# BIOREMEDIATION TECHNIQUES, CATEGORY DEFINITIONS, AND MODE OF ACTION IN MARINE AND FRESHWATER ENVIRONMENTS

(Originally compiled to update and revise US EPA/NRT Bioremediation Fact Sheet and RRT IV Bioremediation Spill Response Guidance, *Types of Bioremediation* section and *Bioremediation Response Plan Appendix G*, in coordination with RRT VI and their Science and Technology Committee, who called for revisions in this material)[1]

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[1]. Submitted to RRT VI Science and Technology Committee in August 2012. Although the chair of the committee stated that key portions of this paper would be integrated into the revised guidance, as of the date of this paper, that has not yet taken place. While facts about MC and NA Bioremediation Types have been covered in these NRT and RRT Fact Sheets, these materials completely omit any information and important facts on the NCP-listed Enzyme Additive Bioremediation Category and its mode of action, which are critical to accurate decision making using science-based protocols.

The purpose of this article is to update and supplement the National Response Team (NRT) Science and Technology Committee's *Bioremediation in Oil Spill Response Fact Sheet* published in May 2000 and RRT guidance documents issued for FOSCs and response professionals. Although existing US NRT and RRT guidance covers important facts about bioremediation, existing material does not adequately define and differentiate between the three primary types of bioremediation categories listed on the EPA NCP Product Schedule and their associated modes of action. This is important because their respective efficacies require precise application parameters, which vary between target environments. While the limitations and decision points related to bioremediation usage have been covered extensively in previously issued materials, this information is provided to simplify the decision-making process when presented with the three primary bioremediation categories as options.

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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."[2]

**NCP PRODUCT TYPES LISTED**

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

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

The first type (MC) constitutes a bioremediation process that utilizes nonindigenous (foreign) bacteria. While useful in controlled environments, a prevailing concern with these types of products has been that the introduction of foreign species might cause future problems that may not become apparent for some time. The second type (NA) comprises those agents that contain nutrients or fertilizers to support the microorganisms present in the spill environment. Both have been designated as not applicable to open-water environments. See 2001 EPA Guidance *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands*, which extensively covers the usage of these two product types, so need not be repeated here.

On the other hand, the third type (EA) is appropriate as a first-response tool in

2. Bioremediation [Types MC and NA] for open-water spills is not considered to be appropriate or achievable 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 Type EA, based on extensive field use and testing on fresh and weathered hydrocarbons/oil, which recently demonstrated an 80% rate of PAH degradation on Macondo Block, La., sweet crude containing Corexit, per BP BCST, D. Tsao , LSU R. J. Portier, L. M. Basirico, March 3, 2011, *Laboratory Screening of Commercial Bioremediation Agents for the Deepwater Horizon Spill Response*].

open-water environments. Bioremediation EA Type has evolved in recent years and has been the subject of considerable technological advances, with 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

The primary reason for cleaning up oil spills is to reduce or eliminate the toxic components, thus enabling the survival of fauna and flora, including single-cell organisms, in each niche of the food chain. Although 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 hydrocarbons contained in dispersants. The goal of the bioremediation process is to convert oil/hydrocarbon-based material to $CO_2$ 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, to help federal On-Scene Coordinators (OSC) and federal, state, and local officials, as well as responsible parties, to understand, and make more informed decisions about, bioremediation agents when selecting oil spill response tools.

## CATEGORY TYPE ENZYME ADDITIVE (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,*[3] they do not sufficiently detail the mode of action of *Bioremediation Type EA.*[4] This may be described as follows.

## ENZYMATIC AGENT (EA) DEFINITION:

Enzymatic agents are biocatalysts that are designed to enhance the emulsification and/or solubilization of oil to make it more available to microorganisms as a source of food or energy. These agents are generally liquid concentrates, which may be mixed with surfactants and nutrients manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms.

## EA TYPE MODE OF ACTION:

**Enzyme Additive mode of action is applicable to open/moving water (fresh, salt, and brackish), marsh/estuaries, shoreline, and soil environments.** When applied, the nontoxic converters and biosurfactants in Bioremediation Agent EA Type eliminate the classic appearance of an oil spill by emulsifying and solubilizing the molecular hydrocarbon structure and eliminating the adhesion properties of

3. 2001 *Guidelines for the Bioremediation of Marine Shorelines and Freshwater Wetlands,* http://www.epa.gov/oswer0e1/docs/oil/edu/bioremed.pdf.

4. As of this date, 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 here.

crude oil. This usually takes place within the first 5 to 30 minutes (depending upon temperature). The emulsified oil continues to float near the surface, thereby eliminating a secondary impact to the water column and seabed.

With the toxicity and adhesion properties eliminated, wildlife that may come in contact with the broken-down hydrocarbons will not become coated in oil, and oil adherence to marsh, shorelines, sands, and man-made structures is greatly reduced. Flammability is eliminated rapidly (again, depending upon temperature), helping to protect ports, harbors, and oil/gas platforms from potential explosion hazards associated with fuel spills.

A further action of bioremediation category EA is that its numerous enzymes then attach themselves to hydrocarbons with the biosurfactants, developing protein-binding sites. These sites act as a catalyst to accelerate the bioremediation process by inducing enhanced indigenous bacteria to utilize the detoxified oil/hydrocarbons as a food source. The EA category also contains properties that cause all the constituents to remain in contact with the spilled oil/hydrocarbons in moving waters.

Over ensuing days or weeks (again, depending on temperature), nontoxic nutrients in the Enzyme Additive Type rapidly facilitate an increase in indigenous bacterial populations. The bacteria consume the detoxified hydrocarbon emulsion, digesting the oil and reducing it to $CO_2$ and water—permanently removing the oil/hydrocarbons from the environment—resulting in final water clarification. Without category EA assistance, this natural process may take up to 20 years, based on the Ixtoc and *Valdez* spill studies.

**SHORELINES/MARSHES:**

When a spill makes landfall or contaminates a marsh, category EA can be safely applied to lift the spill off the marsh grass (or sandy beaches or shorelines), limiting the time required for the oil to adversely impact these areas. The use of category EA does not deplete $O_2$ from water, since the oil is buoyant and the enzymes use atmospheric $O_2$ for their biochemical interactions.

There are no known trade-offs, deleterious effects, or collateral damage associated with the EA method.

There is no limited window of opportunity for the application of category EA; it can be used in estuaries, open marine (salt) waters, moving freshwater bodies such as rivers, and in soil. It is effective as a first-response tool and/or when applied days or months after a spill. Category EA can also be applied to oil accumulated on the seafloor, eventually lifting it to the surface and returning the seabed to pre-spill conditions.

As of this date, 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 here.

**CATEGORY TYPE MICROBIOLOGICAL CULTURE ADDITIVE (MC)**

As covered in NRT Science and Technology Guidance,
"… *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. All commercially available agents use naturally occurring microorganisms. Some agents may also contain nutrients to assure the activity of their microbial cultures. 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. Microbial agents are designed to enhance the biodegradation of oil at any location and would be most useful in areas where the population of indigenous oil degraders is small.

## MC TYPE MODE OF ACTION:

Bioremediation Agent Type MC mode of action utilizes nonindigenous bacteria with the objective of digesting oil/hydrocarbons to $CO_2$ and water.

Bioaugmentation is considered to be a product used as a "polishing-up" or "finishing" response, since it cannot be applied to fresh oil. This is because the toxicity levels kill the added oil-degrading bacteria.

When nonindigenous bacteria are placed on or near weathered oil, they attempt to release enough quantities of biosurfactants to detoxify the spill so that the oil-degrading bacteria will not be adversely impacted by the spill's toxicity. This in turn enables the bacteria to use the hydrocarbons as a food source. The oil-degrading bacteria (both indigenous and nonindigenous) produce enzymes to develop protein-binding sites, which permit the bacteria to convert the molecular structure of the hydrocarbons to one which can be used as a food source. This process requires a protracted amount of time.

While bioaugmented bacteria 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 past uncertainty associated with using Bioremediation MC Type as a cleanup methodology.

Nonindigenous bacteria should generally be used where there is very little water movement. Water movement causes the products to become diluted to ineffective levels incapable of staving off natural competition from indigenous bacteria, and thus also incapable of supplying sufficient population numbers to produce enough biosurfactants and enzymes to start the breakdown of the molecular structure of the hydrocarbons. (Laboratory environments do not satisfactorily duplicate this type of competitive environment; hence, particularly in moving waters, the final outcome of treatment is often uncertain.)

Next to the toxicity of the spill, the most difficult aspect of utilizing nonindigenous bacteria in a foreign environment is natural competition from indigenous bacteria already acclimated to the target area. Indigenous bacteria are often competitively superior.

Bioaugmented 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.

The use of nonindigenous bacteria in most countries is not permitted due to the uncertain effects of introducing them into sensitive environments.

## **CATEGORY TYPE NUTRIENT ADDITIVE (NA)**

As covered in NRT Science and Technology Guidance, this next category (NA)—"*biostimulation*"—is a process "*in which nutrients, or other growth limiting 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 (N) and phosphorus (P). The nutrients are distributed in association with a spill and are expected to enhance the population growth of indigenous bacteria.

These bacteria 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 a spill area with toxic oil and enhance bacterial population growth. Many of the indigenous bacteria are destroyed initially by the toxicity of the oil. Because of the oil's toxicity, the nutrients or fertilizers are usually precluded from enhancing the remaining indigenous bacteria.

Supplying nutrients or fertilizers in concentrations necessary to enhance bacteria while not increasing the nitrogen levels to the point where they become toxic to aquatic life is another key problem. In addition, it is difficult to contain the nutrients or fertilizers in the target area with the oil, especially in moving waters.

The process of enhancing indigenous bacteria with nutrients or fertilizers and waiting for them to secrete biosurfactants and enzymes in order to start the bioremediation process takes a protracted period of time. Again, this makes NA Type inappropriate as a first-response agent.

Bioremediation category NA can be effectively used where there is little tidal flush, and where the oil has weathered so its toxicity is reduced to the point that indigenous bacteria can survive. This requires NA to be used only as a polishing-up agent, with limited scope.

## A BRIEF NOTE ON PHYTOREMEDIATION

Phytoremediation is defined as the use of green plants and their associated microorganisms to degrade, contain, or render harmless environmental contaminants.

Phytoremediation of petroleum hydrocarbons generally involves three major mechanisms: (1) degradation, (2) containment, and (3) the transfer of contaminants from the soil to the atmosphere.

For further information on applicability, consult page 87 of http://www.epa.gov/osweroe1/docs/oil/edu/bioremed.pdf.

## 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 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 obviously different.

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