Bioremediation: Successes and Shortfalls

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1 Introduction

Bioremediation is the process of utilising biological agents (bacteria, fungi, higher plants, algae and cyanobacteria) to remove contaminants from the environment in order to remediate land or water (Wackett & Hershberger, 2001). Increasing legislative and economic pressures to develop alternative strategies for the remediation of contaminated land has led to bioremediation becoming an attractive remedial solution. Today, in the UK (and Europe), the principal driver for exploring the use of bioremediation is the increasing cost incurred for sending contaminated waste to landfill following the implementation of the EC Landfill Directive and subsequent enforcement of the regulations (Landfill Regulations, 2004).

The application of a successful bioremediation programme requires a multidisciplinary approach usually led by microbiologists and supported by hydrogeologists, soil scientists and engineers. There is a great diversity of contaminants susceptible to degradation, and of organisms capable of bioremediation. This discussion focuses only on the use of prokaryotic microorganisms for remediating sites contaminated with petroleum hydrocarbons since these are the most common contaminants of concern in the environment (Nathanial *et al.*, 2001), particularly as a result of spillages. It is well documented that a large number of contaminants can be degraded by microorganisms under aerobic and anaerobic conditions (Atlas, 1995, Hollinger & Zehnder, 1996; Ellis *et al.*, 2003; UM-BBD, 2006). In general, aerobic conditions are often considered more favourable than anaerobic owing to the wider range of contaminants that can be degraded, increased rate of degradation for some contaminants, and the efficiency of conversion to innocuous products or complete mineralisation.

A large number of publications highlight the merits of bioremediation at laboratory scale but relatively few peer-reviewed publications detail effective bioremediation in the field and even fewer publications detail unsuccessful applications of bioremediation. This paper provides a brief review of bioremediation and outlines some successes and shortfalls when applying bioremediation in the field.

2 Bioremediation in a risk assessment framework

In the UK, Part IIA of the Environmental Protection Act 1990 defines Contaminated Land on the basis of risk assessment. Both human health and environmental impacts are assessed within a risk assessment framework and this should be site-specific and related to the identified contaminant source, pathway and receptor. In accordance with good practice, the remediation of contaminated sites should only be commenced after at least a qualitative assessment of risks posed by that contamination to the identified receptors (e.g. human health, groundwater or surface water) has been undertaken. The remedial

strategy will be chosen to address those risks identified. As with other remedial techniques, bioremediation should only be applied once all of the potential risks posed by that contamination have been established and it has been determined that bioremediation will address those risks, and will not introduce any additional risk to a particular site. For example, consideration should be given to the possibility that flushing with nutrients or oxygen delivery solutions may increase the migration of a contaminant source or plume, or any potential health effects resulting from the addition of microbial inoculums.

Specifically for oil spills, early response and intervention is key in minimising the risks, contaminant extent and cost of remedial actions. When attempting to clean-up spills, timely and comprehensive source control and associated actions must be implemented. These include, for example: immediate control and cessation of the release; repair or removal of the release source; and removal / recovery of free product. Any remedial action initiated before the source is controlled is likely to be ineffective and has potential for spreading the contamination and increasing the scope of the remedial action required.

3 The use of bioremediation

Vidali (2001) provided a useful overview of bioremediation techniques and the advantages and disadvantages of the technology. Commercially available bioremediation products, including mixtures of oil degrading microbial inoculums and sources of nutrients, have been patented and marketed since the early 1970s (Linn, 1971; Zhu *et al.*, 2004). Rapid commercialisation of bioremedial technologies occurred following the use of various bioremediation techniques after the Exxon Valdez spill in 1989, with venture capitalists taking advantage of growing markets (Macdonald, 1997). Surveys of remedial practitioners operating within the UK, have suggested that the subsequent progression of bioremediation as an effective remediation technology has, in part, been limited by uncertainty regarding the efficacy of the technique, operational constraints and the regulatory permissions required (Environment Agency, 2000; Nathanial *et al.*, 2001). Low confidence in the efficiency of bioremediation, particularly when applied *in-situ*, may be due to the relatively few validated field trials that have been reported. Barriers to the exploitation of bioremediation research were identified by BBSRC (1999). These included low end-user confidence, a lack of focused research and field validation of new techniques. The report concluded future research should be more interdisciplinary and include molecular approaches to examining the microbiology of bioremediation. A number of UK government initiative programmes have subsequently been developed to encourage and stimulate alternative strategies to remediate contaminated land (e.g. C:LAIRE, FIRST Faraday and Link).

3.1 *Ex-situ* and *in-situ* bioremediation

In-situ and *ex-situ* methods utilised for remediation of contaminated soils and waters have been reviewed by Evans (2001); Nathanail *et al.*, (2001); Barr (2002); Khan *et al.*, (2004). In brief, *ex-situ* techniques, which require the excavation of contaminated soils or pumping of contaminated waters prior to bioremediation include: land farming, biopiling (windrows) composting and bioreactor treatment. *Ex-situ* remediation methods have been demonstrated to offer a cost-effective solution to the remediation of contaminated land and their frequency of use is increasing. The removal of contaminated

materials off-site also results in a rapid transfer of liability and more time available for subsequent bioremediation of contaminants. *Ex-situ* methods enable environmental conditions of the contaminated material to be easily modified and monitored, and the efficacy of treatment more easily validated.

Bioremediation techniques may be required to be directly applied *in-situ* where excavation of soils may be undesirable or impractical owing to physical constraints. *In-situ* techniques include bioventing; biosparging; biostimulation; bioaugmentation; and natural attenuation. *In-situ* techniques may require longer time periods for treatment resulting in a slower transfer of liability and extended periods of monitoring. In addition the delivery of the bioremediation enhancing agents (e.g. oxygen, nutrients) can be difficult owing to geological and hydrogeological factors.

Table 1 provides a summary of the available techniques for *in-situ* and *ex-situ* remediation, and illustrates the benefits, limitations and factors that should be considered when applying these techniques.

Table 1: Summary of bioremediation technologies and their benefits, limitations and factors for consideration

Technology	Examples	Benefits	Limitations	Factors to consider
In-situ	Biosparging Bioventing Biostiumlation Bioaugmentation	Most cost efficient. Non-invasive. Relatively passive. Accelerates natural processes. Treats both soils and waters.	Environmental constraints. Extended treatment times. Monitoring difficulties.	Biodegradative abilities of indigenous microorganisms. Presence of metals and other inorganics. Environmental parameters. Biodegradability of contaminants. Chemical solubility. Geological and hydrogeological factors. Distribution of contaminants. Toxic concentrations of contaminants.
Ex-situ	Landfarming Biopiling (windrows) Composting Bioreactor treatment	Cost efficient. Can be carried out on site. Optimize environmental parameters. Effective use of inoculums, surfactants and other additives.	Space requirements. Extended treatment time. Soil requires excavation. Relatively high operating costs.	
Source: Vidali (2001)				

4 Limitations to bioremediation

A number of factors can limit the effectiveness of bioremediation, including:

- No organism is known to degrade all organic wastes, and contaminated sites usually contain mixtures of contaminants;
- High concentrations of the contaminant or component of a complex mixture may be inhibitory or toxic to microorganisms;
- Binding of the contaminant to soil may occur, reducing bioavailability, exacerbated by poor contaminant solubility and pollutant ‘ageing’ to recalcitrant by-products;
- Limited availability of electron acceptors, nutrients and co-metabolites; and
- Build up of toxic transformation products (Vogel & McCarty, 1985).

There is a notable gap between research carried out in the laboratory and the application of effective bioremediation in the field. In the field, contaminants, nutrients and

indigenous micro-organisms are distributed heterogeneously and it is extremely difficult to recreate models that have been demonstrated as being effective in the laboratory. For example, numerous papers detail the qualities of bioaugmentation in laboratory studies (e.g. Fathepure *et al.,* 2005). However, the inability of introduced cultures to establish and proliferate at a contaminated site has been a significant hurdle for this technology (reviewed by Thompson *et al.,* 2005).

4.1 Oxygen delivery

With respect to *in-situ* bioremediation, bioventing and biosparging have been used with some success (CL:AIRE technical report TDP9, Shields *et al.*, 2004). It is essential that sufficient nutrients are available for bioremediation to occur, and efficiency of oxygen delivery is often restricted by ground conditions and the method of oxygen delivery. In general, soils of low permeability, generally consisting of clay minerals, are less permeable to oxygen diffusion than loose, poorly consolidated soils consisting predominantly of sand or gravels. Innovative oxygen introduction systems, such as oxygen diffusion technologies are improving the feasibility of oxygen delivery for *in-situ* bioremediation programmes. Recent advances in oxygen delivery systems include gas infusion systems such as ISOC™ (*in-situ* oxygen curtain, InVentures Technologies Inc. Canada) capable of cost effectively delivering high concentrations of oxygen to groundwater.

Oxygen can also be supplied to soils and groundwater using compounds that release oxygen on contact with water such as magnesium peroxide. Modern formulations have been designed to control the rate of oxygen release. One such example is Oxygen Release Compound (ORC®; Regenesis, UK) that consists of phosphate intercalated magnesium peroxide. A selection of case studies describing the successful use of ORC® in remediating hydrocarbon contaminated sites is provided by Koenigsberg & Norris (1999).

It must be noted that when oxygen is introduced to the subsurface as a terminal electron acceptor, it can react with dissolved iron ($Fe^{(II)}$) to form insoluble iron precipitate, ferric oxide. This precipitate can reduce soil and aquifer permeability and effects of iron precipitation tend to be most noticeable around injection wells, where oxygen concentration in groundwater is highest and can render injection wells inoperable.

The direct oxidation of petroleum hydrocarbons using reactive oxygen intermediates (hydroxyl and superoxide radicals), generated by the Fenton process (Walling, 1975), is an effective strategy for the breakdown of hydrocarbons. This application has also been suggested to result in post-treatment increases in oxygen concentrations within soils, potentially stimulating microbial activity (EPA, 2004). However, the action of oxygen radicals on organic matter is indiscriminate and will also oxidize microbial cellular components leading to cell inactivation. Exposure of microorganisms to oxygen radicals may result in cellular inactivation due to membrane damage (lipid peroxidation), protein denaturation or DNA strand breaks (Waddell & Mayer, 2003; Imlay & Linn, 1988). While aerobic organisms possess enzymes such as catalase and superoxide dismutase that protect the organisms from oxygen radicals generated by normal metabolic processes, the

concentrations of oxygen radicals generated by chemical oxidation treatments are likely to far exceed that which the organisms can withstand. In addition, obligate anaerobic organisms may not contain such protective enzymes and may be more susceptible to nucleophilic attack. For example, *Pseudomonas sp*. are commonly isolated hydrocarbon degrading bacteria (Atlas, 1992). However, Elkins *et al.,* (1999) reported that the survival of *Pseudomonas aeuroginosa* following exposure to hydrogen peroxide at concentrations of as low as 50 mM was less than 1% after 40 min. Cell inactivation by chemical oxidation treatments may result in a shift in soil microbial community structure and the requirement for repopulation of the hydrocarbon degrading microbial consortia, which may be a potentially lengthy process Stokely *et al.,* (1997).

4.2 Nutrient delivery

For biostimulation to be effective, the stoichiometric relationship between nutrients and contaminants must be estimated to ensure appropriate levels of biostimulants are added. Within a laboratory controlled microcosm, it is possible to accurately monitor and control nutrient ratios, whereas in the field (especially *in-situ*), the efficiency of delivery mechanisms, heterogeneity of soils affecting distribution of nutrients and electron acceptors, and nutrient wash-out rates from soil, all influence the concentration of nutrients available for bioremediation (e.g. Greer *et al.,* 2003).

Optimal nutrient conditions for the growth of microorganisms are rarely sustained or achieved in the natural environment. Consequently microorganisms may pass through frequent cycles of growth and dormancy. The presence and distribution of nutrients in soils and waters is not uniform due to variations in soil type, organic content, weathering processes and land-use. Furthermore, microorganisms face fierce competition for the limited nutrients that are present. The rate of microbial degradation of hydrocarbons in soils and waters is limited by the availability of inorganic nutrients (Toccalino *et al.,* 1993; Steffensen & Alexander, 1995; Xu & Obbard, 2003 and 2004). Amendment of soils or waters by the addition of nutrients such as nitrogen and phosphorous has been shown to stimulate or increase hydrocarbon degradation by microbial communities (Toccalino *et al.,* 1993; ; Steffensen & Alexander, 1995; Xu & Obbard, 2003).

Large nutrient additions can result in biofouling, limiting the successfulness of remedial programmes through a reduction in soil/aquifer permeability (Lee *et al.,* 1988) resulting in a reduction in oxygen present in the sub-surface, and nutrient additions in the absence of electron acceptors will result in the failure of bioremediation. The use of high nitrate fertilisers as amendments may also be in appropriate in areas that are designated as nitrate vulnerable zones and can cause eutrophication of water bodies. Restrictions also exist on the concentration of electron acceptors that can be added for bio-stimulation purposes. For example, concentrations of nitrate entering groundwater are usually restricted to less than 50 mg l^{-1} and lower concentrations are targeted in sites designated under directive 91/676/EEC as nitrate vulnerable zones (Beeson & Cook, 2004).

4.3 Health and safety concerns

Given that commercially available inocula are feely available for bioremedial applications, there is still little guidance or regulation in the use of microbes for

remediation, especially with regard to health and safety. This is surprising, given that physical works involved in the bioremediation of soils may be performed by a variety of workers (groundworkers, engineers etc) and not necessarily by microbiologists. Therefore, it is important that all those intending to perform bioremediation are aware of the risks associated with the use of microorganisms and are adequately trained in the use of protective equipment and hygiene practices. For example, microbial inoculums may be provided as dry powders which may be inhaled. In addition, there is an increasing interest in the use of nutrients and waste products as soil amendments to stimulate bioremediation (Vasudevan and Rajaram, 2001). However, the use of such amendments, particularly in batch *ex-situ* techniques, may provide a suitable environment for the considerable growth of potentially harmful molds and fungi. Other potential risk factors include the metabolic products of microbial activity since partial or incomplete degradation of hydrocarbons that may result in compounds that are more harmful or toxic than the original compounds (Vogel and McCarty, 1985).

Concern has also been expressed that microorganisms isolated from soils that have considerable potential for metabolizing hydrocarbon substrates also pose human health risks in terms of opportunistic infections and allergenicity. Most notably, the potential use of organisms such as *Burkholderia* (previously *Pseudomonas*) *cepacia* has come under scrutiny due the serious nature of disease this organism can cause in cystic fibrosis and immunocompromised individuals (Holmes *et al*., 1998; Berg *et al*., 2005). Furthermore, Holmes *et al*., (1998) suggest that potential exists for the evolution of multiple antibiotic resistant pathogenic organisms through horizontal gene transfer and that further work is required to establish the risks of widespread use of *B. cepacia* in agriculture and bioremediation.

5 Case studies

Specific examples are detailed here to highlight the importance of carrying out bioremediation within a risk-based framework and having a comprehensive understanding of the science involved. Given the commercially confidential nature of the products and sites discussed in the following sections, some details can not be provided.

5.1 Case study one - *In-situ* biostimulation through nutrient addition

A diesel spill occurred from the feed pipe of an above ground storage tank (AST) in the grounds of an office building, neighbouring a property development worth approximately £700,000. Concentrations in soils of the principal contaminants of concern, diesel range organics (DRO), were approximately 70,000 mg kg^{-1}. An initial site investigation indicated that bioremediation may be a viable option, with evidence of degradation products and contamination found in both the unsaturated and saturated zones. The remedial target concentration for DRO in soils was 250 mg kg^{-1} and 250 µg l^{-1} DRO for the groundwater beneath the site.

Stringent time-frames for the remediation programme were in place because of health and safety concerns associated with the contamination and the neighbouring development, which was postponed throughout remedial works. The employed ‘remediation

consultants' predicted that the targets could be achieved through bioremediation within 3 to 6 months by the addition of a commercially available bacterial inoculum.

Following 6 months treatment, the average concentration of DROs in soils remained above 2,000 mg kg^{-1}. There were a number of contributing factors leading to the failure of the bioremedial programme in the agreed timeframe. Consideration had not been given to the proliferation of the added bacterial inoculum; the availability of nutrients; the concentrations of contaminants in relation to toxicity to micro-organisms; the bioavailability of the contaminants; and, overall ground conditions (e.g. soil permeability, soil temperature). Furthermore, the potential of the indigenous microflora to metabolise hydrocarbons had not been investigated.

In addition, the operators failed to effectively validate the site and therefore concluded the site was remediated when there were DRO concentrations of up to 7,000 mg kg^{-1} still present. No contingency plan was in place for the event of inadequate treatment, so when bioremediation failed, there was no mechanism in place to contain the contamination.

5.2 Case study two – *Ex-situ* biostimulation through nutrient addition

An estimated 4,000 litres of kerosene was released to the ground surface following the failure of an oil feed pipe running between an AST and the main house on a domestic property, in Jersey, Channel Islands, UK.

A site investigation indicated that soils beneath the spill origin contained concentrations of total petroleum hydrocarbons (TPH) of approximately 4,330 mg kg^{-1}. Given the extent of the contamination and the risk to the householders and the environment, it was considered that the excavation and removal of contaminated soils from the site was a priority. However, at the time of the remediation there was no licensed landfill site on Jersey that would accept such waste. Furthermore, the export of hazardous waste from Jersey to the UK (or other EU State) was not an option, since Jersey is not a signatory to the Basel Convention on the Control of Transboundary Movement of Hazardous Wastes and their Disposal or a member of the EU, and as such there was no legal mechanism in place for shipments to take place. The legislation governing the disposal and movement of contaminated soils in Jersey required that the concentrations of petroleum hydrocarbon within the soils were considerably reduced to enable reuse or disposal of the soils at a non-hazardous landfill site. For the latter, a target concentration of 500 mg kg^{-1} TPH was agreed with the States of Jersey Environment Department. Therefore, soils excavated from the site required some form of remedial treatment to reduce TPH concentrations to acceptable levels prior to disposal.

Laboratory feasibility studies indicated that the addition of inorganic nutrients and a nutrient source derived from a waste product of a food manufacturing process enhanced the degradation of hydrocarbons by the indigenous microorganisms. Therefore, it was decided to treat the soils with nutrient amendments using *ex-situ* bioremediation techniques prior to disposal. Contaminated soils from the site were excavated and placed into lined containers and amended with a fertilizer (Nitrate:Phosphate:Potassium (N:P:K); 7:7:7) and the nutrient-rich waste product. Kerosene degradation was also

examined in contaminated soils that had not been amended with nutrients (control). The soils were incubated for 93 days and aerated by turning every two weeks with a mechanical excavator. At monthly intervals, the concentration of TPH in soils was determined in triplicate. In addition, individual hydrocarbon components were identified and quantified.

The concentrations of kerosene range petroleum hydrocarbons in the nutrient amended soils and un-amended soils were reduced from initial concentrations of over 4000 mg kg^{-1} to concentrations considerably lower than the target concentration of 500 mg kg^{-1} within 93 days using this bioremediation technique. An accurate assessment of the effect of the type of soil amendment on the rate of petroleum hydrocarbon degradation during this study was limited by the observations that concentrations of TPH compounds in separate treatment vessels was highly variable.

Significant TPH concentrations have been observed in materials not derived from petroleum products such as leaves and grass (Environment Agency, R& D Technical report P5-080/TR1 (2003)). The organic matter fraction of soil contains a vast array of hydrocarbon molecules derived from the decomposition of plant and animal origin and that of microbial activity or humic substances (Chefetz *et al*., 2002). Hence, the solvent extraction method employed for the determination of TPH concentrations in soils may result in the extraction of hydrocarbons that do not necessarily originate from the fuel spilt. In this study a high concentration of non-kerosene range organic compounds was present in all soil samples prior to treatment. These compounds resulted in elevated TPH concentrations and could have potentially been misconstrued as being derived from the fuel spill. Speciated analysis and study of the Gas Chromatography (GC)-chromatographs allowed elimination of higher molecular weight compounds and was used to more accurately assess the extent of contamination derived from kerosene.

Treatment of soils with the waste product alone resulted in temporary increases in TPH concentrations and incubation of the waste product alone for 93 days resulted in the greatest TPH concentrations. The waste product used is reported to comprise approximately 10% ether extractable organic material, which includes fatty acids, and phospholipids. Compounds such as phospholipids and fatty acids and their degradation products (due to microbial activity) may potentially interfere with the total concentration of extractable hydrocarbons.

In an attempt to further eliminate compounds interfering with petroleum derived hydrocarbon yield, solvent extractable hydrocarbons were separated by silica column chromatography prior to analysis using Gas Chromatography – Flame Ionisation Detection (GC-FID). The effect of removing polar hydrocarbons by silica column chromatography prior to GC-FID analysis on the TPH concentrations of soils resulted in considerable decreases in the concentrations of “reportable TPH”. Following the sample clean-up step with the silica column, TPH concentrations were reduced to approximately 1% of the value reported by solvent extraction alone.

In this study, assessment of the efficacy of the biostimulatory amendment was complicated by the observation that standard and commonly employed analytical procedures to determine TPH concentrations could not be used to determine TPH concentrations without additional complex and costly analysis. Careful and detailed interpretation of the analytical data was required to illustrate to the regulator that the risks from the kerosene contaminated soils had been mitigated by the successful bioremediation programme within the 93 day incubation period.

5.3 Case study three – *In-situ* biostimulation through oxygen addition

Oxygen can be supplied to soils and groundwater using compounds that release oxygen on contact with water. Modern formulations have been designed to control the rate of oxygen release allowing slow dissolution of oxygen.

Following a release of an estimated 1600 litres of kerosene to ground at a domestic residence as a result of actions of contractors working in the vicinity of an oil feed line from an AST, emergency remedial action was required in order to remove free phase kerosene from groundwater on a nearby private well. A detailed investigation of the site was undertaken involving the drilling of boreholes to 40 m below ground level (approximately 10 m below rest water level) and geophysical logging (i.e. wire-line logging including temperature, conductivity, calliper and resistivity logging) of all available boreholes to characterise the fracture network in the Chalk.

The geology underlying the site comprised minimal superficial clay soils (up to a maximum of 0.3 m) overlying White Chalk. Free phase hydrocarbons were removed from the impacted well using pump and treat methods, and approximately 6 months after the initial incident when no free phase hydrocarbons were evident in the impacted well and surrounding monitoring wells, a programme of bioremediation using a compound that releases oxygen when in contact with water, was instigated at the source area to further reduce the concentrations of dissolved phase hydrocarbons in groundwater.

Shallow injection wells (5 m to 12 m) were drilled at the spill origin and the compound was injected as a slurry into the wells, with drainage pipes inserted within the wells in order to allow the re-circulation of water from the impacted well through the compound in an attempt to allow the continued dissolution of oxygen. The system was designed to allow the flow of oxygenated waters through the same flow paths as those taken by the original contaminant in order to enhance the degradation of the residual kerosene.

Only 5 days after the initial injection of the compound into the injection wells, free phase hydrocarbons were noted in the originally impacted abstraction well and in adjacent monitoring boreholes and a new abstraction borehole drilled up hydraulic gradient of the spill origin. Following the discovery of free phase hydrocarbons, the operation of the system was ceased as bioremediation of the kerosene was considered to be negligible given the likely toxicity posed by the free phase kerosene to any micro-organisms present.

The lesson learned from this case study was that *in-situ* treatment of contaminants using injection of biostimulants was ineffective as a result of the complex fracture network of the underlying Chalk. The distribution of both light non-aqueous phase liquids (LNAPL's) and dense non aqueous phase liquids (DNAPLS's) in fractured rock is highly complex (e.g. Hardisty *et al.,* 2003; Davison *et al.,* 2002). The site in question was also located on an interfluve and a seasonal fluctuation in groundwater level of in excess of 15m has subsequently been observed. This results in a complex distribution of kerosene and prediction of the flow paths of water and contaminants in the unsaturated and saturated zone of the Chalk is likely to prohibit the successful application of bioremediation in this environment.

6. Conclusions

Bioremediation, when used within a risk-based framework, can offer a cost-effective and sustainable remediation strategy. However, there is a danger that the increase in commercial "solve-all" approaches may jeopardize clients' confidence that bioremediation is a credible alternative to other remediation strategies. This is, in part, due to a lack of reporting of in depth/peer reviewed field trials and also an increase in the use of bioremediation products without sufficient initial investigation and monitoring or validation. The case studies presented highlighted the following:

- Case study one highlighted that even where initial investigation indicates bioremediation is a viable option, additional factors may impede progress, and monitoring is required so that this can be identified early in the remedial programme.

- Case study two demonstrated a successful field programme but highlighted the difficulties associated with trying to obtain high-resolution contamination data to validate the efficacy of *ex-situ* bioremediation. Heterogeneity of samples and interferences (such as natural organic matter) can contribute to difficulties in validation monitoring particularly in relation to TPH analysis.

- Case study three demonstrated the importance in prior investigation before the application of bioremediation, and illustrated that where contamination is associated with a highly heterogeneous environment (e.g. Chalk aquifer) bioremediation may not be the most appropriate option as flow path prediction for water and contaminants can be difficult to predict and can result in an increase in the extent of contamination if not managed carefully.

Remediation of contaminated sites should only be commenced after at least a qualitative assessment of risks posed by that contamination to identified receptors (e.g. human health, groundwaters or surface waters). In this context, bioremediation should also only be applied once all of the potential risks posed by that contamination have been established and it has been determined that bioremediation will address those risks and will not introduce any additional risk to a particular site (e.g. increasing the migration of a contaminants source by flushing or the introduction of foreign pathogens).

In-situ bioremediation is a very site-specific process and treatability tests should be carried out prior to initiating bioremediation at a contaminated site to ensure the technology is appropriate for use. Thompson *et al.*, (2005) states that it is not practical, for example in the instance of bioaugmentation, to tailor bacteria for each site both in terms of financial and time constraints but bioremediation should not be exploited without an appreciation of the underlying mechanisms. Following implementation of bioremediation, detailed monitoring is required to follow the progress of the bioremediation and a contingency plan should be made to implement in the instance of failure.

RAW Consulting is investing in research to combine bioremediation with other engineering techniques, such as electrokinetics, in order to overcome some of the shortfalls of in-situ bioremediation that are frequently observed. It is recognised that bioremediation can offer a cost-effective, efficient solution when used as a hybrid process, combining both biological and engineering based technologies. Such as the incorporation of engineering techniques e.g. permeable reactive barriers (PRBs), to impede migration of contaminants during bioremediation. Research is also being carried out by the authors to develop bioremediation products that will increase remedial timescales for *in-situ* and *ex-situ* bioremediation further increasing the successful application of this technology.

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