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Combating Manuals/Guidelines

Draft Guidance Document for Decision Making and Implementation of Bioremediation in Marine Oil Spills

Submitted by France

SUMMARY

Executive summary:	This document entitled, Draft Guidance Document for Decision Making and Implementation of Bioremediation in Marine Oil Spills aims to provide users with clear and cost-effective criteria to enable them to evaluate the circumstances in which they might consider the use of bioremediation for shoreline clean-up
Action to be taken:	To note the information
Related documents:	MEPC 47/5/2

1 This document entitled, Draft Guidance Document for Decision Making and Implementation of Bioremediation in Marine Oil Spills aims to provide users with clear and cost-effective criteria to enable them to evaluate the circumstances in which they might consider the use of bioremediation for shoreline clean-up.

2 MEPC 47/5/2 should be read in conjunction with this document.

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ANNEX

DRAFTGUIDANCE DOCUMENT FOR DECISION MAKING AND IMPLEMENTATION OF BIOREMEDIATION IN MARINE OIL SPILLS

31 August 2001

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SUMMARY

PREFACE

Major incidents such as the *Amoco Cadiz* (France, 1978), the *Exxon Valdez* (USA, 1989), the *Braer* (UK, 1993), the *Sea Empress* (UK, 1996) and the *Erika* (France, 1999) have provided the stimulus for the development of alternative response techniques to tackle oil pollution both at sea and on the shoreline. One such technique is bioremediation. Although recognised as a potential response option 30 years ago, it is receiving renewed attention as more environmentally acceptable clean-up methods are sought and as new claims of the potency of bioremediation are made.

The public often sees bioremediation as the ‘environmentally friendly’ response to an oil spill since it converts the oil into harmless products such as carbon dioxide and water. It has been used successfully for a number of years to enhance the natural degradation of oil in, *ex-situ* methods as landfarming, composting and biopiling. However, the use of bioremediation *in situ* to remove residual oil directly from the shoreline, approaches, remains somewhat controversial.

The benefit of using bioremediation is dependent upon fulfilment of a number of specific criteria. The scientific community is in the process of researching many of these criteria with a view to understanding more fully the processes involved and seeking improvements.

Given the prominence that bioremediation has gained in oil spill response, potential users need guidelines to help identifying scenarios where this technique could be environmentally beneficial and for implementation in their contingency plans. Equally, they need to be aware of situations in which bioremediation will be unsuitable or could cause even greater harm than the oil itself.

With a view to providing responders with a set of practical guidelines the ?? session of the Marine Environment Protection Committee (MEPC) of IMO date? decided that Guidelines for the use of bioremediation should be prepared and published by the IMO. France agreed to act as the lead country in preparing this task, acting through the CEntre de Documentation de Recherche et d’Expérimentations sur les pollutions accidentelles des eaux (CEDRE). A workshop held in Brest (France) on the 17-19th April 2001 prepared a first draft of the Guidelines which was completed through mail exchanges between the experts by the summer 2001. From these guidelines a short version was written to be incorporated in the IMO manual as a separate chapter on bioremediation. The final draft documents (full guidelines and separate chapter) were considered at the ?? session of the MEPC date? by the OPRC Working Group. The committee approved the documents as amended for publication.

The aim of these guidelines is to provide users with clear and cost-effective criteria to enable them to evaluate the circumstances in which they might consider the use of bioremediation for shoreline clean-up. These guidelines are not intended to address the treatment of waste generated at oil spills. They contain a summary of the most important bioremediation processes and decision-making criteria. The various strategies employed are discussed and some suggestions as to how to monitor the effectiveness and check for possible adverse consequences of the technique are made. Wherever possible, reference is made to reliable field trials and studies. Suggestions for further reading are provided at the back of the guidelines for those readers who wish to study this subject in greater detail.

The committee expressed its appreciation to:

- the Government of France and CEDRE for having taken the lead to host the workshop to formulate the guidelines;
- the experts (listed below) who participated in the workshop and participated in the text.

List of participants attending the workshop set to review the guidelines (*Cedre* - Brest, France, 17-19 April 2001)

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Chapter 1: INTRODUCTION TO BIOREMEDIATION

Bioremediation is the active use of biological techniques to mitigate the consequences of a spill using biological processes

1.1. WHAT IS BIOREMEDIATION?

Bioremediation is defined above and refers both to the stimulation of pollutant biodegradation and/or to enhance ecosystem recovery. Biodegradation is the process by which microorganisms, primarily bacteria, breakdown a wide range of organic contaminants such oil that are susceptible to microbial degradation. Enhanced ecosystem recovery may occur in the absence of biodegradation but is still considered to be bioremediation.

In these guidelines the term bioremediation includes those techniques used on site (*e.g.* biostimulation, bioaugmentation; phytoremediation; monitored natural attenuation; composting/biopiling); and the extensions to bioremediation that can be applied through combination with physical or chemical clean-up methods (surf washing, surfactant addition).

1.2. WHY USE BIOREMEDIATION?

There is no single response technique that is suitable for all spill circumstances. Therefore, a contingency plan should include consideration of all current clean-up methods (Chapter 2).

A principal advantage of bioremediation over other more conventional physical and chemical methods is that it can result in the removal of the contaminant by the enhancement of the natural biodegradation process of converting potentially toxic compounds into water and carbon dioxide. Furthermore it can enhance the rate of habitat recovery, for example, by favoring plant growth in wetlands. As such, it is more likely to be acceptable to public than are the more invasive chemical or physical procedure.

Bioremediation like all other methods has advantages and disadvantages. Table 1 shows the pros and cons of using bioremediation in comparison with conventional response techniques.

Table 1: Pros and cons of bioremediation

Pros	Cons
Oil degrading microorganisms are ubiquitous (present everywhere) and therefore it can be used on a range of shoreline type	Will not work in open water due to the dilution
Relatively non-intrusive method for final polishing	Cannot be used on heavily contaminated beaches unless free contaminant has been removed.
Is a natural process	Dependant on prevailing environmental conditions and the nature of the oil (i.e. limitations on heavy fuel oils)
Does not generate waste products	Takes longer than other physical/chemical techniques

Is generally not labour intensive and can be cost effective	Requires thorough planning and detailed monitoring
Has received a positive public response	Concerns exist regarding potentially adverse health effects associated with release of bioremediation agents particularly bioaugmentation products, and those resulting from the metabolic byproducts of biodegradation.

1.3. HOW BIOREMEDIATION WORKS

Organic contaminants that are susceptible to biodegradation include oils (*e.g.* petrol, diesel, heating oil, crude oil, lubricants, and some fuel oils), Polynuclear Aromatic Hydrocarbons (PAHs), oxygenated hydrocarbons (*e.g.* glycols, surfactants, detergents), pesticides, BTEX components (Benzene, Toluene, Ethylbenzene, Xylene), solvents, chlorinated solvents, amines, anilines, and even some explosives. Biodegradation of organic contaminants may follow different mechanisms according to the environmental conditions : presence (or availability) of oxygen, aerobic conditions, or absence of oxygen, anaerobic conditions.

Under aerobic conditions (*i.e.* in the presence of oxygen) many organic molecules are eventually converted to carbon dioxide, water and microbial cell mass (biomass). Under anaerobic conditions, biodegradation is usually much slower and therefore of less operational interest.

In the case of oil this can be represented by the following formula:

$$\mathbf{1\ kg\ HC^{a} + 2.6\ kg\ O_2 + 0.07\ kg\ N + 0.007\ kg\ P \Rightarrow 1.6\ kg\ CO_2 + 1\ kg\ H_2O + 1 kg\ biomass}$$

[a] Hydrocarbon

***Insert picture 1** of bacteria at work (supplied by R Swannel)*

1.3.1. Mechanisms of biodegradation

Petroleum hydrocarbons can be divided into four major classes (and subclasses) whose potential for biodegradation is highly variable; they can be listed in order of biodegradability:

- alkanes, (or saturates)
- aromatics (including Poly Aromatic Hydrocarbons (PAH))
- asphaltenes
- resins or polar compounds

Alkanes (or saturates) Alkanes are degraded rapidly in the presence of oxygen by a wide range of micro-organisms.,. They can be subdivided into normal paraffins (straight-chain compounds, n-alkanes), branched-chain saturates and cyclic saturates (or napthenes or alicyclics). Whereas straight- or branched-chain saturates can degraded quickly and completely (degradation begins with straight-chained compounds), cyclic compounds degrade to less extend and much slower.

Aromatics are compounds with one or more condensed aromatic rings -or benzene rings-; they can also be branched (these includes benzenes, substituted benzenes, two, three, four and even five ringed PAHs).. Whereas light compounds (1 or 2 cycles) degrade quite well (and quickly), heavy compounds (with 5 or 6 cycles) are highly resistant to degradation.

Degradation of aromatic hydrocarbons may be most rapid in aerobic conditions (although the rate of biodegradation is usually a little slower than alkanes degradation). These mechanisms tend to detoxify the aromatic components rather than degrading it.

For asphaltenes and resins which biodegradation has been shown to be slow (and always incomplete) in comparison to the other hydrocarbon components in crude oil. Moreover, both asphaltenes and resins may contain compounds that are the by-products of crude oil degradation, although these chemicals make up a small proportion of petroleum products, they are extremely recalcitrant.

In summary, as soon as oil enters the environment, biodegradation will occur along with other weathering processes. However, biodegradation is only one component of bioremediation which also includes other natural processes such as leaching, adhesion to mineral particles (oil fines interaction) which contribute to the final clean-up process.

1.3.2. Factors affecting bioremediation

Bioremediation is heavily influenced by the nature of the contamined environment and the interactions between microorganisms. As a biological process, factors that impact microorganism growth such as temperature, dissolved oxygen (DO), and nutrient concentrations can also limit bioremediation. Such factors should be taken into account in any decision making process regarding the use of bioremediation (See chapter 3). The potential impacts of some of these factors are as follows:

Temperature

Sea surface temperature range from about ≃ 2°C in the polar regions to ~35°C in tropical areas. Biodegradation rates are significantly lower at lower temperatures. Low temperature also increases oil viscosity thereby reducing bioavailability and volatilisation of the toxic short chain alkanes thus retarding the onset of biodegradation. Temperature has often been shown to be a limiting factor to bioremediation in colder climes.

***Insert picture 2** of cold environment*

Dissolved Oxygen (DO)

Appropriate DO concentrations are vitally important for bioremediation to occur. The surface layers in beach environments are generally sufficiently oxygenated, with DO concentrations usually higher in coastal areas where wave action enhances oxygen supply/transport However, reduced oxygen availability is of greater concern for beaches with fine-grained sediments, such as saltmarshes or mudflats. Here, mass transfer of oxygen may not be sufficient to replenish oxygen consumed by microbial metabolism.

***Insert picture 3, 4 & 5** of sandy beach/saltmarsh or mudflat*

Nutrient limitation

Bioremediation can only be sustained as long as there are sufficient concentrations of nutrients available. With the hydrocarbons supplying carbon, the remaining nutrients must come from the environment for successful degradation to occur. The typical concentration of nitrogen in seawater is 0.5-0.6 mg L^{-1}, which even allowing for efficient water exchange means that nitrogen may be a limiting factor (see section 3.3.8). Nutrient concentrations may also be limiting in pristine areas, in polluted or sediment rich inland waterways, estuaries and coastal waters nutrient concentrations may be sufficient.

***Insert picture 6 & 7** of high and low nutrient sites*

Pollutant accessibility and toxicity

The pollutant accessibility or bioavailability and its potential toxicity are crucial to the success of bioremediation. Bioavailability is influenced by the solubility of the contaminant and its sorption onto organic matter or sediment particles. Research has shown that the longer contamination remains in the sediment (or soil) the less bioavailable it tends to be. Thus, for "weathered" contamination an assessment of the bioavailability of the pollutants is advisable prior to treatment. Moreover, weathering of oil on shorelines will increase viscosity in the longer term rendering it less amenable to biodegradation.

In certain circumstances (if the hydrocarbons concentrations are very high), biodegradation may also be inhibited by the presence of toxic organic molecules such as low molecular weight alkanes (heptane, hexane and pentane, etc.), high levels of BTEX and substituted monoaromatics. This is rarely a problem after an oil spill at sea as these toxic components tend to evaporate rapidly.

***Insert picture 8** of fresh spill*

1.4. WHAT ARE THE MAIN BIOREMEDIATION STRATEGIES?

Choice of bioremediation strategy and its eventual success are dependent on the nature of the contaminated shore, and on whether or not there is a chance that contamination may migrate to an unoiled area and/or impact a sensitive resource. Presented below are those bioremediation strategies that can be used directly on the polluted site (the beach, the beachhead and the back of the beach). This includes *in-situ* and *ex-situ* techniques.

1.4.1. Biostimulation

Biostimulation consists in providing the microbiota sufficient amounts of elements needed to biodegrade the oil -oxygen or nutrients.

As previously stated (formula in section 1.3) oil biodegradation requires oxygen (2,6 times the amount of hydrocarbon to be actually degraded), nitrogen (nitrogen/hydrocarbon = 0.07)and phosphorus, (phosphorus/hydrocarbon= 0.007).

As most porous shorelines (sandy, gravel, pebble and cobble) are carbon limited, where the appropriate biodegrading microorganisms are present they will respond rapidly to the presence of hydrocarbon contamination by proliferation. At low oil concentrations (probably <1 g/kg), the hydrocarbon toxicity will be reduced and the oxygen availability as well as the nitrogen and phosphorus ambient concentrations should be sufficient for rapid oil degradation. In such circumstances, bioremediation should consist of monitoring the natural processes. However, at high oil concentration (probably >5 g/kg) the microbes will eventually become oxygen or nutrient limited, biostimulation may be an appropriate choice of clean-up strategy.

Generally speaking, bioremediation through biostimulation (addition of oxygen or nutrient) takes time, and is used as a polishing technique, (i.e. when the bulk of the oil is already cleaned).

Oxygen stimulation:

Oxygen limitation occurs when the sediment is not permeable enough to let the oxygen to diffuse to the micro-organisms themselves. This lack of permeability can be due to the presence of oil in the sediment which clogs the interstitial spaces. In this case, oxygen can be supplied by aerating the sediment, through periodic raking, tilling or harrowing, in order to restore the sediment permeability and to allow the oxygen in the air enter in it; (however, when moving the sediment, care should be taken not to bury the oil deeper in the sediment).

Insert picture 9 *tilling sand*

Nutrient stimulation :
Nutrient mainly nitrogen can be added in order to maintain enough nutrient concentration; ideally to ensure having no limitation, the ratio between hydrocarbon and nutrient should be up to C:N:P = 100:10:1.

A great variety of nutrient products are available for use as biostimulants. Appropriate inorganic and/or organic nutrient sources may be used as briquettes, granules or liquid fertilizers. Accumulation of nutrients (e.g.) ammonia) must be avoided as it could lead to eutrophication and toxic algal blooms.

Insert 10 *nutrient addition from Exxon Valdez*

Other clean-up procedures that increase the surface area of the oil (surf washing or the use of surfactants)and hence increase the rate of oil degradation could be classified as biostimulation strategy for bioremediation: when oil is spilled in the marine environment microbial attack occurs principally at the oil-water interface Thus, facilitating an increase in the oil-water interface through the addition of chemical dispersant, surface agents or biosurfactants may enhance the rate and extent of biodegradation.

However, precaution must be taken prior such an operation as the use of surfactants (e.g. dispersant) on the shoreline can lead to adverse effect such as ecological impact or driving the oil deeper in the sediment

Insert Picture 11 *surfwashing operation*

1.4.2. Bioaugmentation

If competent degraders are not indigenous to the contaminated site then their addition may be helpful provided they can survive in their new environment (a process termed bioaugmentation). However, a shoreline environment in which there are no recorded hydrocarbon degrading microorganisms has yet to be found. Bioaugmentation has not been used with success on a contaminated shorelines, except when added with nutrients. It should be noted that where indigenous degraders are present competition generally results in the failure of bioaugmentation.

1.4.3. Phytoremediation

Freshwater wetlands and saltmarshes are among the most sensitive of ecosystems and the most difficult to clean. Where traditional clean-up techniques may exacerbate the damage consideration is now being given to the inherent capacity of wetland plant species to stimulate aerobic biodegradation. This process of utilizing plant growth to accelerate the rate of oil biodegradation and/or habitat recovery is called phytoremediation. Furthermore, there is now evidence that some wetland plant species may effectively stimulate aerobic oil biodegradation by aeration of the rhizosphere and the release exudates and enzymes that stimulate microbial activity. Phytoremediation, contaminant degradation associated with plant growth shows promise as an oil spill countermeasure for coastal environment. The procedure is based on the growth stimulation of existing tolerant plants (e.g. fertilisation) or re-planting with plants from the impacted region (preferably those with phytoremediation attributes) when residual oil concentrations have diminished to levels to which the plants are tolerant

***Insert Picture 12** of phytoremediation operation in Ste Croix*

1.4.4. *Ex-situ* techniques

Ex-situ techniques can be conducted on site, close to the contamination. The main *ex-situ* techniques include landfarming, composting, and biopiling. These process are probably most appropriate for dealing with oily waste arising during oil spill treatment and this approach was used extensively after the *Sea Empress* incident in 1996.

Landfarming, is long standing and well understood. Composting involves the formation of large windrows of contaminated material and the addition of a biodegradable additives (*e.g.* nutrients). The windrow can be turned periodically for aeration and homogenization. In essence, this is a slightly more intensive version of landfarming capable of treating more material per unit area.

Engineered biopiling is a more intensive version of composting, where a greater effort is made to optimize the biodegradation processes. Air is either sucked or blown through the pile either continuously or periodically to ensure that the biopile is completely aerated. Suction of air through the biopiles has the advantage of concentrating any volatiles in a fixed volume of air such that they may be treated using other equipment, whereas blowing can lead to the dispersal of volatiles and odors in the atmosphere and cause a nuisance. The biopile may be covered and heated in periods of low temperature, therefore maintaining the optimal temperature range for biodegradation (20-30ºC). The leachate can be collected and sprayed back onto the pile to keep the soil moist.

These ex-situ processes will not be discussed further herein

1.5. SCOPE FOR APPLICATION OF BIOREMEDIATION

In summary, application of the appropriate approach for oil clean-up is dependent on a number of factors. The first question to ask is whether a clean -up strategy is required at all. Oil contaminated sites may recover most rapidly without intervention. A range of factors influence the choice of treatment strategy including: the likely environmental impact of leaving the contaminated site alone; the impact of environmental damage from movement and use of personnel, equipment and chemicals; accessibility of the shoreline, and cost/benefit analyses (Sections 2 and 3).

Where shoreline clean-up is required, a number of different physical and chemical as well as biological techniques have been developed. In heavily populated locations with easy public access to the coast, physical removal procedures may be appropriate. However, when access for personnel or heavy equipment is limited, or when natural clean-up via biodegradation is limited by low oxygen levels or low nutrient concentration, bioremediation may be an appropriate clean-up strategy (Section 4).

Chapter 2: CONTINGENCY PLANNING

2.1. INTRODUCTION

As for other response techniques, bioremediation requires careful planning in order to achieve desired results. When included as an oil spill clean-up measure, the Contingency Plan should contain clear policy statements regarding its use. This document should address the procedures applicable for the approval of bioremediation agents. At present, specific national regulations exist in only few countries (dominantly for the evaluation and approval of bioremedation agents). The implementation of this technique may fall under regulations dealing with waste disposal, agricultural practices or environmental pollution control, and these should be considered when undertaking strategic and local contingency planning. Details for implementation should be specified and dealt in the appropriate local plans (tier 1 and 2; refer to the IMO document (section II; Manual on oil pollution; “Contingency planning” 1995 edition)

As with other oil spill response methods, bioremediation is a complex process that is clearly not suitable for all scenarios and will be effectively applied only for sites satisfying specific criteria. Identification of such sites requires detailed analysis and consideration before inclusion in the Contingency Plan.

2.2. BIOREMEDIATION WITHIN THE OVERALL SHORELINE CLEAN-UP RESPONSE STRATEGY

Response to oil spills usually comprises response operations at sea (containment and recovery, or chemical dispersion), protection of threatened shorelines, and shoreline clean-up. Bioremediation can be effectively applied only on the shoreline and after all free (bulk) oil has been removed using one or more of the standard shoreline clean-up techniques (e.g. manual or mechanical removal, flushing).

Bioremediation should be considered as a technique for complementing such standard shoreline clean-up procedures rather than as a primary response method aimed at the removal of bulk oil.

It can be used to mitigate long term environmental impact of residual oil deposited on the shoreline after an oil spill, and to restore the affected shoreline ecosystems to their original state as far as is practicable. If used in the final stages of shoreline clean-up, bioremediation may significantly speed up the removal of residual oil and hence the time taken for restoration of the shoreline

2.3. SELECTION OF SITES FOR BIOREMEDIATION

Identification of candidate sites suitable for the adoption of bioremediation can be carried out at the planning stage. This level of detail is specific to the local plan. The process of identifying suitable sites can be approached in a stepwise method following the criteria outlined in section 3. The selection process can take time and may require specialist expertise. Section 3 provides a decision tree that should also assist in the identification of suitable sites.

Consideration should be given to how site selection is presented in the contingency plan in order to provide best guidance to the strategic response. It is equally important to identify all of those sections of shoreline where bioremediation would not be an appropriate strategy. Contingency Plan authors may find it useful to consult with bioremediation specialists to assist in the preparation of this part of the plan. Agreement should be sought with all parties who have an interest in the adoption of bioremediation as a response tool.

The most important criteria to examine when determining site suitability are:

- Geomorphological characteristics, i.e. the shoreline substrate.
- Oceanographic features, i;e. local energy regimes.
- Climate, i.e. typical ambient temperatures.
- Tidal range.
- Shoreline usage
- Sensitivities

2.4. MAPPING

Maps are the best way of presenting information in a Contingency Plan. Characterization with mapping of areas that are (or are not) suitable for bioremediation should therefore be carried out as part of the contingency planning process.

National Contingency Plans maps will only indicate the suitability for bioremediation of different coastal areas, while those attached to local plans will provide more detailed information required for its implementation. In addition to the information normally included in such maps (the type of coastal formations, tidal movements, access to the shoreline, shoreline habitats, sensitive areas……), additional more specific information relevant to the application of bioremediation techniques should be included on maps covering areas designated for bioremediation in case of contamination by oil:

- Sediment composition
- Exposure (high/low energy)
- Background nutrient concentrations (mg N / Litre)
- Oxygen availability (dissolved interstitial oxygen)
- Seasonal ambient temperatures
- Tidal range
- Shoreline gradient
- Vegetation
- Nearshore current regimes

It is helpful to supplement maps with photographs of the area concerned. These might be particularly useful for the evaluation of the degree of recovery once such an area was bioremediated after a spill. Photographs should also show seasonal variations in coastal vegetation if appropriate. The use of electronic maps facilitates the integration of images into maps.

The process of the preparation of maps can be long, however the time dedicated to it will be fully justified in the case of an emergency. Fundamental baseline data collected through the mapping process will not only facilitate the decision making process in case of pollution incidents, but also simplify post spill environmental and socio-economic evaluations.

Insert picture 13 *example of mapping*

2.5. LOGISTICS, IDENTIFICATION OF THE REQUIREMENTS FOR THE IMPLEMENTATION OF BIOREMEDIATION

Where the potential for bioremediation has been agreed [at a strategic and local level] the resource requirements should be identified. The contingency plan should include a description on the likely resource requirements and where those resources can be procured. The resources required for the implementation of a bioremediation programme will be largely dependent on the nature and extent of the incident. The plan should include guidance on the procurement of:

- Local [and national] expertise for overall supervision of operations.
- Manpower/operators
- Bioremediation agents (specifically developed products, agricultural fertilizers)
- Laboratory facilities
- Field equipment

2.6. FUNDING AND CLAIMS

It is likely that parts of the contingency plan dealing with funding of clean-up operations and the compensation of costs will address bioremediation as an element of overall spill response efforts and not separately.

However, bioremediation is closely related to the restoration of habitats and environmental recovery of pollution affected areas, potentially controversial issues in the context of compensation of costs. Bioremediation may need more considerations than traditional clean-up techniques.

The costs of bioremediation techniques will be considered for compensation under the present international regimes if the criteria of technical reasonableness have been applied and can be supported by relevant and scientific evidence. During the response to a specific incident, the insurers should be given the opportunity to become involved in the discussions on the use of bioremediation at an early stage if compensation is to be claimed.

As with other treatment methods, the costs of bioremediation techniques will be considered for compensation under the present international regimes only if the criteria of technical reasonableness have been applied and can be supported by relevant technical and scientific evidence.

As the application of bioremediation techniques should be closely supervised and the progress precisely recorded with a view to providing sufficient evidence for potential claims for compensation, the plan should includes information on the analytical resources and the expertise available.

2.7. TRAINING

Implementation of bioremediation techniques requires only basic skills similar to those regularly used in farming. Training of operators can therefore be included in routine oil spill responders training programmes, planned at national and local levels.

On the other hand, the level of training required for the proper supervision of the implementation of bioremediation techniques and controlling their progress is more specific and is considered to be too extensive to be provided through standard training courses for on-scene commanders and supervisors.

Supervisory staff need to have at least a basic scientific training and certain experience in chemistry and/or biology, and ideally should be recruited from institutions specializing in microbiology, environmental chemistry, or related disciplines.

If the required expertise is not available locally, it may be brought in from specialized national or external sources which may include industry. Such an approach may provide the opportunity for the training of local scientific and or technical staff without a background in bioremediation technology.

2.8. HEALTH AND SAFETY

Since bioremediation may involve the use of products that could be potentially impact human health the contingency plan should address the issue of health and safety. This should include the health and safety data sheets for substances likely to be used. Ideally generic activity risk assessments for the implementation and monitoring techniques should be carried out for inclusion in the contingency plan.

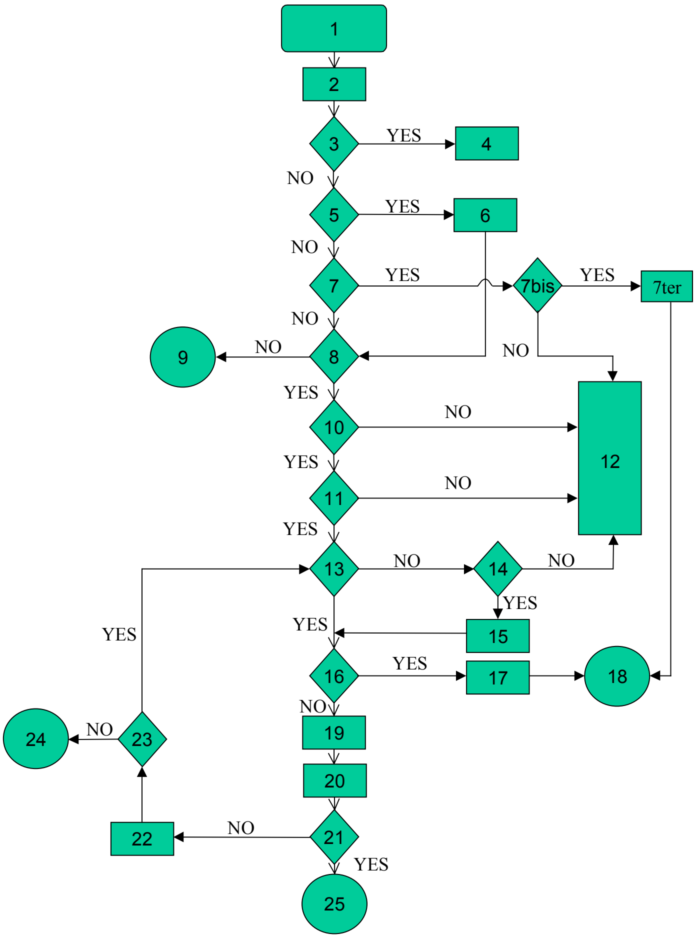
Chapter 3: DECISION MAKING

3.1. INTRODUCTION

In this Chapter the decision making process for determining whether to use bioremediation and selecting the most appropriate approach is explained. A flowchart outlining when, where and how to use bioremediation is given in Section 3.2. Detailed guidance on each decision point is given in Section 3.3.

3.2. WHEN, WHERE AND HOW TO USE BIOREMEDIATION

The decision making process begins at the moment oil impact the shoreline (bioremediation is a long term remedial technique it is only applicable for contaminated shorelines).



Key:

1. OIL ON SHORE
2. DETERMINE CRITERIA FOR SUCCESSFUL REMEDIATION
3. IS THE SITE EXPOSED?
4. LEAVE OIL TO BE REMOVED NATURALLY OR USE STANDARD REMOVAL METHOD?
5. IS FREE OIL PRESENT?
6. APPLY OTHER (REMOVAL) METHODS
7. IS IT A WETLAND? / 7 bis IS PHYTOREMEDIATION SUITABLE? / 7 ter APPLY PHYTOREMEDIATION

8. ADDITIONAL CLEAN UP REQUIRED?
9. TREATMENT COMPLETED
10. IS OIL SUFFICIENTLY BIODEGRADABLE (RATE AND EXTEND)?
11. IS TEMPERATURE HIGH ENOUGH?
12. CONSIDER ALTERNATIVE CLEAN UP OPTIONS
13. IS THE OXYGEN CONTENT SUFFICIENT?
14. IS AERATION FEASIBLE?
15. AERATE
16. IS THE NITROGEN CONTENT SUFFICIENT?
17. LEAVE TO BIODEGRADE NATURALLY
18. MONITOR UNTIL CLEAN UP TARGET IS MET
19. ADD SOURCE OF NITROGEN
20. MONITOR BIOREMEDIATION PERFORMANCE
21. ARE THE CLEAN-UP CRITERIA MET?
22. RECONSIDER CLEAN-UP CRITERIA
23. IS FURTHER BIOREMEDIATION APPROPRIATE?
24. STOP BIOREMEDIATION AND CONSIDER OTHER OPTIONS
25. TREATMENT COMPLETED

3.3. GUIDANCE ON THE DECISION MAKING PROCESS

3.3.1. Determine the objective of Remediation and Criteria for Success (Key 2)

The objective of the remediation is to minimize the damage resulting from the shoreline pollution. This can be achieved by:

- reducing the oiling and/or
- encouraging the habitat recovery.

Whatever the objective, clear criteria and target levels should be established to judge the cleanup success, for example:

- reduction of oil concentration to an agreed level or,
- re-establishing the main structure and functions of the habitat;

These criteria and target levels should be established in consultation with local stakeholders.

3.3.2. Is the Site Exposed? (Key 3)

In practice, bioremediation is considered suitable for sheltered shorelines where the polluted sediments are not likely to be removed by tidal action. These areas are also generally the most environmentally sensitive (Table 3.1).

In certain cases, bioremediation could be used on a partially exposed beach if the pollutant is buried deeply enough to become trapped.

Table 2 – Shorelines Types and Natural Cleaning Times
(Underlined: the most suitable for Bioremediation)

Exposure	Type of coast	Typical Time required for natural cleaning
HIGH ENERGY	Wave-cut cliffs, seawalls and piers	Weeks
	Wave-cut rock platforms	Weeks-Months
	Pebble beaches	Months-Years
	Mixed sand and gravel beaches	Months-Years
	Coarse grained sand beaches	1-5 years
	Fine grained sand beaches	1-10 years
	Coral reefs	> 10 years
LOW ENERGY	Cliffs, seawalls and piers	Months
	Rock platforms	Months
	Pebble beaches	Months-Years
	Mixed sand and gravel beaches	Months-Years
	Coarse grained sand beaches	Months-Years
	Fine grained sand beaches	1-5 years
	Tidal mudflats	1-10 years
	Salt Marsh	> 10 years
	Mangroves	> 10 years

3.3.3. Is Free Oil Present? (Key 5)

Bioremediation is not suitable for shoreline which are saturated with oil. Saturation is clearly indicated by:

- visible significant seepage of oil (not sheen) which can be observed spontaneously when the tide is coming up or at high tide or when mild pressure is exerted on the sediment when submerged (e.g. with a boot or a tool)
- formation of pools of oil along the shoreline.

Oil saturation is very dependant to the sediment type and oil properties but generally speaking occurs at concentrations higher than about 25,000 mg/Kg (or ppm).

In case of oil saturated sediment it is necessary to consider other standard appropriate cleanup methods to remove this free oil and possibly more. Bioremediation technique should be consider at the end of this first cleaning if further clean-up is required or if the impacted site must be restored.

Insert picture 14 & 15 *of sheltered and exposed site*

3.3.4. Is the Site a Wetland? (Key 7)

Wetlands, (salt and fresh water marshes), are particularly sensitive areas. When oiled, there are a limited number of response options:

- Leave undisturbed to recover naturally (often preferred),
- Gentle flooding (or flushing) to remove the free oil,

- Phytoremediation (enhancement of oil degradation and/or habitat recovery by plant growth).

Insert picture 16 *example of wetland*

3.3.5. Is Oil Biodegradable? (Key 10)

If oil reduction is the main objective, bioremediation can only be applied to oils whose biodegradability is significant (in rate and in extent).

Information of biodegradability can be obtained either from the technical literature or directly from chemical analysis. After spillage, oil is subjected to a number of weathering processes (e.g. evaporation, emulsification, photooxidation, dissolution). Thus the potential for bioremediation alters as the oil becomes more highly weathered. Therefore, it may be prudent to analyze chemically samples of oil taken from the shoreline, in order to accurately determine the actual potential for bioremediation, i.e. what can be degraded in a reasonable time. Methods for analysis of oil are given in Appendix 1

(GC methods should quoted), and examples of the extent of biodegradability of a range of crude oils are given in Appendix 2. Appendix 3 indicates the way to assess roughly the biodegradation potential of an oil according to its composition.

As a general rule, oil with a higher proportion of low molecular weight hydrocarbons, (diesel oil, light crudes) will be more easily biodegraded than oils containing a significant amount of high molecular weight compound such as heavy fuel oils and residual oils.

Based on the pollutant's overall biodegradability, a decision can be made about whether or not bioremediation for oil reduction is a worthwhile option. As a guide, if the weathered oil (or mixtures of oils) taken from the shorelines are in the order of <30% biodegradable, then bioremediation is unlikely to be beneficial. On shorelines oil is removed by both physical and biological processes. Thus on sheltered shorelines promoting the removal of >30% of the oil mass through bioremediation may be sufficient to reach the remediation objective more rapidly (see Section 3.2.1).

If the main objective is enhanced habitat recovery, phytoremediation can be advisable in case of vegetated areas (see section 3.3.4).

Oil type	Composition			Ù degraded

Table 3 Examples of potential of biodegradability for typical oil product

3.3.6. Is the Temperature High Enough (>+5°C)? (Key 11)

Bioremediation is sensitive to the ambient (sediment/water) temperature: when the temperature drops, the biodegradation activity slows down; as a rule, bioremediation is less likely to be beneficial if the sediment temperature is below 5°C; at lower temperature the degradation process will run slower but anyway faster than the natural attenuation. Bioremediation in cold conditions may be considered in certain circumstances (e.g. for extensive restoration in remote area with limited resources)

At last, but not the least seasonal temperature variation should be taken into consideration.

3.3.7. Is the Oxygen Concentration Sufficient? (Key 13)

Rapid biodegradation of hydrocarbons is an aerobic process and requires a great deal of oxygen. Therefore, the polluted sediment must be well-oxygenated to sustain optimal biodegradation rates.

Fine sediments (e.g. mud, muddy sand) have a low permeability and are often anoxic. The presence of petroleum in fine-grained sediment often reduces permeability by clogging spaces between sediment particles (polluted sand can thus become impermeable). An estimation method for sediment permeability is given in appendix 4) Organic matter, whether it is from a biogenic source (local plant or animal life) or a petroleum source, (petroleum itself) generally increases the oxygen deficit because the microflora consume oxygen in digesting it. Consequently, oxygen availability is often a limiting factor in biodegradation, and the primary goal of a bioremediation operation in such a case can be to rectify this situation if possible.

The oxygen content of the sediment can be assessed in several ways (methods for assessing these point are given in the appendix 5). In Table 4 some criteria for assessing oxygen limitation are given.

Table 4 Assessing Oxygen Sufficiency

Assessing Oxygen Content	Criteria for Estimating Oxygen Limitation	Decision
Visual and olfactory observation	Black sediment with putrid odour.	Consider the feasibility of overcoming oxygen limitation
Measurement of oxidation-reduction potential (redox)	An Eh of <-50 mV	
Measurement of dissolved oxygen in the interstitial water	DO < 1 mg/litre	

In terms of sediment permeability, a water flow rate of 0.2 ml/min/cm^2 and below beach sediment is insufficient to allow bioremediation without some form of pretreatment (i.e. physical mixing) of the sediment to improve permeability. More, biostimulation by fertilization (addition of nutrient) can enhance an oxygen limitation due to the fact it increases the microbiological activity.

***Insert picture 17** measuring the O_2 level in the interstitial water*

To overcome oxygen limitation the main techniques are mechanical. These methods improve sediment permeability by mixing or agitation e.g. raking, tilling/harrowing, using a rotovator. This must be done carefully to avoid burying the pollutant even deeper in the sediments. Such techniques may also release oil from the sediment (see Section 4).

The use of mechanical aeration in habitats with vegetation (e.g. salt marshes) cannot be considered due to the impact of such technique on the vegetation itself.

The feasibility of aeration techniques depends upon:

- logistical considerations (e.g. equipment availability and accessibility of the site)

- the sediment type (soft i.e. mudflat, sediments cannot be tilled).

If aeration is not feasible then other remedial strategies should be considered (See other IMO publications Volume 4 clean-up methods).

3.3.8. Is the Nitrogen Concentration Sufficient? (Key 16)

Hydrocarbon biodegradation requires significant concentrations of nitrogen to proceed at an optimal rate. Therefore it is important to quantify the amount of nitrogen available for biodegradation. This can be determined by measuring the nitrogen content of the sediment interstitial water. Methods for assessing nitrogen levels are given in Appendix 6.

At a level of 2 mg total N/litre or higher, nitrogen is not considered to be limiting biodegradation; hence, there is no need for fertilization with nitrogen products. Under these conditions, on aerobic shorelines, the oil may be left to remediate naturally. In order to estimate the completion of the bioremediation, monitoring the in situ nitrogen level is required (see Section 4).

If the nitrogen level is lower than 2 mg total N/litre, then fertilization should be considered.

When applying nutrient it is possible that phosphorus limitations may occur. Therefore typically a small amount phosphate is applied with nitrogen – 1:10 ratio – (most fertilizer mixtures however contain phosphorus).

3.4. CONCLUSION ON DECISION MAKING

If as the result of working through the structured decision process flowchart (described in section 3.2), limitations have been clearly identified, some of the following actions can be considered:

- Biostimulation by sediment aeration for lack of oxygen or sediment permeability,
- Biostimulation by fertilisation (Nitrogen addition) for lack of nutrient,
- Phytoremediation in marshes or wetlands.
- Other bioremediation option(s) such as bio-augmentation (section 1.4.2) and enhanced dispersion (section 4.1.5).

Whatever the treatment decision which has been taken, the situation will need to be periodically monitored to take into account changes in conditions resulting from natural processes or consequence of the treatment. The application of bioremediation approaches and monitoring are described in more detail in the next chapter.

Chapter 4: BIOREMEDIATION GUIDELINES IMPLEMENTATION

4.1. BIOREMEDIATION TREATMENT OPTIONS

There are two main approaches towards *in situ* oil spill bioremediation:

1) Biostimulation, the augmentation of oxygen or nutrients or growth-enhancing co-substrates concentrations, or other means of modifying habitat quality, that stimulates the growth of indigenous oil degraders,
2) Bioaugmentation, the addition of oil-degrading bacteria to supplement existing microbial populations, and

4.1.1. Bioaugmentation

There is a perception that marine oil spills may be effectively treated by the addition of oil degrading bacteria. In reality, there is little or no need to add microorganisms to oil contaminated ecosystems. Microbial ecologists have conclusively demonstrated that oil-degrading bacteria within the environment increase in numbers following exposure to oil. Furthermore, field trials have shown that the addition of commercial mixtures or enriched cultures of indigenous oil-degrading bacteria do not significantly enhance the rates of oil biodegradation over that achieved by nutrient enrichment alone.

4.1.2. Biostimulation by addition of nutrients

The potential capability of indigenous microflora to degrade oil is a function of the physical and chemical properties of the seawater and oil, the environmental conditions, and the biota themselves. It is generally accepted that nutrient availability is one of the limiting factors that it is possible to correct. Fertilising with nitrogen and phosphorus offers great promise as a countermeasure against marine spills. The ratios of carbon, nitrogen, and phosphorus to support optimal oil degradation rates have been defined (C:N:P = 100:10:1). Controlled studies suggest that optimal rates of degradation could be sustained by retaining high, non-toxic, renewable concentrations of nutrients within the interstitial pore water.

Field and laboratory beach microcosm studies point to interstitial concentrations of nitrogen of approximately 2 $mg.L^{-1}$ for optimal biostimulation. Liquid inorganic fertilisers had proven to be effective, but they require frequent applications and are comparatively labour intensive and expensive. Field trials have demonstrated the feasibility of applying commercial agricultural fertilisers on a periodic basis as a cost-effective bioremediation treatment. Other advantages of this protocol include product availability and ease of application.

Slow-release briquettes tend to decompose through hydrolysis and tidal action. Because briquettes are moved independent of the oil by tidal action and waves, it is important that the briquettes be of sufficient density and appropriately secured for maximum benefit. Slow-release granules are easily applied, releasing the nutrient when contacted by seawater or rain. However, in energetic tides, small granules may be washed away before dissolving, and so be ineffective. It may be prudent to secure the nutrients to the beach in mesh containers. Slow-release may decrease the cost of fertilisation. The use of agricultural slow release fertilisers decreases the cost of fertilisation procedures.

Proprietary oleophilic nutrient formulations including other organic products have also been developed. These partition preferentially with the oil to promote growth of local microbial hydrocarbons degraders at the oil-water interface

Product availability and environmental conditions must be considered in the selection and application of bioremediation agents. For example, low temperatures (<10°C) were shown to reduce the permeability of the coating of a slow-release fertiliser formulation, effectively suppressing nutrient release.

The table 5 summarises the successive actions to be undertaken to carry out an operation of biostimulation by nutrient addition.

	Table 5 Guidelines for the application of bioremediation products for nutrient enrichment
1	⇐ Is the product pre-approved for use? Check safety data sheet To ensure treatment success and to minimize environmental impacts, use of commercial bioremediation products should be limited to those that have passed regulatory screening procedures for performance and toxicity.
2	⇐ Product testing For products that have not been pre-approved, small-scale test should be conducted under the direction of the responder; check the application rate is below the no toxicity threshold for the chemical.
3	⇐ Determine the quantity to be applied Use manufacturer's recommended dosage. Otherwise, use of 10% amount of nitrogen relative to the mean oil content in the site for the initial application. Total nitrogen concentration in the interstitial water should be approximately to 2 $mg.L^{-1}$.
4	**⇐ Select application equipment** Fertilisers should be applied directly to the surface of the site using standard agricultural procedure. Care should be taken to insure that there is no run off due to over application of liquid fertilisers. Slow release fertilisers may be buried in fine mesh bags to facilitate retention on shoreline.
5	**⇐ Determine schedule and criteria for re-application** Nutrients should be re-applied when the concentration returns to background levels (approximately 2 weeks for water soluble fertilisers and 2 months for slow released fertilisation).
6	**⇐ Check tidal conditions** Tidal conditions should be considered to facilitate nutrient penetration in the sediment. This will most likely be at low and / or falling tide condition.

7	**⇐ Consider preparation of the sediment** The sediment surface may be treated to facilitate nutrient delivery or penetration (e.g. physical treatment, raking).
8	**⇐ Control the application procedure** Applied the product using control application procedures (e.g. constant and controlled application rate).
9	**⇐ Consider post application measures** Re-apply the product as necessary (according to the result of the analytical Nitrogen level monitoring).

4.1.3. Biostimulation by oxygen addition

As microbial oil degradation rates within sediments are very slow under oxygen limited conditions, increasing the concentration and depth of oxygen availability potential (e.g. penetration) by mechanical treatment has been shown to improve either the rate of the natural biodegradation either the efficacy of bioremediation treatments. If field surveys indicate oxygen limitation within the oiled sediment, agricultural procedures (e.g. raking, tilling and disking – rotavator-) can be used to increase the permeability of the sediment.

Precaution must be taken to contain the oil which might be released from the sediment by the mechanical treatment, (the use of floating booms, sorbent…) and/or to avoid transfer of oil to deeper layer of the sediment particularly when those remain anaerobic.

These mechanical treatments are unlikely to be suitable to sensitive habitat with vegetation such as marsh or wetland as their use would result in destroying the vegetation.

The use of chemical oxidant can be also considered for improving oxygen availability. However, care should be taken to use non toxic and/or pre-approved product.

When potential site for these treatment strategies include mudflats, wetland or saltmarshes, monitoring programs must be included to ensure minimal damage from physical disturbance and chemical toxicity.

***Insert picture 18** of beach aeration*

4.1.4. Phytoremediation

Freshwater wetlands and salt marshes are among the most sensitive of ecosystems and the most difficult to clean. Application of traditional oil spill cleanup techniques within this habitat may cause more damage than the oil itself. Consideration is now being given to the inherent capacity of wetland plant species to aerate the rhizosphere as a means of stimulating aerobic biodegradation. Plants also may release exudates and enzymes that stimulate microbial activity. Stimulation of existing tolerant plants or re-planting shows promise as a marine oil spill countermeasure

Freshwater wetlands and salt marshes are among the most sensitive of ecosystems and the most difficult to clean. Application of traditional oil spill cleanup techniques within this habitat may cause more damage than the oil itself. Development of on site remediation procedures for impacted wetland sites is under consideration to control coastal erosion and loss of habitat. Conduct of phytoremediation operations should include the advice of biologists with experience in wetland ecology.

Insert picture 19 *phytoremediation*

4.1.5. Enhanced dispersion

Microbial attack of oil spilled in the marine environment principally occurs at the oil-water interface. Thus, facilitating an increase in the oil-water interface may enhance the rate and extent of biodegradation, as the oil becomes more accessible to nutrients, oxygen and bacteria. Increases in microbial activity and oil biodegradation have been correlated with the addition of chemical dispersants, surface agents, biosurfactants, and the facilitation of oil mineral aggregate formation. Only pre-approved products should be used. Treatment should be made following the manufacturer's recommendations. Application of the product should disperse oil stranded within coastal sediments into the water column at concentrations below the threshold which will cause significant toxic effects. Controlled feasibility studies (i.e. plot studies) should be conducted prior to full response operations to ensure that the chosen procedure will not transport oil deeper into the sediment. For surf-washing operations, where oil dispersion is facilitated by mechanical procedures to accelerate the interaction between oil and mineral fines, consideration must be given to the ecological impacts associated with physical disturbance of the site to be cleaned. For protection of nearshore habitat, a biological monitoring program must be implemented with the use of remedial operations based on enhanced dispersion.

4.2 MONITORING

Monitoring programs are needed to verify ongoing treatment success without detrimental effects on the environment.

Treatment success can be assessed by chemical analysis to illustrate the reduction in residual oil concentrations or changes in composition. Biological studies can be used to show a reduction in oil induced effects. Detrimental effects include any changes to ecosystem structure and function as a result of bioremediation treatment.

Monitoring programs are also needed to identify operational endpoints for the remediation operation. Since some trace of hydrocarbons will be found at all spill impacted sites, regardless of the treatment process used, operational endpoints for bioremediation should be based on evidence of attaining an acceptable level of residual oil and/or habitat recovery. Monitoring programs should document the net benefit of bioremediation over natural attenuation (i.e. leaving the site alone to recover naturally).

Heterogeneity within the natural environment is the major obstacle to overcome in the design of programs to monitor bioremediation success. To ensure that the resultant data accurately reflect reality, it is paramount that all survey/sampling plans are based on standard statistical procedures. Efforts should be made to ensure that an adequate number of samples are taken to resolve significant differences, if any, to illustrate treatment success. Appendix 7 give basic recommendations for sampling plan.

A comprehensive monitoring program will cover changes in environmental factors that can influence bioremediation rates, the efficacy of treatments, evidence of oil biodegradation, toxicity reduction, and habitat recovery. For operational guidance, the monitoring program must be capable of identifying detrimental treatment effects (e.g. toxicity of the bioremediation agent or oil degradation by-products). Ecological significance of the biotests is improved by the use of a multi-trophic level test battery approach (e.g. integration of biotest results with bacteria, plants, invertebrates and vertebrates).

4.2.1. Monitoring of treatments

Analysis of nitrogen can be done either on or off site. For off site analysis, sediment samples should be kept frozen (-20°C) until analysis. In this case, total nitrogen will be measured e.g. using Kjeldahl – nitrogen. For analysis of organic nitrogen, samples of interstitial water should be taken using nutrient wells (see appendix 8). On site measurements can be done using colorimetric kits or electronic probes following manufacturer guidelines. Nutrients should be monitored weekly to determine the time required for nitrogen depletion. The sampling strategy can be modified dependent on results.

Oxygen is measured on-site following the same procedures as for nitrogen. The requirement for oxygen analysis will depend on the treatment. Where oxygen is the limiting factor and aeration is part of bioremediation strategy, levels of dissolved oxygen should be measured to determine effectiveness of aeration (oxygen may be used as also an indicator of microbial activity).

Total Petroleum Hydrocarbon (TPH) can be measured using gravimetry, spectrometry or chromatography methods to determine oil loss. The progress of degradation can be monitored by detailed chemical analysis (GC-MS) on selected sediment samples according to TPH levels. To measure the benefit of the treatments the results should be compared to similar untreated areas. Samples should be taken before treatment and than every 2 months. The samples should be stored frozen until analysis.

In addition to the demonstration of the reduction of contaminant concentrations, it is necessary to demonstrate that bioremediation does not induce significant biological effects that may suppress the rates of natural habitat recovery. Environmental assessments should be conducted to govern the application of the bioremediation strategy chosen. In such assessments ecosystem structure and function must be considered.

Two separate, yet complimentary, approaches have evolved for environmental assessment: bioassessment and bioassays. Consultation with the appropriate regulating bodies and experts is recommended.

For bioassessment, changes in benthic community structure can be used as a means of assessing ecosystem response to contaminated sediments in aquatic ecosystems. Of particular importance are the macrobenthic invertebrates because of their basic longevity, sedentary lifestyles, proximity to sediments, influence on sedimentary processes, and trophic importance. The bioassessment process can readily include potential impacts on vegetation.

If the aim of oil spill bioremediation is to return a site back to its pre-spill condition, recolonization of impacted areas should be a primary process to monitor in bioassessment.

Bioassays are toxicity tests that measure organisms response on exposure to a sample matrix. A single species biotest cannot represent the range of sensitivity of all biota within an ecosystem. To improve ecological relevance, a test battery approach with species from different trophic levels is required. While any living organisms can be used, toxicity tests with fish and macroinvertebrates have been standardised by environmental agencies to assess the hazards of industrial wastes to aquatic systems. Major criteria to consider in the selection of species for sediment toxicity testing include: behaviour in sediment, sensitivity to test material, ecological and/or economic relevance, availability and geographical distribution, taxonomic relation to indigenous animals, acceptability for use in toxicity measurement (e.g., standardised test method) and tolerance to natural sediment characteristics such as grain size. In general, assays using whole sediment samples and larval or juvenile life stages are the most sensitive and controlli recommended.

Insert picture 20 *of sediment sampling*

4.2.2. Operational endpoints for bioremediation

Bioremediation treatments should be terminated when it is deemed that the contaminant concentrations are reduced to acceptable levels (according to the usage and environmental specificity of the site) or if detrimental effects from the treatment strategy are identified. Cost-benefit analysis should be considered in the decision of the acceptable level. Like most oil spill counter measures, it is futile to expect bioremediation techniques to remove all traces of residual hydrocarbons. In terms of ecological relevance, clear evidence of habitat recovery such as toxicity limits within regulatory guidelines and return of original community structure should suffice. For methods see section above.

FURTHER READING

to be proposed by each participants

APPENDIX LIST

Appendix 1
Methods for analysis of oil (GC methods)

Appendix 2
Examples of the extent of biodegradability of a range of crude oils

Appendix 3
The way to assess roughly the biodegradation potential of an oil according to its composition

Appendix 4
Estimation method for sediment permeability

Appendix 5
Methods for assessing the oxygen content

Appendix 6
Methods for assessing nitrogen levels

Appendix 7
Basic recommendations for sampling plan

Appendix 8
Sampling method for sediment interstitial water

List of pictures, Table and Drawing missing

# picture	subject	provider	Comment
1	bacteria at work	AEAT R Swannel	
2	cold environment	Sintef (P Svein)	
3	sandy beach	Cedre (F Merlin)	Sheltered sandy beach
4	saltmarsh	Cedre (F Merlin)	Polluted salt marsh in the Arabian gulf
5	mudflat	AEAT?	
6	high nutrient site	AEAT	
7	Low nutrient site	AEAT	
8	Fresh spill	Cedre (F Merlin)	Fresh spill for which any bioremediation can be considered when the bulk of the standed oil has been removed by controlli means
9	traking sand	Cedre (F Merlin)	Raing the sad to improve its permeability and the depth of oxygen penetration
10	nutrient addition from E. Valdez	Total Elf Fina (A Basseres)	
11	surfwashing operation	DFO (K Lee)	
12	phytoremediation in Ste Croix	DFO (K Lee)	
13 (drawing)	example of mapping	??????????????	
14	sheltered site	AEAT?	
15	exposed site	AEAT?	
16	example of wetland	Cedre (F Merlin)	Polluted wetland during Amoco Cadix incident
XX (table)	Examples of potential of biodegradability for typical oil product	MNHN (J Oudot)	
17	Sampling interstitial water	Cedre (F Merlin)	Sampling intertitial water for controlling the oxygen and nutrient levels
18	Tilling operation on sandy beach	Cedre (F Merlin)	Tilling a sandy beach using agricultural means
19	phytoremediation	DFO (K Lee)	
20	sediment sampling	Cedre (F Merlin)	Sediment sampling to monitor the oil concentration

List of appendix

#	Title	Provider
1	Methods for measurements and analysis of hydrocarbons in marine sediments	see below proposed version
2	Examples of the extent of biodegradability of a range of crude oils	see below proposed version
3	The way to assess roughly the biodegradation potential of an oil according to its composition	see below proposed version
4	Estimation method for sediment permeability	see below proposed version
5	Methods for assessing the oxygen content	see below proposed version
6	Methods for assessing nitrogen levels	see below proposed version
7	Basic recommendations for sampling plan	see below proposed version
8	Sampling method for sediment interstitial water	?????? missing

Appendix 1

Methods for measurements and analysis of hydrocarbons in marine sediments

Total Hydrocarbons THC
After drying of the sample at <60°C *(optional)*, HC are extracted by sonication or in Soxhlet apparatus with dichloromethane DCM.

Following an optional purification on a florisil column that retains most biogenic lipids THC can be determined by :

-microgravimetry

-infrared spectrophotometry

-gas chromatography using FID detection *(total area of the chromatogram estimated relatively to a known amount of added internal standard)*

Biodegradation
Biodegradation extent and rate can be determined by GC-FID or GC-MS in reference with a biodegradation-resistant oil compound *(constitutive internal standard)* like nor-hopane or hopane (m/z 191).

GC analyses can be performed on total residual oil or after fractionation *(column chromatography on activated silica-gel 60-100 mesh, successive elution with hexane⇒ saturates, hexane – DCM 3/2 V/V ⇒ aromatics and methanol ⇒ polar fraction, i.e resins + asphaltenes + biogenic lipids)*. The saturated and aromatic fractions can be analysed by GC-FID and/or GC-MS.

Biodegradation of total HC, molecular classes or individual compounds are expressed in percent of the initial values in the original oil product.

Appendix 2

Examples of the extent of biodegradability of a range of crude oils

Table 1– Biodegradability of oil products

Gasoline	**100 %**
Jet fuel	**100 %**
Diesel oil	**85 %**
Crude oil	**30-70 %**
Heavy fuel	**10-20 %**
Asphalts	**<5 %**

Appendix 3

The way to assess roughly the biodegradation potential of an oil according to its composition

Biodegradation extent and rate can be determined by GC-FID or GC-MS in reference with a biodegradation-resistant oil compound *(constitutive internal standard)* like nor-hopane or hopane (m/z 191).

GC analyses can be performed on total residual oil or after fractionation *(column chromatography on activated silica-gel 60-100 mesh, successive elution with hexane⇒ saturates, hexane – DCM 3/2 V/V ⇒ aromatics and methanol ⇒ polar fraction, i.e resins + asphaltenes + biogenic lipids)*. The saturated and aromatic fractions can be analysed by GC-FID and/or GC-MS.

Biodegradation of total HC, molecular classes or individual compounds are expressed in percent of the initial values in the original oil product.

Table 1 - Theoretical biodegradation percentages for a crude oil (Arabian light type)

Composition / families		**Composition (%)**	**Biodegradation Rate**	**Biodegradability**
Saturates	Aliphatics (GC-resolved peaks)	17	100	17
	alicyclics (UCM)	20	50	10
Aromatics	GC-resolved peaks	8	100	8
	unresolved (UCM)	34	50	17
Resins-Asphaltenes		21	15	3
OVERALL		100	-	**55**

Appendix 3

The way to assess roughly the biodegradation potential of an oil according to its composition

The composition of a petroleum product gives an indication of the biodegradation rate that can realistically be obtained within a reasonable time frame (about one to two years).

For saturates and aromatics, identified compounds (peaks) can be completely degraded (100%), but only 50% degradation can be achieved for the unresolved complex mixture (UCM). For resins and asphaltenes, the maximum rate of biodegradation is about 15%.

Based on the pollutant's overall biodegradation rate, a decision can be made about whether or not bioremediation is a worthwhile option (Table 1: calculation of theoretical biodegradation percentages for a crude oil).

N.B. If the hydrocarbon concerned is already highly degraded, the prospects for biodegradation are of course very poor, and the evaluation method proposed above cannot be applied. If there are few or no tall peaks on the chromatogram of saturated fractions (tall peaks correspond to straight-chain ***linear*** saturated fractions which are preferentially biodegraded by micro-organisms), this generally indicates that the hydrocarbon is already quite degraded.

The illustration on the following page shows the change in the chromatograms of saturated and aromatic fractions of a petroleum product as a function of biodegradation status (Figure 1: chromatograms of saturated and aromatic fractions before and after biodegradation).

EXAMPLE

a) Result of partitioning of a petroleum product: saturates 37%, aromatics 42%, resins and asphaltenes 21%.
b) Chromatographic analysis of saturates: peaks 45%, UCM 55%.
c) Chromatographic analysis of aromatics: peaks 18%, UCM 82%.
d) Composition of the petroleum and estimate of whether it is biodegradable within a "reasonable" time frame.

Table 1 - Theoretical biodegradation percentages for a crude oil

Composition / families		**Composition (%)**	**Biodegradation Rate**	**Biodegradability**
Saturates	linear (peaks) cyclic (UCM)	17 20	100 50	17 10
Aromatics	resolved (peaks) Unresolved (UCM)	8 34	100 50	8 17
Resins-Asphaltenes		21	15	3
OVERALL		100	-	**55**

Appendix 4

Estimation method for sediment permeability

The permeability of sediment at and above the polluted layer can be estimated by digging small holes in the sediment and checking how much time it takes them to refill with interstitial water or how much time it takes the water to escape after the holes are filled with water.

A more detailed estimate can be obtained by using a small cylinder (about 10 cm in diameter and height) that is open at both ends. The cylinder must be pushed into the sediment up to its midpoint, while keeping the cylinder straight and disturbing the sediment as little as possible. A known volume (e.g., 250 cm^3) of water should be poured into the cylinder (taking care not to disturb the sediments), and the time it takes the water to flow through the sediment should be measured; water flow relative to the surface area unit gives an idea of the sediment's permeability.

These analyses should be carried out at several locations on the site, especially if there are variations in the sediment composition or grain-size or if runoff is thought to be occurring
When sediment permeability is lower than 0.20 $ml/mn/cm^2$, it can be considered insufficient.

Appendix 5

Methods for assessing the oxygen content

The oxygen content of the sediment can be assessed in several ways:

1) Observation: During digging in fine-grained sediment, if a black layer of sediment is clearly observed, along with a nauseating odour, this points to anaerobic conditions (sulphate-reducing activity). In a case like this where an oxygen shortage is noted, it is useful to determine the depth of the anoxic sediment layer. However, it must first be confirmed that the black colour is not due to the petroleum itself. The odour of the sediment is a good indicator in this regard.

Except in clear-cut situations (muddy and sand-mud environments), it is insufficient to observe that the oxygen supply is low, and measurements are required to assess the degree of oxygenation.
0
Sediment (generally fine-grained) that is black and gives off a putrid odour is a clear indication of anaerobic conditions

2) Measurement of oxidation-reduction potential: the oxidation-reduction potential (or redox potential) of sediment indicates its degree of oxygenation (aerobic - anaerobic conditions); it is based on the proportion of reduced or oxidized compounds present in the sediment. The redox potential is measured at various depths between an AgCl reference electrode placed directly in the water above the sediment and a platinum electrode pushed into the sediment to a depth encompassing the polluted layer.

By taking measurements at various locations, profiles of redox potential are obtained for the surface sediment (covering tens of centimetres, say 20 cm). A negative potential is considered to indicate a lack of oxygen.

3) Dissolved oxygen in the interstitial water: This parameter can be measured with an oxygen meter. The probe is placed in the interstitial water which has filled a small hole previously dug in the sediment. To properly measure the oxygen in the interstitial water, it is best to take the measurement after allowing the hole to fill up again with water (This can be done by removing the water with a syringe and allowing the hole to fill up again); it is also important to avoid mixing the water in the hole with the probe so that the measurement will not be skewed. Finally, the presence of petroleum in the water can pollute the membrane of the oxygen meter; it is recommended that, between each measurement, a reading be taken in the open water in front of the site to check whether instrument drift is occurring. In addition, the probe and its membrane should be rinsed periodically using the spray from a pipet of water. An oxygen concentration of 0.2 mg/l or less in the interstitial water indicates that oxygen is a limiting factor

These analyses should be carried out at several locations on the site, especially if there are variations in the sediment composition or grain-size or if runoff is thought to be occurring

Appendix 6

Methods for assessing nitrogen levels

In the coastal environment, nitrogen is available to microflora in mineral form, mostly nitrate (NO_3^-) and ammonia (NH_4^+), as well as in organic form (organic nitrogen) and possibly nitrite (NO_2^-).

Nitrate concentrations in sea water range from 0 to 50 µM, with the highest concentrations found in deep water layers. In the surface layer of the ocean, wide seasonal variations occur which are linked to phytoplankton growth: low concentration in summer (lower than the method detection limits), high concentration in winter (up to 40 µM).

Ammonia nitrogen (NH_4^+) comes from animal excretions and bacterial decomposition of organic nitrogen compounds; it serves as an effective tracer for urban and agricultural pollution.

Near the coast, nutrient concentrations can be appreciably higher because of inputs from zones with a high biological productivity (algal beds, marshes, etc.) or from human activities, predominantly agricultural and possibly urban. For example, high nitrogen and phosphorous concentrations (several dozen µM) are a sign of nutrient enrichment associated with household and agricultural effluents and can cause eutrophication. An assessment should be made of the nutrient levels at the site, in the interstitial water and possibly in the sediment, and especially in the open water near the site, to determine whether nitrogen (and phosphorus) availability is a limiting factor for biodegradation.

- The following analyses should be performed on the interstitial water and the open water surrounding the site: determination of nitrates, ammonia nitrogen and total nitrogen (Kjeldhal), that is, organic nitrogen and ammonia nitrogen).
- Phosphorous and nitrite levels may be determined as well.

At a level of 2 mg/l or higher (that is, 140 µM), nitrogen is not considered to be limiting for biodegradation; hence, there is no need for fertilization with nitrogen products.

Note: Owing to seasonal variations, nutrient concentrations may sometimes be limiting in summer and excessive in winter. Potential variations of this type should be taken into account in determining whether bioremediation through fertilization would be useful or not.

Note: Nitrates are transformed fairly slowly in the natural environment and are not particularly toxic. By contrast, ammonium ions and nitrites are toxic and are rapidly converted in the natural environment. As a result, substantial concentrations of nitrite and ammonium are rarely encountered in nature, in contrast with nitrate ions which may sometimes accumulate. However, if the nitrite and/or ammonium ion levels are high, their toxicity may have a limiting effect on the activity of microflora and this possibility should be considered; NH_3 is the most common form of ammonia nitrogen and also the most toxic form for aquatic life; however, ammonia nitrogen concentrations can be as high as several dozen micromoles per litre without reaching a toxicity threshold.

Phosphorus is rarely a limiting nutrient since demand for phosphorus is 10 times lower than that for nitrogen (minimum concentration in the order of 2 µM).

Appendix 7

Basic recommendations for sampling plan

Many of the elements of information required in order to decide whether to implement a bioremediation operation, and to plan the operation and subsequent monitoring, are derived from measurements and sampling done at the site.

To ensure that the resultant data accurately reflect reality, it is paramount that the measurements and sampling be conducted in an intelligent matter, in accordance with a pre-defined and well-designed survey or sampling plan.

In theory, the more extensive the sampling and analyses (measurements / sample collection using a grid encompassing the entire site), the better the information that is obtained. In fact, the greater the variability in the parameter being measured, the finer the grid needs to be to obtain an accurate picture of the site.

However, owing to constraints of cost and feasibility, the number of measurements and samples has to be limited.

The most objective method consists in <u>randomly</u> selecting within the grid a certain number of measurement and collection points (random survey plan). It is important that these points be objectively selected in a random manner (the co-ordinates should be randomly located within the grid) to avoid any operator bias. The measurements obtained in this way will be used to determine the mean value and the dispersion of observed values about the mean.

The optimum number of measurement points can be determined by tracking changes in the dispersion of measured values relative to the number of measurements made. The number is gradually increased by calculating the standard deviation of all the measurements on a regular basis. When the increase in the number of measurement points no longer produces an associated decrease in the standard deviation, it is not worthwhile continuing since additional measurements will not permit greater precision.

Often when the targeted parameters present considerable spatial variability, it may be worthwhile dividing the site into more homogeneous subunits, which will be measured or sampled the same way but independently (stratified random survey plan). For example, when the petroleum content in sediments needs to be determined, and a major part of the pollution is concentrated in a band situated near the high-water mark, the site should be divided into two parts for evaluation purposes: the upper beach and the rest of the site. For each of these zones, both the mean petroleum concentration and its dispersion can be determined.

In practice, it is convenient to do this work in two stages:

1) carry out an initial survey aimed solely at obtaining a rapid, general idea of how the parameter of interest is distributed, and divide the site into smaller subunits if necessary. This initial step can be carried out whenever possible, either through simple observation when the parameter being studied is visible (e.g., colour of petroleum-impregnated sediment), or by using rapid, inexpensive field measurement methods (e.g., an infrared spectrophotometer to measure sediments impregnated with a

colourless refined petroleum product). When observations cannot be made and no practical field measurement technique exists, the investigator can use his or her judgement and experience in gauging the potential range of variability and determine whether and how the site should be divided (For example, to study nutrient concentrations in interstitial water, factors related to potential variability, such as runoff from upstream, sediment type and permeability, are taken into account in dividing the site into more homogeneous subunits).

2) every subunit is then accurately measured and sampled (suitable measurement or analysis method) according to a random survey plan (random measurement / sample co-ordinates on a pre-defined grid). For every subunit, the mean value and dispersion are determined (standard deviation with 95% confidence limits) for the parameter under study.

Note: when sampling for further investigations, according to the type of analyses to be carried out, specific storage conditions for samples can be required to preserve their quality.