Standard Guide for Consideration of Anaerobic Bioremediation as a Chemical Pollutant Mitigation Method on Land[1]

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1. Scope

1.1 This guide covers recommendations concerning the application of anaerobic bioremediation to mitigate chemical pollutants (see Appendix X1, Table X1.1).

1.2 This is a general guide only, assuming the bioremediation to be safe, effective, available, applied in accordance with manufacturers recommendations and in compliance with relevant environmental regulations.

1.3 This guide addresses the application of anaerobic bioremediation alone or in conjunction with order technologies, following chemical pollution of terrestrial environments.

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

1.5 This guide applies to all terrestrial environments. Specifically, it addresses available information concerning anaerobic bioremediation of chemical products and wastes of industrial processes.

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:* [2]

F 1481 Guide for Ecological Considerations for the Use of Bioremediation in Oil Spill Response- Sand and Gravel Beaches
F 1600 Terminology Relating to Bioremediation
F 1693 Guide for Consideration of Bioremediation as a Oil Spill Response Method for Land
E 1943 Guide for Remediation of Ground Water by Natural Attenuation at Petroleum Release Sites

3. Terminology

3.1 For additional information relating to bioremediation, see Terminology F 1600.

3.2 *Definitions of Terms Specific to This Standard:*

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

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

3.2.3 *anaerobic reactor*—an engineered system designed to maintain a high anaerobic microbial population to effectively biodegrade chemical contaminants.

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

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

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

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

3.2.8 *cometabolism*—transformation of the contaminant is the result of an incidental reaction catalyzed by enzymes involved in the normal cell metabolism or special detoxification reactions.

3.2.9 *indigenous*—native to a given habitat or environment

3.2.10 *nutrient*—a substance that supports organismal growth.

3.2.11 *remediation by natural attenuation (RNA)*—a remedy where naturally occurring physical, chemical, and biological processes will effectively achieve remedial goals and can be

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[2] For referenced ASTM standards, visit the ASTM website, www.astm.org, or contact ASTM Customer Service at service@astm.org. For *Annual Book of ASTM Standards* volume information, refer to the standard's Document Summary page on the ASTM website.

demonstrated through monitoring. The application of natural attenuation processes as a remedial action also has been described by a variety of other terms, such as intrinsic remediation, intrinsic bioremediation, passive remediation, passive bioremediation, etc.

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

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

4. Significance and Use

4.1 The purpose of this guide is to provide guidance on a means (called anaerobic bioremediation) of effectively cleaning up specific chemical pollutants on and below terrestrial surfaces.

4.2 This technology has been shown to be applicable to the mitigation of the chemical pollutants presented in Appendix X1, Table X1.1.

5. General Considerations for Anaerobic Bioremediation Use

5.1 Bioremediation technologies attempt to accelerate the natural rate of biodegradation. *Ex situ* and *in situ* anaerobic bioremediation technologies can be employed. The use of adequate controls in preliminary field studies, or results of previously reported studies, will assist in determining to what extent microorganism or nutrient, or both, and additional amendments are necessary to obtain the desired rate of degradation.

5.2 Bioremediation performance depends particularly on the efficiency of the chemical degrading indigenous bacteria or bioaugmented bacteria. Performances also depends on the availability of rate-limiting nutrients, physical characteristics, and the susceptibility of the target chemical product to anaerobic microbial degradation.

5.2.1 Chemicals shown to be effectively biodegradable by anaerobic microbes and processes are included in, but not necessarily limited to, several categories: explosives, dyes, pesticides/insecticides, herbicides, and chlorinated solvents. All are generally stable in nature except under conditions that favor anaerobic biodegradation.

5.2.1.1 Chemicals shown to be anaerobically biodegradable are listed in Appendix X1 (Table X1.1) by UN Number, CAS registry number, chemical name, and source.

5.2.2 Several defined anaerobic species are responsible for the degradation of the chemicals. Various texts and scientific studies describe biodegradability and biodegradation rates of the chemical listed in Appendix X1, Table X1.1 (**1-3**)[3].

5.2.2.1 Several groups of bacteria usually cooperate in the anaerobic degradation of one compound. Different substrates may be used for energy and for carbon metabolism (**3**).

5.2.2.2 Aerobic bacteria may further metabolize anaerobic metabolites (**3-5**). For example, as the degree of halogenation diminishes, the susceptibility to aerobic degradation increases.

5.2.2.3 Growth yields of anaerobic bacteria are low due to energy yields compared with aerobic bacteria; 10 % versus 50 % of the substrate carbon can be incorporated as cell matter (**3**). Anaerobic reactors that either retain or recycle the active biomass maintain substrate conversion rates competitive with aerobic processes.

5.2.3 Temperature has also been shown to play a role in anaerobic biodegradation, from 10 to 40°C (**3**). For example, optimum biodegradation of TNT-contaminated soil was achieved at 25 to 30°C (**4, 6**).

5.2.4 Special conditions are favorable for anaerobic bacterial growth. For example, aerobic microorganisms that adhere to surfaces which come in contact with oxygen, utilize the oxygen creating conditions that promote anaerobic growth (**3**). In addition, metal sulfide precipitates that form cover the surface and act as barriers to oxygen, provide a redox buffer, and help balance the availability of trace elements (**3**).

5.2.5 Clay content and the sizes of soil particles and aggregates affect the microbial activity (**7**). Bulk density is affected by the pore size. Water retention for sandy loam soil ranges from 15 to 30 %, and for clays ranges from 40 to 45 %. The difference between the total pore space and the water content represents the air-filled space. A minimum air-filled pore space of 10 % by volume is considered necessary for adequate aeration of aerobic microorganisms. Therefore if the water content were to exceed 30 % for sandy loam soils, the soil may not have enough void space for aeration, and the system would promote growth of anaerobic microorganisms.

5.3 Anaerobic bioremediation should be carried out 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 have been presented in previous documents (**3, 5, 8, 9**). Additional information can be found in Guide F 1481 and Guide F 1693.

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

6.2.1 *Anaerobic Reactors/Ex Situ Treatment*:

6.2.1.1 Systems are designed to maintain a high anaerobic microbial population. These anaerobic digestors are engineered to accumulate both digestible and nondigestible organic and inorganic solids. The solids may contribute to the prevention of oxygen diffusion and provide a large surface area to support adhesion of anaerobic microbial communities.

6.2.1.2 Basic types include: septic tanks; conventional anaerobic digestors; anaerobic contact process reactors; upflow anaerobic sludge blanket reactors; anaerobic filters; and, anaerobic fluidized bed reactors.

6.2.1.3 Anaerobic reactors have been successfully used at commercial scale.

6.2.2 *In Situ Anaerobic Bioremediation*

[3] The boldface numbers given in parentheses refer to a list of references at the end of the text.

6.2.2.1 *In situ* bioremediation is engineered to circulate nutrients or microbes, or both, that enhance anaerobic biodegradation (**9**). Sites amenable to treatment allow solutions to be transported to the contaminated soil and water. Treatment is most likely to be successful if the soil is relatively uniform and if the hydraulic conductivity is greater than 10^{-4} cm/s.

6.2.2.2 Anaerobic bioremediation has been demonstrated at the laboratory and pilot-scale levels. Success at the commercial scale is currently undergoing evaluation for *in situ* anaerobic treatment.

6.2.3 *Remediation by Natural Attenuation (RNA)*:

6.2.3.1 Intrinsic bioremediation requires no intervention and depends on native microbial activity to degrade the contaminants (**9**). Amenable sites have a adequate groundwater flow, natural buffering capacity, anaerobic conditions and sufficient levels of nutrients.

6.2.3.2 Field demonstrations should be performed to confirm laboratory tests. There should be a documented loss of contaminants owing to the action of microorganisms.

6.2.3.3 RNA has been established at the laboratory, pilot-scale and commercial levels.

6.2.3.4 For additional information on RNA, refer to Guide E 1943.

6.3 Compounds amenable to anaerobic biodegradation are produced as solvents, herbicides, insecticides, dyes, and nitroaromatic explosives.

6.3.1 Halogenated aliphatic compounds are degreasers and solvents. Highly chlorinated compounds can be completely dechlorinated to relatively nontoxic compounds, which are biodegraded by aerobic microbes. Less halogenated ethenes are destroyed by cometabolism when certain aerobic bacteria are supplied with methane, phenol, or toluene (**3, 18**). As the degree of halogenation in aliphatics decreases, susceptibility to aerobic biodegradation increases.

6.3.2 Halogenated aromatic compounds are used as solvents, pesticides, herbicides, and in electrical transformers. Anaerobic microbes can remove chlorine atoms from the highly halogenated aromatics. As halogen atoms are replaced by hydrogen atoms (dehalogenation), the molecules become more susceptible to aerobic biodegradation.

6.3.3 Nitroaromatic compounds are used in production of explosives and dyes. Anaerobic microbes transform nitroaromatics to nontoxic volatile organic acids which are aerobically biodegraded. Anaerobic and aerobic metabolic pathways affect the fate of nitroaromatic compound catabolism (**6, 12-18**).

7. Bioremediation Technology Selection Assessment

7.1 Treatability studies have provided data to support treatment selection and are performed prior to reagent selection. The data indicate whether cleanup goals can be met and further determine the optimal operating conditions for remediation project design. The level of study chosen depends on available literature information, technical expertise, and site-specific considerations. In addition, treatability study design and interpretation for anaerobic biodegradation remedy screening has been addressed (**19**).

7.2 Various federal, provincial and state agencies require support documentation. Studies are available through databases developed by groups sponsored by the USEPA and Environmental Canada.

7.3 Federal, provincial, state, and other governmental agencies regulate the use of bioremediation agents. The role of these regulatory agencies varies.

7.4 There are many advantages associated with the various bioremediation applications. In general, compared with other technologies, advantages may include cost effectiveness, reuse, reduced personnel exposure to hazardous conditions, and reduced intrusion by response personnel (short and long term) into affected areas.

8. Recommendations

8.1 Anaerobic bioremediation should be considered as an option to mitigate certain chemical pollutants (Appendix X1, Table X1.1) in terrestrial chemical spills.

8.2 Treatability studies and available supporting literature should be used to provide data to support treatment selection, indication 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 anaerobic bioremediation field application. This information should be provided through treatability studies and/or published reports.

8.4 The effects of biostimulation should be compared with the effects of bioaugmentation. Consideration must be given to time, predictability, regulatory and public opinions, cost, and clean-up requirements for process selection.

8.5 Implementation of bioremediation technology is site- and chemical contaminant-specific, and should be utilized appropriately in accordance with local, state, provincial and federal agencies.

9. Keywords

9.1 aerobic; anaerobic; anaerobic reactor; bioaugmentation; bioremediation; biostimulation; cometabolism; *ex situ*; *in situ*; remediation by natural attenuation (RNA); terrestrial; treatability

APPENDIX

(Nonmandatory Information)

X1. SELECTED CHEMICALS

TABLE X1.1 Selected Chemicals Known to be Amenable to Anaerobic Biodegradation[A]

Chemical Name	Source	CAS Number	UN Number
DDT	Pesticides	50-29-3	1846
2,4-Dinitrophenol	Pesticides, explosives, dyes	51-28-5	1320 1599
Carbon tetrachloride	Steel manufacturing, paint, ink, petroleum	56-23-5	1846
T-Hexachlorocyclohexane	Insecticide	58-89-9	2761
1, 1, 2, 2-Tetrachloroethane	Degreasers, paint, varnish, rust, removers	79-34-5	1702
Dinoseb	Herbicide, insecticide, miticide	88-85-7	0077
Nitrobanzene	Aniline production	98-95-3	1662
2, 4, 6-Trinitrotoluene	Explosives	118-96-7	0388
2, 4-Dinitrotoluene[B]	Dyes, explosives, propellants	121-14-2	2038 1600
RDX (Hexahydro-1, 3, 5- tri-nitro-1, 3, 5-triazine)	Explosives	121-82-4	0391
Methyl parathion	Pesticides	298-00-0	2783 1967
Aldrin	Insecticide	309-00-2	2761
a-Hexachlorocyclohexane	Insecticide	319-84-6	2729
β-Hexachlorocyclohexane	Insecticide	319-85-7	2729
4, 6-Dinitro-o-cresol	Insecticide	534-52-1	1598
2, 6-Dinitrotoluene[B]	Dyes, explosives, propellants	606-20-2	2038 1600
Heptachlor epoxide	Insecticides	1024-57-3	NA[C]
HMX (Octahydro-1, 3, 5, 7-tetraazocine)	Explosives	2691-41-0	0226
Tetrachloroethylene	Solvent	127-18-4	. . .

[A] These chemicals have been shown to be preferentially degraded under anaerobic conditions. The absence of a compound from this list does not mean that it is not amendable to anaerobic biodegradation.
[B] Biodegrades in both aerobic and anaerobic conditions.
[C] NA; Not Applicable.

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