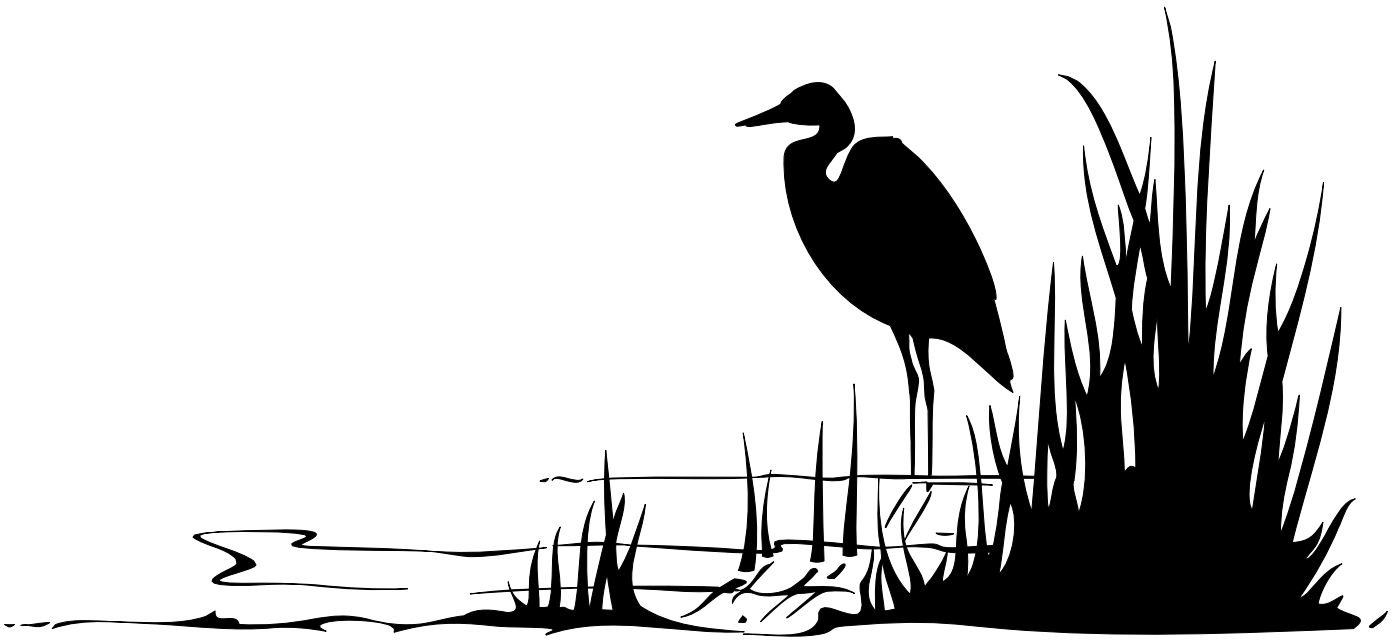
REGION IV
REGIONAL RESPONSE TEAM

BIOREMEDIATION
SPILL RESPONSE PLAN

AUGUST 1997



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INTRODUCTION

Biodegradation is a natural process in which microorganisms chemically alter and breakdown organic molecules into other substances - such as fatty acids, carbon dioxide and water - in order to obtain energy and nutrients. The basis for this process is relatively simple: microorganisms require minerals and sources of carbon, as well as water and other elements, to survive and function. The process can involve one step or a series of steps that proceed through the formation of molecules with successively fewer carbons. Generally, the extent to which a particular organic molecule is biodegradable and the rate of degradation depend on the molecule's structural characteristics (chain length, amount of branching, number and arrangement of rings, stereochemistry) and the environmental conditions (temperature, available oxygen, substrate).

Bioremediation is a treatment technology that utilizes biodegradation to reduce the concentration and/or toxicity of chemical substances such as petroleum products and other hydrocarbons. Because microbes capable of degrading hydrocarbons are commonly found in nature, most untreated hydrocarbon spills eventually are removed from the environment by microbial degradation and other processes. Enhanced bioremediation, however, seeks to accelerate natural biodegradation processes by applying specially chosen nutrients and/or microbes to spilled substances. Although microbes have been used extensively and successfully for many years to treat wastes and wastewater in controlled facilities, their potential as a tool for responding to spills of oil and hazardous substances in uncontrolled environments has only more recently received significant interest. (For additional information on bioremediation, refer to Appendix G.)

This document presents a plan for considering and implementing bioremediation, through either natural attenuation or nutrient/microbe enhancement, as a supplemental response tool for spills in US Environmental Protection Agency (EPA) Region 4. It was developed through the coordinated efforts of EPA's Subcommittee on National Bioremediation Spill Response and the members of the Region 4 Regional Response Team (RRT), using EPA's Interim Guidelines for Preparing Bioremediation Spill Response Plans.

PURPOSE

This document has a threefold purpose:

To outline a process by which Federal On-Scene Coordinators (OSCs) in Region 4 may request authorization to use bioremediation in response to spills of oil or hazardous substances (the authorization procedures presented are consistent with the National Oil and Hazardous Substances Pollution Contingency Plan (NCP));

To define the types of information necessary to determine if bioremediation is

feasible, provide as much of this information in advance as possible, and outline a mechanism for capturing information on bioremediation use for future decision making; and,

To describe how to implement a bioremediation activity and determine if bioremediation is working.

The document is intended to guide decision makers in evaluating the appropriateness of bioremediation in the cleanup strategy for a spill and in undertaking a bioremediation activity. Ultimately, decisions regarding the use of bioremediation must be based on the OSC's best judgment given the particular circumstances of the spill incident.

The RRT's Response Technology Committee will examine, on an as needed basis, the information in this plan, consider any new advances in and additional experience with bioremediation, and revise the plan as appropriate. Recommendations for revisions should be submitted to the Region 4 RRT for approval. Upon, approval by the RRT, revisions should be incorporated into the Region 4 RCP and other local plans, as appropriate.

APPLICABLE REGULATIONS

Legislation at both the federal and state level may affect decisions to use bioremediation. Existing regulations and policies that govern the use of bioremediation agents in response to spills in Region 4 are summarized in Appendix A.

ROLES AND RESPONSIBILITIES

This section discusses issues relevant to managing the response to a spill, with particular emphasis to managing bioremediation activities.

On-Scene Coordinator (OSC)

As per 40 CFR Section 300.120, USCG and EPA provide pre-designated OSCs that have overall responsibility for oil spill responses in the coastal and inland zones respectively. When considering or actually using bioremediation as a response tool, the OSC shall be responsible for ensuring that the requirements set forth in this plan are properly followed and implemented. This includes notification, planning, documentation and monitoring of all bioremediation activities. Thus, the OSC, in conjunction with his/her contractors or a responsible party, will be directly involved in the cleanup effort.

Federal Agencies

US Environmental Protection Agency - EPA, with their extensive technical expertise in

bioremediation, may lend themselves to the OSC as a technical advisor. This expertise includes information on the ability of various bioremediation treatment techniques to degrade oil, their relative toxicity to a habitat and the expected rate of degradation. Typically, EPA provides the Scientific Support Coordinator for inland zone spills. In addition, EPA maintains laboratory facilities that may be used to run bioremediation related studies and analyses.

US Coast Guard - The USCG supplies expertise in oil spill response technology and incident command. Response support, through manpower or equipment, can be provided by the Strike Teams and the National Strike Force Coordination Center. Additionally, the USCG can assist with cost tracking and funding support from the Oil Pollution Trust Fund.

National Oceanographic and Atmospheric Administration - NOAA/HAZMAT provides Scientific Support Coordinators (SSCs) and their support teams. The SSC provides scientific advice to support the Federal OSCs in operational decisions that will protect the environment effectively, mitigate collateral harm, and facilitate environmental recovery. The NOAA/HAZMAT Scientific Support Team has extensive expertise in all scientific aspects of spill response and mitigation and vast experience with oil spill response and several applications of bioremediation in both operational and experimental use. Their expertise in biology, geomorphology, chemistry, and physical and coastal processes and their support can assist in the appropriate selection of bioremediation as a response technique and in its proper application. NOAA/HAZMAT also provides the Department of Commerce RRT member. The DOC RRT member provides advice and access to NOAA and DOC resources and expertise and serves as the point of contact for DOC/NOAA trustee issues.

Department of Interior - DOI has direct jurisdiction for the protection of resources on its own lands, as well as trustee responsibilities for certain natural resources, regardless of location. They can provide information concerning the lands and resources related to geology, hydrology, minerals, fish and wildlife, cultural resources and recreation resources. The DOI natural resource trusteeship also includes migratory birds, anadromous fish and endangered or threatened species and their critical habitats.

State and Local Agencies

State and local agencies have a distinct role and perspective during a response that impacts their own resources. Typically, these agencies can provide valuable information on the latest regulations, guidelines, water resource conditions, environmentally sensitive areas and public concerns. Therefore, any response effort should be carefully coordinated with impacted State and local agencies.

Responsible Parties (RP)

Since the RP has firsthand information concerning the spilled material, the RP may request OSC approval for the use of bioremediation or the application of a bioremediation enhancing agent. The RP can initiate a bioremediation activity after the request is approved by the OSC following concurrence from RRT 4 and consultation with the impacted natural resource trustees. The OSC's request, on behalf of the RP, shall be accompanied by a completed Bioremediation Use Authorization Form. Maximum cooperation and participation should be expected from the RP throughout the entire response and bioremediation activity.

DECISION TOOLS

Spills may be good candidates for bioremediation treatment based on characteristics of the spill and environmental sensitivities of the spill location. To assist OSCs and the RRT in evaluating spills for bioremediation treatment and to document the basis for response decision making, the following are provided: (1) a diagram outlining the decision process that OSCs should follow when deciding whether to use bioremediation, and (2) a form for obtaining authorization to use bioremediation that specifies information which should be collected for presentation to the OSC and RRT. This form, the Bioremediation Use Authorization Form, is presented in Appendix B.

Decision Process

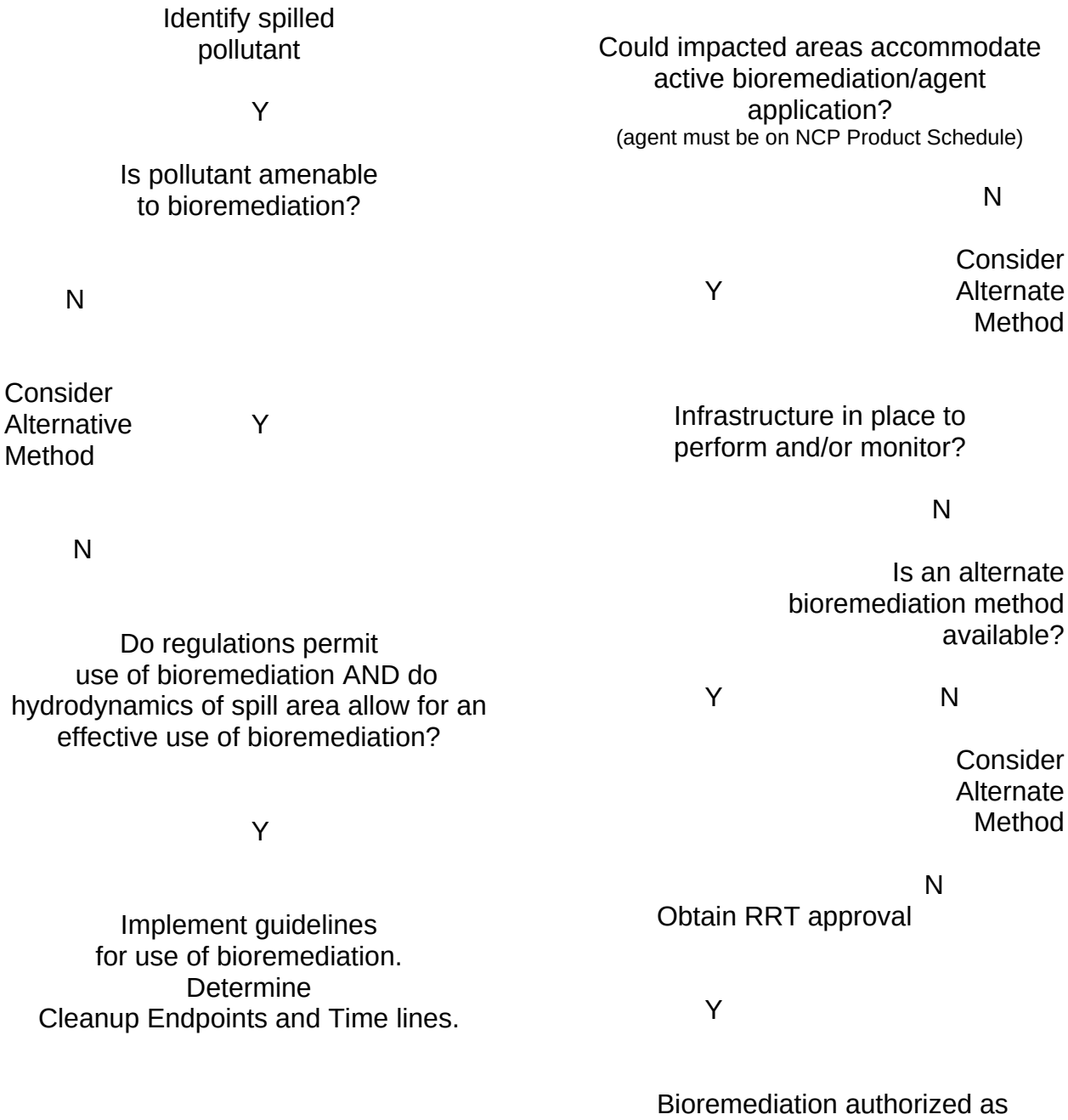
Decisions to use bioremediation should be made after applicable regulatory policies, potential environmental impacts, operational feasibility, logistical coordination, and other pertinent issues have been evaluated. The process to determine whether bioremediation may be feasible for a particular spill is illustrated in Diagram 1. Details for addressing the specific issues are outlined in the section Feasibility Assessment Criteria.

Bioremediation Use Authorization Form

A Bioremediation Use Authorization Form that specifies the minimum information requirements necessary to support decisions regarding the use of bioremediation is included in Appendix B of this plan. The form requests details of the spill incident, bioremediation details, bioremediation Work plan and monitoring plan. Once the form has been completed, it should provide pertinent information needed to make a decision regarding the use of bioremediation.

A completed authorization form should be transmitted to the RRT for the required authorization to proceed with bioremediation treatment. The RRT shall approve or disapprove the use of bioremediation within *24 hours* of receiving a completed form from an OSC.

DIAGRAM 1: DECISION TREE FOR CONSIDERATION OF BIOREMEDIATION



FEASIBILITY ASSESSMENT CRITERIA

Assessing the feasibility of bioremediation is basically a two-stage process. The first stage determines whether a particular spill is a candidate for bioremediation treatment. The second stage determines whether bioremediation can be implemented effectively, given the logistics of application and monitoring.

Incident Characteristics

The characteristics of a spill incident provide indications of the extent to which bioremediation treatment will be safe and effective against the contaminant spilled in a particular location. To aid in assessing bioremediation as a response option in several different habitats, bioremediation advisability information has been provided in the following sections. The matrix provides general guidelines regarding the advised use of bioremediation in different habitats based primarily on concerns for preserving habitats and minimizing harm to the indigenous flora and fauna.

Characteristics of Spilled Oil

The possibility and practicality of using bioremediation against the type of oil or petroleum product spilled should also be evaluated. That is, the extent to which the remaining chemical constituents of the spilled oil (which characterize that oil) are expected to be biodegradable needs to be assessed before bioremediation treatment is considered further. Biodegradation is typically useful on moderately to heavily oiled substrates, after other techniques have been used to remove as much oil as possible and on lightly oiled shorelines where other techniques are destructive or not effective. When used on diesel-type and medium oils that do not have large amounts of high molecular weight, slowly degrading components, bioremediation is most effective. On thick oil residues it is least effective. However, bioremediation should not be considered for gasoline spills, which will be completely removed by evaporation at faster time frames than by microbial degradation. Generally, oils can be divided into the following categories *(to further assist in making this determination see Appendix C, "Evaluating Biodegradation Potential of Various Oils".)* :

<u>Group I: Very Light Refined Products</u> *(gasoline, naptha, solvents)*

- very volatile and highly flammable
- complete removal by evaporation likely
- high acute toxicity to biota
- can cause severe impacts to water-column and intertidal resources
- specific gravity less than 0.80
- will penetrate substrate, causing subsurface contamination
- **not considered for bioremediation due to high evaporation rates**

Group II: Diesel-like Products and Light Crude Oils *(no.2 jet fuel oil, jet fuel, kerosene, marine diesel, West Texas Crude, Alberta Crude)*

- moderately volatile; persists in environment for an increasing period of time as Aweight@ of material increases
- light fractions will evaporate to no residue
- crude oils leave residue after evaporation
- moderate to high toxicity to biota
- can form stable emulsions
- tend to penetrate substrate; fresh spills are not adhesive
- specific gravity of 0.80-0.85; API gravity of 35-45
- **bioremediation most effective on lower molecular weight oils, with faster degrading components; aromatic portions less susceptible to degradation**

Group III: Medium-grade Crude Oils and Intermediate Products *(North Slope crude, South Louisiana crude, no. 4 fuel oil, lube oils)*

- moderately volatile
- up to one third will evaporate in the first 24 hours
- moderate to high viscosity
- specific gravity of 0.85-0.95; API gravity of 17.5-35
- variable acute toxicity, depending on amount of light fraction
- can form stable emulsions
- variable substrate penetration and adhesion
- **bioremediation most effective on lower molecular weight oils, with faster degrading components**

Group IV: Heavy Crude Oils and Residual Products *(Venezuela crude, San Joaquin Valley crude, Bunker C, no. 6 fuel oil)*

- slightly volatile
- very little product loss by evaporation
- very viscous to semisolid; may become less viscous when warmed
- specific gravity of 0.95-1.00; API gravity of 10-17.5
- low acute toxicity relative to other oil types
- can form stable emulsions
- little substrate penetration; can be highly adhesive
- **higher molecular weight and fewer number of straight-chained hydrocarbons makes bioremediation less effective than on medium oils**

Group V: Very Heavy Residual Products

- very similar to all properties of Group IV oils, except that the specific gravity of the oil is greater than 1.0 (API gravity less than 10). Thus, the oil has greater potential to sink when spilled.

Characteristics of Affected Habitats

After evaluating the spilled oil's susceptibility to biodegradation, the habitats impacted by the spilled contaminant and the background level of nutrients in the impacted area should be identified and characterized. For each of the following habitats, the recommended approach is provided; **O** for *Optional*, **NA** for *Not Advisable*. [NOTE: NA does not preclude the OSC from conducting a Pilot Test to determine the effectiveness of bioremediation in an area. The harmful effects of the oil must be balanced against the potential effects of bioremediation.] The listed habitats are appropriate for marine, estuarine and riverine settings.

Open Water (NA)	Off-shore Waters (NA)
Tidal Inlets (NA)	Water Intakes (NA)
Small Lakes/Ponds (NA)	Small Rivers/Streams (NA)
Exposed Man-made Structures (NA)	Sheltered Man-made (NA) Structures
Exposed Scarps in Clay (O)	Wave-cut Clay Platforms (O)
Fine-grained Sand Beaches (O)	Sandy Banks (O)
Mixed Sand and Shell Beaches (O)	Shell Beaches or Banks (O)
Exposed Rip-rap (O)	Sheltered Rip-rap (O)
Exposed Tidal Flats (NA)	Sheltered Tidal Flats (NA)
Salt to Brackish-water Marshes (O)	Freshwater Marshes (O)
Freshwater Swamps (O)	Mangroves (O)

Open Water, Off-shore, Tidal Inlets and Water Intakes **NA**

Bioremediation is not effective for the time-frames of concern, relative to the potential of transport of the oil to areas where it could affect more sensitive resources. Thus, bioremediation treatment is not advisable for these habitats or areas.

Small Ponds, Lakes, Rivers and Streams **NA**

Not applicable for gasoline and light oils due to their rapid evaporation. There is insufficient information on impacts and effectiveness for other oil types, however there are special concerns about nutrient overloading in small, restricted water bodies.

Solid Man-Made Structures: Exposed and Sheltered **NA**

Oiling of exposed sea walls usually occurs as a band at the high-tide line. This type of oiling is not amenable to bioremediation because of difficulty of application and low effectiveness.

Exposed Scarps in Clay and Wave-Cut Clay Platforms **O**

Because of their erosional nature, removal of lightly oiled sediments may not be recommended on these habitats. Bioremediation may be an option whereby the oil could be treated in place.

Fine-grained Sand Beaches or Sandy Banks **O**

On outer beaches with low recreational use, bioremediation may be an option, particularly for light oiling or residual oil left after other countermeasures have been completed.

Fine-grained sand beaches also occur along bay margins and dredge spoil banks. Sandy banks occur along rivers. These habitats typically occur in more sheltered areas, where natural removal of residual oil by wave or current action will be slower then along exposed beaches. They are often not amenable to mechanical removal, thus manual removal of heavy accumulations of oil or oiled wrack may be conducted. Bioremediation may be considered for sites with light oiling or residual oil left **after** manual removal efforts have been terminated.

Mixed Sand and Shell Beaches and Shell Beaches or Banks **O**

For lightly or moderately oiled beaches and banks, particularly where mechanical cleanup may result in removal of large amounts of sediment or be logistically difficult, bioremediation or Ano action@ may be considered. This option is best considered for sites without significant recreational use.

Riprap: Exposed and Sheltered **O**

Oil on riprap can occur as a coating on the boulders or as persistent accumulations of oil in the void spaces between the boulders. Neither type of oil is amenable to effective removal by bioremediation techniques under most conditions. Thus, bioremediation treatment would be optional.

Exposed Tidal Flats and Sheltered Tidal Flats **NA**

Both of these habitats are inundated daily by high tides which results in rapid dilution and flushing of applied nutrients. Bioremediation is not likely

to be effective under these conditions. There are significant toxicity concerns for use of bioremediation agents in shallow, poorly flushed areas, such as sheltered tidal flats, or subtital habitats where there are concentrations of sensitive life stages of fish and shellfish, such as sea grass beds and oyster reefs.

Salt to Brackish-water Marshes, Freshwater Marshes, Freshwater Swamps and Mangroves **O**

There are very few cleanup options which do not cause significant impacts to these sensitive habitats. Most often, Ano action@ is the preferred option. However, there may be conditions under which bioremediation may be considered, particularly for lighter oils. In wetlands with shallow, poorly mixed water bodies, the potential increase in eutrophication and ammonia caused by aggressive bioremediation needs to be considered.

LOGISTICAL CONCERNS

Characteristics of a spill incident, including characteristics of affected habitats and spilled pollutant, should determine whether a spill is a candidate for bioremediation treatment. If, based on these factors bioremediation has not been eliminated as a response alternative, then the logistical feasibility of implementing an appropriate bioremediation action plan should be evaluated. Implementation considerations include the proposed scale of a bioremediation activity, the availability of the bioremediation agent(s) proposed for application (if used), and the availability of the resources necessary to conduct the application and monitoring recommended for the agent(s) proposed for use in each affected habitat. (The latter two considerations are highly dependent on the first.)

Scale of Bioremediation Response

The first step in assessing the logistical feasibility of bioremediation is to determine the scale of the bioremediation response. The scale of the bioremediation response refers to the extent to which bioremediation will be involved in the cleanup, particularly in terms of the size of the area. The scale of the bioremediation response effort will determine the amount of agent(s) *(if any)*, the number of personnel, and the equipment resources necessary to complete the chosen treatment technique and monitoring of the bioremediation response effort.

Agent Availability

Once the proposed scale of the bioremediation response activity has been determined and agent alternatives have been identified, the availability of these agents for use at the spill location should be assessed. If an agent is not available in quantities necessary to complete the bioremediation response activities, the scale of the

bioremediation response should be reevaluated, a different bioremediation technique should be considered, or bioremediation should be eliminated as a response alternative.

Application and Monitoring Resources

Several application methods are generally available for bioremediation agents and each method may have unique resource requirements for its implementation. To determine whether requirements for application methods will preclude or limit the use of a particular method, the habitat(s) where bioremediation is being considered for cleanup should be evaluated to determine which method is most appropriate.

Next, the types and supply of available equipment and personnel adequate to implement and monitor the bioremediation response effort, as well as access to laboratory facilities for sample analyses, should be evaluated. (Refer to the Biomonitoring Plan section for recommended monitoring activities and monitoring resource requirements.) If the desired bioremediation response requires more resources than are currently available or attainable, the scale of the bioremediation response may need to be reduced.

IMPLEMENTATION

Before initiating bioremediation treatment, several steps shall be completed. First, the OSC shall notify RRT 4 that the use of bioremediation is being proposed by transmitting the completed Bioremediation Use Authorization Form. Second, a Bioremediation Work Plan and Bioremediation Monitoring Plan shall be developed to address issues necessary to ensure an efficient and effective bioremediation spill response.

RRT Notification

After finalizing the selection of a bioremediation treatment technique and the appropriate method for each affected habitat to receive treatment, the completed Bioremediation Use Authorization Form shall be transmitted to the affected State(s), EPA Region 4, the appropriate USCG District and the Federal Trustees for concurrence and consultation with the decision. If applicable, the appropriate Federal Land Manager (e.g., DOI) should also be notified.

If use of bioremediation in the spill area has been pre-approved or pre-authorized by RRT 4, this concurrence is not necessary. However, the OSC must still notify RRT 4 of the decision to use bioremediation. In the event RRT 4 pre-authorizes an area for the use of bioremediation, such areas will be included in the plan by addendum.

BIOREMEDIATION WORK PLAN

Work plans are important to ensure the safe, coordinated, and well documented implementation of bioremediation. Work plans are comprised of systematic procedures and guidelines that clarify and resolve issues such as worker and public safety, documentation requirements, response personnel roles and responsibilities, treatment technique agent application protocols, and application control and oversight considerations. Complete Work plans must include spill and site specific considerations. It is essential in a response that every incident or event be managed according to a plan and bioremediation is no exception. The Work plan shall provide:

- A clear statement of objectives and actions.
- A basis for-measuring work effectiveness and cost effectiveness.
- A basis for measuring work progress and for providing accountability.

Plans should be prepared for specific time periods or operational periods. These periods can be of various segments of time. Decisions on the length of the operational period or time segments may be affected by the length of time available/needed to achieve objectives, the availability of resources, environmental considerations, and safety considerations. Essential parts of any Work plan are:

1. **Statement of objectives -** Statement of what is expected to be achieved. Objectives must be measurable.

2. **Organization -** Describes what organization will be in place. This will describe in detail the specific roles and responsibilities of the participants in a bioremediation treatment technique. This will also describe the interaction of one entity to another.

3. **Tactics and assignments -** Describes tactics and control operations and what resources will be assigned. If the application is a large one, resource assignments may be done by groups.

4. **Supporting material -** Examples include a map or sketch of the area(s) to be treated, communications, traffic plan, weather data, special precautions, and safety information.

All supervisory personnel must be familiar with the plan and any changes which develop throughout the life of the project. This can be accomplished through briefings and by distributing copies of the written plan.

The Work plan must include an avenue to provide for ongoing evaluation of the plan's effectiveness. Supervisors should regularly assess work progress against control operations called for in the plan. If deficiencies are found, improved direction or additional staffing may be required, tactical operations may need to be modified, and/or changes may need to be reflected in planning for the next segment of time.

Demobilization activities, although often overlooked, are an integral part of the

Work plan. As the project begins to wind down, everyone will be anxious to leave the scene and return home. Demobilization planning helps to assure a controlled, safe, efficient, and cost effective demobilization process.

Organization

The response structure or organizational framework identifies the participants in a response, their general areas of responsibility, and the lines of authority among them. A chart illustrating the participants in a bioremediation response activity in Region 4 and their inter-relationships would be very helpful in summarizing this information. In developing this section, the following questions should be addressed:

- Who will manage the overall bioremediation activity?
- Who will be the likely participants (e.g. federal and state agencies) in the activity for the Region? What are the general roles?
- Who will be the likely participants, if any, from outside the Region? What are the general roles?
- Who will manage the monitoring portions of the activity?
- Who will develop an appropriate Work plan for the bioremediation activity?
- Who will perform specific treatment method or agent(s) application(s)?
- Who will perform monitoring?
- Who will perform public outreach?

Describe in detail the specific roles and responsibilities of the likely participants (RRT, federal and state agencies, international governments/agencies, non-governmental organizations, responsible parties, etc.) in a bioremediation activity in Region 4. The information in this section should coincide with the information presented above on the regional response structure.

Tactics and assignments

Tactical direction includes determining the tactics and operations necessary for the selected strategy and determining and assigning the appropriate resources.

Resource assignments should be made for each specific work task. Such assignments should consists of the kind, types and numbers of resources available and needed to achieve the desired outcomes.

Personnel and logistical support factors must be considered in determining tactical operations. Lack of logistical support can mean the difference between success and failure in achieving objectives.

Supporting Material

Public Safety/Information - Public safety is paramount in any bioremediation project. The following are some suggested actions which should be taken during a spill response to ensure public awareness and protection:

> Provide news releases and updates to newspapers, radio, television stations, and neighboring areas that could potentially be impacted by bioremediation activities. Be prepared to discuss details regarding the chosen treatment technique in simple layterms so the affected public will have an understanding of exactly what to expect and what the expected benefits are.

Site/Worker Safety - Worker health and safety is always the foremost concern during any spill response action. Since all oil spill response actions require a health and safety plan and the bioremediation application is merely a facet of the total spill response effort, the existing heath and safety plan should be used for the bioremediation application and augmented with the specific safety hazards associated with the bioremediation treatment method or agent application. A section referred to as biological hazards should be included in all health and safety plans associated with oil spill responses where biological agents are used as a response tool. This section should discuss the specific health and safety concerns associated with possible exposure to biological agents and include material safety data sheets (MSDS) for all agents being used. At a minimum, the health and safety plan should address the following aspects of the bioremediation treatment method/monitoring program:

1. minimum health and safety concerns,
2. potential hazards during application and monitoring,
3. evaluations of those identified hazards,
4. actions described to minimize the potential hazards, and
5. response(s) needed if hazard does effect worker(s).

The following documents contain guidance on the preparation of health and safety plans:

1. OSHA 1910.120 and EPA 40 CFR 311,
2. USEPA, OERR ERT Standard Operating Procedures,
3. NIOSH/OSHA/USCG/EPA Occupational Health and Safety Guidelines,
4. ACGIH Threshold Limit Values, and
5. existing local and area contingency plans.

To avoid disturbances to the treated area after treatment, all treated and control sites should be secured by the best achievable means. To avoid possible injury, post

warning signs or secure the treated area to differentiate the site from surrounding localities.

BIOMONITORING PLAN

Bioremediation is assumed to enhance the biodegradation of oil or hazardous substances without increasing adverse impacts to human or ecological health. Until there is defensible documentation from actual field use to confirm this assumption, however, bioremediation effectiveness and safety need to be monitored through a sound program of applied science. Therefore, an associated biomonitoring program shall be conducted when bioremediation treatment (either natural or enhanced) is used as a response tool. The plan outlining the biomonitoring program will be referred to as the biomonitoring plan.

Objectives

The principal objectives of the monitoring program and the elements of each objective are listed below.

1. Determine the efficacy of the selected bioremediation treatment method as it relates to the degradation of the spilled material.

   To continue to use biological degradation, the response community must compile data which shows that the use of bioremediation accelerates the breakdown of oil in the environment at a faster rate than if the oil was left to breakdown and degrade naturally. If there is no proven acceleration of the breakdown, then the risks and costs associated with the use of biological methods may outweigh the advantages.

2. Measure the environmental impact, if any, resulting from the biotreatment of an area, throughout the response activity to ensure against the harmful effects from the response. Especially, monitor any increases in eutrophication or ammonia caused by bioremediation.

   The monitoring of water quality parameters throughout the bioapplication is essential due to the potential for algae blooms, dissolved oxygen depletions, elevated available toxins in the water column, all of which may result in a critical impact to aquatic and vegetative life.

3. Determine if the bioremediation end points have been reached.

   With the use of all response tools it is important to determine at what point the tool is no longer effective or at what point it has achieved its objective. Thus biomonitoring end points must be developed prior to the initiation of the application, keeping in mind that these end points may need to be modified as the program progresses.

4. Ensure the comparability of data collected from all bioremediation response efforts conducted within Region 4 through compliance with USEPA Region IV=s Sampling Standard Operating Procedures.

This is done in order that the data may be used to enhance our understanding of bioremediation as an oil spill response tool. Properly collected, validated and interpreted data will provide critical information to assess the efficacy and environmental impact of bioremediation treatment and related response activities. Such documentation is needed to identify and correct problems in the biological treatment process, to determine whether bioremediation endpoints have been reached, to ensure that biotreatment is less environmentally harmful than the spilled pollutant and to support cost recovery and other legal actions.

Secondarily, the data can be used for developing regional and national data bases, interfacing with natural resource trustees, preparing interim and final reports, and revising this biomonitoring plan.

Quality Assurance

The quality of environmental data used to support OSC decision-making is critical to a spill response that considers or uses bioremediation. The primary goal of the quality assurance (QA) program is to ensure the accuracy of the environmental data considered by the OSC and RRT 4. It is the QA policy of RRT 4 that all activities associated with data collection and derivation are to be documented thoroughly. A monitoring program manager should be selected to specify procedures for ensuring the quality of data generated through the monitoring program and for providing sufficient resources for QA of collected data.

Biomonitoring Plan Design

Each biomonitoring program, in large part, will be event/site specific; however, pre-event planning and standardization of collection/analysis methods is encouraged. The design of the biomonitoring program is two-fold: (1) to document any impact to water quality which might result from the treatment or application and (2) to provide for the evaluation of the effectiveness of the treatment method or applied agent(s).

Conducting biomonitoring does not preclude the OSC/RP from conducting any other required monitoring associated with the spill event.

Project planning and site reconnaissance are essential activities conducted prior to the design of the biomonitoring plan. The OSC/RP may wish to refer to the area contingency plan (ACP) for existing shoreline or site assessment procedures developed by the area committees. The purpose of site reconnaissance activities are to gather information sufficient to:

- Determine that the objectives of the biomonitoring plan are consistent with the features of the site selected for application;
- Identify the type and quantity of existing historical water quality data for the area selected for the application, such as nutrient loading trends and physical water parameters;
- Define the geographic area of the spill targeted for application, for physical and chemical characteristics important to the design and execution of the biomonitoring plan;
- Determine the distribution, abundance, and seasonality of habitats, in the area to be considered for application;
- Project weather forecasts, meteorological and hydrogeological trends in the potential application area, for the proposed application time period;
- Determine equipment needs based on operational logistics; and
- Develop procedures designed to document sample collection methods and procedures.

The extent of the biomonitoring program should be directly proportional to the complexity and sensitivity of the area(s) chosen for biological degradation. The more diverse and sensitive the effected environment, the more complex and extensive the biomonitoring program should be. The volume of material spilled is not the driving factor in determining the extensiveness of the biomonitoring program; however, the larger the spill, in general, the more area affected and the greater the potential for affecting sensitive ecosystems. Thus, large spills generally will require a more extensive biomonitoring program. The OSC/RP should refer to the ACP and incorporate any and all required monitoring as directed by the ACP.

Because one spill event may affect several different morphological environments or habitats, bioremediation treatment techniques may be applied in several different habitats. The supporting biomonitoring program must be designed to accommodate inherent differences which are present in each habitat. Thus, each discrete habitat, within an application area, may require its own monitoring program.

Monitoring Activities

Biomonitoring plans should ensure that observations and samples be collected and analyzed from the following areas - within each discrete habitat(s):

<u>Untreated areas</u>

1. uncontaminated, untreated source areas (this will serve as background information and may not require the same intensity of sampling as the other areas),
2. contaminated, untreated source areas, and

<u>Treated area</u>

3. contaminated, treated areas

In order to evaluate the effectiveness of the bioremediation treatment technique

the biomonitoring plan should provide for the comparison of replicate data from treated and untreated areas for the duration of a project.

Within each discrete habitat which is a part of the bio application project, treated and untreated sites that exhibit similar chemical and physical characteristics should be chosen. Their similarity will support the comparability of the data generated. During their selection the following criteria should be considered, (1) environmental parameters, (2) physical habitat and geomorphology, and (3) oil loading and the probability of further oiling. Site variability should be limited as much as possible in order to generate data which is comparable.

Other physical variances which may effect the integrity of the data collected are wave action, tidal flushing, currents, boat traffic, and exposure to wind or other external forces.

Because efficacy analyses focus on evaluating relative changes in the concentration of the constituents of oil between treated and untreated sites, it is important to ensure that uncontaminated source areas remain uncontaminated for the duration of the monitoring program and contaminated areas are not reoiled for the duration of the monitoring program.

Monitoring should take in place in two forms:

1. Qualitative - serves as real time feedback for response decision and is usually in the form of visual observations, supported by photo documentation.

2. Quantitative - serves as the basis for longer term analysis of the success of the project and is in the form of sample collection and analysis.

Although visual observation is considered subjective, there is no substitute for this type of "real time" or fast feedback. Observers must be assigned to the project and trained to monitor morphological changes which may occur to the oil as it breaks down and any changes in organism behavior, such as the occurrence of algae blooms and fish kills.

All sample collection and analysis begins with a sampling plan. The sampling plans should include, at a minimum, the following:

- Implementation schedule (monitoring should be expected to take place over 3-4 months or until end points are reached)
- List of objectives
- Tasks to be conducted
- Description of project management
- Identification of sensitive areas included in/adjacent to the sample location areas
- Identification of sample locations, frequency, and collection methods
- Description of sample chain of custody procedures and QA/QC procedures
- Description of water quality history (if available) of the affected area or procedure for

determining background values for the affected area if historical data does not exist

The environmental characteristics and measurements that should be assessed and the samples that should be taken as part of the biomonitoring are presented in Table 2, along with a schedule for performing these activities. Sampling at each site, water depth (as appropriate), and time, should be performed in *duplicate for 10% of the samples collected*. Although the mix of samples collected should be based on the requirements of the analytical methods, minimum sample sizes are recommended as 1 liter for water samples and 4 - 16 oz for sediment or shoreline materials. All samples should be placed in precleaned jars or bottles with Teflon lined caps, as appropriate.

The monitoring parameters should involve a tiered approach which utilizes relatively inexpensive techniques such as total petroleum hydrocarbons (TPH) for screening and more sophisticated methods that target individual petroleum constituents to confirm biodegradation efficacy in *at least 25% of the samples analyzed*. The latter would include GC/MS analysis of target aliphatic and aromatic hydrocarbons which have been identified as marker compounds for tracking oil degradation and weathering, such as the normal alkanes, the isoprenoids, pristane and phytane, and the conservative biomarker hopane. Water quality measurements should include nutrients, dissolved oxygen, biological oxygen demand (BOD), TOC and COD. Refer to Appendix E for methodologies and recommended procedures.

All data is subject to review by the OSC or a delegate and will be made available upon request. This data will support further response decisions and to provide the response community with a better understanding about the use of bioremediation as an oil spill response tool.

DOCUMENTATION AND REPORTING

During the course of a bioremediation activity and accompanying monitoring effort, the following reports shall be prepared and submitted to the OSC:

Activity reports -- provide descriptions of the bioremediation activity area, weather, unique observations, and activities undertaken, as well as the names and affiliations of persons on site. Activity reports should be prepared whenever activities on a site are undertaken.

Analytical reports -- provide laboratory analysis results of environmental and control samples. Lab results should be analyzed, interpreted and a brief summary report prepared within a reasonable time agreed to by all parties.

After action report -- provide a description of the overall bioremediation activity and accompanying monitoring effort, including results of both field and laboratory activities. A draft should be submitted within 30 days after the end of the monitoring

effort. A final report, (incorporating comments from those the draft was submitted to, as well as photos) should be submitted within 60 days after submission of the draft.

In addition, at the time the final after action report is submitted, all field notes, including those of contractors, should be submitted to the OSC.

To facilitate information transfer and the development of a data base on bioremediation use and bioremediation agents, the Bioremediation Use Follow-Up Form in Appendix F should be completed at the end of the bioremediation activity.

PLAN REVISION

The monitoring plan and suggested procedures outlined in this section should be implemented and modified, as necessary, based on the cumulative experience and knowledge gained from conducting bioremediation field activities and associated laboratory activities. Recommendations for revisions should be submitted to the Region 4 RRT for approval.

TABLE 1
FIELD-MONITORING PARAMETERS

Parameter	Sample Size[1]	Assessment/ Collection Location	Assessment/Collection Frequency[2]
Visual observations (mortality, behavioral effects, appearance changes, oil distribution)	N/A	All test sites	Daily to the extent possible; at least each day that water, sediment, and/or shoreline material sampling is performed
Temperature (air, water)	N/A	All test sites	Days 0, 1, 7, 14 and every week thereafter
Salinity	N/A	All test sites	Days 0, 1, 7, 14 and every week thereafter
Dissolved oxygen	N/A	All test sites	Days 0, 1, 7, 14 and every week thereafter
Sea state	N/A	Activity area	Days 0, 1, 7, 14 and every week thereafter
Current	N/A	Activity area	Days 0, 1, 7, 10 and 20
Wind velocity	N/A	Activity area	Days 0, 1, 7, 14 and every week thereafter
Efficacy (water, sediment, and/or shoreline material)	1 liter water; 20 grams sediment or shoreline material	All test sites and, as appropriate, all water depths	Days 0, 1, 7, 14 and every week thereafter
Toxicity[3] (water, sediment, and/or shoreline material)	8 liters water; 20 grams sediment or shoreline material	All test sites and, as appropriate, all water depths	Days 0, 1, 7 for Microtox and at same intervals for every reapplication of agent, for long term amphipod days, 0, 1, 7, 14 and every week thereafter

[1]N/A means "Not Applicable".
[2]Frequency is relative to the time of agent application.
[3]Sample size, location and frequency for toxicity testing are recommendations. Actual parameters shall be determined based upon conditions of the spill event.

APPENDIX A

APPLICABLE FEDERAL AND STATE REGULATIONS

Legislation at both the federal and state level may affect decisions to use bioremediation. Existing regulations and policies that govern the use of bioremediation treatment techniques and agents in responses to spills in Region 4 are summarized below.

Federal Regulations

At the Federal level, Subpart J of the NCP governs the use of chemical and biological agents -- which include bioremediation agents -- in responding to oil spills. Specifically, the Subpart:

Restricts the use of chemical and biological agents that may affect US waters to those listed on the NCP Product Schedule;

Specifies technical product information that must be submitted to EPA for an agent to be added to the Schedule; and

Establishes conditions for obtaining authorization to use chemical or biological agents in a response action.

If EPA determines that the required data were submitted, EPA will add the agent to the Schedule. Note, however, that listing of an agent on the NCP Product Schedule does not constitute approval of that agent for use or confirmation of any claims regarding the agent's safety or effectiveness.

Data on agents listed on the NCP Product Schedule are available through EPA's Emergency Response Division in Washington, DC.

The OSC, with concurrence of RRT 4, including the RRT representative from the State with jurisdiction over the waters threatened by the spill, may authorize the use of any agent listed on the Product Schedule. In addition, when practicable, the OSC should consult with the Department of Commerce (DOC) and Department of Interior (DOI) representatives to the RRT before making a decision to bioremediate a spill. If the use of particular products under certain specified circumstances is approved in advance by the State, DOC, and DOI representatives to the RRT, **and** such preapproval is specified in the Regional Contingency Plan, the OSC may authorize bioremediation without consulting the RRT.

State Regulations and Policies

The following States do not currently have set policies regarding the use of bioremediation during a spill event. For approval or information, contact the State=s representative to the Region 4 RRT.

Alabama	**Georgia**
Kentucky	**Mississippi**
South Carolina	**Tennessee**

Regulations and Policies in the State of Florida

The State of Florida does not have any regulations that specifically address the use of bioremediation as a spill response tool. However, regulations do specify that any person discharging a pollutant shall immediately undertake actions to contain, remove, and abate the discharge (Chapter 376.305(l), Florida Statutes) to the satisfaction of the Department of Environmental Protection (DEP). The DEP does not encourage bioremediation as a primary response countermeasure, but instead it may be used in conjunction with other conventional remedial actions. The exception to this is when the option of doing nothing is considered or conventional cleanup/treatment methods are not feasible. In those cases, in-situ bioremediation can be an effective substitute for traditional cleanup technologies.

The DEP has developed a set of guidelines to assist the state OSC or first responder with bioremediation decisions and proper use. The AGuidelines for the Use of Bioremediation as a Cleanup Technique@ apply to spills of less than 50 gallons of petroleum on inland areas or in non-navigable waters of the state. The DEP has not established any guidelines or policies regarding the use of bioremediation for coastal spill response. In these cases, the DEP will work closely with the Florida Marine Research Institute, the federal OSC and the RRT to identify areas where bioremediation would be considered.

The use of bioremediation is prohibited for petroleum contaminated site (inland UST sites) remedial actions unless specifically approved by the DEP Bureau of Waste Cleanup, Technical Support Section. The DEP has established petroleum contaminated soil cleanup criteria (Chapter 62-770, Florida Administrative Code) and publishes AGuidelines for the Assessment and Remediation of Petroleum Contaminated Soil@ to clarify the DEP=s position concerning petroleum contaminated soil remedial actions.

Regulations and Policies in the State of North Carolina

The State of North Carolina=s Department of Environment, Health, and Natural Resources regulates the use of bioremediation for response to spills. When requesting an evaluation to utilize bioremediation the following information must be submitted to:

Dr. Luanne Williams
North Carolina Department of Environmental, Health and Natural Resources
Occupational and Environmental Epidemiology Section
PO Box 29601
Raleigh, NC 27626-0601
(919) 715-6429

Required General Information

1. Division of Environmental Management (DEM) contact person and phone number.
2. Current or future use of site with site contact person, address & phone number.
3. Contractor applying product, contact person, address & phone number.
4. Distance and impact to public or private wells used for drinking, industrial processes, cooling, agriculture, etc. and is area served by public water supply? Verification must be provided by the regional Groundwater and Public Water Supply Sections. Send responses to Dr. Luanne Williams.
5. Detailed specifications of the contamination present in the soil and/or groundwater.
6. Approximate distance & name of nearest surface water body (provide map).

Required Product/Process-Specific Information (All information submitted will be maintained as proprietary and not disclosed to other parties.)

1. Product manufacturer name, address, phone number and contact person.
2. Genus/species/strain of microorganism(s) contained in product
3 Identity of specific ingredients and concentrations of ingredients contained in the product and purpose of each.
4. Documentation of evidence from authoritative technical references (i.e. Bergey=s Manual of Systematic Bacteriology, Bergey=s Manual of Determinative Bacteriology or other existing references) that the microorganism(s) are not pathogenic to animals or humans.
5. Documentation (i.e. references) of whether or not the microorganism(s) are naturally-occurring in the immediate or similar environment.
6. Documentation (i.e. references) of specific degradation products expected.
7. Documentation (if available) of migratory potential of microorganisms and degradation products in soil and groundwater.
8. Complete description of the bioremediation process on a site (e.g. application of the product to soil and/or groundwater, aeration of soil, procedures needed to maintain growth and chemical degradation).

The risk evaluation will be forwarded to the designated contact person within the company, site owner, manufacturer, consultant applying the product, DEM contact person and Groundwater Section contacts--Linda Blalock (Federal Trust Fund) and Brian Wagner

(Operations Branch).

APPENDIX B

BIOREMEDIATION USE AUTHORIZATION FORM

The following questions should be answered, if known, and presented to the OSC who will review them and present them to the RRT for consideration. A question left unanswered will not automatically result in a no-go decision, but EVERY effort should be made to present accurate and timely information. The RRT will use the information provided below to assist in making the decision for use of bioremediation.

The form consists of two parts, incident characteristics and feasibility assessment criteria. Additionally, a Bioremediation Work plan and Biomonitoring Plan must be prepared and submitted to the OSC or his designee for review. (Note: Many of the items requested in the feasibility assessment criteria section can and should be included in the bioremediation Work plan.)

Incident Characteristics

Time and date of release:

Product spilled:

Quantity spilled:

Status of spill:

Location of incident:

Description of incident:

Properties of spilled product:

- specific or API gravity
- viscosity, cp
- pour point, at temp, F
- sulfur content, %w

Responsible party information:

- company
- address
- telephone
- contact person
- telephone

Feasibility Assessment Criteria

Specific location proposed for treatment:

What are the characteristics of the spill environment?

- type of environment, habitat
- marine, brackish, freshwater
- past spill history

Amount of weathering spilled product has undergone:

Description of impact(s):

Has ownership of land been determined:

Has written permission from landowner been obtained:

Bioremediation agent proposed for use:

- Name of product.
- Type of agent (microbial, nutrient, microbial + nutrient, etc.).
- Is agent listed on NCP?
- Has EPA data been reviewed by the SSC?
- To what tier has the agent been formally evaluated?
- Does the agent or responsible party have any previous first hand experience with the use of the proposed bioremediation agent, or have any corroborated (laboratory or field) data indicating it enhances biodegradation and is not toxic to affected spill environment?
- Has this agent been used on previous oil spills?
- What were the characteristics of the oil and the spill environment in each case?
- Are degradation results (based on oil chemistry and microbial tests) available for review?
- Is a reference available?

Supply:

- source of supply
- amount available
- ETA to site

Application:

- estimated amount of agent(s) needed
- who will apply the agent (vendor personnel, response contractor personnel, or other contractor)
- method to be used in applying agent
- impacts of proposed application method
- time to prepare agent for application
- has application equipment been calibrated for this particular application
- planned rate of application
- how long will application take
- will product have to be reapplied
  - how frequently

Bioremediation Work plan

Has a bioremediation Work plan been prepared?
Has the plan been reviewed?

Biomonitoring Plan

Has a biomonitoring plan been prepared?
Has it been reviewed?

Project Management

Bioremediation application project manger:
contact number:
address:

This bioremediation application has been approved:

Federal On-Scene Coordinator	State On-Scene Coordinator	Environmental Protection Agency
Department of Commerce	Department of Interior	

APPENDIX C

EVALUATING BIODEGRADATION POTENTIAL OF VARIOUS OILS

APPENDIX D

BIOREMEDIATION AGENTS AND AGENT SELECTION

This section describes the various types of bioremediation agents, a procedure for evaluating them, and guidelines for selecting the appropriate agent for use in a particular spill situation.

Background

Section 311 of the Clean Water Act requires that the US Environmental Protection Agency (EPA) prepare a schedule of dispersants and other chemicals that may be used in preparing for and responding to discharges of oil and releases of hazardous substances, as provided for in the National Oil and Hazardous Substances Pollution Contingency Plan (NCP), 40 CFR Part 300. This schedule is known as the NCP Product Schedule. The Schedule lists agents that may be authorized for use on oil discharges in accordance with the procedures set forth in Section 300.910 of the NCP. (Authorization of use requires that the Federal On-Scene Coordinator (OSC) considering the use of a dispersant or other agent, such as a biodegradation enhancing agent, seek the concurrence of the Regional Response Team prior to the agent's application.) Any agent considered for application to an oil spill should be listed on the NCP Product Schedule.

The NCP Product Schedule currently divides chemical and biological agents into five categories:

1. dispersants,
2. surface collecting agents,
3. biological additives,
4. burning agents, and
5. miscellaneous oil spill control agents.

Most bioremediation agents, including those that are solely nutrients, are listed as biological additives, as the designed purpose of these agents is to enhance the rate of oil biodegradation by increasing microbial activity. There are also bioremediation agents listed as dispersants; these agents are water-based products that claim to enhance the rate of oil biodegradation by emulsifying spilled oil thereby making it more "bio-available." Additionally, other products that do not fit a current regulatory definition because of their unique nature may be listed as miscellaneous agents. Use of any of these agents should be consistent with the Regional Response Team's general guidelines for their application and use.

Types of Agents

The number and type of agents which claim to enhance the rate of biodegradation has broadened to fill the current perceived market. Although there are no current regulatory definitions for every type of bioremediation agent, the following are broad definitions for those currently available:

Microbial Agents -- concentrated cultures of oil-degrading microorganisms grown on a hydrocarbon-containing medium that have been air- or freeze-dried onto a carrier (e.g., bran, cornstarch, oatmeal). In some cases, the microorganisms may be grown-up 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.

Nutrients -- agents containing 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. This type of product has been used in Prince William Sound, Alaska and Pall's Island, New Jersey to reduce the amount of oil on contaminated beaches. [For information on uses in Alaska, refer to Pritchard and Costa's article entitled 'EPA's Alaska Oil Spill Bioremediation Project' in *Environmental Science & Technology* (Vol. 25(3), 1991), and the article by Chianelli *et al.,* entitled "Bioremediation Technology Development and Application to the Alaskan Spill" *in Proceedings: 1991 Oil Spill Conference.*]

Enzymatic - bio-catalysts designed to enhance the emulsification and/or dispersion of oil and 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, that are manufactured through fermentation. This type of agent is intended to enhance biodegradation by indigenous microorganisms.

Other Agent -- include agents that do not fall under the above definitions, such as application mechanism agents that are designed to have an affinity for oil and bring together the elements needed for enhanced oil degradation. Examples of application mechanism agents include time release capsules, liposomes, timed-release fertilizers (e.g., Custom blend), and agents that make oil more hydrophilic.

Agent Evaluation Procedure

In considering bioremediation agents listed on the NCP Product Schedule or

proposed by agent vendors for potential use in spill cleanup, it is important that response decision-makers evaluate the various characteristics of agents, particularly their safety and efficacy. From the perspective of planning for bioremediation use, the most appropriate time to evaluate agents whether performed by EPA, product vendors, or contractors - is before a spill occurs. Provided below is a procedure designed specifically to aid in such an evaluation, which is directed ultimately at identifying bioremediation agents that will be safe and effective in field applications. There may be circumstances, however, under which there is not adequate time to perform thorough agent evaluations before a decision regarding bioremediation use must be made. In these instances, the procedure below should be used as a guide to determine whether existing information on individual agents is adequate to support further consideration of their use.

The procedure follows a "tiered" approach (a "Base Tier and four subsequent tiers) whereby bioremediation agent performance data is gathered as a means to predict the safety and efficacy of agent applications in various field settings or habitats where oil spills may occur. The proposed procedure is intended as a standard methodology for assessing the effectiveness and safety of different bioremediation agents. Following the procedure will not assure that a tested agent will be effective in spill cleanup, however, following the procedure should increase the level of confidence that use of an oil spill bioremediation agent will be effective and safe.

Base Tier -- "Go"/"No Go". Requirements and Information

Information on a bioremediation agent should be collected from the agent vendor and an initial screening of the information performed. Objectives of this screening are to:

- Ensure that the agent is listed on EPA's NCP Product Schedule.
- Obtain basic information on a bioremediation agent's makeup;
- Ensure satisfaction of minimal regulatory approvals that may be required;
- Certify whether the agent contains pathogenic, carcinogenic, or hazardous substances or microorganisms normally considered unacceptable for release into the environment; and,

Information needed from the agent vendor to perform this initial screening includes the agent's exact chemical and biological makeup as well as formulation characteristics, and proof of the agent's listing on the NCP Product Schedule.

Tier I -- Feasibility Assessment

Additional vendor information on a bioremediation agent should be collected to

support an assessment of whether use of the agent is feasible. The objectives of this tier and assessment are to obtain an understanding of a vendor's capabilities; an agent's availability, contents, and proposed method of use; and an agent's history of use, where applicable. Agent information needed from the vendor to perform this assessment includes the following:

- Application rates and methods;
- Mode of biodegradation and calculated efficiency;
- History of use at previous cleanups;
- Chemical properties, fate and persistence, and potential toxicity or bioaccumulation for humans, mammals, and birds based on a review of published literature and chemical databases;
- Acute or chronic toxicity to one marine or freshwater fish and invertebrate species selected from US EPA's "Effluent Monitoring Program"; and, where available,
- Effectiveness in enhancing biodegradation over a baseline standard or control demonstrated by descriptions and quantitative analytical results of any laboratory or field studies performed (such as results of gas chromatographic analyses of treated and untreated samples for alkanes and/or aromatics).

A description of the management structure and qualifications of the vendor's organization is also needed.

Tier II - Laboratory-Scale Data

Standard laboratory methods should be used to develop data on an agent's toxicity and its ability to stimulate the biodegradation of a standard oil. The specific objectives of this tier are to evaluate the relative ability of a bioremediation agent to degrade oil, or stimulate the rate of biodegradation, under defined and controlled laboratory conditions and to determine the potential toxicity associated with the agent's use through the performance of standard toxicity tests. Analytical methods developed by EPA should be used to perform these laboratory studies.

The approach to evaluate an agent's relative effectiveness at degrading oil should:

- Provide sufficient information to indicate with a firm degree of confidence that the agent is degrading oil constituents;
- Provide an indicator of total microbial activity; and
- Assure the viability of the culture being tested, where applicable.

The approach should include temperature, salinity, and nutrient testing to document the conditions under which an agent's ability to degrade a standard type of oil

was determined.

The approach to evaluate an agent's toxicity should be conducted for specific fresh-water or marine species on the agent alone and the agent and standardized oil combined. Seven-day chronic estimator methods should be performed using daphnia (Ceriodaphnia) and fathead minnows (Pimephales) for fresh water, and mysids (Mysidopsis) and silversides (Menidia) for marine applications. These are standard tests; additional tests specific for Regional species may be desirable. Mammalian toxicity of agent constituents should be reviewed in existing data to determine whether any precautions need be taken with regard to application methods, rates, or timing to protect persons applying agents as well as indigenous wild life.

Tier Ill - Simulated Field Test Demonstration

Based on findings of previous tiers, microcosm systems should be used to perform simulated field test demonstrations on a bioremediation agent, as appropriate. The objective of this tier is to predict a bioremediation agent's effectiveness at degrading oil or petroleum products in specific field settings or habitats.

Although EPA-approved microcosm systems for performing simulated field test demonstrations are still under development at the time of this writing, the approach for performing these tests is to use microcosm systems that simulate actual biodegradation field kinetics. This approach will aid in determining the relative effectiveness and toxicity of an agent under conditions that cannot be modeled in standard laboratory methods, such as those proposed in Tier 11 of the procedure. Microcosm systems that should be considered for simulated field test demonstrations of agents include:

1. cobble beaches, both marine and fresh water;
2. open water, both marine and fresh, warm and arctic;
3. marshes and wetlands, both marine and fresh water;
4. inland shoreline;
5. sandy beaches, both marine and fresh water; and,
6. land/soil.

Tier IV -- Limited Field-Scale Demonstration of the Agent

Depending on the results of the simulated field test demonstration in Tier III, a limited field scale demonstration of a bioremediation agent should be conducted. The objectives of this field demonstration are to test the effectiveness and toxicity of the bioremediation agent in actual field tests and to verify the accuracy of Tier Ill laboratory results in predicting field efficacy using the actual field monitoring data obtained. The approach for performing these demonstrations is to collect information during active field testing to support an evaluation to confirm the bioremediation agent's estimated environmental safety and efficacy.

At this time, EPA-approved protocols for performing limited field-scale demonstrations in various settings are still under development. Until such protocols become available, the guidelines provided in Section 6 for monitoring field applications

of bioremediation agents could be used for evaluating limited field-scale demonstrations of agents.

Agent Selection

Due to a lack of specific bioremediation agent research and agent testing standards, the selection of a bioremediation agent that will enhance the rate of oil biodegradation must be based on best professional judgment. For most of the bioremediation agents currently on the NCP Product Schedule, there are only limited comparative data by which to measure their relative efficacy and safety. Some of the agents have been tested by EPA according to the procedure described above; however, these agents are not necessarily better than ones that have not been tested by these methods. Therefore, agent selection will remain largely a subjective process until a larger and more complete database of standard test data on agents can be assembled.

To the extent possible, the selection of bioremediation agents for potential use in oil spill cleanup against specific oils or petroleum products should take place in anticipation of an oil discharge, when time is not a critical factor. For areas where the potential for an accidental spill is high or where there has been a high frequency of spills (assuming the use of bioremediation agents is allowed in these areas), specific plans should be developed that outline the most likely petroleum products to be spilled and the alternative bioremediation agents that could be used to perform cleanup of those products in these areas.

APPENDIX E

LABORATORY ANALYSIS PARAMETERS

Parameter	Sample Matrix	Methodology	Recommended Methods
Oil hydrocarbons (C17, pristane, C18, Phyl	Water, Sediment or shoreline	GC + GC/MS	ASTM Method D3328
NH_3	Water, Sediment or shoreline m	Spectrophotometric	EPA Method 350.1, 350.2 or 350
NO_3	Water, Sediment or shoreline m	Spectrophotometric	EPA Method 353.2 or 353.3
NO_2	Water, Sediment or shoreline m	Spectrophotometric	EPA Method 354.1
PO_4	Water, Sediment or shoreline m	Spectrophotometric	EPA Method 365.1, 365.2 or 365
Toxicity	Water, Sediment or shoreline m		

Sampling is to be conducted in accordance with an approved sampling plan and should utilize a justified random approach where the individual sites are selected based on appropriate habitat-types within treated and untreated zones. Within a site, individual sampling stations should be randomly chosen. Dependent on habitat-type, the site may be further divided such that specific zones within the site are monitored such as the upper and lower intertidal zones or stream-side and back marsh areas. Sediment grab samples may be collected using a variety of standard techniques. Core sampling is preferred for most intertidal and subtidal areas since it consistently allows for a highly reproducible volume of sample to be collected. Typically the core depth should exceed the depth of contamination if applicable and the core should be sectioned by 5 cm increments. Scoop-type grab sampling is applicable but great care is required to ensure that consistency is maintained. The sampling plan should provide exact guidance as to the width and depth of each sample.

Adjacent subsurface water samples may be collected using standard grab techniques. Caution should be exercised to prevent surface oil from contaminating the collection vessel as it is lowered to the specified sampling depth. Water grab sample will typically be collected at 1-3'depth.

Analytical methods used for bioremediation monitoring should be consistent with standard methods utilized for oil weathering and degradation studies. Analytical guidance being developed by the EPA and NETAC for laboratory testing of bioremediation agents should be adopted for field monitoring studies.

Field and laboratory blanks should be specified in the monitoring plan and should represent at least 10% of the samples analyzed. To assess environmental variability, 10% of the sample stations should be sampled and analyzed in triplicate. Since no

certified reference material is currently available for oil bioremediation monitoring, a reference sample of the spilled oil should be analyzed periodically to verify laboratory consistency. Quantitative values for the reference oil should not vary by more than 20% for selected analytes. Good laboratory practices should be employed that are consistent with the objectives of the biomonitoring plan.

Accurate sample identification and proper control of samples is essential. A chain of custody procedure will be established and implemented which will ensure integrity of the samples and proper handling of the samples.

APPENDIX F

INFORMATION FEEDBACK:
BIOREMEDIATION USE FOLLOW-UP FORM

Lessons learned from a spill cleanup operation are most useful when others, particularly those not personally involved in the original cleanup operation, can benefit from them by drawing upon the original responders' experiences. Region 4 has established a program to facilitate the collection and transfer of information on uses of bioremediation that is intended to provide decision makers with case data upon which future decisions regarding bioremediation may be based. Particularly because response officials have very limited experience with bioremediation in uncontrolled environments, such as open water and other marine areas, this program is expected to be a valuable resource for supporting informed decisions regarding bioremediation.

The principal objective of this bioremediation information feedback program in Region 4 are as follows:

To gather relevant, accurate, descriptive, and complete information from sites - where bioremediation has been used for spill response; and

To provide that information via an accessible network to future decision makers who are considering the use of bioremediation.

The Bioremediation Use Follow-Up Form on the following pages has been provided to guide information collection efforts in support of this program. A separate form should be completed for each unique bioremediation activity. Because certain information may not have been anticipated when the form was developed, feel free to provide any other information deemed appropriate regarding the use of bioremediation in a particular response action.

BIOREMEDIATION USE FOLLOW-UP FORM

A. SPILL INFORMATION

1. Spill event
2. Date
3. Location (e.g., offshore, wetlands, coastal)
4. Product(s) spilled
5. Amount of spill
6. Reason(s) for using bioremediation
7. Age of oil when bioremediation agents applied

B. BIOREMEDIATION AGENT INFORMATION

1 . First Treatment or Application:
   a. Type of agent applied (e.g., nutrient, microbial, enzyme)
   b. Name of agent
   c. Agent listed on the NCP Product Schedule?
   d. Vendor
   e. Vendor address and phone number
   f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score
      Visual observation
      Oil chemistry
      Method used (e.g., GC, GC/MS, TPH)

2. Second Treatment or Application (complete if different from above):
   a. Type of agent applied (e.g., nutrient, microbial, enzyme)
   b. Name of agent
   c. Agent listed on the NCP Product Schedule?
   d. Vendor
   e. Vendor address and phone number
   f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score
      Visual observation
      Oil chemistry
      Method used (e.g., GC, GC/MS, TPH)

3. Third Treatment or Application (complete if different from above):
   a. Type of agent applied (e.g., nutrient, microbial, enzyme)
   b. Name of agent
   C. Agent listed on the NCP Product Schedule?
   d. Vendor
   e. Vendor address and phone number
   f. Rate effectiveness (compared to control site) on a scale of 1 to 10, 10 being the highest score
      Visual observation
      Oil chemistry
      Method used (e.g., GC, GC/MS, TPH)

C. <u>SITE CONTROLS</u>

1. Size and number of test site(s)
2. Size and number of control site(s)
3. Site security measures taken

D. TREATMENT AREA LOCATION

1 . On water (latitude and longitude)
2. Shoreline (latitude and longitude)
Shoreline type (e.g., sand, shell, cobble)
Shoreline zone (e.g., intertidal, surge, storm/overwash) Depth of shoreline oiling

E. APPLICATION INFORMATION

1. Microbial counts before application
2. Microbial counts after application
3. Applications performed by (names and titles)
4. Application method(s) used
5. Application date(s)
6. Application conditions (e.g., winds, waves)
7. Agent concentration and rates (e.g., gal/acre)
8. Additional information on re-applications

F. MONITORING

1 . Schedule and duration (e.g., weekly for 3 months)
2. Method (e.g., foot, by air, boat)
3. Monitoring performed by (names and titles)
4. Toxicity noted

G. PROBLEMS ENCOUNTERED (e.g., weather, site security, application)

H. LESSONS LEARNED

1. CONTACTS
    1. OSC (name, address, and phone)
    2. SSC (name, address, and phone)
    3. Form completed by (name, title, and agency)

APPENDIX G

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