CRC for Contamination Assessment and Remediation of the Environment

National Remediation Framework

Technology guide: Bioremediation

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National Remediation Framework

The following guideline is one component of the National Remediation Framework (NRF). The NRF was developed by the Cooperative Research Centre for Contamination Assessment and Remediation of the Environment (CRC CARE) to enable a nationally consistent approach to the remediation and management of contaminated sites. The NRF is compatible with the *National Environment Protection (Assessment of Site Contamination) Measure* (ASC NEPM).

The NRF has been designed to assist the contaminated land practitioner undertaking a remediation project, and assumes the reader has a basic understanding of site contamination assessment and remediation principles. The NRF provides the underlying context, philosophy and principles for the remediation and management of contaminated sites in Australia. Importantly it provides general guidance based on best practice, as well as links to further information to assist with remediation planning, implementation, review, and long-term management.

This guidance is intended to be utilised by stakeholders within the contaminated sites industry, including site owners, proponents of works, contaminated land professionals, local councils, regulators, and the community.

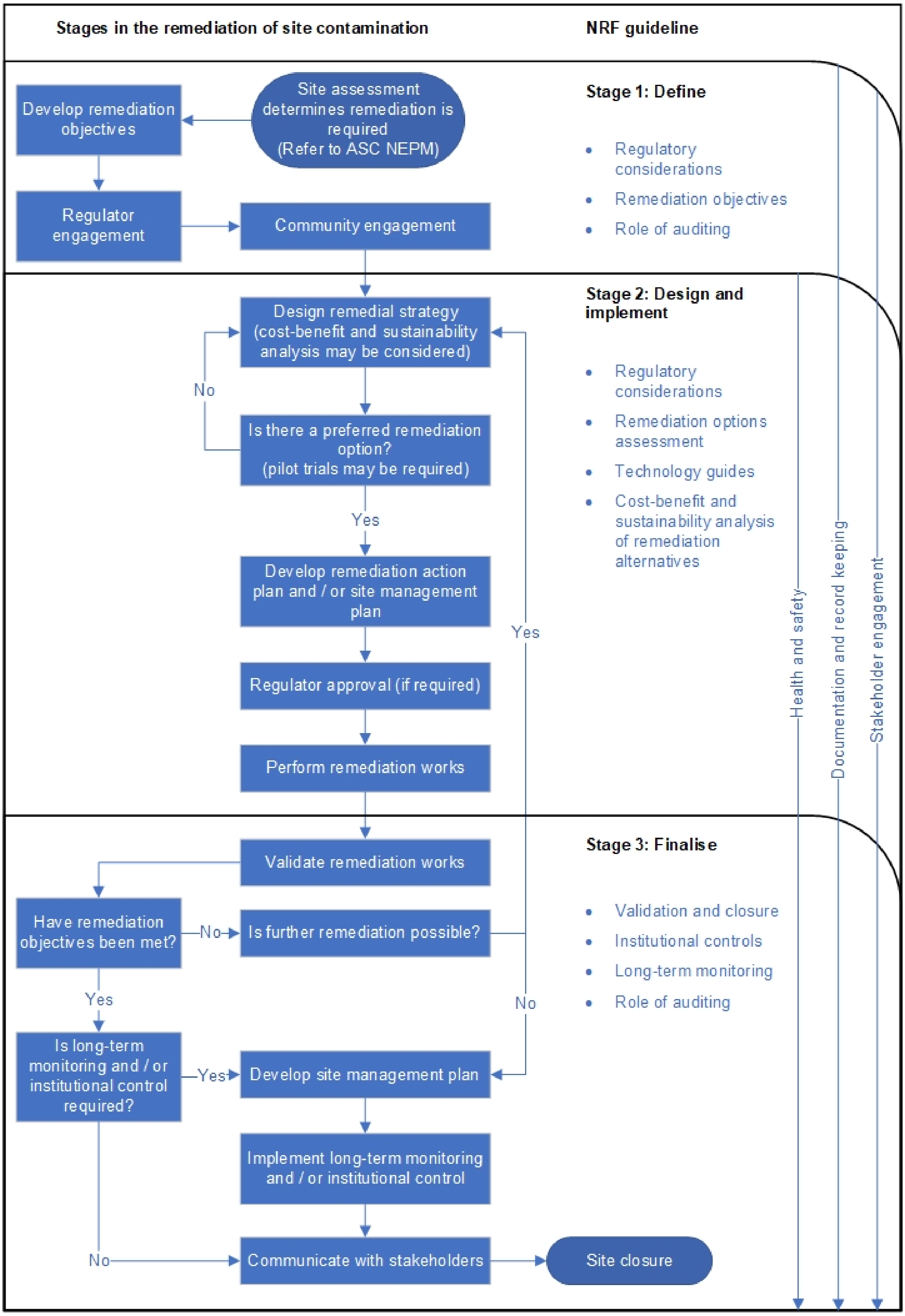
The NRF is intended to be consistent with local jurisdictional requirements, including State, Territory and Commonwealth legislation and existing guidance. To this end, the NRF is not prescriptive. It is important that practitioners are familiar with local legislation and regulations and note that **the NRF does not supersede regulatory requirements**.

The NRF has three main components that represent the general stages of a remediation project, noting that the remediation steps may often require an iterative approach. The stages are:

- Define;
- Design and implement; and
- Finalise.

The flowchart overleaf provides an indication of how the various NRF guidelines fit within the stages outlined above, and also indicates that some guidelines are relevant throughout the remediation and management process.

It is assumed that the reader is familiar with the ASC NEPM and will consult other CRC CARE guidelines included within the NRF. This guideline is not intended to provide the sole or primary source of information.


Stages in the remediation of site contamination
NRF guideline
Stage 1: Define
Develop remediation objectives
Site assessment determines remediation is required (Refer to ASC NEPM)
Regulator engagement
Community engagement
Regulatory considerations
Remediation objectives
Role of auditing
Stage 2: Design and implement
Design remedial strategy (cost-benefit and sustainability analysis may be considered)
No
Is there a preferred remediation option? (pilot trials may be required)
Yes
Develop remediation action plan and / or site management plan
Regulator approval (if required)
Perform remediation works
Yes
Regulatory considerations
Remediation options assessment
Technology guides
Cost-benefit and sustainability analysis of remediation alternatives
Health and safety
Documentation and record keeping
Stakeholder engagement
Stage 3: Finalise
Validate remediation works
Have remediation objectives been met?
No
Is further remediation possible?
Yes
Is long-term monitoring and / or institutional control required?
Yes
Develop site management plan
No
Implement long-term monitoring and / or institutional control
No
Communicate with stakeholders
Site closure
Validation and closure
Institutional controls
Long-term monitoring
Role of auditing

Executive summary

Bioremediation techniques are designed to break down contaminants via the stimulation of microorganisms which use the contamination as an energy source for development and growth. Various bioremediation techniques can be applied in situ or ex situ and under aerobic or anaerobic conditions. Soil, groundwater and vapour are all able to be bioremediated.

Successful design and implementation of a bioremediation program for soil, for example, is dependent on the following key technical considerations:

- Physical properties of the soil to be treated.
- Chemical composition of the soil to be treated.
- Chemistry and concentrations of contaminants.

Moisture content, available nutrients, contaminant mass and distribution and physiochemical parameters are also important factors to consider in assessing whether soil bioremediation will be effective. For groundwater-related bioremediation, hydrogeological factors such as aquifer permeability and water quality will play an important role.

If there is uncertainty as to whether bioremediation will achieve the required outcome, treatability studies may need to be conducted to resolve the issues. Treatability studies can be undertaken in three stages – the first stage is feasibility testing to assess the ability of bioremediation techniques to meet the remediation objectives. This is typically conducted as a series of bench tests. The second, more detailed stage is to evaluate the application of the method under the specific site conditions, usually conducted on site as a pilot trial. The information obtained in stages 1 and 2 is generally sufficient to enable formulation of a remediation action plan. However, where additional data are required to enable the remediation system to be designed a third stage of treatability testing should be undertaken to determine specific operating requirements and performance criteria to enable completion of a RAP.

References to case studies are provided in **Appendix A**.

A number of sources of information were reviewed during the formulation of this document to compile information on potential technologies. These are listed in references, and provide an important resource to readers.

Abbreviations

CRC CARE	Cooperative Research Centre for Contamination Assessment and Remediation of the Environment
NRF	National Remediation Framework
ORC	Oxygen Releasing Compounds
PAH	Polycyclic Aromatic Hydrocarbons
PCBs	Polychlorinated Biphenyl
PCDD/F (several)	Tetrachloroethylene
PCE	Tetrachloroethylene
PCP	Pentachlorophenol
PFOS	Perfluorooctanesulfonic Acid
pH	Power of Hydrogen
PPE	Personal Protective Equipment
RAP	Remediation Action Plan
Redox potential	Reduction/Oxidation Potential
SVOCs	Semi-Volatile Organic Compounds
TCE	Trichloroethylene
VOCs	Volatile Organic Compounds

Glossary

Aerobic	A process that occurs in the presence of free oxygen.
Amendment	A substance added to a soil to encourage microbiological activity and degradation
Anaerobic	A process that occurs in the absence of free oxygen. In this case it refers to degradation in the absence of oxygen
Bioaugmentation	The addition to the environment of microorganisms that can metabolise and grow on specific organic compounds
Biodegradation	The transformation of a substance or chemical by micro- organisms such as bacteria or fungi, resulting in a change in chemical structure mass within the environment.
Biopile	A bioremediation technology that utilises the action of soil microorganisms to remove contamination. Excavated soils are mixed with soil amendments, formed into compost piles and enclosed to allow the microorganisms to metabolise the contamination.
Bioremediation	Destruction of contaminants via the stimulation of microorganisms which use the contamination as an energy source for development and growth.
Biostimulation	A process that increases activity of microorganisms biodegrading contaminants. For example, addition of nutrients, oxygen, or other electron donors and acceptors
Bioventing	The process of supplying oxygen in-situ to oxygen deprived soil microbes by forcing air through unsaturated contaminated soil at low flow rates. This stimulates biodegradation and minimises volatilisation.
Concentration	The amount of material or agent dissolved or contained in unit quantity in a given medium or system.
Conceptual site model	A representation of site-related information including the environmental setting, geological, hydrogeological and soil characteristics together with the nature and distribution of contaminants. Contamination sources, exposure pathways and potentially affected receptors are identified. Presentation is usually graphical or tabular with accompanying explanatory text.
Contaminant	Any chemical existing in the environment above background levels and representing, or potentially representing, an adverse health or environment risk.

Contaminated site	A site that is affected by substances that occur at concentrations above background or local levels and which are likely to pose an immediate or long-term risk to human health and/or the environment. It is not necessary for the boundaries of the contaminated site to correspond to the legal ownership boundaries.
Contamination	The presence of a substance at a concentration above background or local levels that represents, or potentially represents, a risk to human health and/or the environment.
Electron acceptor / donor	A chemical capable of accepting / donating electrons during oxidation-reduction reactions.
Enhanced bioremediation	The addition of microorganisms or nutrients to the subsurface environment to accelerate the natural biodegradation of contaminants.
Environment(al) protection authority / agency	The government agency in each state or territory that has responsibility for the enforcement of various jurisdictional environmental legislation, including some regulation of contaminated land.
Ex-situ	A Latin phrase that translates literally to "off site" or "out of position". It refers to remediation that is performed on the contamination following removal, ususally the excavation of soil.
Fungi	A group of diverse and widespread unicellular and multicellular eukaryotic organisms. Some species are important in the decomposition of plant litter.
Groundwater	Water stored in the pores and crevices of the material below the land surface, including soil, rock and fill material.
Indigenous	Naturally occurring at that location
In-situ	A Latin phrase that translates literally to "on site" or "in position". It refers to remediation that is performed on the contamination while it is in place, without excavating soil.
Land farming	A remediation method that involves tilling the contaminated soil, with or without additives, to aerate the soil and encourage biological activity to remediate the contamination.
Mycoremediation	Destruction of contaminants via the stimulation of fungi which use the contamination as an energy source for development and growth.
Oxidant	A chemical that oxidises other substances, causing them to loose electrons.

Phyto-accumulation	The uptake of contaminants by vegetation roots and transfer of the contaminants to the vegetation shoots and leaves.
Phyto-degradation	The metabolism of contaminants in vegetation tissues
Phytoremediation	Remediation utilising the natural process of phytoaccumulation by encouraging growth of vegetation that will uptake the contaminant of concern. Particularly used for remediation of heavy metals.
Phyto-stabilisation	The production of chemicals by the vegetation that immobilises contaminants at the interface between the roots and the soil.
Practitioner	Those in the private sector professionally engaged in the assessment, remediation or management of site contamination.
Proponent	A person who is legally authorised to make decisions about a site. The proponent may be a site owner or occupier or their representative.
Redox	This is short for reduction / oxidation, and describes a chemical reaction in which electrons are transferred between chemicals.
Remediation	An action designed to deliberately break the source-pathway-receptor linkage in order to reduce the risk to human health and/or the environment to an acceptable level.
Rhizosphere	The zone of soil around a plant root where the biology and chemistry of the soil are influenced by the root.
Risk	The probability that in a certain timeframe an adverse outcome will occur in a person, a group of people, plants, animals and/or the ecology of a specified area that is exposed to a particular dose or concentration of a specified substance, i.e. it depends on both the level of toxicity of the substance and the level of exposure. 'Risk' differs from 'hazard' primarily because risk considers probability.
Site	A parcel of land (including ground and surface water) being assessed for contamination, as identified on a map by parameters including Lot and Plan number(s) and street address. It is not necessary for the site boundary to correspond to the Lot and Plan boundary, however it commonly does.
Solvent	A chemical that is able to dissolve other substances. Solvent contaminants include a range of hydrocarbons used in industrial processes due to their dissolving properties.

Surfactant	A substance that chemically bonds to both oils and water, and transfers oils into the aqueous phase where they are more readily remediated.
Treatability studies	A series of tests designed to ascertain the suitability of the treatment for the contaminants under the site conditions
Windrow	A long line of soil or similar material heaped up by a machine to aerate, dry out or encourage biological activity through composting.

Measurements

Unit or symbol	Expansion
^{o}C	Degrees Celsius
m bgl	Metre(s) below ground level
mg/kg	Milligram per kilogram
mV	Millivolts

Table of contents

1. Introduction

The purpose of this guideline is to provide information on bioremediation as a treatment technology for the remediation of contaminated sites to assist with selection of remediation options. The document contains information to inform remediation planning and aid compilation of a remediation action plan (RAP).

While soil, groundwater and vapour are all able to be bioremediated, this document predominantly provides guidance on the application of bioremediation as a remediation technology to treat contaminated soil. Readers are directed to the NRF *Technology guide: Monitored natural attenuation* for more information on the bioremediation of contaminated groundwater.

This guidance is primarily intended to be utilised by remediation practitioners and those reviewing practitioner's work, however it can be utilised by other stakeholders within the contaminated sites industry, including site owners, proponents of works, and the community.

Bioremediation is one of many technologies available for contamination remediation, and other technologies may be more appropriate. It is assumed that the information presented within will be used in a remediation options assessment to identify and select the preferred technologies for more detailed evaluation. This guideline provides information for both initial options screening and more detailed technology evaluation. This guideline does not provide detailed information on the design of bioremediation systems as this is a complex undertaking and should be carried out by appropriately qualified and experienced practitioners. Readers are directed to the NRF *Guideline on performing remediation options assessment* for detailed advice on assessing remediation options. In addition, the remediation objectives, particularly the required quality of the soil after treatment, are a critical matter and it is assumed that these have been determined and considered in the remediation options assessment and selection process. Readers are directed to the NRF *Guideline on establishing remediation objectives* for more detailed advice.

References to case studies are provided in **Appendix A.**

2. Technology description and application

Bioremediation techniques aim to break down contaminants via bioaugmentation and/or biostimulation of microorganisms that use the contamination as a food and energy source for development and growth. This is done via the introduction of oxidising or reducing agents, and moisture and/or nutrients to increase the favourability of the environment for microorganisms. Bioremediation technologies can be conducted ex-situ or in-situ.

2.1 Types of bioremediation

There are several techniques that fall under the broad heading of bioremediation, including:

- Bioventing;
- Enhanced bioremediation;
- Phytoremediation;
- Mycoremediation;
- Biopiles or windrows;
- Composting;
- Land farming; and
- Slurry phase biological treatment.

Each of these techniques is described in the section below.

2.1.1 *Bioventing*

Bioventing is an in-situ remediation technology involving the injection (and sometimes extraction) of air into the subsurface to enhance microbial activity and facilitate biodegradation of organic contaminants adsorbed to soils in the unsaturated zone. Bioventing is different from air sparging, which is typically conducted at higher flow rates to promote *volatilisation*, rather than *biodegradation* of volatile organic compounds.

During bioventing, air is injected at low rates to increase the oxygen content in the sub-surface and promote oxidation reactions. Other gases, such as methane or propane, can also be injected (at concentrations below the lower explosive limit) to promote the degradation of organic contamination under reducing conditions.

Where high concentrations of contaminants are present, it is possible that the soil pores can become clogged with additional biomass generated during bioventing, reducing the oxygen levels. Pulsed air injection can be useful to increase the oxygen levels under these conditions.

2.1.2 *Enhanced bioremediation*

Enhanced bioremediation is an in-situ remediation technology involving the addition of a chemical to the subsurface to enhance microbial activity and facilitate biodegradation of organic contaminants adsorbed to soils in the unsaturated zone.

Aerobic enhancement comprises the addition of oxygen (an electron acceptor) to the subsurface to increase the population of microbial organisms to assist with the biodegradation of contaminants in the soil or groundwater.

Although the introduction of oxygen releasing compounds (ORC) is more commonly used to enhance aerobic bioremediation of groundwater, ORC can also be applied to the unsaturated zone. The ORC can be a proprietary oxidant, or substances such as hydrogen peroxide or ozone.

Anaerobic enhancement comprises the addition of an electron donor (such as hydrogen or hydrocarbons) to the subsurface to increase the population of microbial organisms to assist with reductive dechlorination processes (anaerobic degradation) in groundwater. The direct addition of hydrogen is rare, as during anaerobic biodegradation hydrogen is normally indirectly generated via fermenting organic matter.

Other nutrients such as nitrate and sulphate can be added to groundwater to enhance anaerobic biodegradation of petroleum hydrocarbons.

2.1.3 *Phytoremediation*

Phytoremediation is an in-situ remediation technology that involves the use of plants to remove or stabilise contaminants in soil and, to a lesser extent, groundwater. An example of phytoremediation in the wastewater industry is the use of reed beds for on-site biological treatment of sewage effluent.

The following mechanisms are used in the process of phytoremediation:

- Enhanced rhizosphere biodegradation: the release of natural substances from plant roots to supply nutrients to microorganisms which increases biological activity;
- Phyto-accumulation: the uptake of contaminants by plant roots and transfer of the contaminants to the plants shoots and leaves;
- Phyto-degradation: the metabolism of contaminants in plant tissues; and
- Phyto-stabilisation: the production of chemical by the plant that immobilises contaminants at the interface between the roots and soil.

Hardy species, such as eucalyptus, fern, rye and fescue grasses, are often selected for phytoremediation in Australia due to their fast growing and robust nature and ability to survive in saline and water-logged soils.

2.1.4 *Mycoremediation*

Mycoremediation is a form of in situ bioremediation that uses fungal material (mycelium) to accumulate and degrade contaminants to remediate contaminated soils and groundwater. Mycelium is the dense network of branching white hyphae that make up the fungi. The mycelia deliver the enzymes required to break down the contamination; as such, the reaction is extra-cellular (outside rather than within the fungi). Fungi can be effective in breaking down petroleum hydrocarbons and some chlorinated compounds and are able to stimulate native microbes and enzymes in-situ. Heavy metals can also bioaccumulate in fungi and the contamination can be removed during harvesting.

The type of fungi used in mycoremediation is affected by the temperature, soil pH and the availability (or lack) of oxygen. Typically, a mycelium-treated substrate such as wood chips and straw is spread over contaminated soils which produce enzymes capable of decomposing contaminants over time.

Some of the common fungi used in mycoremediation and the contaminants they can treat are presented in Table 1 below.

Table 1: Common fungi used in mycoremediation

Type of fungi	Target contaminants
Shaggy Mane	• Arsenic, • Cadmium, and • Mercury
Elm Oyster	• Dioxins, • Wood preservatives
Phoenix Oyster	• Cadmium, • Mercury, • Copper
Pearl Oyster	• Polychlorinated biphenyls (PCBs) • Polycyclic aromatic hydrocarbons (PAHs) • Cadmium, • Mercury, • Dioxins
Shitake	• PAHs, • PCBs, • Pentachlorophenol
Turkey Tail	• PAHs, • Organophosphates, • Mercury
Button Mushrooms	• Cadmium
King Stropharia	• E-coli and other biological contaminants

2.1.5 *Biopiles or windrows*

Biopiles or windrows are an ex situ application of bioremediation where petroleum hydrocarbon impacted soils are excavated and placed in a treatment area where agents are usually mixed into the contaminated soils to enhance the degradation process. The soil can be placed in stockpiles (biopiles) or rows (windrows). The excavated soil needs to be aerated and moisture, temperature, oxygen and pH can be adjusted to make the process more effective. A leachate barrier and collection system is required to avoid contamination leaching into the soil and groundwater below the treatment area.

Biopiles can also be engineered and contain ventilation piping and blower, irrigation piping and/or sump and pump systems to facilitate aeration and drainage to maximise degradation rates.

2.1.6 *Composting*

Composting is an ex-situ remediation technology that involves the biological decomposition of wastes under controlled conditions to a state in which it can be handled, stored and / or applied to land without adversely affecting the environment. Contaminated soils are added to the compost process, and the contaminants are degraded together with the degradable waste material into humus and inert by-products (such as carbon dioxide, water and salts).

Composting is a special type of decomposition for which the conditions are established to allow for optimal microbial activity. Conditions that are important include the correct proportions of carbon and minerals in the compost mix (e.g. carbon to nitrogen ratio), good aeration and adequate moisture content. When the conditions are right, microbial (bacteria, including actinomycetes, and fungi) activity is very rapid and a large amount of heat is produced and the temperature rises.

It is generally accepted that if the whole of the composting mass has been held at 55^{o}C or more for three consecutive days, the compost can be termed a pasteurised product with significantly reduced numbers of plant and animal pathogens and plant propagules. The composting process can be extended to produce a mature product with a lower level of phytotoxicity and a higher degree of biological stability than pasteurised compost.

When the material is heavily contaminated or odorous, different systems will be required, such as enclosed trenches or rotating drums where odours can be captured during the composting process and treated. All systems require air to be drawn through the contaminated medium to provide suitable conditions for the microorganisms to survive.

In the initial stage, microbial activity will be at its peak and the temperatures will be highest, and the most degradable contaminants will be consumed. Following this initial stage, the temperatures will drop until heat is no longer generated and the material is now a compost product. The material has a high microbial diversity (higher than healthy fertile soils), and this expedites degradation of the contaminants.

2.1.7 *Land farming*

Land farming is an ex-situ remediation technology that involves spreading impacted soils in thin layers across a prepared surface and regularly turning the material to enable air flow through the soil matrix (introducing oxygen to facilitate degradation). The soil material is placed on a lined surface, with drainage control and bunding, to minimise the potential for leaching and run-off of contaminants. The soil conditions are controlled to maximise the degradation rate, including moisture content (via irrigation/spraying), aeration (by tilling) and pH (buffered to neutral by adding acid or alkali).

Where land farming is carried out in the open and volatile contaminants (such as petrol) are involved, volatilisation can be a significant contributor to loss of contaminants. Where volatile emissions and odours are possible, the requirements for emission management must be addressed as part of such remediation works. If the process involves only volatilisation without degradation, some regulatory agencies will not accept land farming as an acceptable treatment option.

Land farming can also be conducted in-situ to treat soils up to approximately 1 m bgl. Soils are mechanically agitated to introduce oxygen to the subsurface and facilitate the addition of nutrients and lime to reduce the soil acidity.

2.1.8 *Slurry phase biological treatment*

Slurry phase biological treatment is an ex-situ remediation technology that is performed in a reactor to remediate a mixture of water and excavated soil. The soil is mixed with water to a slurry that is determined by the proportions of the contaminants in soils, the rate of biodegradation, and the physical nature of the soils. If the soil is prewashed, the contaminated fines and wash water are treated in the reactor. Readers are directed to the NRF *Technical guide: Soil washing* for more information on soil washing.

The slurry contains between 5% and 40% solids by weight depending on the nature of the biological reactor. The soil is suspended in a reactor vessel and mixed with nutrients and oxygen. Microorganisms, acid or alkali may be added depending on treatment requirements. When biodegradation is complete, the soil slurry is dewatered and the liquids filtered and clarified.

2.2 Comparison of technologies

The advantages and disadvantages of various bioremediation technologies, along with the soil types and conditions for which each technology may be suitable, are listed in Table 2.

Table 2: Comparison of bioremediation technologies

Bioremediation technology	Advantages	Disadvantages	Treatable medium/applicable conditions
Bioventing	• Fast degradation rates (in comparison to other bioremediation methods) • No excavation required	• Contaminants may volatilise during treatment posing a potential vapour exposure risk and increased greenhouse gas emissions	• Permeable soils • Unsaturated soils • Can be applied under aerobic and anaerobic conditions
Enhanced bio-remediation	• Can support rapid degradation rates • May be used concurrently to address groundwater contamination • Low cost • Minimal exposure • No excavation required	• Requires correct oxygen/hydrogen and nutrient dosing and may need several trial stages	• Can be applied to soils with high and low permeability • Can be applied under aerobic or anaerobic conditions (groundwater) • May require addition of dehalococcoides bacteria for reductive dechlorination • Can be applied in situ or ex situ

Bioremediation technology	**Advantages**	**Disadvantages**	**Treatable medium/applicable conditions**
Phytoremediation	• Generally low operation & maintenance cost • Capable of treating large soil and wastewater volumes • Environmentally sustainable – can improve air quality • Potentially more agreeable to stakeholders or adjacent land users (no equipment, low maintenance and high aesthetic outcome)	• Where the process involves uptake and concentration of contaminants in the plant, the resulting plant matter (following remediation) is another waste stream that requires treatment • May not be suitable for high contaminant concentrations, or if contaminants toxic to plants are present • Slow process • Weather/season dependent (less effective during winter months) • Will not be able to treat contamination at significant depth as plant roots are generally contained to shallower soils	• Low permeability soils • Saturated/high moisture content and water retaining soils
Mycoremediation	• Low cost and minimal maintenance required • Environmentally sustainable • Minimal disturbance (agreeable to stakeholders) • Delivers fast results (odours often immediately mitigated) and is generally completed in weeks/months	Technology still in development – comparatively small number of successful case studies available	• Can be applied to near surface soils with high and low permeability • Aerobic application

Bioremediation technology	**Advantages**	**Disadvantages**	**Treatable medium/applicable conditions**
Biopiles/windrows	• Generally low operation & maintenance cost	• Potential exposure risks during excavation. Potential odour and air emissions may require management • Leachate may be an issue and base liner and/or bunding may be required to prevent migration of contaminants to the water table	• Permeable soils • Aerobic application
Composting	• Low cost • Generates heat (naturally)	• Bulking agents necessary • Potential exposure risks during excavation • Residual contamination will require treatment • Leachate may be an issue and base liner and/or bunding may be required to prevent contamination migration to the water table • Treated material may not be suitable for reuse or building over if retained on-site (dependent on physical properties at completion)	• Permeable soils • Aerobic application

Bioremediation technology	Advantages	Disadvantages	Treatable medium/applicable conditions
Land farming	• Low cost. • Simple design and set up.	• May not be suitable for high contaminant concentrations. • Potential dust, odour and vapour exposure during spreading of the soil and aeration. • Needs a large treatment area (reducing treatable volume). • Runoff collection facilities must be constructed and monitored. • Leachate may be an issue and base liner may be required to prevent contamination migration to the water table.	• Permeable soils. • Aerobic application.
Slurry phase biological treatment	• Operational parameters can be adjusted easily. • Fast degradation rates.	• High cost • Treatable volume (and rate) limited by size of equipment used. • Potential exposure risks during excavation.	• Can be applied to soils with high and low permeability. • Can be applied under aerobic or anaerobic conditions. • Surface contamination.

3. Feasibility assessment

Key site-specific considerations that will often determine the feasibility of bioremediation include:

- Whether the contaminants are sufficiently biodegradable and there is confidence that the remediation targets will be met within an acceptable timeframe.
- Whether the bioremediated material will be suitable for future use or disposal, taking into account the amendment material added, other contaminants present, and the byproducts and residuals of the treatment.
- Whether the extent and distribution of contamination is sufficiently well known.
- Whether the physiochemical composition and heterogeneity of the soil will allow sufficient uniformity of treatment to meet the remediation targets.
- Whether biodegrading organisms are naturally present or need to be added.

Appropriate remediation data must be collected to evaluate the applicability of a bioremediation technology. If there is reasonable confidence that the selected bioremediation method will achieve the required treatment outcome, then other issues will need to be considered to determine if it is likely to be an appropriate technology for the site. These include:

- Are there sufficient microorganisms present and is the contaminant bioavailability sufficient to enable degradation?
- Will the relevant regulatory agencies accept the bioremediation technology as a viable means of remediation?
- Will it be able to be confirmed that the contaminants have degraded, and have not been simply diluted by the material added or mixing operations, or volatilised and lost in ambient air, if these loss mechanisms are not acceptable to the regulatory agency?
- Are there planning or regulatory approvals required to use these technologies?
- Will the treated material be of a form and with contaminant concentrations that will allow the material to be reused as backfill on the site or as clean fill elsewhere, or will subsequent treatment (e.g. stabilisation) or landfill disposal be required? Could there be remnant biodegradable material present that would give rise to methane or carbon dioxide concerns, or a geotechnical concern (physical stability).
- What are the break-down products of the parent compound/s? Are they more toxic than the parent compound/s and does this risk require additional assessment? Does the breakdown product require a different treatment method (such as the production of vinyl chloride during reductive dechlorination of PCE)?
- Is there any risk of contamination migrating to other environmental segments through the use of this technology (e.g. incorrect controls during land farming resulting in transfer of contaminants from soil to the atmosphere)?

- Is it likely that other stakeholders (such as local government or the public) will accept the use of the technology, particularly those stakeholders that can have a significant bearing on whether the technology is applied at the site?
- Are there sensitive sites nearby that would not be compatible with the proposed operation?
- Is there a time constraint, and can the bioremediation application meet this constraint?
- Is the expected order of cost of treatment acceptable?

3.1 Data requirements

Successful implementation and design of a bioremediation system, whichever approach is used, is likely to be dependent on the following key technical considerations:

- The physical properties of the soil;
- The chemical composition of the soil; and
- The chemistry, concentrations and distribution of contaminants within the soil materials.

3.1.1 *Physical properties*

The physical composition of the material to be treated needs to be well characterised. Important factors include:

- Soil type and heterogeneity: differing grain sizes and the presence of coarse fragments of material (such as concrete or bricks) will impact upon air, water and contaminant migration pathways and can prevent the distribution of oxygen or nutrients through the contaminated soils;
- Organic matter: Where there is a significant quantity of organic matter present, this can result in depleted oxygen for microorganisms, slowing the biodegradation (in aerobic applications); and
- The permeability and plasticity of the material: low permeability soils will hinder the migration of oxygen and nutrients, both vertically and horizontally. In situ applications are unlikely to be appropriate for low permeability soils.

3.1.2 *Chemical composition*

The composition of the material to be treated needs to be well characterised. Important factors include:

- The distribution, concentrations and mass of contaminants in the soil at the site, as the requirement will be to locate and treat contamination that exceeds certain concentrations;
- Range of contaminants, their concentrations and physical form and their ability to degrade, volatilise or inhibit the rate of microbial degradation. Volatility is important for slurry phase biological treatment where the contaminants could volatilise in the reactors before degradation. Certain contaminants (such as heavy metals) can have a toxic effect on microorganisms, and inhibit degradation. The form of the contaminant can be important as to whether biodegradation will occur (e.g. non-aqueous phase liquid will not sustain

biodegradation, whereas free phase contamination that is distributed as an adsorbed phase may sustain biodegradation);

- Ion exchange and filtration mechanisms of the soil to be treated to assess whether it will be necessary to improve the transport of water, electron acceptors (such as oxygen), nutrients and microorganisms to assist the biodegradation process (via injection wells or pumping etc); and
- Physicochemical parameters – pH, electron acceptors, nutrients, temperature and toxicity to assess what effect these will have on microorganisms and which strain will be most effective at treating the contaminants present, such as:
  - pH can affect contaminant solubility and bioavailability of nutrients for microorganisms in the degradation process. Bioremediation processes generally perform optimally in the pH range 6 to 8
  - Redox potential and oxygen content indicates whether conditions are oxidising or reducing
  - Availability of nutrients that are required for microbial growth (and cell division) – these can be added where needed
  - Contaminant bioavailability depends on the contaminants ability to sorb to solids and be diffused in soil macropores (bioavailability for microbial reactions is lower for contaminants that are strongly sorbed to soils, or are within macropores and are less bioaccessible)
  - Temperature affects the rate of microbial metabolism and degradation. The biodegradation rate generally increases with temperature.

3.1.3 *Maximum allowable concentrations*

The maximum allowable concentration and variation in concentration of the contaminants and by-products of treatment in the treated soil needs to be determined. If very stringent clean up criteria are applicable, then bioremediation technologies may not be sufficient to meet the criteria and additional ‘polishing’ stages of treatment may be required. For example, criteria for substances such as chlorinated organics may be very stringent (e.g. < 1 mg/kg of total chlorinated organics) and may not be readily achievable by bioremediation. In such circumstances, additional cycles of treatment may be required resulting in prohibitive cost, or the requirement for subsequent treatment by another technique that, by itself, could achieve the criteria more cost effectively than biodegradation. Specific concentration considerations include the following:

- The maximum allowable concentrations of contaminants (such as heavy metals or asbestos) that will remain after treatment, and which could preclude the intended use or disposal of the treated soil or inhibit the biodegradation processes;
- The maximum allowable concentrations of volatile components, so that volatiles will not cause an environmental or human health risk during excavation or treatment;
- The maximum allowable quantities and concentrations of other material such as contaminated concrete or plastic clay that may restrict the distribution of microorganisms, or the movement of gases or nutrients in the subsurface

which are needed to facilitate biodegradation, and hence affect the uniformity (and completeness) of treatment;

- The maximum allowable concentrations of reagents and biodegradable material that are added to the soil to facilitate biodegradation, and which will remain after treatment (e.g. will the residual concentrations or gases that result (such as methane or carbon dioxide) affect the intended use or disposal of the treated material);
- Whether there is a concern regarding the microorganisms that will be present during or after treatment and could pose a risk to persons or the environment, and the maximum allowable concentrations that could apply; and
- If phytoremediation is under consideration, the contaminant concentrations and their depth will be a key factor – high concentrations of certain contaminants may inhibit plant growth, limiting the viability of phytoremediation to achieve the remediation objectives. If contamination is deep and extends beyond the root zone, then remediation of this material may not occur.

3.1.4 *Regulatory requirements*

The regulatory agencies (particularly the agencies responsible for protection of the environment, town planning, and licensing treatment facilities) should be consulted to determine the specific requirements that relate to obtaining the necessary approvals and licences, and controls that can be expected. This is particularly relevant where emissions to the environment are planned or possible.

3.2 Treatable contaminants

Bioremediation technologies are potentially able to treat a wide range of:

- Volatile organic compounds;
- Semi-volatile organic compounds; and
- Petroleum hydrocarbons.

Bioremediation of PAHs is possible in engineered biopiles or windrows with the addition of compost, nutrients, and surfactants to release contaminants to the aqueous phase. High molecular weight PAHs and aged petroleum products are of low bioavailability and are therefore not generally suitable for treatment by bioremediation, particularly phytoremediation, unless only minor reductions in concentration are required.

Some higher-boiling-point halogenated compounds such as PCBs, dioxins and furans, and fluorinated compounds such as PFOS can be very difficult to degrade and are unlikely to be suitable for treatment by bioremediation.

Information on the biodegradability of contaminant groups and the preferred conditions (anaerobic or anaerobic) is summarised in Table 3.

Table 3 Contaminant Biodegradability and the Preferred Conditions

	Contaminant	Microbial Degradability			Preferred conditions
		High	Low	No	
Mineral oil hydrocarbons	Short-chain mineral oil hydrocarbons	X			Aerobic
	Long-chain/branched mineral oil hydrocarbons		X		Aerobic
	Cycloalkanes		X		Aerobic
Monoaromatic hydrocarbons	(Mono)aromatic hydrocarbons	X			Aerobic
	Phenols	X			Aerobic
	Cresols		X		Aerobic
	Catechols	X			Aerobic
Polycyclic Aromatic Hydrocarbons	2 to 3 ring PAHs (e.g. naphthalene)	X			Aerobic
	4 to 6 membered ring PAHs (e.g. benzo(a)pyrene)		X		Aerobic or Anaerobic/Aerobic
Chlorinated Aliphatic Hydrocarbons	Tetrachloroethene, tricloroethane	X			Anaerobic
	Trichloroethene	X			Anaerobic
	Dichloroethane, dichloroethene, vinyl chloride	X			Anaerobic/Aerobic
Chlorinated Aromatic Hydrocarbons	Chlorophenols (superchlorinated)		X		Anaerobic
	Chlorophenols (low chlorinated)	X			Anaerobic/Aerobic
	Chlorobenzenes (super chlorinated)		X		Anaerobic
	Chlorobenzenes (low chlorinated)	X			Anaerobic/Aerobic
	Chloronaphthalene	X			Anaerobic/Aerobic
	Polychlorinated biphenyls (PCBs) (super chlorinated)*		X		Anaerobic
	Polychlorinated biphenyls (low chlorinated)	X			Anaerobic/Aerobic

	Contaminant	Microbial Degradability			Preferred conditions
		High	Low	No	
Nitroaromatic compounds	Mono- and dinitroaromatics	X			Anaerobic/Aerobic
	Trinitrotoluene (TNT)	X			Anaerobic/Aerobic
	Trinitrophenol (picric acid)		X		Anaerobic/Aerobic
Nitroaliphatic Compounds	Glycerol trinitrate	X			Aerobic
Pesticides	g-hexachlorocyclohexane (lindane)	X			Anaerobic/Aerobic
	b-hexachlorocyclohexane**		X		Anaerobic/Aerobic
	Triazines	X			Aerobic
Dioxins	PCDD/PCDF		X		Anaerobic/Aerobic
	2,3,7,8-PCDD/PCDF			X	Anaerobic/Aerobic
Inorganic compounds	Free cyanides		X		Aerobic
	Complex cyanides		X		Aerobic
	Ammonium	X			Aerobic/Anaerobic
	Nitrate	X			Anaerobic
	Sulphate***	X			Anaerobic

* The degradation process and susceptibility to degradation are different for planar and non-planar highly chlorinated PCB congeners
** Microbially transformable but not degradable
*** Activity of sulphate-reducing bacteria results in precipitation of metal sulphides or production of hydrogen sulphide gas

4. Treatability studies

If it is uncertain whether bioremediation will achieve the remediation objective or be applicable for the specific site conditions, it may be necessary to conduct a treatability study. Treatability studies also allow estimates of remedial costs and technology efficiency to be refined.

Designing the treatability study may require input from a number of technical specialists including environmental scientists/engineers, chemical engineers, mechanical engineers and air quality specialists to ensure that the study is designed to obtain the data required to enable the most appropriate implementation strategy to be developed.

The requirements for additional information should be determined through a review of the specific bioremediation technology being proposed. If the technology has been widely applied and the results are readily available, it may be possible to extrapolate information from previous case studies to avoid duplication and reduce project costs.

If it is determined that treatability testing is required to adequately assess the application of bioremediation or to assist in cost appraisal, consideration needs to be given to the staging and level of detail of the testing. Typical stages of testing can be directed to remediation screening, remediation selection and remediation design, irrespective of whether aerobic or anaerobic bioremediation is involved.

The process involved in each of these stages is outlined below, for the case of aerobic bioremediation, and this process is generally applicable to anaerobic degradation.

The objectives of the treatability testing should be determined at the outset and, although these will be site specific, treatability testing should determine:

- Will the proposed bioremediation technology meet the remediation objectives in a timely and cost effective manner?
- Are there sufficient contaminant degrading microorganisms present?
- What are the optimum moisture, nutrients and pH conditions to facilitate contaminant degradation?
- That any reported decreases in contaminant concentrations are the effect of the bioremediation treatment and not a result of volatilisation, leaching of contaminants from the soil or adsorption.

References to treatability case studies are presented in **Appendix B.**

4.1 Bench testing

Initial screening or bench scale treatability studies comprise the first stage of treatability testing, to assess whether biodegradation is a viable option to treat the specific contaminated material.

The screening tests are generally low cost and relatively fast to complete (approximately a few weeks or a few months to obtain the results). The initial screening tests are carried out using simple equipment in a laboratory, such as shake flasks, soil pans or slurry reactors, and are undertaken using saturated and unsaturated soils, slurries and various aqueous solutions. The microorganisms can be indigenous to the specific site, cultured, a commercially available mixture, or a combination of all of these.

Various parameters can be adjusted during the initial screening, including pH, contaminant loading rates, and oxygen and nutrient availability, to improve the potential success rate.

Setting the data quality objectives for the screening treatability testing at the outset is vital to obtain the desired results. Usually the main goal of this preliminary treatability testing is to establish whether biodegradation will occur in the specific contaminated material. The objectives of the initial screening tests do not normally include assessing whether the remediation clean up criteria can be met. The testing is normally concluded after a few weeks when it is evident that the contaminant concentrations have decreased by a significant percentage, though not necessarily to the level required to meet the specific clean up criteria.

4.2 Pilot trial

The next stage of treatability testing is to evaluate the application of biodegradation for the specific site conditions and to establish whether the remediation criteria and clean up goals are likely to be met. The information obtained in the second stage of testing is usually sufficient to enable development of the remediation action plan (RAP).

The key objectives for the second stage of treatability testing are:

- Assess contaminant concentrations achieved following treatment (to determine whether the nominated remediation criteria can be met);
- Determine microorganism populations, oxygen input method and nutrient load etc. required for efficient treatment; and
- Characterise the composition and physical nature of the final material to confirm that it can be expected to be suitable for the intended reuse or disposal.

This stage requires more effort (and cost) than the initial screening testing and generally takes several months to plan and implement. These tests have the objective of more closely replicating the physical and chemical parameters of the site under investigation and the specific bioremediation technology being considered. The tests should therefore be undertaken using the particular soils to be treated in the full scale remediation program. However, it should be noted that given the small amount of material used in the tests (in comparison to the actual volume to be treated), full scale treatment conditions are likely to differ, particularly where in situ application is being considered.

At complex sites where in-situ application is being considered, this stage of treatability testing may be undertaken on-site, ideally in a small section of the area to be remediated using methods and equipment similar to those proposed for the full scale application to enable an accurate estimate of whether biodegradation will work and can meet the remediation criteria.

4.3 Finalising design

If additional data is required to enable the remediation system to be designed, a third stage of testing may be necessary to establish specific requirements and performance criteria and provide sufficient information to enable completion of a RAP.

The key objectives of the third stage of treatability testing may be, for example:

- Obtain all data and information required to enable the remedial program to be designed;
- Refine the remediation cost estimate;
- Confirm the rate of biodegradation and the concentrations of the treated medium (to ensure they meet the remediation criteria); or
- Confirm that the composition and physical nature of the treated material will allow its reuse or disposal.

These studies are usually costly and may take many months to complete, so the benefit of obtaining more specific operating design parameters and cost estimates should be weighed against the cost of the overall remedial program.

The tests are usually conducted using a mobile treatment unit brought onto site. The equipment used should be designed to ensure that the data obtained can be extrapolated for the full scale unit.

4.4 Anaerobic bioremediation

The process of treatability testing for anaerobic bioremediation can involve a similar staged approach to that outlined above. In the case of anaerobic treatment, the available oxygen must be removed (or reduced). This can be achieved by supplying excess electron donors (reducing agents) to the microorganisms which will consume oxygen that may be present.

Different electron acceptors (nitrate, carbonate or sulphate) are required to enable bacteria to facilitate anaerobic, de-nitrifying, sulphate reducing, and methanogenic conditions, and different by-products and metabolic intermediates will be produced. Anaerobic tests depend largely on the type of microorganisms that will be used to achieve biodegradation.

The redox potential of the medium to be treated needs to be reduced to approximately -150 mV to -350 mV at pH 7 to facilitate the growth of most anaerobic organisms. The exact redox potential required and the concentration of dosing agent to achieve such a concentration is dependent upon the specific anaerobic organism.

The redox potential will determine which microorganisms are present and active in the medium and, as such, the specific anaerobic organisms to be stimulated in the detailed treatability testing should be determined in the initial screening tests.

5. Validation

The following information describes the specific validation appropriate for bioremediation, to assist validation planning within the RAP. Readers are directed to the NRF *Guideline on validation and closure*, which among other things, provides further information on each of the lines of evidence.

Recommended lines of evidence for the validation of bioremediation in soils or groundwater include:

- The measured decrease in contaminant concentrations;
- An assessment of the microbiota present to break down the contamination; and
- The geochemistry and biochemistry of the environment

The latter two lines of evidence help demonstrate whether contaminant mass loss is a result of biodegradation processes or whether it is attributable to non-destructive processes such as sorption, dilution, or dispersion.

In-situ and ex-situ bioremediation typically require different lines of evidence to validate:

5.1 In-situ bioremediation

In addition to the COC, amendments such as nutrients as well as carrier fluids injected into the subsurface should be monitored.

Depending on the nature of the facility, surface and groundwater monitoring may be required for the duration of the bioremediation operation and for a period post-completion to verify that contaminants have not migrated during the remediation process (e.g. displaced by the injection of carrier fluids).

The validation of *in-situ* groundwater remediation should comprise groundwater monitoring:

- In the plume,
- Up- and down-gradient of the plume;
- Sufficiently long term to account for seasonal variations.

Depending on the remedial method used, validation monitoring of bioremediation in groundwater may additionally comprise an assessment of the air distribution and pressure in an aquifer, oxygenation of the groundwater and vadose zone, changes in fluid pressures and changes in groundwater chemistry. It is recommended that cross-sectional contour plots oriented along the path of groundwater flow are prepared as part of the validation process to understand the distribution and reduction of contaminants.

It is essential to analyse not only concentrations of the COC, but also biodegradation products, to establish that the primary contaminants are degrading rather than simply moving into areas of lower hydraulic conductivity. Testing of biochemical parameters such as volatile fatty acids and dehydrogenase activity may be useful to determine whether biodegradation has occurred.

5.2 Ex-situ bioremediation

The number of samples collected and analysed for validation purposes should be adequate to provide statistically reliable results, taking into account the intended end use of the soils .

Where soil remediation involves a static or turning pile (e.g. biopile), sampling should avoid shallow locations as they may not be representative of the pile. Whilst heterogeneity is likely to be significant, this will be reduced if the pile is regularly turned.

Following the removal of the soils from the treatment location, the underlying area should be validated to confirm that contamination has not migrated vertically through the underlying liner. Where ex-situ treated soils are reinstated on the site, they must first be validated to ensure that they meet land use and/or validation criteria relevant to the site and its setting. Where treatment occurs off site, the material must be validated prior to import back onto the site.

It is noted that monitoring, appropriate cover and potential treatment of vented gases and leachate should be undertaken. Also excess nutrients where these have been added.

6. Health and safety

Bioremediation projects can expose site workers to safety and health hazards via exposure to the biological agents involved, exposure to vapours, noise, and slip/trip hazards. The specific remediation technology and design will determine the specific risks which should be assessed as part of the RAP.

Some of the hazards associated with bioremediation and control mechanisms are outlined in Table 4. The list is intended to provide an indication of the hazards potentially associated with soil washing application. They will vary significantly from site to site and the list is not intended as a substitute for a detailed hazard assessment of the operation, which should be provided in the RAP.

Readers are directed to the NRF *Guideline on health and safety* for further information on health and safety on remediation sites, including risk assessment, the hierarchy of controls and suggested documentation.

Table 4 Common bioremediation hazards and controls

Hazard	Sources of exposure	Suggested controls
Process Chemicals	• Splashing or leaking chemicals used to facilitate the biodegradation process • Responding to an emergency release of process treatment chemicals or fuel (for excavators etc)	• Use appropriate storage containers • inspect containers for leaks and damage • Install eye wash and emergency shower • Prepare and train for spill containment • Appropriate bunding to assist with containing any spills • Ensure use of personal protective equipment (PPE)
Site Contaminants	• Off-gassing or releasing contaminants during excavation and spreading/storage and handling of soil to be remediated	• Work 'up-wind' of disturbed soil, when possible • Segregate treated feedstock until tested • Routinely monitor work areas; some contaminants require an initial assessment of exposure (e.g. lead) • Ensure use of PPE
Dust	• Releasing dust while excavating soils and placing in treatment area/zone, and in the addition of reagents • Release of pathogens during or after treatment	• Spray water or use dust suppressants on storage piles • Do not operate earth moving equipment during high winds • Ensure use of PPE
Ergonomic Risks	• Lifting or performing any other movement with too much force and/or in an awkward position, or repeating the lift/movement too often	• Provide conveniently located equipment for the job, like correctly sized tools • Train workers on ergonomic risks and prevention

Hazard	Sources of exposure	Suggested controls
Slips, Trips and Falls	• Storing construction materials or other unnecessary items on walkways and in work areas • Creating and/or using wet, muddy, sloping, or otherwise irregular walkways and work surfaces • Constructing and/or using improper walkways, stairs, or landings or damaging these surfaces • Creating and/or using uneven terrain in and around work areas • Working from elevated work surfaces and ladders • Using damaged steps into vehicles	• Keep walking and working areas free of debris, tools, etc • Keep walking and working areas as clean and dry as possible • Perform a Job Hazard Analysis • Ensure use of PPE, including fall arrest systems • Train workers on fall hazards and use of ladders • Use an observer (spotter or signal person) when visibility is limited
Moving Vehicles	• Moving and stockpiling untreated and treated soils using earth moving equipment	• Train equipment and vehicle operators on limitations of equipment and drivers • Train equipment and vehicle operators in safe operation • Set acceptable speed limits and traffic patterns. Ensure that equipment has, and workers use, back-up alarms, mirrors, and seat-belts • Set parking brake and if on incline, chock wheels • Ensure equipment has required roll-over equipment • Establish vehicle inspection schedules and procedures • Do routine maintenance

Appendix A – Case studies

The following case studies illustrate implantation of bioremediation:

- Transforming Ebbw Vale, Wales, UK:
  - *http://www.wrap.org.uk/sites/files/wrap/case%20study%20-%20Ebbw%20Vale.pdf*
- Windrowing on Former Industrial Land, Wales, UK:
  - http://www.churngold.com/case-studies/remediation/in-situ-and-ex-situ-bioremediation-of-hydrocarbons-and-japanese-knotweed-treatment.html
- Biopiling at a Former Dairy, England, UK:
  - http://www.churngold.com/case-studies/remediation/pcb-mechanical-segregation-and-ex-situ-bioremediation-of-hydrocarbons-heating-oil.html
- Penny's Bay Dioxin Site, Hong Kong:
  - *http://www.thiess.com.au/projects/pennys-bay-dioxin-site/detail*

Information on the following case studies can be found in US EPA (1997):

- Land Treatment at the Burlington Northern Superfund Site, Brainerd/Baxter, Minnesota;
- Composting at the Dubose Oil Products Co Superfund site, Cantonment, Florida;
- Slurry Phase Bioremediation at the Southeastern Wood Preserving Superfund site, Canton, Mississippi;
- Cost report: Windrow Composting to Treat Explosives-Contaminated Soils at Umatilla Army Depot Activity (UMDA);
- In situ Bioremediation Using Horizontal Wells, US Department of Energy, M Area, Savannah River Site, Aiken, South Carolina; and
- Lasagna Soil Remediation at hat the US Department of Energy Cylinder Drop Test Area, Paducah, Kennedy.

Information on all the following (and more) US based case studies can be found in the Federal Remediation Technologies Roundtable case studies database, available at http://costperformance.org/search.cfm:

- In situ Bioremediation and Soil Vapour Extraction at the Former Beaches Laundry & Cleaners (2010);
- In situ Bioremediation of Perchlorate and Nitrate in Vadose Zone Soil using Gaseous Electron Donor Injection Technology in Sediment at Hunters Point Shipyard Parcel F, San Francisco Bay, California (2010);
- In situ Bioremediation of Chlorinated Solvents Source Areas with Enhanced Mass Transfer at the Fort Lewis, Pierce County, Washington (2009);
- Enhanced In situ Anaerobic Bioremediation of Chlorinated Solvents at FF-87, Former Newark Air Force Base, Ohio (2007);

- Enhanced In situ Anaerobic Bioremediation of Chlorinated Solvents at the Hangar K Site, Cape Canaveral Air Force Station, Florida (2007);
- Pump and Treat and In situ Bioventing at Onalaska Municipal Landfill Superfund Site, Onalaska, Wisconsin (2006);
- In situ Bioremediation Using Hydrogen Release Compound (HRC®) at Four Dry Cleaner Sites, various locations (2005);
- In situ Bioremediation Using HRC® at a Former Industrial Property, San Jose, California (2004);
- Ex Situ Bioremediation at Two Dry Cleaner Sites, various locations (2001);
- In situ Bioremediation Using Oxygen Release Compounds (ORC®) at an Active Service Station, Lake Geneva, Wisconsin (2001); and
- In situ Bioremediation at the Texas Gulf Coast Site, Houston, Texas (2000).

Appendix B – Treatability case studies

Table 5, presented overleaf, includes a summary of documents that may be useful in conducting treatability studies. References are available in Appendix C.

Table 5: Summary of treatability case studies

Technology	References	Comments
Bioventing	• US EPA (1995a) • US EPA (1995b) • USAF CEE (2004)	• Field studies may include an in-situ respiration test and a soil gas permeability test: • In situ respiration test: determines whether microbial activity is occurring and whether oxygen is limited. • Soil gas permeability test: assists with determining the permeability and radius of influence and provides additional in situ respiration data • If the results of such field tests are positive the installation of an air injection and/or extraction system may be considered.
Enhanced Bioremediation	• USAF CEE (2004) • US ACE (2010a) • US ACE (2010b)	• These documents discuss site selection, design, and performance criteria for enhanced bioremediation as a remedial alternative, select specific enhanced bioremediation approaches that are suitable for achieving remedial goals, and track the cost and performance of enhanced bioremediation applications. • The addendum involved the evaluation of 15 case studies for system design, operation, and performance and outlines advances made in the field of enhanced in situ bioremediation of chlorinated solvents, and provides resources and references that can be used to identify and mitigate the limiting factors and challenges that practitioners face when applying the technology.

Technology	References	Comments
Phytoremediation	• US EPA (2000)	• Results of studies pertaining to a wide range of site conditions, plants and contaminants have been assessed under laboratory and field testing conditions. Information is provided on undertaking site-specific laboratory tests and small scale field trials, including the growing of plants in contaminated soil and water collected from the site, rather than from soils that have been spiked with a known concentration of a contaminant. Pilot studies can include a number of plant species, soil pH and several chelates, which assists with determining the best combination of variables. • In general, treatability studies should provide information regarding the following experimental factors: reduction in contaminant; phytotoxicity of contaminants at the site; growth of plant species under site specific conditions and the rate and level of clean up.
Mycoremediation	• Singh (2006)	This book provides information on techniques to identify, select, and apply fungi for the remediation of contaminated sites, including degradation of specific waste streams and classes of contaminants such as: industrial wastewaters; distillery and brewery wastes; dyes; pulp and paper mill effluents; petroleum hydrocarbons; polychlorinated biphenyls and dioxins; phenols, chlorophenols, and pentachlorophenol; polycyclic aromatic hydrocarbons; and pesticides. Information is also provided on fungal biosorption of heavy metals and mycorrhizal fungi in rhizosphere remediation.
Biopiles / windrows	• NAVFAC (1996)	Information is provided on the assessment, application and design and operation of biopile systems. Information is included on determining the feasibility of biopile remediation, including the data required and a selection process.
Composting	• Vallini et al (2002)	An overview is provided on the potential use of composting in organic waste recycling and decomposition of contaminants in contaminated soils and sediments. In the latter case it is important that the degradation proceeds to the fullest extent with formation of innocuous end products, and information is provided on evaluating the conditions that can hinder the process in different situations.

Technology	References	Comments
Landfarming	• NSW EPA (2014) • MDA (2005) • NEZ Perce Tribe (2009)	• The NSW document outlines the requirements for landfarming, including requirements for a treatability study. • The Bioremediation Treatability Study Fact Sheet includes information on the requirements for treatability studies for bioremediation including landfarming. • The NEZ Perce Tribe Soil Landfarming guidance includes information on treatability studies.
Slurry Phase Biological Treatment	• US EPA (1993) • US EPA (1995)	Information is provided on the planning and carrying out biodegradation treatability studies, including slurry phase biological treatment.

Appendix C – References

ICSCS, 2006, *Manual for biological remediation techniques*, International centre for soil and contaminated sites, Dessau, Germany.

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US EPA, 2000, *Introduction to phytoremediation*, EPA/600/R-99/107, United States Environmental Protection Agency, Washington, D.C.

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VALLINI, DI GREGORIO, PERA & CUNHA QUEDA, 2002, Exploitation of composting management for either reclamation organic waste or solid-phase treatment of contaminated environmental matrices, *Environmental Reviews,* Vol. 10(4)**,** pp 195-207.

WILLIAMS & KEEHAN, 1992, Bioremediation using composting, *Proceedings of 1992 National Waste Processing Conference : fifteenth biennial conference : solid waste processing into the 21st century* Solid Waste Processing Division, American Society of Mechanical Engineers, New York, N.Y.