Response—Sand and Gravel Beaches[2]

3. Terminology

3.1 *Definitions:*

3.1.1 *aerobes*—organisms that require air or free oxygen for growth.

3.1.2 *anaerobes*—organisms that grow in the absence of air or oxygen and do not use molecular oxygen in respiration.

3.1.3 *bioaugmentation*—the addition of microorganisms (predominantly bacteria) to increase the biodegradation rate of target pollutants.

3.1.4 *biodegradation*—chemical alteration and breakdown of a substance, usually to smaller products, caused by microorganisms or their enzymes.

3.1.5 *bioremediation*—enhancement of biodegradation.

3.1.6 *bioremediation agents*—inorganic and organic compounds and microorganisms that are added to enhance degradation processes, predominantly microbial.

3.1.7 *biostimulation*—the addition of microbial nutrients, oxygen, heat, or water, or some combination thereof, to enhance the rate of biodegradation of target pollutants by indigenous species (predominantly bacteria).

3.1.8 *ecosystem*—organisms and the surrounding environment combined in a community that is self-supporting.

3.1.9 *identification*—the process of establishing the identity of an unknown organism by comparing the properties with respect to known organisms.

3.1.10 *indigenous*—native to a given habitat or environment.

3.1.11 *methemoglobinemia*—an acquired blood disorder leading to oxygen deprivation, stupor, and death from exposure to nitrates in drinking water.

3.1.12 *nutrient*—a substance that supports the growth of organisms.

3.1.13 *refined petroleum products*—products derived by means of various treatment processes from crude oil, a highly complex mixture of paraffinic, cycloparaffinic, and aromatic hydrocarbons that contains a low percentage of sulfur and trace amounts of nitrogen and oxygen compounds. Hydrocarbon products made by refining crude oils are specified in Section 5 of the *Annual Book of ASTM Standards* **(1)**.[3]

3.1.14 *risk*—the probability or likelihood that an adverse effect will occur.

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[2] *Annual Book of ASTM Standards*, Vol 11.04.

[3] The boldface numbers in parentheses refer to the list of references at the end of this guide.

3.1.15 *species*—a taxonomic category characterized by individuals of the same genus that are mutually similar and are able to interbreed.

3.1.16 *terrestrial*—consisting of land, as distinguished from water.

3.1.17 *toxicity*—the property of a material, or combination of materials, to affect organisms adversely.

4. Significance and Use

4.1 The purpose of this guide is to provide remediation managers and spill response teams with guidance on an alternate means (called bioremediation) of treating oil spills safely and effectively on and below terrestrial surfaces.

4.2 Bioremediation is one of many available tools and may not be applicable to all situations. This guide can be used in conjunction with other ASTM guides addressing oil spill response operations, including Guide F 1481, as well as options other than bioremediation.

5. General Considerations for Bioremediation Use

5.1 Bioremediation technologies attempt to accelerate the natural rate of biodegradation. In situ, solid-phase, and slurry-phase represent the major bioremediation technologies used. These technologies may be unnecessary in those cases in which the natural rate of biodegradation suffices. The use of adequate controls in preliminary field studies, or the results of previously reported studies, will assist in determining the extent to which microorganism or nutrient amendments, or both, are necessary to obtain the desired rate of degradation.

5.2 Bioremediation performance depends on the efficiency of the petroleum hydrocarbon degrading indigenous microorganisms or bioaugmentation agents. Performance also depends on the availability of rate-limiting nutrients and the susceptibility of the target crude oil or refined product to microbial degradation.

5.2.1 In general, aerobic bioremediation systems degrade oil more rapidly than anaerobic systems.

5.2.2 Numerous microorganisms, represented by hundreds of species, are responsible for the degradation of the oil. Various texts describe the biodegradability and biodegradation rates of a variety of organic compounds present in oil **(2,3)**.

5.2.3 The biodegradation of saturated hydrocarbons in the absence of molecular oxygen is limited to a few species. In general, shorter chain hydrocarbons are less effectively degraded in anaerobic conditions compared with aerobic conditions. However, anaerobic degradation is possible **(4)** if there is at least one double bond on the hydrocarbon molecule, in an appropriate position.

5.3 Bioremediation must be conducted under the guidance of qualified personnel who understand the safety and health aspects of site activities.

6. Background

6.1 General background information concerning approaches to bioremediation are presented in this guide, as well as discussed in Guide F 1481. Pertinent information from that guide is included in 6.1.1 through 6.1.4, as follows:

6.1.1 Approaches to bioremediation for oil spill response include biostimulation, the addition of nutrients, oxygen, heat, or water, or combination thereof, to stimulate indigenous microorganisms, and bioaugmentation, the addition of oil-degrading microorganisms, which may be used in combination with biostimulation **(5-15)**. As a precaution, it should be noted that nutrient components may be toxic or harmful to plants, animals, and humans, and that non-indigenous species may alter the indigenous microbial ecological balance at least temporarily. Water effluent nitrate levels, which can affect drinking water sources, should be minimized to diminish risks of anemias such as methemoglobinemia. Similarly, excessive ammonium levels should be avoided because they can affect fish and invertebrates, since many are immobile and cannot avoid the treated area. Therefore, nitrogen and other nutrient levels should be monitored. Instructions to ensure safety and effective product use should be established by the manufacturer or supplier for each commercial microbial product, and specific instructions should be followed by the product user.

6.1.2 Biostimulation has been shown to enhance the biodegradation of terrestrial oil spills. Biostimulation uses the addition of appropriate nutrients (for example, nitrogen, phosphorus, potassium, micronutrients, and so forth), oxygen, heat, or water, which may have been limiting factors. If microbial degraders of the target oil contaminants are present in the soil or contaminated waters, this approach may lead to increases in the rate of degradation. In some cases there may not be a sufficient indigenous oil-degrading population to stimulate. This may be the case in environments in which the degrader population has not developed. Alternately, the toxic nature of the petroleum product may diminish or eliminate microorganisms. Also, the excavation of soil from anoxic zones and subsequent relocation to an oxygen-rich environment may result in a lack of microbial degraders due to the drastic change in conditions **(16)**. The microbial response to biostimulation may include a lag period (weeks to months) for the growth or natural selection of degraders to occur. Microorganisms, as well as oil contaminants, should be monitored throughout the process to establish efficacy and safety. Comparisons with databases that include soil and water microorganisms may be used to identify microbes.

6.1.3 Bioaugmentation may use commercial microbial products, on-site production of microbes from stock cultures, or laboratory isolation, characterization, and subsequent production of microbes from the particular site (or another site similar in soil and contaminant characteristics). This approach may increase soil microbe concentrations rapidly. Microbes selected must be nonpathogenic and must metabolize the oil contaminant(s), reducing toxicity. Growth requirements of the microbes need to be well understood. Their growth rate is controlled by the limiting growth conditions of temperature, pH, nutrients, water, oxygen, the contaminated medium (soil, sludge, and water), and oil. Microorganisms as well as oil components should be monitored to establish efficacy and safety.

6.1.4 While apparently safe and effective in the laboratory setting, genetically engineered oil-degrading microorganisms have not yet been authorized for environmental release **(16)**.

6.2 There are several bioremediation technologies available. It is important to understand the potential use of these systems when assessing their applicability for full-scale implementation. Costs are determined by the size of the site, soil properties, type and level of oil contaminant(s), goals, time allowed for attaining the goals, and testing requirements.

6.3 *In situ* bioremediation occurs without excavation of the contaminated soil. This technology relies predominantly on the enhanced degradation of oil by bacteria following the addition of nutrients, air, oxygen or oxygen-releasing compounds, and moisture. This has been demonstrated through the use of indigenous as well as augmented microorganisms. Groundwater treatment may be achieved simultaneously or through pump and *ex situ* treatment methods. Anaerobic biodegradation systems can also be promoted; however, their utility is limited. Since soil is not excavated, volatile release is limited, and the risks and costs associated with excavation and treatment are reduced.

6.3.1 Bioventing involves the introduction of air under pressure to the unsaturated zone of contaminated soil. The process pulls or pushes air into the soil for use by the aerobic microorganisms. Although the purpose is to deliver oxygen required by the microbes, the flow of air will desorb some of the more volatile components from the soil (for example, gasoline-contaminated soil), and the exhaust gases may have to be treated. Successful treatment requires adequate soil porosity, moisture, nutrients, and microorganisms with the appropriate biodegradation abilities. Additives may be provided at or near the surface to percolate through the treatment zone.

6.3.2 Biosparging is similar to bioventing except that air is injected directly into the ground below the water table in the saturated zones. Although the purpose is to deliver oxygen required by the microbes, vacuum pumps may be used to recover vapors that may also have to be treated prior to discharge. Nutrients and microbes may be added in the injection well to stimulate and augment biodegradation.

6.4 Solid-phase bioremediation treats soils above ground, primarily in contained treatment cells or tanks. Techniques similar to landfarming are used, including irrigation, tilling, and nutrient and microbe additions. As with *in situ* bioremediation, treatment can involve biostimulation or bioaugmentation. Abiotic losses through volatilization and leaching can be minimized through treatment design and implementation. The contaminated soil is contained, preventing leaching, and is defined with respect to the volume and concentration of the oil, especially as the soil is homogenized during processing. The defined nature of the soil and its oil contaminants allow predictability in the remedial process.

6.4.1 A comprehensive contaminated materials handling plan (CMHP) should be developed prior to excavation and treatment when using systems that require excavation. It may include the designation of a materials staging area present within the treatment facility and equipment decontamination within delineated exclusion zones.

6.4.2 A comprehensive health and safety program should be in effect throughout the remediation project. This program may include medical examinations of employees, contact and respiratory protection, and air, soil, and water monitoring.

6.4.3 The treatment facility, or biopad, should contain an appropriate rainfall event protection (for example, 10 years, 24-h rainfall event). After the appropriate soil moisture content is determined for the specific treatment, a water budget should be calculated. This should maintain the proper moisture content balance between moisture added by irrigation and rainfall, and moisture lost through evaporation, transpiration, and percolation.

6.4.4 Solid heaping (biopiles or soil piles) involves piling the contaminated soil to several meters, usually over a network of perforated piping that may be layered throughout. Nutrients, water, and microorganisms are added through simple irrigation techniques, and air is drawn through pipes by vacuum. The vacuum system exhaust may be treated prior to discharge, effectively removing airborne volatile or semi-volatile components. Advantages include a requirement for less space and less material handling compared with solid-phase treatment (landfarming), and diminished volatile losses. Leachates are collected and treated, recirculated or discharged.

6.4.5 Composting promotes biodegradation in stored wastes by means of supplementation with bulking agents (biodegradable or non-biodegradable) that enhance soil permeability. The biologic decaying process is often thermophilic, thus limiting the types of microbes and associated degradation rates. Three basic systems have been used. “Open windrow” stacks the waste in long piles that are aerated through constant excavation and reconstruction.“ Static windrow” is similar to heap methods, laying the soil over a network of perforated pipes that aerate through forced air. “In-vessel” methods enclose the soil in a closed reactor that aerates and mixes the soil both physically and by means of forced air. The material remaining after treatment, the humus, can serve as a source for fill, cover, and landscaping material.

6.5 Slurry-phase bioremediation combines contaminated solids (soil, sludge, sediment) and liquids to form a slurry suspension. The slurry is supplemented with nutrients, air or oxygen-releasing chemicals, or microorganisms, or a combination thereof, in a bioreactor system. The slurry is stirred or agitated to enhance contact between the oil, nutrients, and microbes. The system may be designed as a batch or continuous flow process. Frequently, slurry-phase bioremediation is combined with other (physical, chemical or other biological) processes in a treatment train. These systems treat contaminated soil and water. Bioreactors promote optimal conditions while minimizing volatile losses, resulting from the design of the system and the type and amount of oil in the solid or liquid matrix. Compared with solid phase treatment, time is generally reduced when using bioreactors. The slurries are comprised of approximately 10 to 30 % w/v soil in water, and may require multiple batch treatments to process the total soil volume depending on reactor size and number. Hydrocarbons in high concentrations, as well as fines from soil washing can be treated. Slurry phase systems can be cumbersome and require extensive mechanical and technological construction and design, and maintenance. The costs for slurry phase treatment are generally higher in comparison to solid phase treatment.

7. Bioremediation Technology Selection Assessment

7.1 Treatability studies provide data to support treatment selection and are performed prior to remedy selection. The data indicate whether treatment goals can be met and further determine the optimal operation conditions for remediation project design. For example, a document prepared by the U.S. Environmental Protection Agency (U.S. EPA) mentions three levels of treatability studies **(17)**. The level of study chosen depends on available literature information, technical expertise, and site-specific considerations. In addition, treatability study design and interpretation for aerobic biodegradation remedy screening has been addressed **(18)**.

7.2 Various government agencies require support documentation. Studies are available through databases developed by groups sponsored by the U.S. EPA and Environment Canada. One such database, which includes innovative cleanup technologies, is the U.S. EPA's Vendor Information System for Innovative Treatment Technologies (VISITT) **(19)**. This database, made available since 1992, focuses on treatment of ground water, soil, sludge, sediments, and solid wastes, excluding the traditional technologies of incineration, solidification, stabilization, and pump-and-treat groundwater systems. The U.S. EPA Office of Emergency and Remedial Response (OERR), in conjunction with the Office of Research and Development (ORD), developed a new Contaminated Soil and Debris (CS&D) database. It contains information concerning the effectiveness of a given technology to remediate soils, sludges, and debris contaminated with hazardous waste. This database, available in 1992, supports technology transfer through easy access searches by title, site, scale, author, content, and other keywords. The data are used in developing standards for treating CS&D (40 CFR 261) under land disposal restriction (LDR) that may not be treatable to the best developed available technology (BDAT) levels established by 40 CFR 261.

7.3 Governmental agencies may regulate the use of bioremediation agents. The role of these regulatory agencies varies **(16)**.

7.4 There are many advantages as well as disadvantages associated with the various bioremediation options. Table 1 describes these attributes for the derivations discussed in Section 6 of this guide. In general, compared with other technologies, selection advantages include cost effectiveness,

TABLE 1 Selection Assessment

Bioremediation Technology	Advantages	Disadvantages
In situ	Since soil is not excavated, volatile release is limited, and the risks and costs associated with excavation and treatment may be reduced. Less intrusive than other technologies. Cleanup of the source may help prevent contamination of the ground water. Applicable in emergency response situations.	The impact on ground water may necessitate hydrogeologic monitoring. Representative soil monitoring is difficult to achieve, and treatment requires adequate soil porosity, moisture, nutrients, heat, oxygen, and biodegrading microorganisms. The time frame to achieve cleanup may be uncertain.
Ex situ and *in situ* solid-phase landfarming	The contaminated soil is contained, preventing leaching, and volatilization can be controlled. The material being treated is defined with respect to the volume and concentration of the oil, especially as the soil is homogenized during processing. If the pH is not within physiological levels for bacterial growth, it can be balanced prior to further treatment. The toxic nature of some oil can be diluted with clean soil or bulking agents (for example, yard waste). The defined nature of the soil and its oil contaminants allow predictability in the remedial process. Treatment of similar wastes, achieved through appropriate placement, may help to accelerate the process.	Airborne volatile or semi-volatile components released during excavation and tilling operations occur. Material handling is associated with treatment. Aerobic biodegradation pathways are favored, requiring continuous air supply by means of tilling or chemical treatment. Depth of soil is limited to the tilling apparatus, ~0.5 m. Treatment requires adequate soil porosity, moisture, nutrients, and biodegrading microorganisms.
Ex situ solid-phase soil heaping	Less space and less material handling are required compared with *ex situ* solid-phase treatment (landfarming). Diminished volatile losses due to vacuum capture. Leachates are collected and treated and recirculated or discharged. Treatment of similar wastes, achieved through appropriate placement, may help to accelerate the process.	There are limitations in soil pile height and therefore soil volume. Some pile manipulation may be required to ensure thorough treatment, and this may affect the predictability of cleanup. Energy requirements add to overall costs.
Ex situ solid-phase composting	Compared with stockpiling, these methods accelerate degradation. The material remaining after treatment may serve as a source for fill, cover, and landscaping material.	The biologic decaying process is often thermophilic, thus limiting the types of microbes and associated degradation rates.
Ex situ slurry-phase	The simultaneous ability to treat contaminated soil and water. Bioreactors promote optimal conditions while minimizing the release of volatiles through system design and the type and amount of oil in the solid or liquid matrix. Compared with solid phase treatment, time is usually reduced when using bioreactors. No dewatering is required prior to treatment. Hydrocarbons in high concentrations, as well as fines from soil washing, can be treated.	Slurry-phase systems are engineered treatment systems requiring design, fabrication, and maintenance similar to above-ground biological wastewater treatment processes. Emissions may have to be controlled. Slurries are comprised of 10 to 30 % w/v of soil in water and may require multiple batch treatments to process the total volume, depending on reactor size and number. Costs are generally higher in comparison to solid-phase treatment. Dewatering after treatment is energy demanding, resulting in water and solid fractions that may have different cleanup criteria for subsequent disposal.

reuse, and reduced intrusion by response personnel (short and long term) into affected areas.

8. Recommendations

8.1 Application of bioremediation in terrestrial oil spill treatments should be considered as an alternative to other techniques.

8.2 Treatability studies should be used to provide data to support treatment selection, indicate whether cleanup goals can be met, and optimize operational conditions for remedy design.

8.3 Safety and efficacy data should be substantiated prior to any bioremediation field application. This information should be provided and should be substantiated through treatability studies and published reports.

8.4 Biostimulation of indigenous microorganisms should be weighed against bioaugmentation with selected microorganisms. Consideration must be given to time, predictability, regulatory and public opinions, cost, and cleanup requirements for process selection.

8.5 Implementation of bioremediation technology is site- and oil contaminant-specific and must be used appropriately in accordance with government agencies.

9. Keywords

9.1 bioaugmentation; bioremediation; biostimulation; composting; in situ; landfarming; oil; oil spill response; soil heaping; solid-phase; slurry-phase; terrestrial; treatability

REFERENCES

(**1**) *Annual Book of ASTM Standards*, Vols 05.01, 05.02, 05.03, 05.04, 05.05, and 11.04, ASTM, Conshohocken, PA.

(**2**) *Handbook of Environmental Fate and Exposure Data for Organic Chemicals*, P. H. Howard, ed., Lewis Publishers, Inc., Chelsea, MI, Vols I–III, 1989–1991.

(**3**) *Handbook of Environmental Degradation Rates*, P. H. Howard, ed., Lewis Publishers, Inc., Chelsea, MI, 1991.

(**4**) Schink, B., "Principles and Limits of Anaerobic Degradation: Environmental and Technological Aspects," *Biology of Anaerobic Microorganisms*, J. B. Zehnder, ed., John Wiley & Sons, New York, NY, 1988, pp. 771–846.

(**5**) *Bioremediation of Petroleum Contaminated Sites*, E. Riser-Roberts and C. K. Smoley, eds., CRC Press, Boca Raton, FL, 1992.

(**6**) *Hydrocarbon Contaminated Soils*, Vol II, P. T. Kostecki, E. J. Calabrese, and M. Bonazountas, eds., Lewis Publishers, Chelsea, MI, 1992.

(**7**) *Petroleum Microbiology*, R. M. Atlas, ed., Macmillan Publishing Co., New York, NY, 1984.

(**8**) *Microbiological Decomposition of Chlorinated Aromatic Compounds*, M. L. Rochkind-Dubinsky, G. S. Sayler, and J. W. Blackburn, eds., Marcel Dekkar, Inc., New York, NY, 1987.

(**9**) *Bioremediation of Hazardous Wastes*, EPA/600/R-92/126, Office of Research and Development, U.S. Environmental Protection Agency, August 1992.

(**10**) *Bioremediation of Hazardous Wastes*, EPA/600/9-90/041, Office of Research and Development, U.S. Environmental Protection Agency, September 1990.

(**11**) *Bioremediation of Contaminated Surface Soils*, EPA/600/9-89/073, Office of Research and Development, U.S. Environmental Protection Agency, August 1989.

(**12**) *Proceedings of the Joint CSCE-ASCE National Conference on Environmental Engineering*, R. N. Yong, J. Hadjinicolaou, and A. M. O. Mohamed, eds., McGill University, Montreal, Quebec, July 12–14, 1993.

(**13**) Rowell, M. J., Ashworth, J., and Qureshi, A. A., *Bioremediation of Petroleum Hydrocarbons in Soil Environments*, Document EE-141, Environment Canada, Environmental Protection Directorate, River Road Environmental Technology Centre, Ottawa, Ont.

(**14**) *Understanding Bioremediation: A Guidebook for Citizens*, EPA/540/2-91/002, Office of Research and Development, U.S. Environmental Protection Agency, February 1991.

(**15**) *Applied Bioremedial Technology: Basic Concepts and Technical Considerations*, A. K. Kaufman and K. Devine, eds., The Applied Biotreatment Association, Fall 1990.

(**16**) Hoeppel, R. E., and Hinchee, R. E., "Enhanced Biodegradation for On-Site Remediation of Contaminated Soils and Groundwater," *Hazardous Waste Site Soil Remediation: Theory and Application of Innovative Technologies*, D. J. Wilson and A. N. Clarke, eds., Marcel Dekker, Inc., New York, NY, 1994, pp. 311–395.

(**17**) *Guide for Conducting Treatability Studies Under CERCLA*, Interim Final, EPA/540/2-89/058, Office of Research and Development and Office of Emergency and Remedial Response, U.S. Environmental Protection Agency, December 1989.

(**18**) *Guide for Conducting Treatability Studies Under CERCLA: Aerobic Biodegradation Remedy Screening*, Interim Guidance, EPA/540/2-91/013A, Office of Research and Development, Office of Emergency and Remedial Response, and Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, Risk Reduction Engineering Laboratory, July 1991.

(**19**) *VISITT 3.0 Bulletin*, EPA 542-N-94-003, Office of Solid Waste and Emergency Response, U.S. Environmental Protection Agency, June 1994.