

BIOREMEDIATION SYSTEM FOR OIL SPILL RESPONSE

FACT SHEET

**Bioremediation Techniques,
Category Definitions, and Modes of Action
in Marine and Freshwater Environments**



The following material is an excerpt from the Lawrence Anthony Earth Organization, Science and Technology Committee research paper entitled:

A Call for a Twenty-first Century Solution in Oil Spill Response — Updated September 2014. While it is recommended that the complete research paper is studied, this excerpt is being made widely available as critical information for educational purposes.

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Lawrence Anthony Earth Organization (LAEO)
P.O. Box 2287
Toluca Lake, California 91610

*The complete research paper can be ordered online at
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BIOREMEDIATION TECHNIQUES, CATEGORY DEFINITIONS, AND MODES OF ACTION IN MARINE AND FRESHWATER ENVIRONMENTS FACT SHEET

(Originally compiled to update and revise RRT IV Spill Response Guidance, *Types of Bioremediation* Section and *Bioremediation Response Plan Appendix D*, in coordination with RRT VI Science and Technology Committee, who called for revisions of this material. Original NRT/RRT material quoted herein is *italicized* to differentiate from proposed revisions or additions.)

Recommended Revisions in consultation with:

Steven Pedigo, (acting as RRT 6 Science and Technology Sub-Committee Consultant)

Marynette Herndon, Environ Eng, REM, CHMM

Paul W. Sammarco, PhD

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The original purpose of this Fact Sheet was to update and supplement the US National Response Team (NRT) Science and Technology Committee's *Bio-remediation in Oil Spill Response Fact Sheet* published in May 2000 and Regional Response Team (RRT) Bioremediation Response Plan guidance issued for On-Scene Coordinators and oil spill response professionals. Although existing NRT and RRT technical information covers important facts about bioremediation, this material does not adequately define and differentiate among the three primary types of bioremediation categories and their attendant modes of action. This is particularly important because their respective efficacies require precise application parameters, which vary between target environments and types of oil/hazardous spills to which they are applied. The last U.S. EPA bioremediation guidance was issued 15 years ago, which is missing considerable information on later advancements. This fact sheet updates and fills data gaps covering the advance mode of action for a unique biological agent category listed as Bioremediation Enzyme Additive Type (EA category). With its multifaceted mode of action, EA Type is clearly appropriate for and should be designated as a first response tool for spills of significance with much broader capabilities than the other categories.

The following information is provided to clarify and simplify the On-Scene Coordinator's decision-making process when considering the three bioremediation categories and evaluating their appropriateness in the cleanup strategy for a spill.

NCP PRODUCT TYPES LISTED

The three Bioremediation Agent Types listed on the US NCP Product Schedule are designated as follows:

Microbiological Cultures (MC)	
Nutrient Additives (NA)	
Enzyme Additives (EA)	

The first type (MC) constitutes a bioremediation process that utilizes *nonindigenous* bacteria. While useful in controlled or contained environments, a prevailing concern with these types of products has been that the introduction of foreign species into a given eco system is unpredictable and might cause future problems that may not become apparent for some time. Additionally, as noted in NRT's May 2000 Fact Sheet, "*there is usually no reason to add hydrocarbon degraders unless the indigenous bacteria are incapable of degrading one or more important contaminants*". The second type (NA) comprises those agents that contain nutrients or fertilizers to support indigenous microorganisms already present in the spill environment. Both MC and NA types have been correctly regarded as inappropriate for use in open-water environments. See 2001 EPA Guidance *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands*, which extensively covers appropriate usage of these two agent types. That information will not be repeated here except to provide definitions and mode of action summaries for comparison purposes to differentiate the more complex and advance mode of action of *EA Type*.

The third type (EA) *is* appropriate as a first-response tool in open water, intertidal zones and sensitive estuary ecosystems, as well as for soil, ground water remediation and contained environments. Experience with Bioremediation *EA Type* in the field on actual spills has evolved in recent years with considerable technological advances in usage protocols giving it a wide applicability for oil spill response in fresh, brackish, and marine environments, under temperature conditions as low as 28°F. The mode of action of this type will be reviewed in detail here.

CONTEXT

“Many compounds in crude oil are environmentally benign, but significant fractions are toxigenic or mutagenic. The latter are the ones we are most interested in removing or destroying in an oil spill. Bioremediation is a technology that offers great promise in converting the toxigenic compounds to nontoxic products without further disruption to the local environment.”

The primary reason for cleaning up oil spills is to reduce or eliminate the toxic and/or harmful components, thus enabling the survival of flora and fauna, including single-cell organisms, in each niche of the food chain. Although chemical dispersants commonly used today eliminate the visual and other damaging aspects of the spill on the surface, the spill's toxicity problem remains in the environment and at times, is worsened by the adding of chemicals contained in dispersants. The goal of the bioremediation process is to convert toxic compounds in oil/hydrocarbon-based material to nontoxic such as CO₂ and water, thereby permanently removing oil/hydrocarbons from the environment and returning the affected spill area to pre-spill conditions.

Herewith, the three main types of bioremediation are further defined, along with their modes of action:

Essential facts stated in the ***May 2000 NRT SCIENCE AND TECHNOLOGY COMMITTEE Fact Sheet: Bioremediation in Oil Spill Response:***

“Several factors influence the success of bioremediation, the most important being the type of bacteria present at the site, the physical and chemical characteristics of the oil, and the oil surface area...”

“Effective bioremediation requires that

- 1) nutrients remain in contact with the oiled material, and*
- 2) nutrient concentrations are sufficient to support the maximal growth rate of the oil-degrading bacteria throughout the cleanup operation.”¹*

CATEGORY TYPE

MICROBIOLOGICAL CULTURE

ADDITIVES (MC)

As covered in NRT Science and Technology Fact Sheet, “... *Bioaugmentation* is the process by which “oil-degrading bacteria are added to supplement the existing microbial population.”

DEFINITION

“Microbial agents are concentrated cultures of oil-degrading microorganisms grown on a hydrocarbon-containing medium, which have been air or freeze-dried onto a carrier (e.g., bran, cornstarch, oatmeal). In some cases, the microorganisms may be colonized in bioreactors at the spill site. This type of agent is intended to provide a massive inoculum of oil-degrading microbes to the affected area, thereby increasing the oil-degrading population to a level where the spilled oil will be used as a primary source of food for energy. Addition of oil-degrading bacteria has not been shown to have any long-term beneficial effects ...over and above bio-stimulation of already present oil degraders.”

1. Bioremediation (Types MC and NA) for open-water spills is not considered to be appropriate because of the above two requirements. When nutrients are added to a floating slick, they immediately disperse into the water column, being diluted to near-background levels (with the exception of NCP-listed EA Type which binds to fresh or weathered hydrocarbons/oil, and has recently demonstrated an 80 percent rate of reduction on Macondo Block, La., sweet crude containing Corexit, per BP Biochem Strike Team leader D. Tsao, LSU R. J. Portier, and L. M. Basirico, *Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response*, March 3, 2011).

MC TYPE MODE OF ACTION

Bioremediation Agent Type MC mode of action utilizes non-native cultures of microorganisms to address a spill.

Bioaugmentation is considered to be a process used as a “polishing-up” or “finishing” response tool because the microbial action is too slow at converting fresh oil to less harmful components since its toxicity concentrations are initially too high.

When foreign microorganisms are exposed to an oil or hazardous spill, they attempt to release enough quantities of biosurfactants to detoxify and insulate themselves from the spill so as not to be adversely impacted by the spill’s toxicity. The oil-degrading bacteria (both indigenous or nonindigenous) produce enzymes to develop protein-binding sites, which permit the bacteria to convert the molecular structure of the hydrocarbons to one that can be used as a food source.

While bio-augmented bacteria are taking their time to acclimate to the newly available oil, temperature of the environment, pH, and available nutrients, other environmental factors may produce adverse conditions that can forestall the breakdown action. These factors, along with the unknown time frames associated with their acclimation process, are at least partially responsible for the uncertainty associated with using Bioremediation *MC Type* as a first response cleanup methodology.

MC Type should only be used where there is very little water movement in a contained environment. Water movement causes the agent to dilute to ineffective levels incapable of supplying sufficient population numbers to produce enough biosurfactants and enzymes to start the breakdown of the molecular structure of the hydrocarbons.

Next to the toxicity of the spill, and a questionable ability to compete with indigenous bacteria already acclimated to the target area, indigenous bacteria are often competitively superior. The use of nonindigenous bacteria in most countries

is not permitted due to the uncertain effects of introducing them into sensitive environments.

Bio-augmented bacteria developed specifically for fresh water must be used in freshwater settings only. Products containing saltwater bacteria can only be utilized in salt water.

MC Type bioremediation is best used on closed and/or controlled environments and should not be considered effective in open-water environments.

CATEGORY TYPE

NUTRIENT ADDITIVES (NA)

As covered in NRT Science and Technology Guidance, this next category (NA)—“*bio-stimulation*”—is a process “*in which nutrients, or other growth limiting [sic], (suggest ‘enhancing’) substances, are added to stimulate the growth of indigenous oil degraders.*”

DEFINITION

Nutrient Additives are bioremediation agents that “*contain nitrogen and/or phosphorous as the primary means to enhance the rate of growth of indigenous oil-degrading microorganisms. This type of agent is intended to increase the oil-degrading biomass already present in an affected area to a level where the oil will be used as a primary source of food or energy. Because the natural environment may not have sufficient nutrients to encourage bacterial metabolism and growth, extra nutrients may be required. The purpose of this type of agent, therefore, is to provide the nutrients necessary to maintain or increase microbial activity and the natural biodegradation rate of spilled oil.*

NA TYPE MODE OF ACTION

The NA mode of action involves the general use of nutrients or fertilizers that contain various volumes of nitrogen and phosphorus. “*Effective bioremediation requires nutrients to remain in contact with the oiled material... ”.*

Given the nutrients remain at high enough levels, the native microbes enhanced by them will need time to secrete biosurfactants to attack the molecular structure of the spill by solubilizing

the oil/hydrocarbons, emulsifying the spill and increasing the oil-water interface. This helps to detoxify the hydrocarbons to a point where enriched indigenous bacteria can utilize the spill as a food source.

It can be difficult to apply nutrients or fertilizers to enhance oil-eating microbe population growth in a spill area containing toxic oil. Many microbes indigenous to the spill environment are initially weakened and or killed by the toxicity of the oil. And, because of the oil's toxicity, the nutrients are usually precluded from enhancing the remaining indigenous microbes.

Further, supplying nutrients or fertilizers in concentrations necessary to enhance oil-degrading bacteria without increasing the nitrogen levels to the point where they become toxic to aquatic life is a major problem. It is also difficult to contain or bind the nutrients or fertilizers with the oil in windy and otherwise undesirable weather conditions that generate wave and turbulent water motion.

The process of enhancing indigenous organisms with nutrients or fertilizers with the expectation that they will secrete biosurfactants and enzymes in sufficient quantities to catalyze the bioremediation process is unpredictable and often takes a protracted period of time.

Bioremediation category NA can be effectively used where there is little tidal flush and where the oil has weathered reducing its toxicity to the point that indigenous bacteria can survive.

CATEGORY TYPE **ENZYME ADDITIVES (EA)**

Although the NRT and RRT guidance documentation addresses the MC and NA bioremediation types in the 2001 *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands*,² this guidance does not sufficiently detail the mode of action of *Bioremediation EA Type*.

DEFINITION (EA TYPE)

"Enzymatic agents are biocatalysts that are designed to enhance the emulsification and/or solubilization of hydrocarbon-based chemicals/ crude oil to make it more available to microorganisms that can utilize such as a source of food or energy. These agents are generally liquid concentrates that may be mixed with biosurfactants and nutrients manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms."

EA TYPE MODE OF ACTION³

Enzyme Additive Bioremediation is a system appropriate for use in open/moving water (fresh, salt, and brackish), marsh/estuaries, shoreline, and soil environments. Its complex mode of action begins by detoxifying the oil and eliminating the harmful characteristics of an oil spill by employing naturally derived biosurfactants which act to rapidly emulsify the contaminants enabling multiple enzymes to move in creating binding sites on the contaminant. The indigenous bacteria then feed on the nutrients included in the *EA Type* formula enhancing their growth and colonization process.

The biosurfactant action eliminates the adhesion properties of the oil, usually within the first 5 to 30 minutes (depending upon temperature and specific gravity). The emulsified oil will continue to float near the surface, thereby eliminating any secondary impact to the water column and seabed and completely avoiding any dissolved oxygen (DO) risks during the process. With the toxicity and adhesive properties eliminated, wildlife that may come in contact with the emulsified hydrocarbons will not become coated in oil, and oil adherence to marsh, shorelines, sands, and man-made structures is greatly reduced. Flammability is eradicated rapidly, protecting ports, harbors, and oil/gas platforms from potential explosion hazards associated with fuel spills.

2. 2001 EPA Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands, <http://www.epa.gov/osweroe1/docs/oil/edu/bioremed.pdf>. Note that this guidance does not cover EA Type use specifically, nor does it address open water application of such, hence it inadvertently precluded it from consideration by OSCs.

3. As of September 2014, there is only one product on the NCP list that falls under this Bioremediation Agent Type EA classification: B-53—EA—OIL SPILL EATER II; thus, descriptions above regarding the mode of EA interaction at this time are related solely to this EA product. Any newly added EA Type listings would require review and validation for being categorized under this mode of action.

DILUTION FACTOR, WAVE MOTION AND CURRENTS

A further difference in Bioremediation *EA Type* is that its numerous enzymes and other constituents attach themselves to the hydrocarbon molecules forming protein-binding sites and eliminating dilution issues from wave motion and current-prone environments. These sites become safe mediums where oil-eating microorganisms can reside. The multiple enzymes also act as a catalyst to accelerate the biodegradation process by inducing the indigenous bacteria to more rapidly ingest the detoxified oil/hydrocarbons as a food source. The EA category contains ingredients that cause the agent's constituents to remain in contact with the spilled oil/hydrocarbons during the remediation process.

FATE OF EA TYPE

Over ensuing days or weeks (again, depending on temperature), nutrients in the agent rapidly facilitate an increase in indigenous bacterial populations. The enhanced microorganisms consume the detoxified hydrocarbon emulsion, digesting the oil and converting it to CO₂ and water—permanently removing the spill from the environment. As covered in the NRT/EPA May 2000 Bioremediation Fact Sheet, when microorganisms break down petroleum hydrocarbons, progressive oxidation takes place leading to a reduction of the different toxic compounds that make up oil. The combination of biosurfactants, nutrients and more than 150 different types of enzymes in the *EA Type* agent form a system that attract native microorganisms to the hydrocarbons which eventually convert the spill to a harmless carbon dioxide and water. The complex process could be likened to biological processes associated with the human or other life forms' digestion of food. As oxygen, combined with the other agent ingredients is added to the oil compounds, they become more water soluble, less toxic and, finally, break up their molecular structures and are fully digested. This process will not cause environmental damage or toxic effects at any stage to nearby organisms. Furthermore, the rate of dilution from the tidal or open water

wave and current motion is so great that any amounts of the benign constituents entering the food chain are likely to be negligible. Thus, the effect of biochemical end products from the easily metabolizable compounds in oil will be insignificant in the environment.

Without category EA support, this natural process may take up to 30 years or more to reach the end point of a complete degradation of an oil spill; and the lingering toxicity of the oil would remain in the environment for that duration.

SHORELINES / MARSHES

An additional benefit is that one can use the EA agent on near shore waters and environments as well as deep water locations, unlike dispersants, which are usually restricted to three miles off shore in waters 10 meters deep or greater due to their toxicity. When a spill makes landfall or contaminates near shore areas, *EA Type* can be safely applied to lift the spill off the marsh grass (or sandy beaches or shorelines), greatly reducing the time that sensitive ecosystems and people are exposed to the toxic compounds in oil.

The use of *EA Type* does not deplete O₂ since the oil it is applied to remains buoyant (able to float) and the nutrient and enzyme ingredients use atmospheric O₂ for their biochemical interactions.

There are no known trade-offs, deleterious effects, or collateral damage associated with the EA method. And, as is the case with chemical dispersants, there is no limited time window for its application to be effective.

CLOSING COMMENT

The three types of bioremediation and their modes of action (described above) have been detailed here to help responders understand how these agents will interact with a spill. The different types and their modes of action are clearly independent of each other, even though their stated end point, in principle, is the same. The ability to reach that end point, and the amount of time it takes to do so, is observably different.

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