25 years of advancement in understanding biological processes in remediation: the impossible becomes possible

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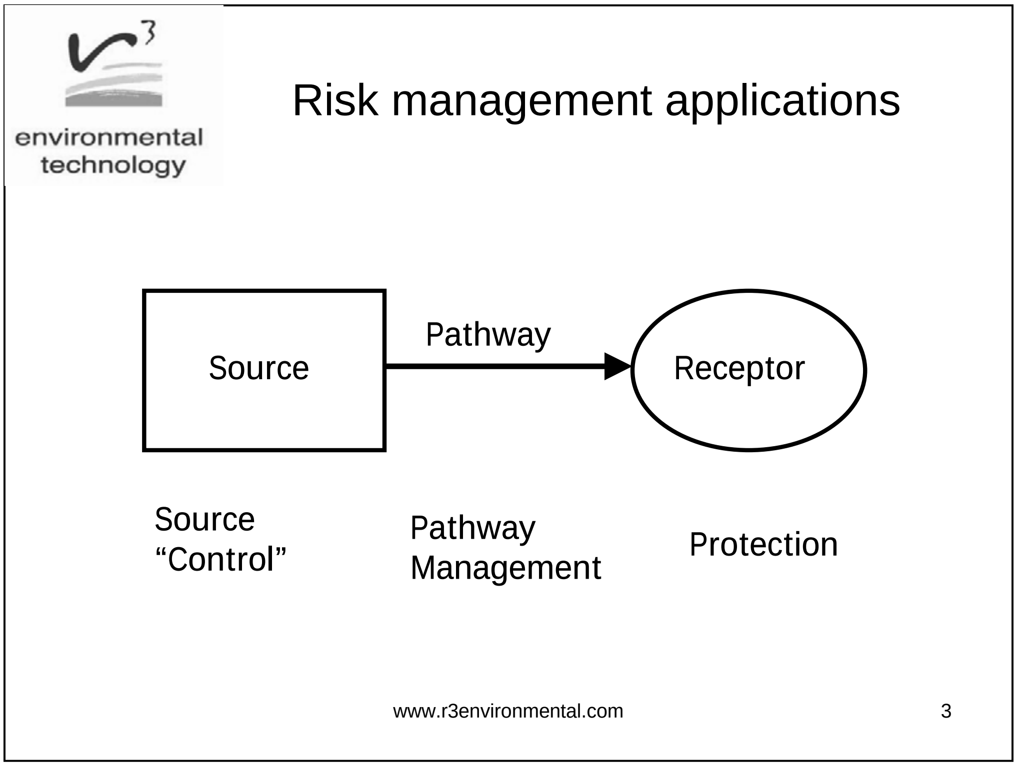
It was not so long ago when the state of the art in bioremediation was represented by the manipulation of biodegradation by aerobic micro-organisms. Initial technologies included landfarming using rotavation of treatment beds dosed with inocula and nutrients intended to support biodegradation, or the injection of hydrogen peroxide into the subsurface to provide oxygen to *in situ* biodegradation.

The technology, its rationale and its application have since come a long way. Initially regarded with deep scepticism by the remediation fraternity, advances in fundamental knowledge have shifted the bioremediation paradigm, and ultimately resulted in a wide range of practical solutions now in general use. Who knows what the next 25 years might bring?

This brief look back highlights some of the key developments that have ushered in modern bioremediation, able to deal with problems thought untreatable just 25 years ago.

The intention of this paper is to provide a basic microbiological perspective, in particular for those from other disciplines involved in contaminated site management. A list of more detailed references has been provided for those who would like to find out more.



The adoption of a risk based approach to contaminated land management has had a major impact on the application of bio-remediation, allowing a more strategic and effective use of biological techniques.

25 years ago some major contaminated soil and groundwater problems surfaced all over Europe. For instance in the Netherlands the Volgermeerpolder, a waste deposit site, with dioxins and chlorophenols, triggered the start of a large investigation and remediation program. In 1984 the number of Dutch sites was estimated at 6000 of which 1600 needed to be remediated. Meanwhile, status 2004, the number has increased to 600.000 sites of which approximately 10% needs to be remediated. Initially, the expected costs kept pace with the number of sites but with time several shifts in thinking occurred: people adopted the concept of risk based management of contaminated soil and in situ remedial techniques such as bioremediation became accepted.

The adoption of risk based decision making has had a major impact across the contaminated land management sector. Remediation has come to be seen as an activity that is employed in managing risks from historically contaminated soil. For a risk to be present there needs to be a linkage between a source (hazard), a pathway (a route along which a hazard can migrate) and a receptor (something that can be affected by the hazard). The structure of this linkage also offers a clue to how risks can be managed, i.e.:

* Source control (e.g. *in situ* bioremediation of diesel contaminated soil)

* Pathway management (e.g. by removing or destroying the contaminants migrating along a pathway, or by preventing the transport pathway operating)

* Protecting the receptor (e.g. installing an alternative water source, preventing site access, restricting land use).

Risk based approach

- Allows a more strategic and effective use of biological techniques
  - Deal with components of the pollutant linkage rather than everything at once
  - Different components may have different solutions
  - Integration along pollutant linkages, especially with MNA
- Environmentally, economically and socially realistic remediation goals = sustainable development

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Dealing with the components of the pollutant linkage, rather than tying to tackle the whole problem at once, allows a more structured and cost effective approach to be taken to remediation, and allows deliverable goals to be set for each component of the remediation process. It allows the principal risks to be identified and remedied, and avoids excessive effort on less important problems.

Before the adoption of such a structured approach a common goal might have been to reduce groundwater concentrations of a contaminant to undetectable levels, or a drinking water related target. In practical terms such a treatment result was (and most often still is) unobtainable or prohibitively expensive. Using a structured approach problems can be managed effectively, ensuring the receptor is protected without needless expense or environmental impact.

Management of Biological Processes (1)

- Better investigation tools:
  - 25 years ago: direct / viable counts and activity measurements
  - Now
    - *field samples and laboratory studies* that do not rely on viable cell cultures (gene probing and protein and fatty acid analysis)
    - specific stable isotope analysis to demonstrate degradation
    - field based process measurements (e.g. *in situ* respiration)

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Until comparatively recently the environmental microbiologist' main tools were direct observation of micro-organisms in soil extracts dried on slides, or cultivated as "viable counts", and a series of indirect measurements of microbial activity such as estimates of microbial biomass, ATP and enzyme activity. These measurements could be difficult to interpret and were known to have serious limitations . For example, direct counts on slides commonly exceed viable counts by a factor of anywhere from 100 to 1000. Clearly not all soil organisms can be cultivated in vitro.

Two groups of developments in biology have greatly enhanced our ability to visualise microbial processes that might be applied in remediation. Advances in other disciplines, such as improved chemical analyses of organic compounds have undoubtedly supported advances in environmental microbiology.

1. Analyses of field samples and laboratory studies that do not rely on viable cell cultures, but help identify microbial types and activity directly in situ, such as the use of gene probing and protein and fatty acid analysis have allowed a much more rapid identification of organisms and activities of interests. The detection of specific bacterial species or groups and their spatial dispersion over the plume provide strong evidence for degradation of specific compounds. Some examples are the detection of Dehalococcoides ethenogenes a bacterium that, strictly reduced conditions, completely degrades chlorinated solvents such as PCE and TCE to ethene and the naphthalene degrading Pseudomons putida G7.

2. Yet, recently an even more promising technique has seen wider application: that of monitoring stable isotopes. Recent research has shown that compound specific stable isotope analysis can be used to demonstrate degradation at contaminated sites. Carbon, hydrogen, and chlorine isotope ratios are used to assess degradation. The theory behind this is that organic contaminants have a characteristic isotope ratio (e.g. 13C/12C-ratio or ı C) that changes during biodegradation. In microbial degradation processes, light isotopes are preferentially used and the heavier isotopes are enriched in the residual substrate fractionation. This process is called fractionation. Data have been published on the use of compound specific stable isotope analyses for mono-aromatic compounds (e.g. BTEX), chlorinated ethenes (PCE, TCE DCE, and VC) and MtBE.

Field based techniques, such as in situ respiration measurements or in situ microcosms, and improved chemical analyses of contaminants, intermediates and products of biodegradation have enabled a better monitoring of the progress of in situ processes as they are operated in the field.

Management of Biological Processes (2)

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- A better understanding
  - role of consortia
- Exploiting microbial consortia
  - ensuring each member has the conditions suitable for growth
  - perhaps multiple treatment zones, each tailored to the appropriate community member
  - e.g. anaerobic and aerobic zones to complete biodegradation certain chlorinated solvents.

PCE
↓
TCE
↓
cis-1,2-DCE
↓
VC
↓
Ethene

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These techniques have enabled a much better understanding of how communities of micro-organisms work as consortia to effect biological change in the subsurface. This new understanding has been of major significance in widening the range of effective opportunities for optimising in situ biological processes in practical bio-remediation.

Often organic substances are not completely degraded by single organisms, and are degraded by a consortia of organisms. Microbial ecology is complex and inter-connected. Recognition of this inter-connectedness has allowed a better understanding of how biodegradation actually takes place in the subsurface, and a route to understanding why expected degradation may not be occurring at particular sites.

An increasingly important example of this is in the biodegradation of chlorinated solvents. Many bacteria are poorly suited to reductive metabolism of chlorinated ethenes, but are capable of oxidising a range of other organic molecules under anaerobic conditions and releasing hydrogen as a product. De-halorespiring bacteria are well suited to the rapid reduction of chlorinated ethenes, but require simple primary substrates for energy, including hydrogen. Thus the ability of fermentative bacteria to produce hydrogen for the halorespiring population allows many organic substrates to indirectly support bioremediation of chlorinated ethenes. Key to exploiting microbial consortia is ensuring each member has the conditions suitable for its growth. This can mean multiple treatment zones, each tailored to the appropriate community member, for example anaerobic and aerobic zones to complete biodegradation certain chlorinated solvents.

Increased understanding of the factors controlling biodegradation in groundwater and improvements in the design of laboratory microcosms have demonstrated the natural attenuation of certain contaminants originally thought to be recalcitrant in aquifers.

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Carriers

- Carriers “flow” in gaps in the subsurface and exchange with solids, mainly:
  - water - moving water through the subsurface
  - air - moving water through the subsurface
- More efficient “use” of the carrier must be made by the treatment than the pollutant linkage!
- Treatments exploit carriers
  - affect the behaviour of a carrier in a localised way (e.g. an injection well)
  - affect the behaviour of a carrier over an area and at different depths, (e.g. via a series of wells)
  - affect the availability of a carrier in an overarching way (e.g. lowering the water table)
- Strongly affected by discontinuities (natural / man-made)

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Carriers is not a widely accepted term. We have used it as a short-hand in this paper. In situ approaches for the most part depend on the movement of air and/or water in the subsurface, whether to exert a treatment effect or to recover contaminants for treatment or disposal above ground. In other words air and water are carriers of the treatment process. An example of an in situ treatment is supplying oxygen in air to micro-organisms and so facilitate biodegradation.

Many of the engineering aspects of in situ bioremediation, particularly below the groundwater table, have perhaps developed rather less rapidly than some of the underpinning biological sciences and there may still be particular difficulties in ensuring adequate distribution of supplements introduced to the subsurface for in situ bioremediation, especially in heterogeneous formations such as fractured/fissured systems.

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What carriers have to achieve

Contaminants

Treatment effect

Same time (?)

Same place (m to μm)

Amendments

Suitable conditions: pH, surfaces, redox

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In situ treatment zones

- Better definition and process control, e.g. PRBs
  - in-ground reactive zones
  - hydro-fracture zones
  - Funnel and Gate™
- Control planes
  - transects across aquifers, perpendicular to the dominant direction of flow e.g.:
    - using sparge curtains
    - Sequential anaerobic / aerobic zones?

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Taking advantage of exploiting carriers to deal with contamination in limited volumes is conceptually simple and enormously powerful means of circumventing the engineering problems of in situ bioremediation in some cases. This idea of in situ treatment zones is most powerful when combined with the concept of risk management as means of controlling contaminants moving along a pathway, for example in permeable reactive barriers or in the control planes concept. In the treatment zone concept the movement of carriers (primarily groundwater) conveys contamination to the treatment zone, which can be more effectively managed and optimised than trying to treat contamination across an entire pathway, which may be a massive volume. More details of these approaches are given below:

o Permeable reactive barriers are a form of reactive containment, which treats groundwater as it passes through a barrier containing an in situ treatment zone.

o Control planes are transects across aquifers, perpendicular to the dominant direction of flow. They can be used for quantification of contaminant concentrations and contaminant mass flow rates. As an essential part of the integral investigation methods, depth integrated or multi-level concentration-time series are measured and inversely interpreted at control planes downgradient of a source zone, or a suspected groundwater contamination. Based on spatial integration of the acquired data, groundwater risk assessment at a high level of certainty can be achieved in particular at large contaminated areas with complex contaminant mixtures. Additionally, the integral investigation allows the delimiting of areas where contaminant source zones are present or absent by application of numerical calculation procedures and under consideration of reactive transport mechanisms.

Electron acceptors

- Biodegradation is directly or indirectly mediated by redox reactions
  - 25 years ago emphasis was on aerobic processes – remember H_2O_2 injection
  - Now anaerobic processes are also seen as very important
  - 25 years ago we knew about degradation as a substrate
  - Now we know about degradation through use as an electron acceptor

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Biodegradation in the subsurface is directly or indirectly mediated by redox reactions. Other biological effects can be useful for example, the release of acids and ligands. However, these effects are ultimately powered by redox, as the engine by which an organism obtains usable energy. Organisms “breathe” compounds such as oxygen, nitrate, sulphate even CO2 and use the energy to degrade contaminants. This ability can be exploited in treatment zones.

Initially efforts were focused on aerobic biodegradation, where oxygen is used by micro-organisms, commonly referred to as oxygen being the ‘electron acceptor’. Technologists aimed to make oxygen more available. Chance observations of elevated carbon dioxide in the emissions from soil venting projects led to the recognition that the passage of air through the vadose zone could accelerate aerobic biodegradation. Indeed, venting in many cases could be optimised so that the emissions were mainly CO2 rather than volatile organic compounds so reducing environmental impacts and air treatment costs, and so bioventing was born. The same effect took place with air sparging, and so biosparging became a technology for subsurface in situ groundwater treatment.

Other interventions have been less successful. The 1980s saw great interest in the pumping of hydrogen peroxide solutions for increasing the availability of oxygen in the saturated zone. Unfortunately H2O2 treatments were not found to be greatly successful. Little of the H2O2 added makes it to the microbial populations whose activity it was desired to stimulate. H2O2 rapidly decomposes in the soil, as just about every soil surface catalyses its decomposition. A large amount of the oxygen liberated is consumed in oxidising natural soil components, and H2O2 itself is toxic to microbial activity and hazardous to use. It has found a new “lease of life” in in situ chemical oxidation.

Over time the importance of other electron acceptors (e.g. nitrate, sulphate, iron III) has become recognised in bioremediation for the so called “anaerobic” degradation processes. However, perhaps the most interesting observation has been that contaminants themselves may serve as electron acceptors and that this may be an effective treatment.

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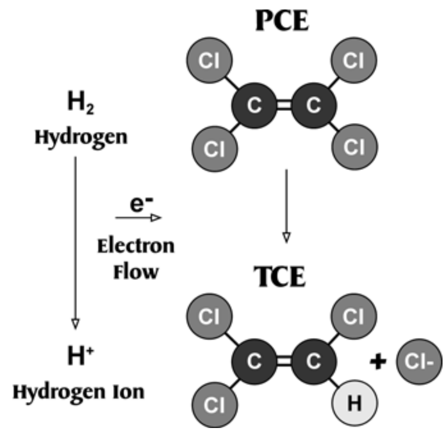
Dehalorespiration

- *dehalorespiration:* use of chlorinated compounds as respiratory substrates by micro-organisms in the absence of oxygen



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The applied use of “dehalorespiration” is the major practical application of this new understanding of microbial use of electron acceptors. Not so long ago contaminants such as perchloroethylene (PCE) were seen as untreatable by biological means by many specialists. The reasons for this were two-fold. Firstly, such compounds were thought to contain too little energy to be degraded as a fuel for microbial respiration. Secondly, these anthropogenic substances were seen as unlikely to have led to the evolution of secondary metabolic pathways, e.g. for their detoxification. Both statements are now known to be untrue. Most importantly, it is now known that chlorinated ethenes, chlorinated benzenes and some other compounds can be used as respiratory substrates under anaerobic conditions (dehalorespiration and can, under suitable conditions, be converted to fully dechlorinated products (for example chlorinated ethenes can be converted to ethene). This metabolic process is now widely used as the basis for bioremediation of chlorinated solvent contamination in groundwater.

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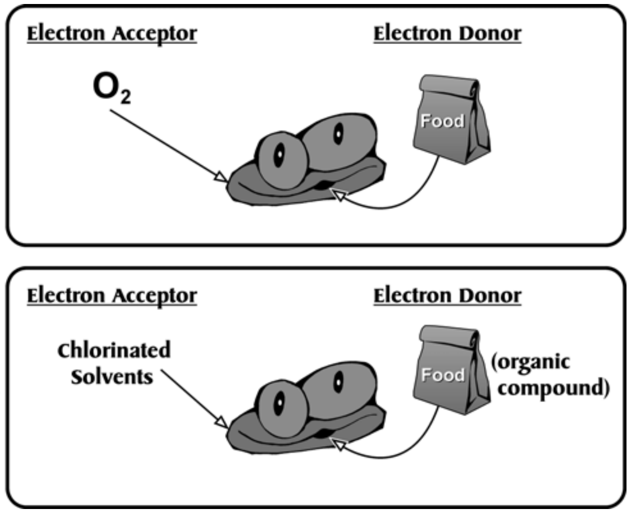
Respiration using oxygen

dehalorespiration



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Monitored Natural Attenuation (MNA)

- Risks decrease as a result of natural processes
- MNA monitors their effects to confirm that risk is likely to decrease within a specified time scale
- Risk reduction may be as a result of a decrease in contaminant concentration, volume, mobility or toxicity.
- MNA processes include
  - dispersion, dilution, sorption, volatilisation, biodegradation, chemical or biological stabilisation, transformation or destruction of contaminants. The contaminants remain in place whilst the processes operate.
- Now widely used with detailed guidance available

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As it turned out that, even without additional stimulus, microbial activity had a great impact on the decrease in concentration of contaminants on soil and groundwater, it became the basis of a 'new' in situ technique named Monitored natural Attenuation.

Monitored natural attenuation (MNA), the application of un-enhanced natural processes in the subsurface to manage the risk posed by contaminated sites has become increasingly common, underpinned by many of the fundamental techniques described above and a wide range of case histories for petroleum hydrocarbons, chlorinated solvents, aromatics and many other contaminants (National Academy of Sciences, 2000). This development has been encouraged by the publication of numerous protocols and guidance documents on MNA, including examples from the UK, USA and the Netherlands.

Fungi and Plants

- Practical use of eukaryotes in bio-remediation is not well advanced, but may further widen the range of biologically treatable problems
  - Revegetation (containment)
  - Phyto-remediation
    - Limitation of extractive / biobarrier techniques is contaminant dispersal (to atmosphere or in biomass)
  - Fungal approaches
    - Non-specific biodegradation of recalcitrant compounds
    - Plant supported via ectomycorrhizae

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So far this paper has focused on prokaryotic organisms (bacteria, actinomycetes etc). However, there has been longstanding interest in the use of eukaryotic organisms such as plants, fungi and even earthworms in bio-remediation. The practical use of eukaryotes in bio-remediation is not as well advanced as microbially based techniques, but may further widen the range of risk management problems that are treatable biologically.

The use of plants in land restoration is long established, for example the revegetation of mining areas. However, revegetation is now being used in a risk management context. The revegetation can be used to limit the flow of contaminants to receptors, for example by creating conditions where contaminants are stabilised so migration is prevented, and also be altering the hydraulic regime in a contaminated area to prevent the migration of contaminated groundwater.

More speculative applications of biological techniques include the use of trees in “biobarriers” and the use of fungi. The use of plants to “extract” contaminants such as metals has also been widely trialled, and in some cases applied in practical projects. However, phyto-extraction has a number of problems associated with it. The first is that the phyto-extraction tends to be slow and incomplete. The second is that the process creates a waste requiring treatment and disposal: biomass. The sustainability of re-using biomass from phyto-extraction as a fuel has been questioned, as has the use of ligands to increase metal mobility in the soil and hence plant uptake.

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Bring land back into economic use

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Poplar Biobarriers: The use of poplars as a barrier to the migration of VOCs in groundwater is now reasonably well established in the USA, although there is still debate on the relative merits of using an approach that allows substantial liberation of VOC to the atmosphere via the poplar leaves. The extent to which plants can directly biodegrade contaminants, or the use of plants to remove contaminants such as metals from ground, remains somewhat contentious.

Fungi The potential role of other organisms than bacteria in bioremediation has always been of great interest . Basidiomycete fungi (such as white rot fungi) have the potential to play a major role in the degradation of recalcitrant organic compounds such as higher PAHs and PCBs. This is because the method they employ to degrade their usual substrates (such as wood) employs a non-specific and highly aggressive system, which releases free radicals containing oxygen, along with non specific enzymes. As well as degrading lignin, this system is also capable of degrading high molecular weight insoluble organic compounds with low solubility in water, and also highly chlorinated organic compounds. Fungal based systems have seen only relatively limited use in the field which have been hard to verify, which is in part connected with the difficulty in propagating fungi and then stimulating their lignase systems “on demand”. It has also emerged that some ectomycorrhizal fungi also have lignolytic activity. Effective practical applications of fungal treatments are relatively few in number to date and further applied research is required to work out how develop this approach into a widely exploitable technology.

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Conclusions

- Only 25 years ago most contaminated soil was dealt with by removal. Now we have alternatives, the most frequent of which are biological techniques
- This change was made possible through a better scientific understanding of biological processes, innovation in how to exploit subsurface conditions, and the use of risk management as a strategic tool in remediation design
- The use of fungi and plants as bioremediation agents does appear to have great potential, in practice they are used only in niche applications

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Only 25 years ago the preferred technique to treat contaminated soil was to remove and dispose. During the past years we great progress has been made in finding alternatives, and the most widely used alternative is now perhaps bio-remediation. This change was made possible through a better scientific understanding of biological processes, innovation in how to exploit subsurface conditions, and the use of risk management as a strategic tool in remediation design.

To date much of this advance has centred on microbially mediated processes. While the use of fungi and plants as bioremediation agents does appear to have great potential, in practice they are used only in niche applications, although some like phyto-stabilisation are of enormous significance.

Putting ourselves in the position of futurologists 25 years ago, we are confident that we would not have guessed all of the advances that have taken place, although we may have identified one or two. We are therefore reluctant to hazard a guess on the next 25 years, but our money is on:

o A wider range of eukaryotic bio-remediation agents,

o Increasing use of an ecological “community” based approach to using biological processes in risk management

o Improved in situ methods for treating contamination and wider exploitation of these, and

o An even longer list of contaminants that can be biodegraded by microbes.

These developments will need some more investment in underpinning science. This investment is likely to provide a good return given the cost-effective nature of many of the existing bioremediation approaches.

The next 25 years?

- A wider range of eukaryotic bio-remediation agents,
- Increasing use of an ecological “community” based approach to using biological processes in risk management
- Improved *in situ* methods for treating contamination and wider exploitation of these, and
- An even longer list of contaminants that can be biodegraded by microbes.
- These developments need investment in underpinning science.

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