**Designation: F1693 – 21**

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

This standard is issued under the fixed designation F1693; 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, health, and environmental 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.*

1.8 *This international standard was developed in accordance with internationally recognized principles on standardization established in the Decision on Principles for the Development of International Standards, Guides and Recommendations issued by the World Trade Organization Technical Barriers to Trade (TBT) Committee.*

## 2. Terminology

2.1 *Definitions:*

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

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

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

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

2.1.5 *bioremediation*—enhancement of biodegradation.

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

2.1.7 *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).

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

## 3. Significance and Use

3.1 The purpose of this guide is to provide remediation managers and spill response teams with guidance on bioremediation.

3.2 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.

## 4. General Considerations for Bioremediation Use

4.1 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, such as for thin films. The use of adequate controls in preliminary field studies, or the results of previously reported studies, will assist in

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determining the extent to which microorganism or nutrient amendments, or both, are necessary to obtain the desired rate of degradation.

4.2 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. As oil consists of hundreds or more compounds, many of which require different conditions or different microorganisms to degrade, oil biodegradation should not be considered a single process. Oil biodegradation should at least consider the aliphatics separate from the aromatic compounds. Some compounds may degrade to other compounds which may be toxic or less biodegradable. Other classes of compounds often degrade to a lesser degree, these classes include resins, asphaltenes, large aliphatics and large aromatics (**1**, **2**)[2].

4.2.1 In general, aerobic bioremediation systems degrade oil more rapidly than anaerobic systems, and adequate aeration may be the most promising approach in many cases.

4.2.2 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 (**3**, **4**, **5**).

4.2.3 The biodegradation of aliphatic and aromatic hydrocarbons in the absence of molecular oxygen is generally slower than under aerobic conditions. Anaerobic biodegradation has been characterized under sulfate-reducing, nitrate-reducing and methanogenic conditions (**6**, **7**).

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

## 5. Background

5.1 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 (**8-17**). 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. Indigenous microbes have been found to be more effective than non-indigenous microbes (**14**, **18**). Water effluent nitrate levels, which can affect drinking water sources, should be minimized to diminish risks of health issues. 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.

5.1.1 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, these approaches usually 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. 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.

5.1.2 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. Addition of non-indigenous microbes has not been found to be highly effective (**14**, **18**).

5.1.3 While apparently safe and effective in the laboratory setting, genetically engineered oil-degrading microorganisms have only rarely been authorized for environmental release (for example,**19**).

5.2 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.

5.3 *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 usually been demonstrated through the use of indigenous microorganisms. Ground-water treatment may be achieved simultaneously or through pump and *ex situ* treatment methods. Anaerobic biodegradation systems can also be promoted; however, their utility has been limited to date. Since soil is not excavated, volatile release is limited, and the risks and costs associated with excavation and treatment are reduced.

[2] The boldface numbers in parentheses refer to a list of references at the end of this standard.