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# ENHANCED BIOREMEDIATION AS A COST EFFECTIVE APPROACH FOLLOWING THERMALLY ENHANCED SOIL VAPOUR EXTRACTION FOR SITES REQUIRING REMEDIAITON OF CHLORINATED SOLVENTS

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## ABSTRACT

Thermally enhanced bioremediation can be a more cost-effective alternative to full scale in-situ thermal treatment especially for sites contaminated with chlorinated solvents, where reductive dechlorination is or might be a dominant biological step.

The effect of Thermally Enhanced Soil Vapour Extraction (TESVE) on indigenous microbial communities and the potential for subsequent biological polishing of chlorinated solvents was investigated in field trials at the Western Storage Area (WSA) – RSRL (formerly United Kingdom Atomic Energy Authority - UKAEA) Oxfordshire, UK.

The WSA site had been contaminated with various chemicals including mineral oil, chloroform, trichloroethane (TCA), carbon tetrachloride and tetrachloroethene (PCE). The contamination had affected the unsaturated zone, groundwater in the chalk aquifer and was a continuing source of groundwater contamination below the WSA.

During TESVE the target treatment zone was heated to above the boiling point of water increasing the degree of volatilization of contaminants of concern (CoC), which were mobilised and extracted in the vapour phase. A significant reduction of concentrations of chlorinated solvent in the unsaturated zone was achieved by the full-scale application of TESVE – In Situ Thermal Desorption (ISTD) technology. The rock mass temperature within target treatment zone remained in the range of $35^{o}$-$44^{o}$ C, 6 months after cessation of heating. The concentration of chlorinated ethenes and other CoC were found to be significantly lower adjacent to the thermal treatment area and 1 to 2 orders of magnitude lower within the thermal treatment zone. Samples were collected within and outside the thermal treatment zone using BioTraps® (passive, in- situ microbial samplers) from which the numbers of specific bacteria were measured using quantitative polymerase chain reaction (qPCR) methods of analysis. High populations of reductive dechlorinators such as *Dechalococcoides spp.* and *Dehalobacter spp.*, were found within the zone that was subjected to thermal remediation and moderate levels of *Dehalobacter sp* were found outside the treatment area. These results confirm dehalogenating bacteria are present within the site and suggest populations have bounced back following thermal treatment. The thermally treated zone showed a greater number of active indigenous bacteria – indicating that the conditions following TESVE treatment selectively promote the growth of desirable bacteria. This might result from elimination of micro-organisms competing for hydrogen as an electron donor, increased biovailability of CoC or a reduction in its inhibiting properties.

This paper aims to show the potential for biologically mediated contaminant reduction in assisting thermal remediation projects. During and post active thermal remediation this approach can help reduce total treatment costs by providing an inexpensive final polishing step or by being a complementary process within the perimeter of heated zone and inside hot-spots during the cool-down phase.

## INTRODUCTION

Microbiological activity can increase at elevated temperatures and consequently in-situ thermal remediation could potentially enhance the biodegradation rate of hydrocarbons. Thermally Enhanced Soil Vapour Extraction (TESVE) technology was adapted to remediate the residual contamination within the unsaturated zone at a former waste disposal site at Harwell,

Oxfordshire. Thermal conductive heating was utilized to bring the soil mass temperature of the unsaturated zone to the boiling points of contaminants of concern (CoC). The CoC identified within the contaminated area compromised a mixture of chlorinated aliphatic and aromatic hydrocarbons, including tetrachloroethene (PCE), trichloroethene TCE, (1,1 and cis1,2) dichloroethene (DCE), carbon tetrachloride and chloroform. The target temperature was engineered to be above the boiling points of main CoC and was in the range of 80-100$^{o}$C. In simple terms contaminants are boiled off in a controlled environment and extracted from the unsaturated zone of the aquifer by means of a vacuum extraction system. A vacuum was applied through an array of vertical wells concentrated around the Target Treatment Zone (TTZ) of the impacted area. Reaction kinetics are increased by the utilisation of thermal enhancement. This facilitates an increase in the contaminant mass removal rate which itself depends on various transport and removal mechanisms (USEPA 1997) including; chemical partitioning to the vapour phase, sorption of contaminant on soil/rock surfaces, gas advection, contaminant vapour diffusion and biodegradation.

Figure 1 summarises the changes in physical properties that occur during heating for water, TCE, and PCE. While chlorinated solvent density, viscosity, surface tension, and solubility varies slightly, vapour pressure and Henry's law constants increase dramatically with temperature.

Drying of the soil/rock mass during thermal enhancement increases the air permeability and therefore, particularly in relatively low permeability soils, increase the process of transport of contaminant vapours by gas advection as air is drawn through the soil by vacuum extraction.

Other mechanisms include enhanced dissolution, hydrolysis and aqueous phase oxidation. However, vaporisation is dominant for most chlorinated solvents.

In circumstances where non aqueous phase liquid (NAPL) is present, the combined vapour pressure of the NAPL and pore water determine the boiling temperature and co-boiling of the two liquids occurs at temperatures less than the boiling point of water. The concentrations of hydrocarbon contaminants in vapour extracted from a thermal treatment zone will increase with temperature. The boiling temperature of a liquid mixture is the temperature at which its total vapour pressure is equal to atmospheric pressure. Since the total vapour pressure is the sum of partial pressures of all of the components of the mixture, the boiling point of the mixture (eutectic point of the azeotropic mixture) can be achieved at a lower temperature than any of the boiling points of any of the separate components. This phenomenon is called co-distillation. The implication for TESVE is that many contaminants can be easily removed in vapour at steam temperature, even if their boiling temperatures are greater than 100$^{o}$C. Table 1 below illustrates selected NAPL compounds and steam co-distillation points (UFC 2006).

In situations where concentration gradients are present between pores under vacuum and within an air stream but contaminants are also present away from the flowing air, contaminants will move by diffusion towards the air stream. Gas phase diffusion is generally much slower than advection in relatively low permeability strata and is often a significant performance limiting factor. In such situations increasing the temperature enhances the rate of vapour transport from low permeability zones to areas of higher permeability. Furthermore steam produced from the boiling of pore water during thermal enhancement and the steam's pressure-driven flow to zones of high vapour flow and will mobilise contaminant vapours from low permeability zones at a much higher rate (Heron et al 2005).

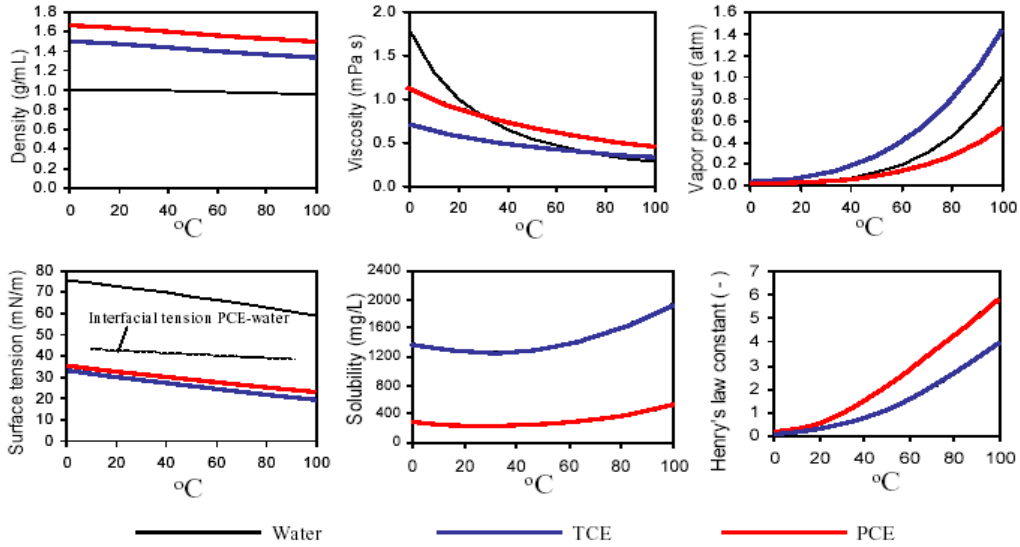


**Figure 1: Properties of water, PCE and TCE as a function of temperature (Heron et al, 2008)**

**Table 1: Boiling Points and Co-Distillation Points of selected NAPL compounds (UFC, 2006).**

| NAPL mixture | Component Boiling Points (°C) | Co-Distillation Point (°C) Eutectic point |
|---|---|---|
| Carbon Tetrachloride<br>Water | 76.8<br>100 | 66.8 |
| Chloroform<br>Water | 61.2<br>100 | 56.3 |
| 1,2 Dichloromethane<br>Water | 83.5<br>100 | 72 |
| 1,1,2 Trichloroethane<br>Water | 113.7<br>100 | 86.0 |
| Trichloroeethene<br>Water | 87.1<br>100 | 73.1 |
| Tetrachloroethene<br>Water | 121<br>100 | 88.5 |

The high temperature environment present during in-situ thermal remediation can also potentially be an inhibiting factor to several species of micro-organisms but at the same time create more favourable conditions for several organisms. There is also a possibility that, further biodegradation of by-products of thermal destruction and breakdown of semi volatile organic hydrocarbons (SVOCs) occurs during and following thermal treatment (UFC, 2006). The temperature has a decisive influence on the biological activity within the impacted area. Microbial degradation rates have an optimum temperature range between 30 and 40°C for most organisms (UFZ, Ulf Roland), especially those responsible for anaerobic breakdown of chlorinated hydrocarbons. The treatment of solvent contaminated areas (pits) during few subsequent years with the application of TESVE technology have demonstrated the high heat capacity of the rock mass and the elevated temperature within the range of 35°-44°C some six months after cessation of heating at the perimeter of the thermally targeted zone. Where conventional soil vapour extraction was undertaken in the different areas, previously subjected to thermal treatment, the VOCs concentration detected within the off gas stream were lower than those detected at the last phase of the cool down period. The continuous assessment of groundwater quality within monitoring wells located within and outside the immediate zone of thermal influence also showed a descending trend in the concentrations of chlorinated ethenes detected.

Chlorinated ethenes can be degraded under anaerobic conditions in the presence of a suitable electron donor through reductive dechlorination. This process is a dechlorination reaction catalyzed by micro-organisms in which a chlorine atom is replaced by hydrogen (the electron donor) on an organic compound. PCE, which contains four chlorine atoms, is degraded sequentially from PCE to TCE to DCE to vinyl chloride (VC) to ethene. Micro-organisms capable of biodegrading chlorinated solvents in oxygen deficient (anaerobic) environment and high redox potential conditions include *Dehalococcoides sp.* and *Dehalobacter sp.* However *Dehalococcoides spp.* is the only organisms known to be able to convert VC to ethane. Micro-organisms capable of biodegrading chlorinated solvents in oxygen deficient (anaerobic) environment and high redox potential conditions include *Dehalococcoides sp.* and *Dehalobacter sp*

It was therefore proposed to undertake a programme of groundwater sampling and analysis at the site to evaluate the extent of microbiological activity and assess changes that occur to the microbiological population prior, during and post the thermal treatment process. One of the objectives of the research was to compare the quality of groundwater within the treatment zone and outside the targeted treatment zone; especially with respect to the potential trends in chlorinated ethene concentrations. In addition to this the study aimed at applicability of thermally enhanced bioremediation as a complementary process to in-situ thermal remediation.

The study also aimed to establish the zone of influence of thermal remediation of the unsaturated zone on groundwater quality within the target treatment zone. This was undertaken by determining if the *Dehalococcoides* and *Dehalobacter* strains of bacteria were present within the site and therefore establishes the potential for microbiological reductive dechlorination, both within and outside the thermally targeted treatment zone.

## MATERIALS AND METHODS

**Site description** The study is located in Western Storage Area (WSA) located at a RSRL site. The Western Storage Area site occupies an area of approximately 1Ha at RSRL, Harwell. The site was originally licensed under the 1974 Control of Pollution Act. Twenty five unlined pits excavated into the Chalk to a maximum depth of 4m below ground level (bgl) at the WSA (Figure 1) were used for the disposal of various untreated chemical wastes including chlorinated solvents and other organic chemicals. (Figure 2). The chlorinated solvents were disposed between 1970 and 1977 with later arisings being bulked up and sent for incineration off-site. The site is now licensed as a closed landfill by the Environment Agency. The disposal of materials in these pits has caused chemical contamination of groundwater in the Chalk aquifer and was a continuing source of groundwater contamination below the WSA. Following the discovery of groundwater contamination in late 1989, a programme of work was implemented to delineate, contain and then remediate the groundwater contamination and its sources. Site investigations have confirmed a diverse range of chemicals, including mineral oils, chlorinated hydrocarbons (including chlorinated solvents and PCBs), pesticides and metals in the chalk at the site. A major ground contamination investigation at the WSA documented the presence of VOCs in chalk pore water samples collected from cored boreholes adjacent to the WSA disposal pits. Non-aqueous phase liquids (NAPL) containing percent levels of chlorinated solvents were also detected as oil films on fissures in the chalk. Light NAPL has been identified periodically in groundwater monitoring wells in the vicinity of the disposal pits. The primary source material, which comprised laboratory waste and vessels which contained the solvents, was excavated and removed from the pits in 2004 to a depth of approximately 150mm below the original depth of the pits. The pits were

backfilled with granular subsoil and a bentomat layer was installed approximately 1m below ground surface. To date four chemical disposal pits underwent final or partial thermal remediation. Pit No. 3 and 18 underwent complete thermal treatment This research programme was mainly undertaken within the vicinity of Pit No. 18 thermal zone of influence area, however initial investigation was also conducted at Pit No.3.

**Stratigraphy and Hydrogeology** The WSA site is underlain by the Grey Chalk Subgroup (formerly essentially known as 'Lower Chalk') of the Cretaceous Chalk Group. The Grey Chalk strata comprised interbedded layers of moderately weak, moderately strong and strong, locally weak and very strong light grey argillaceous chalk with variable clay content. The rock matrix itself has a relatively low effective permeability but high porosity (25%) and retains pore water The Chalk also has a secondary porosity of 1 to 2% of the volume of the rock made up of fractures and rubble zones (highly fractured layers). Some of these fissures in the rock are highly permeable to fluid flow. A relatively soft weathered chalk is present to a depth of approximately 6 to 8m bgl and a laterally extensive horizontal layer of chalk marl at a depth of 30m forms the base of the unconfined Chalk aquifer. The unconfined aquifer has an average estimated transmissivity of 600m$^2$day.
Groundwater elevation data collected by RSRL since 1991 indicate that groundwater levels in fissures vary seasonally with significant recharge occurring in the winter. The water level also varies year to year due to drought or exceptionally wet conditions, with a historical range of approximately 18m from 5 to 23m bgl (115 to 97m AOD). The groundwater flow direction in the unconfined Chalk aquifer at the WSA is to the north and north east during periods of high groundwater level and to the east and south east during times of low groundwater level. Groundwater flow in chalk fractures in the WSA is controlled by a series of abstraction wells arrayed around the perimeter of the WSA from the south east to the north.

**Mesophilic Bacteria Enumeration.** In order to identify and compare the populations of micro-organisms present in groundwater adjacent to and outside the effective thermal treatment zone at Pit No.18, a "Total Viable Colony Count" (TVC) was undertaken on groundwater samples collected before, during and after thermal remediation. The samples were taken from wells located immediately adjacent to the target treatment zone. The TVC count by pour plate method was applicable for the enumeration of viable aerobic micro-organisms in all types of water. The target count was in the range of 0 to 300 organisms per ml of water when 1 ml sample volumes were analysed. Three reference temperatures were applied to count the mesophilic, viable colonies at 25°C, 30°C and 37°C. Most bacteria capable of growth in potable water and natural surface waters in temperate climates will grow better in culture media at 25°C than at higher temperatures. Organisms that grow best at 37°C usually grow less readily in groundwater and are likely to have gained an external source of nutrients or co-metabolites (such as biodegradation by-products). The three groups of organisms were counted separately and used to assess the general quality of groundwater and give an indication of microbial activity occurring in the aquifer.

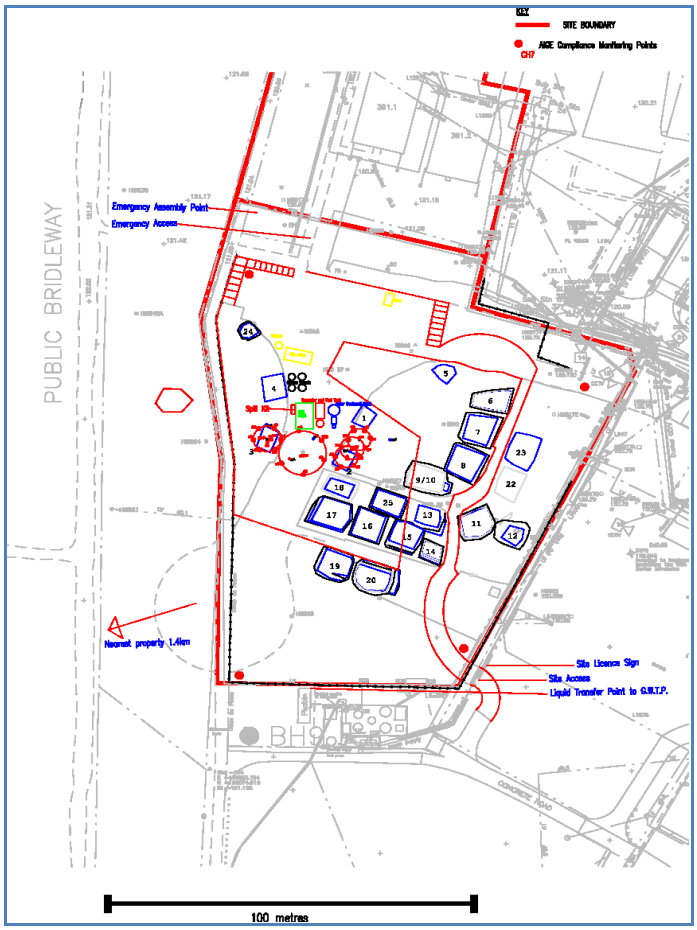


**Figure 1: WSA site layout.**

**Figure 2: Example of a waste disposal pit.**

**Thermophilic Bacteria Enumeration.** Bacteria enumeration at different temperature ranges was performed to identify and compare the temperature tolerance of micro-organisms living in groundwater adjacent to and outside thermal target treatment zone. This process also included the enumeration of biologically active micro-organisms that potentially take part in the biodegradation processes and exclude vegetative biologically inactive forms.

The differentiation between aerobic and anaerobic thermophilic species was important in defining and specifying the consortia of colonies being active in elevated temperature ranges and their particular oxygen demands needed for metabolic processes.

Further analysis of the groundwater samples was undertaken to enumerate thermophilic spores and thermophilic vegetative cells, as below.

The enumeration of thermophilic spores and vegetative cells present in the groundwater samples was undertaken through the following methodology:

*Thermophilic Vegetative Cells Enumeration* – TVC by the Pour Plate Method at 55°C – gives a total number of microorganism – survivals of the high temperature incubation including micro-organisms that might not be biologically active.

*Thermophilic Aerobic Spores Enumeration* – The biologically active aerobic spores are quantified by a Pour Plate Method. The aerobic vegetative cells present in the sample are thermally destroyed, during the incubation and sample preparation procedure, which concurrently activates the thermophilic spores. The sample is distributed between molten medium tubes. The temperature of the molten medium is not less than 80$^{o}$C. The inoculated tubes are placed afterwards in a steamer at approximately 100$^{o}$C for 120 minutes, and allowed to set for 30 minutes, followed by incubation at 55-60$^{o}$C for 72-96 hours. The number of tubes is counted in which the growth occurred, which is normally observed as gas bubble in the medium.

*Thermophilic Anaerobic Spores Enumeration* – The anaerobic vegetative cells present in the sample are thermally destroyed, during the incubation and sample preparation procedure, which is undertaken in an oxygen deficient environment, which concurrently activates the anaerobic thermophilic spores. The biologically active thermophilic anaerobic spores are identified and enumerated.

**Dehalococcoides and Dehalobacter Species Identification.** Reductive dechlorinators bacteria cannot be quantitatively detected by traditional culturing techniques. Therefore the Quantitative Polymerase Chain Reaction (qPCR) method was applicable to identify the presence of specific DNA sequences of *Dehalococcoides spp*. and *Dehalobacter spp*. species within the collected sample and enabled their enumeration by the introduction of fluorescent dyes in the PCR reaction. The functional genes encoding VC and TCE reductases were also targeted for qPCR scanning. Analysis for methanogens, sulphate and iron-reducing bacteria was additionally conducted in order to investigate the preferential pathways for deriving electron donors for metabolic activities. Groundwater samples obtained from investigated boreholes and BioTrap® (Figure 3) in-situ passive diffusion sampling devices were utilized for DNA extraction and qPCR analysis. The BioTrap® is a cartridge filled with 2-3mm diameter beads engineered from a composite of aramid polymer and powdered activated carbon (PAC). The adsorption capacity of PAC allows nutrients and contaminants present within the aquifer to be collected onto the bead matrix. The beads provide a large surface area (~600 $m^2/g$) for the microbes to colonise over a longer period of time and therefore deliver more acclimatised microbial population. The BioTrap® sampler was deployed in a groundwater monitoring well at the depth 2 to 4m below the water table for the period of 30 days to allow growth of bacteria colonies. The groundwater samples for DNA extraction and qPCR analysis were sampled only on one occasion, in order to provide the final post remedial results.

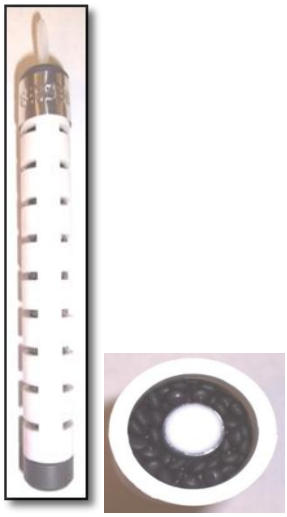

**Figure 3: BioTrap® Sampler**

**Groundwater chemical testing.** Standard Gas Chromatography Mass Spectrometry (GC-MS) chemical testing for volatile organic hydrocarbons (VOCs) and semi volatile organic hydrocarbons (SVOCs) concentrations was performed on groundwater samples collected outside and adjacent to the thermally enhanced target treatment zone. Additional analytical testing was recommended to support the microbiological interpretation, these were:

*Biological Oxygen Demand (BOD)* – biodegradability test, showing the oxygen consumption rate as an indication of degradation potential of substance by oxidation process. *Chemical Oxygen Demand (COD)* – chemical oxidation test, showing the mineralisation potential of contaminants within groundwater. During the test the consumption of dichromate in a sulphuric acid solution is measured. The consumed dichromate amount is equivalent to amount of oxidizable carbon within the sample. The biodegradation potential of the contaminated groundwater was interpreted using the COD to BOD ratio. Table 2 below defines the COD to BOD quotient interpretation.

**Table 2: COD to BOD ratio interpretation (Sontheimer)**

| Ratio | Interpretation |
|---|---|
| **COD/BOD ratio <1.7** | Indicates very good biodegradability potential. |
| **COD/BOD5 ratio between 1.7 and 10** | Indicates moderate biodegradability potential. |
| **COD/BOD5 ratio >10** | Indicates very low to non-biodegradability potential due to slow microbial acclimatisation, non-biodegradable substances, inhibition due to toxic substances. |

In-situ groundwater field measurements were conducted using a HI 9828 Hanna Mulimeter Probe and included the following parameters: pH, red-ox, dissolved oxygen (DO), electrical conductivity (EC) and temperature (T).

The lateral and vertical temperature variation within the rock mass in the vicinity of Pit No.18 was monitored by means of thermocouples connected to a digital thermometer. Thermocouples were installed within the designated extraction wells (Figure 4 below) at three elevations (5m, 10m, and 15m bgl) within the centre of Pit No. 18 (18I and 18E), within the eastern boundary (18A) and extending horizontally 4m (17C) from the northern boundary of Pit No.18). The thermally targeted zone (TTZ) was between 5m and 15m bgl, hence the temperature monitoring points were installed to demonstrate the rock mass temperature cross-section.

**Sampling location.** The groundwater monitoring and sampling programme was undertaken within the immediate vicinity of thermally enhanced zone of Pit No. 18, from wells 17A, 17B and 17C (as depicted on the Figure 4 below) In addition monitoring and sampling was also undertaken from the standard groundwater monitoring borehole (HWS27) located outside the zone of direct thermal influence, however in close vicinity to 18M extraction well. The BioTrap® samplers were immersed during the course of the remediation phase at Pit No. 18 in wells: 17A, 17B and 17C. Subsequent temperature monitoring was also undertaken in these locations.

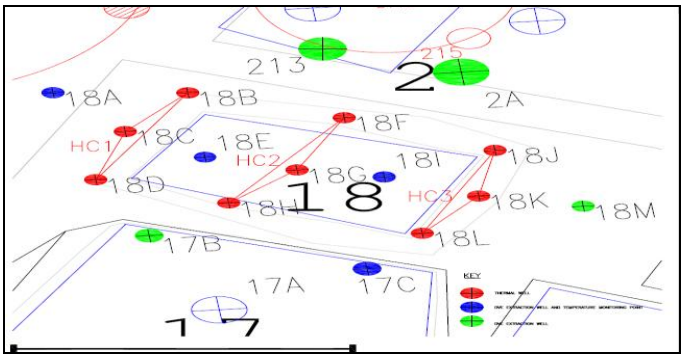


**Figure 4: Pit 18 layout.**

Location of the microbiological sampling points. Red circles indicate heater well location(18B, 18C, 18D, 18F, 18G, 18H, 18J, 18K, 18L); green circles indicate the vapour extraction wells (213, 2A, 17B, 18M); and blue circles indicate the vapour extraction and temperature monitoring wells (18A, 18E, 18I, 17C).

Comparative groundwater analysis was carried out on samples from well SVE1, located within the central point of Pit No.3, which was subjected to thermal in-situ remediation during the initial remediation phase at WSA. The potential of enhanced attenuation was primarily observed at this location. The SVE1 well location, which served as a vapour extraction well, surrounded by thermal wells and temperature monitoring points is presented on Figure 5 below.

The BioTrap® sampler was deployed in SVE1 well following completion of the thermally enhanced treatment. The groundwater monitoring was also completed from standard monitoring borehole (HWS58) located outside of the zone of direct thermal influence, however in close vicinity of Pit No.3.

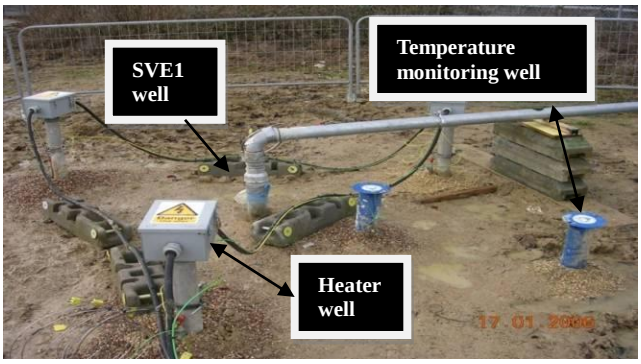


**Figure 5: Pit 3 and SVE1 well location.**

## RESULTS AND CONCLUSIONS

**Temperature Tolerance of Microorganisms.** The difference in microbial populations recorded during the course of thermal remediation was investigated at Pit No 18. Table.3 below presents the results of thermophiles and mesophiles species enumeration for wells: 17A, 17B, 17C and HWS27 investigated within the fourth month period, prior to and during thermal treatment at Pit No.18, referenced as time: T1 to T5. The temperature of the rock mass within the centre (18I) and on the northern boundary (17C) of TTZ, compared against the groundwater temperature at the same monitoring location (17C) corresponding to the ground water levels are plotted on Figure 6 below. Groundwater samples taken from wells: 17A, 17B and 17C located within the close vicinity of the thermally enhanced zone showed similar levels of mesophilic viable colonies prior to commencement of the heating process, when compared to HWS27, located outside the effective radius of thermal influence (T2, Table 3). An ascending trend in thermo-tolerant species (thermophiles) was observed within the groundwater samples taken during the course of thermally enhanced phase, when the groundwater temperature achieved on average 19 -30 $^{o}$C (T3 to T5, Table 3). The mesophilic colonies also showed incremental trend within wells 17A and 17B and were approximately 2 orders of magnitude higher than those recorded at HWS27, at the final stage of thermal enhancement.

The temperature profile showed a similar trend for both groundwater and rock masses recorded at 15m depth (17C-M rock and 17C-GW series, Figure 6 below). The groundwater levels (dashed line, Figure 6) represented asymptotic, but reverse trend to the groundwater temperature profile.

**Table 3: Thermophiles and Mesophiles Enumeration: Wells 17A, 17B, 17C and HWS27**

| Sampling Time | T1 (30/07/09) | | T2 (13/08/09) | | | T3 (08/09/09) | | | T4 (07/11/09) | | | T5 (20/11/09) | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling period | unenhanced SVE | | unenhanced SVE - final | | | TESVE initial | | | TESVE middle | | | TESVE final | | |
| Sampling ID | 17B | 17C | 17B | 17C | HWS27 | 17B | 17C | HWS27 | 17A | 17B | HWS27 | 17A | 17B | HWS27 |
| Type of micro - identification | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g | cfu/g |
| TVC 25°C Mesop. | 2.00E+05 | 2.30E+05 | 2.50E+06 | 9.00E+03 | 2.90E+04 | 2.10E+06 | 1.20E+05 | 3.50E+04 | 4.00E+05 | 1.30E+05 | 3.10E+04 | 1.00E+05 | 2.10E+06 | 9.90E+04 |
| TVC 30°C Mesop. | 1.90E+05 | 1.10E+05 | 3.00E+05 | 1.00E+03 | 9.00E+03 | 1.80E+06 | 4.70E+04 | 2.30E+04 | 4.00E+05 | 9.30E+04 | 2.90E+04 | 1.60E+06 | 1.80E+06 | 1.10E+05 |
| TVC 37°C Mesop. | 3.40E+04 | 8.00E+03 | 4.30E+04 | 1.40E+03 | 990.00 | 1.20E+06 | 1.10E+04 | 2.00E+03 | 7.70E+04 | 8.90E+04 | 3.00E+03 | 1.00E+06 | 1.20E+06 | 3.20E+04 |
| TVC 55°C Mesop. | <10 | 30.00 | 50.00 | <10 | <10 | 340.00 | 350.00 | 250.00 | 90.00 | 360.00 | 10.00 | 280.00 | 340.00 | <10 |
| Thermophilic Aerobic Spores 55°C | <1 | <1 | <1 | <1 | <1 | 100.00 | 577 | 583 | 95.00 | 225.00 | 13.00 | 101.00 | 100.00 | 19.00 |
| Thermophilic Aerobic Spores 60°C | <1 | <1 | <1 | <1 | <1 | 104.00 | 1332 | 1077 | 166.00 | 194.00 | 14.00 | 98.00 | 104.00 | 12.00 |
| Thermophilic Anaerobic Spores 55°C | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 1/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) |
| Thermophilic Anaerobic Spores 60°C | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 1/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 0/3 (Pass) | 2/3 | 1/3 (Pass) | 0/3 (Pass) |

Note:
Unit (cfu/g) - Colonies forming unit per gram of water sample analysed.

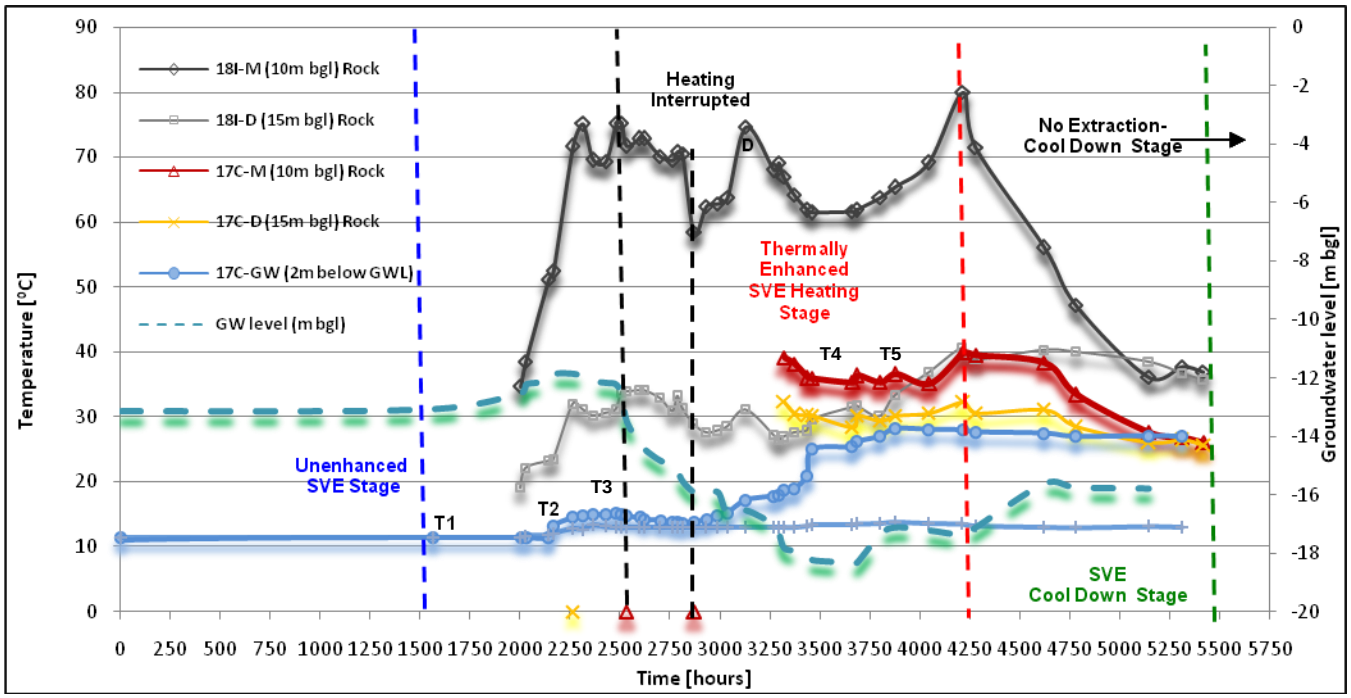


**Figure 6: Rock mass temperature, groundwater levels and temperature profile against time during remediation process at Pit No.18**
T1 to T5 points marked on the plot, indicate reference Time 1 – 5, when the groundwater sampling for microbiological analysis was undertaken. As referenced in Table 3 above.

**Dehalococcoides and Dehalobacter Species.** Quantitative PCR analysis of microbial DNA extracted from The BioTrap® samplers showed that the thermal treatment increased both total microbial populations and the population of reductive dechlorinators including *Dehaloccoides sp*. Moderate levels of total bacteria (qEubac) were detected in BioTrap® samples, deployed between June and July 2008 in wells SVE1 (within Pit No. 3, zone that has been subjected to thermal treatment) and 17B (at Pit No. 18 prior thermal treatment). Moderate to high levels of *Dehalobacter sp* were detected at both locations, *Dehaloccoides sp.* were not found in well 17B prior to TESVE, although the low levels of *Dehaloccoides sp.* were detected at SVE1, which had been previously been treated by TESVE. BioTrap®s subsequently incubated within well 17B during both the final stages of thermally enhanced SVE and the cool down stage did not indicate the presence of *Dehaloccoides sp*. However, an increased level of total bacteria (qEubac) was detected within the sample incubated during the cool down period. (Table 4). Further investigation was conducted in order to investigate the preferential pathways for deriving electron donors for microbiological activities. Results indicated the presence of both methanogens and sulphate & iron reducing bacteria. The presence of these organisms indicated suitable reducing conditions exist for the growth of reductive dechlorinators. Although they are known to inhibit reductive dechlorination by competing for available hydrogen; the levels of methanogens and sulphate reducers detected are unlikely to cause inhibition. (Table 4). Additional groundwater sampling from wells 17B and 17A, located within close vicinity of TTZ, was conducted some six months after cessation of heating at Pit No. 18. A BioTrap® sampler was deployed at the testing well 17B for a period of five months, to provide a well-balanced, in-situ colonised trap. The increase in total bacteria (qEubac) was apparent following the cool down phase; and low levels of both *Dehaloccoides sp.* and *Dehalobacter sp.* were detected at both locations 17A and 17B. However the analysis for the functional genes encoding VC and TCE reductases did not show any concentrations above the limit of detection.

These results confirmed that the dehalogenating bacteria are present within the investigated area and that both locations within the vicinity of Pit No. 3 and 18 harbour an active population of indigenous bacteria

The increase in *Dehaloccoides sp*. and *Dehalobacter sp* following thermal treatment indicates that thermal enhancement creates more favourable conditions for dehalogenating bacteria to adapt and potentially reduces the number of well established colonies competing for available electron donors.

It is also important to emphasize that the dehalogenating bacteria belong to an anaerobic group of organisms; therefore they establish best in oxygen deficient environments. The utilisation of vacuum extraction system, normally used in conjunction with thermal treatment, to remove the volatilised organic chemicals, may also be an inhibiting factor for *Dehaloccoides sp.* and *Dehalobacter sp* to develop undisturbed. The thermophiles and mesophiles TVC enumeration by the Pour Plate Method results (Table 3 above) also indicated that dominant species detected within the groundwater samples are aerobic organisms.

It is anticipated that the low levels of *Dehaloccoides sp.* detected would not be expected to support a high rate of reductive dechlorination and the nutrient enhanced conditions may effectively stimulate the reductive dechlorination following thermal treatment.

**Groundwater chemical testing results.** Sequential transfer from PCE to DCE was apparent for all sampling points located within the direct zone of thermal influence at well 17B and for groundwater samples taken from well HWS27 located outside the immediate vicinity of the thermally enhanced zone. The graphical representation of dechlorination trend is depicted on Figure 6 and 7 for well 17B and HWS27 respectively.

Groundwater sampling was undertaken from well HWS27 before and during thermal treatment and six months following the final remediation works at Pit No.18. The accelerated kinetics of the dechlorination reaction was observed during the thermal and cool down phases. Dichloroethenes were accumulated as the end product of reductive dechlorination, and no further transformation to VC occurred. The substantial reduction of PCE and TCE was evident for all sampling locations. The dechlorination reactions within the zone of direct thermal influence was potentially driven by thermal destruction and desorption processes, whereas the driving force for reductive dechlorination was most likely an effect of microbiological activity of reductive dechlorinators. .

**Table 4: Dehalococcoides, Dehalobacter, Methanogens, Sulphate and Iron Reducers spp, DNA qPCR results.**

| Incubation/Sampling Period | Well | Total Bacteria (qEuBac) Copies/bead | Dehalococcoides sp. (qDHC) | | Dehalobacter sp.(qDHB) | | Methanogens sp.(qMGN) | | Sulphate & Iron Reducers (qIRBSRB) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Copies/bead | % of total qEubac | Copies/bead | % of total qEubac | Copies/bead | % of total qEubac | Copies/bead | % of total qEubac |
| 28 June – July 2008 | SVE1 | 9.04E+06 | 7.7E+02 | 0.009% | 1.3E+04 | 0.14% | - | - | - | - |
| | 17B | 6.84E+06 | <1E+02 | Not detected | 1.19E+04 | 0.17% | - | - | - | - |
| 20 Nov - 20 Dec 2008 | 17B | 4.24E+06 | <1E+03 | Not detected | 3.36E+03 | 0.08% | 3.59E+04 | 0.847% | 5.64E+03 | 0.08% |
| 20 Dec 08 – 22 Jan 09 | 17B | 6.08E+06 | <1E+03 | Not detected | <1E+03 | Not detected | - | - | - | - |
| 22 Jan – 30 May 09 | 17B | 1.78E+06 | 1.49E+03 | 0.0837% | 1.18E+01 | 0.0007% | - | - | - | - |
| 30 May 09 GW sample | 17B | 6.65E+07 | 9.41E+03 | 0.0142% | 6.41E+03 | 0.00965 | - | - | - | - |
| 30 May 09 GW sample | 17A | 1.43E+07 | 1.58E+03 | 0.0110% | 6.19E+03 | 0.0433% | - | - | - | - |

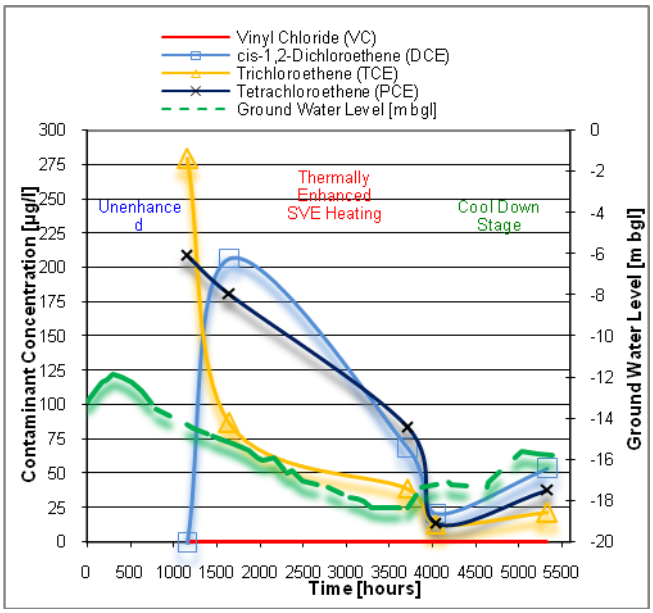


**Figure 7: Groundwater chlorinated ethenes concentration trend for well 17B (located within TTZ).**

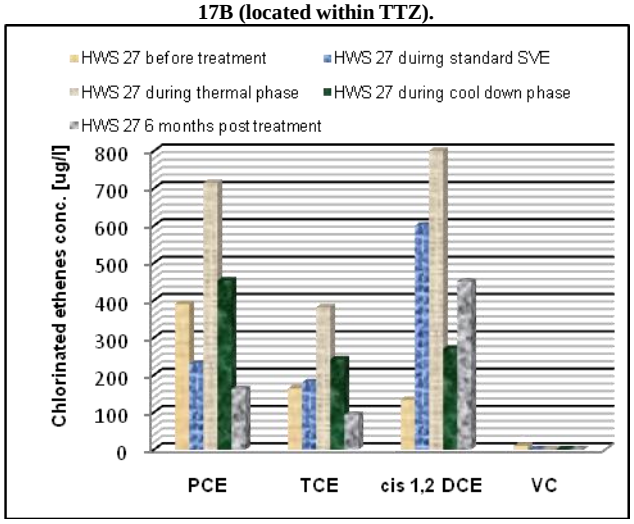


**Figure 8: Groundwater chlorinated ethenes concentration trend for well HWS27, before during and following thermal remediation at Pit No.18.**

Based on the COD to BOD ratio interpretation, the biodegradability potential for groundwater sampled from wells 17B and 17C located within the immediate vicinity of the thermally enhanced zone have increased following the thermal treatment, (Table 5). However the most significant change in biodegradability was observed for groundwater samples taken from well HWS27 located outside the zone of direct thermal influence (Table 5). Samples taken during the initial phase of thermal enhancement showed very low or no biodegradability potential, indicating the presence of non-biodegradable substances and likely inhibition due to toxic chemicals. The COD/BOD ratio at the end of the thermal enhancement phase was approximately 8, which is classified as a moderate biodegradability potential of groundwater.

## ECONOMIC CONSIDERATIONS

**TESVE versus Thermally enhanced Monitored Natural Attenuation (TEMNA).** It is important**,** that the cost analysis is compared on a "like-for-like" basis achieving a similar level of environmental performance. A realistic cost of implementation of TESVE technology for any potential site will vary depending on the nature of CoC and treatment volume. The volumetric cost is highly dependent on the type of contaminants present, which will directly influence the level of target treatment temperature to achieve the desired remediation effect. Consequently this will be reflected in the amount of energy consumed per volume of material undergoing thermal remediation. The thermally enhanced scale and configuration of the treatment volume are two important factors, which have an impact on the economic feasibility of TESVE technology.

The most significant cost of a TESVE project is the capital cost associated with power supply and installation of TESVE units and array of equipment, which includes stainless steel heater rods, carbon steel casings, TESVE control panel, cabling, thermocouples, design and installation. TEMNA has the advantage that a significant increase in the degradation rates can be achieved even with a relatively small energy input (heating up to about 35-40$^{o}$C). Therefore costs associated with fuel/energy consumption can be substantially reduced, since the duration of the heating will probably be limited to approximately a two week period, nevertheless the initial cost associated with installation of TESVE units still have to be taken into consideration.

**Table 5: COD to BOD ratio for groundwater samples detected within wells: 17C, 17B and HW**

| Sample ID | Well 17C | | | Well 17B | | | | HWS 27 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Sampling Date | 13/8/08 | 8/9/08 | 7/1/09 | 13/8/08 | 08/9/08 | 20/11/08 | 14/1/09 | 28/8/08 | 8/9/08 | 7/11/08 | 20/11/08 | 7/1/09 | 14/1/08 |
| **BOD** | - | - | 3.4 | 5 | 10 | 10 | 4 | 4 | 6 | - | 5 | 12.6 | 6 |
| **COD** | 0 | 0 | 15 | 20 | 18 | 120 | 16 | 20.2 | 33 | - | 40 | 6 | 17 |
| **COD/BOD** | 10 | 10 | 7 | 4 | 1.8 | 12 | 4 | 5.05 | 27 | - | 8 | 0.48 | 2.83 |

Operation and maintenance (O&P), which is typically the most significant cost for non-thermal remediation technologies, together with TESVE become more cost-effective due to a short duration of the TESVE project. The TEMNA will not normally generate an excessive O&P cost, however cost associated with the initial O&P of thermal plant and extended time for sampling and monitoring should be considered. Whereas items like commissioning/decommissioning, for both technologies, remain practically similar. Limited off gas treatment will still be required in the initial and final stage of TEMNA. However under ideal conditions a complete conversion of the hydrocarbons to carbons dioxide and water occurs, making cleaning of the off gas limited or unnecessary. Validation and testing is the least cost efficient item for TEMNA. The approximately 10-12 samples per visit and minimum 6 to 8 visits per annum would be required. The testing would involve an initial trial defining whether MNA is applicable in the investigated site, the cost of BioTrap® samplers, DNA extraction and analysis for qDHC, qDHB, qIRSRB and qMGN, chemical analysis of groundwater, site visits and monitoring.
Table 6 below illustrates the potential cost benefits that may be achieved from adoption of TEMNA over TESVE at a site.

**Table 6: Cost Comparison Matrix between TESVE and TEMNA technology implementation.**

| | Thermally Enhanced MNA | TESVE Technology |
|---|---|---|
| **PROJECT DURATION** | **120 weeks (2years)** | **15 weeks (3 months)** |
| **Fixed Cost** | | |
| 1. Mobilisation and demobilisation | ~£20,000 | ~£20,000 |
| 2. Installation | ~£30,000 | ~£60,000 |
| 3. SVE Equipment | ~£30,000 | ~£30,000 |
| 4. TESVE Equipment | £10,000 | ~£15,000 |
| 5. Operation and Maintenance | ~£30,000 | ~£30,000 |
| **Variable Cost** | | |
| 6. Waste GAC (Off Gas Treatment) | ~£800 | ~£3,000 |
| 7. Waste GAC (Condensate Treatment.) | ~£0 | ~£1,500 |
| 8. Energy Consumption | ~£5,000 | ~£38,000 |
| 9. Validation/Testing | ~£25,000 | ~£6,000 |
| **Tonnes Removed** | ~2-3 Tonnes | ~2.8 Tonnes |
| **Area Treated** | **~3000-3500m$^3$** | **~3000-3500m$^3$** |
| **TOTAL COST** | **£150, 800** | **£203,500** |

**Notes:**
**Project Duration-**project duration may vary depending on the CoC, the cost analysis study was conducted based on the treatment of VOCs in particular chlorinated solvent.
**Area Treated –** the radius of influence of TEMNA may potentially expand to larger volumes of soil/groundwater undergoing biological treatment.
**Tonnes Removed –** the mass removed with the utilisation of TEMNA may be difficult to be quantified, because the biodegradation occurs mainly in the dissolved phase.

## DISCUSSION

TESVE technology has been successfully utilised to remediate hydrocarbon contamination, located beneath a former licensed waste disposal site at the WSA.

The elevated temperature environment has effectively stimulated the microbial populations providing further reduction in chlorinated solvents through a reductive dechlorination process within the immediate vicinity and outside the effective zone of thermal influence.

The increase in *Dehaloccoides sp.* some six months post thermal treatment indicated that thermal enhancement created more favourable conditions for dehalogenating bacteria to adapt and reduced the number of well established colonies competing for available electron donors.

The sequential transfer from PCE to DCE was apparent following the thermal treatment and the accelerated kinetics of the dechlorination reactions was observed during the cool down phase and six months after cessation of the heating process , when the rock mass and groundwater temperature remained in the range of 35-40$^o$C.

The innovative, in-situ passive diffusion sampling device, BioTrap®, proved to be a robust microbial sampling tool, which allowed precise sampling of a well acclimatised and biologically active microbial population present in the real site environment.

The most advanced testing method in molecular biology – qPCR was applied to detect and quantify the presence of specific DNA sequences of *Dehalococcoides sp*. and *Dehalobacter sp.* responsible for reductive dechlorination process.

TEMNA can be an economic and sustainable technology in many circumstances. Sites with the following constraints would be worthy of appraisal for the utilisation of TEMNA technology:

- Mainly VOCs (chlorinated solvents) affected sites where reductive dechlorination is the dominant biological step.
- Excavation is expensive or impractical.
- LNAPL sites where aerobic degradation is the domination biological step.
- Rapid remediation is not required.

Generally TEMNA has the advantage that a significant increase in degradation rates can be achieved with a relatively small energy input (heating up to about 35-40$^o$C). Therefore costs associated with fuel/energy consumption can be substantially reduced, since the duration of the heating could be limited to approximately a two week period. However where remedial targets need to be achieved within a shorter time frame or other

 

site characteristics like a mixture of highly persistent hydrocarbons, the applicability is reduced and TESVE will be the preferred approach.

## NOMENCLATURE

| | |
|---|---|
| AOD | Above Ordnance Datum |
| bgl | Below ground level |
| BOD | Biological Oxygen Demand |
| CoC | Contaminant of Concern |
| DCE | Dichloroethene |
| DNA | Deoxyribonucleic Acid |
| DNAPL | Dense Non Aqueous Phase Liquid |
| DO | Dissolved Oxygen |
| COD | Chemical Oxygen Demand |
| EC | Electrical Conductivity |
| GAC | Granular Activated Carbon |
| GC-MS | Gas Chromatography - Mass Spectrometry |
| ISTD | In-Situ Thermal Desorption |
| LNAPL | Light Non Aqueous Phase Liquid |
| NAPL | Non Aqueous phase liquid |
| MNA | Monitored Natural Attenuation |
| PAD | Powdered Activated Carbon |
| PCBs | Polychlorinated Biphenyls |
| PCE | Tetrachloroethene |
| qDHC | Quantitative Dehaloccoides |
| qDHB | Quantitative Dehalobacter |
| qIRBSRB | Quantitative Iron and Sulphate reducers |
| qMGN | Quantitative Methanogens |
| qPCR | Quantitative Polymerase Chain reaction |
| qEubac | Quantitative Total Eukaryotic bacteria |
| SVE | Soil Vapour Extraction |
| SVOCs | Semivolatile Organic Compounds |
| TCA | 1,1,1-trichloroethane |
| TCE | Trichloroethene |
| TEMNA | Thermally Enhanced Monitored Natural Attenuation |
| TESVE | Thermally Enhanced Soil Vapour Extraction |
| TTZ | Target Treatment Zone |
| TVC | Total Viable Colony |
| RSRL | Research Sites Restoration (former UKAEA) |
| USEPA | United States Environmental Protection Agency |
| VC | Vinyl Chloride |
| VOCs | Volatile Organic Compounds |
| WSA | Western Storage Area |

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