Assessing the Potential for *In Situ* Biological Remediation

Gregg Williams (Business Development Manager)
EnviroGene Ltd , Tredomen Innovation and Technology Centre
Tredomen Business Park, Ystrad Mynach, Hengoed CF82 7FQ
Email: gregg.williams@envirogene.co.uk
Telephone: 08452 584368
Mobile: 07809 496383 • Fax: 01443 819052

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Monitored Natural Attenuation (MNA) - Allowing natural processes such as microbial degradation to remove pollutants, offers a low cost, sustainable method to treat large diffuse plumes of organic pollutants with minimal disturbance. For example, using a natural attenuation approach to remediate a site contaminated with petroleum might cost less than a tenth of the cost of excavation to landfill (*Table 1*). An additional benefit is that the approach does not require a fleet of trucks driving through the local area to dispose of the contaminated soil. Following implementation of the EU Landfill Directive (1999/31/EC), *in situ* approaches are being used more and more frequently as there are fewer landfill sites licensed to take hazardous wastes, making landfill an expensive and difficult to source option. Bioremediation can also be used in conjunction with more active treatment options such as soil heating, soil vapor extraction, chemical oxidation and "pump and treat" to provide a final polishing stage. As the concentrations of pollutants decrease, it becomes increasing uneconomical to use these active systems. Switching off the active system as the asymtrophic point is reached and relying on biological process to continue to bring the contaminant concentrations down below the final desired compliance level can save a considerable amount of cost and effort.

Technique	Cost in Euros per m³ treated
Off site incineration	885
Off site landfill	231
In situ immobilization	128
Off site biological treatment	167
On site soil washing	116
In situ enhanced bioremediation	73
In situ Monitored Natural Attenuation	20

Table 1. Comparison of the costs of various treatment options.

Unfortunately, biological processes are not always suitable, particularly where high concentrations of pollutants are present or if the site needs to be remediated quickly. The rates of degradation may be low if the indigenous bacteria are not active under the prevailing conditions. The key to predicting whether natural process will be able to reduce contaminant concentrations over a suitable time frame is to confirm whether there are bacteria present that are able to degrade the pollutants and whether they are active under the conditions within the site. If the current conditions do not support a suitable bacterial activity it may be possible to enhance bioremediation by adding nutrient or modifying the conditions in the site to promote the growth of degradative bacteria.

In general MNA will only be considered an acceptable approach where the contaminant plume is shrinking or stable and where there is no significant risk of impact to receptors. Most authorities require lines of evidence to support an MNA approach which include:

Historical contaminant data to demonstrate a trend of reduced concentration down-gradient of the source.

Geochemical and biochemical indicators which demonstrate the natural processes that are resulting in reduction in contaminant concentration.

Microbiological data to support the occurrence of biodegradation.

"Guidance on the Assessment and Monitoring of Natural Attenuation of Contaminants in Groundwater" R&D publication 95 available online from the UK Environment Agencies web site.

New molecular tools based on analysis of DNA and lipids extracted from samples are now available that can now be used to identify exactly what organisms are present and whether or not they have the ability to degrade the pollutants. For example, a technique known as quantitative polymerase chain reaction (qPCR) can be used to measure the numbers of bacteria or specific catabolic genes involved in the break down of pollutants. The technique is highly specific and can distinguish between different groups of bacteria such as ammonia oxidizers, sulphate reducers, methanogens and specific organisms such as *Geobacter sp.* which are known to degrade organic pollutants. Some common gene assays are listed in *Table 2*.

The microorganisms degrading pollutants in contaminated aquifers tend to grow in biofilms attached to submerged surfaces within the aquifer and are not easily sampled. A novel technique that has recently been developed to overcome many of the limitations of groundwater samples is to use BioTrap® sampling devices. These are based on similar principles to passive diffusion samplers and can be placed directly in a sampling well to collect microbes over time. The key to the Bio-Trap® sampling approach is contained in the unique properties of the Bio-Sep® beads which are used as the sampling matrix. The beads are 2-3 mm in diameter and are engineered from a composite of Nomex and powdered activated carbon (PAC). The adsorption capacity of the PAC allows nutrients and contaminants present within the aquifer to be collected onto the bead matrix and the beads provide a large surface area (~600 m²/g) for the microbes to colonize. The Bio-Traps® are normally deployed in a ground water monitoring well and incubated for between 30 to 90 days, depending on the conditions within the site, to allow the formation of a mature biofilm. The traps are then removed and DNA and microbial lipids can be extracted from the beads and analysed. These samplers integrate the microbial response over time and provide a way to compensate for the inherent variability of groundwater samples.

Figure 1. Bio-Traps® samplers available from EnviroGene Ltd, allow the collection of active microorganisms that form biofilms on submerged surfaces.

Bioremediation of Chlorinated Solvents – A case study

Bio-Traps® in conjunction with qPCR assays can be used confirm whether the indigenous microbial population will degrade pollutants, predict and monitor the impact of amendments on the microbial community and monitor changes in the microbial community as remediation proceeds. For example, Bio-Traps® were used to investigate the potential for *in situ* bioremediation at a dry cleaners site that had been contaminated by spills of chlorinated solvents including tetra

Assay	Application
	Catabolic genes
qNAPD	Napthalene dioxygenase indicative of bacteria able to degrade petroleum hydrocarbons and PAHs
qTOLD	Toluene dioxygenase dioxygenase indicative of bacteria able to degrade BTEX
qHCM	Indicative of organisms able to degrade MTBE
qVCR	Vinyl chloride reductase indicative of organisms able to dehalogenate chlorinated solvents
	Bacterial groups
qEubac	Total bacteria
qAOB	Ammonia oxidizers
qIRBSRB	Sulphate reducers
qMGN	Methanogens
	Specific organisms
qGeo	*Geobacter sp.* – (known petroleum hydrocarbon / BTEX degrader)
qPM1	PM1 - known MTBE degrader
qDHC	*Dehalococcoides sp.* known to dehalogenate chlorinated solvents
qDHB	*Dehalobacter sp.* known to dehalogenate chlorinated solvents

Table 2. Examples of qPCR assays commonly used to measure microbial populations.

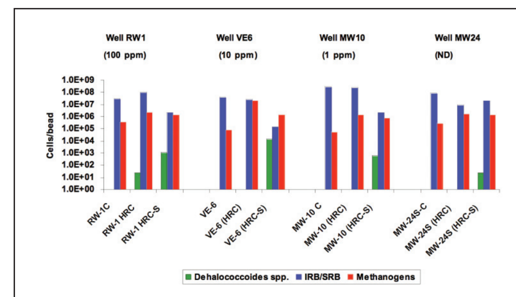

Fig 2. Use of Bio-Trap® samplers loaded with HRC or HRCS to choose the optimum compound for injection to stimulate Dehalococcoides sp. (DHC) in a PCE contaminated aquifer. Data provided by Microbial Insights, USA.

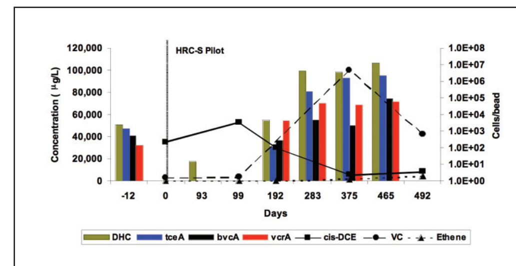

Fig 3. Monitoring the effect of HRC-S injection on the population of Dehalococcoides spp and the concentration of breakdown products resulting from reductive dehalogenation. Data provided by Microbial Insights, USA.

chloroethene (PCE). Chlorinated solvents are toxic, sparingly soluble and denser than water. The spilled solvents sink through the aquifer to form pools of dense non-aqueous phase liquids (DNAPL) that can be difficult to treat and slowly leach into the groundwater. Under anaerobic (oxygen free) conditions these compounds can be attenuated by a natural process called reductive dehalogenation in which the chlorine is progressively removed in the pathway below to produce ethene which is non-toxic and not considered an environmental pollutant.

Tetrachloroethane (PCE) -> Trichlorothane (TCE) -> cis-Dichloroethane (DCE) -> vinyl chloride (VC) -> ethene

Several bacteria such as *Dehalobacter sp.* and *Desulphitobacterim sp.* can reductively dehalogenate PCE and TCE but only *Dehalococcoides sp.* is known to complete the process by dehalogenating vinyl chloride to ethene. The numbers and activity of *Dehalococcoides* is often the critical factor determining whether chlorinated solvents will attenuate over a reasonable time frame. If *Dehalococcoides* is not detected in the site, or suitable anaerobic conditions are not present the process will probably either not occur or stall at vinyl chloride.

Due to the location of the site *in situ* bioremediation was preferred to excavation. Unfortunately, monitoring of contaminant concentrations in groundwater suggested biological breakdown was not occurring. Four wells with different PCE concentrations were choosen for study with standard Bio-Traps® (without amendments). The analysis showed that *Dehalococcoides* was not initially present in any of the wells. Bio-Traps® loaded with various concentrations of either Hydrogen Releasing Compound (HRC) or Low Sulphate Hydrogen Releasing Compound (HRCS) were used to predict whether injection of these compounds would stimulate dehalogenation. It was found that whilst both HRC and HRC-S stimulated the growth of *Dehalococcoides* at high contaminant concentrations (100 ppm) but only HRC-S stimulated *Dehalococcoides* at the lower PCE concentrations (*Figure 2*). HRC-S was subsequently injected in a pilot study resulting in a significant increase in the population of *Dehalococcoides* and subsequent conversion of PCE through VC to ethene (*Figure 3*).

The pilot study was extended with the result that the site was successfully remediated within a two year period. Biotraps® and associated qPCR analysis as described in this article are available from EnviroGene Ltd. (www.envirogene.co.uk)