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ANAEROBIC BIOREMEDIATION OF CHLORINATED SOLVENTS CONTAMINATED AQUIFERS IN THE PRESENCE OF DNAPL: THE RHO TEST SITE PROJECT

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ABSTRACT

This paper illustrates some of the results of a research project aimed at developing enhanced biological in-situ treatment technologies for the remediation of chlorinated solvent contaminated aquifers in the presence of DNAPL. Full scale implementation of effective biologically-based remediation is currently limited by the lack of reliable techniques for the identification and characterization of DNAPL distribution as well as lack of knowledge of biological degradation and related physical-chemical effects at DNAPL-water interface. Investigation of such aspects, requires specific information on contaminant composition and distribution, site hydrogeology and microorganism distribution and should then be accomplished directly on a test site. A comprehensive approach resulting from the combination of laboratory and field activity has been developed and applied to a test site where former industrial activities have produced soil and ground water contamination. Preliminary results together with some general considerations are discussed.

KEY-WORDS: *reductive dechlorination, field test, microcosms, DNAPL, chlorinated ethanes, chlorinated ethenes*

INTRODUCTION

Groundwater (GW) contamination by the release and transport of chlorinated aliphatic hydrocarbons (CAHs) in the subsurface is a common problem at many industrial sites posing serious environmental dangers. In subsurface environments, CAHs are often encountered in the form of dense nonaqueous phase liquids (DNAPLs), which can act as long-term sources of GW contamination by slowly dissolving into the GW (JOHNSON & PANKOW, 1992). As a result remediation is generally found to be quite expensive and can be expected to be long-lasting if the adopted technologies are only able to deal with the dissolved plume (i.e. Pump & Treat) rather than be based on contaminant mass reduction at the source. However mass reduction in the source-zone can only be achieved if DNAPLs are correctly detected and specific processes are implemented. Recent research has proposed many feasible options to enhance DNAPL dissolution or destruction such as in situ chemical oxidation or reduction (LI & SCHWARTZ, 2004 and SMITH *et al.*, 2006), alcohol and surfactant flushing (ABRIOLA *et al.*, 2005 and RAMSBURG *et al.*, 2005), thermal treatment (FRIIS *et al.*, 2005 and COSTANZA *et al.*, 2005), and bioremediation via anaerobic reductive dechlorination (RD) (ADAMSON *et alii*, 2003).

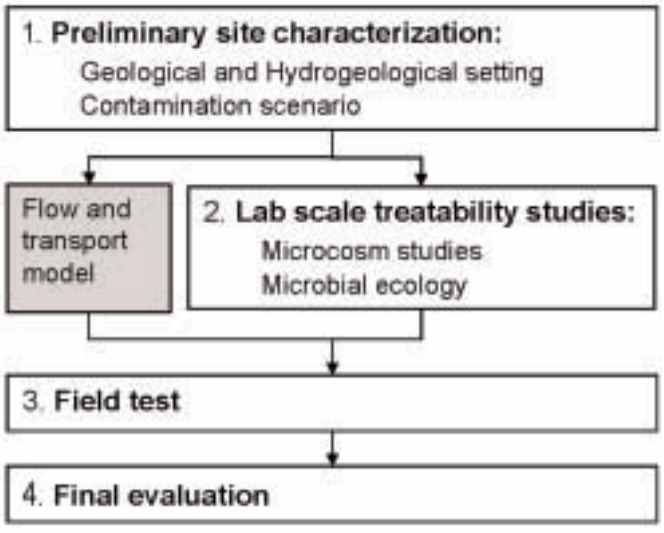

Fig. 1 - Research layout

Among those bioremediation is particularly appealing since it is merely based on the stimulation of naturally occurring processes. Indeed chlorinated solvents can be biologically degraded into harmless compounds as a result of the metabolic activity of some specific microorganisms which, under strictly anaerobic conditions, are capable of using them as terminal electron acceptors in their energy metabolism (FENNELL & GOSSETT, 2003). Such process, commonly referred to as anaerobic Reductive Dechlorination (RD), requires availability of a suitable electron donor, which is generally not naturally encountered in the GW environment and thus requires to be added as part of an engineered remedial action. Despite this relatively simple conceptual scheme, full scale application of this technique is currently limited since its success relies on a deep knowledge of the biological degradation process and the related physical-chemical effects occurring at the DNAPL-water interface on the one side and on the quality

of information on contaminant composition and distribution, site hydrogeology and microbial ecology at the site, on the other side.

The former aspects are typically investigated at the lab scale while the latter instead can only be studied directly at the field.

Therefore, in order to assess whether RD might be a feasible option for reducing the strength of chlorinated DNAPL source-zones, a key point seems the development of a tiered approach able to combine laboratory studies with field activities using mathematical models as the basic tool for successful process up-scaling. The proposed approach is based on a modification of the RABITT protocol (MORSE *et al.,* 1998) and its schematic structure is described in Fig. 1, where the main aims of each single stage are outlined.

Although, at some extents, it might look similar to the standard protocols set by regulatory acts for contaminated sites characterization and remediation it differs significantly in that here characterization efforts are not merely aimed at defining a contamination scenario but rather intended to gain such site specific information needed to correctly implement an appropriate bioremediation strategy.

In the following paragraphs a demo application of the procedure presented above at a test site is described in order to point out those critical issues to be considered when attempting to implement a bioremediation strategy at the full scale and draw some general conclusions.

SITE CHARACTERIZATION

A former chemical facility located in Rho (Milano, Italy), mainly involved in the production of synthetic dyes, was identified as the likely source of a chlorinated solvent plume that extends over approximately 0.4 km^2. The plume possibly originated from the leakage of a "storage basin" where exhausted chlorinated solvents were discharged during at least 50 years of industrial activity. In 1982, local authorities undertook an emergency containment action, which consisted in the lateral and superficial isolation of the zone formerly occupied by the storage basin. The encapsulation system was based on a tiny clay layer (about 1 m thick) which separates an upper shallow aquifer (from 5 to 12 mbgs) from a deeper one (from 15 to 40 mbgs). This action was not completely successful, probably because of discontinuities in the clay layer. GW-monitoring downgradient the source area was carried out on the already existing network of wells properly increased by realization of additional wells as a step of the research activity in order to obtain denser information in the presumed GW flow direction (NW to SE) (Fig. 2). Some wells were screened as to obtain depth-averaged GW samples from the shallow aquifer, while others from the deeper one.

Samples collected over a period of 15 years, indicated the almost stable presence of high concentrations of chlorinated solvents (e.g. up to 180 mg/l trichloroethylene, TCE, and 50 mg/l 1,1,2,2-tetrachloroethane, later referred to as TeCA) in the deep aquifer whereas the shallow one shows quite lower concentrations.

GW and site characterization data are consistent with a scenario

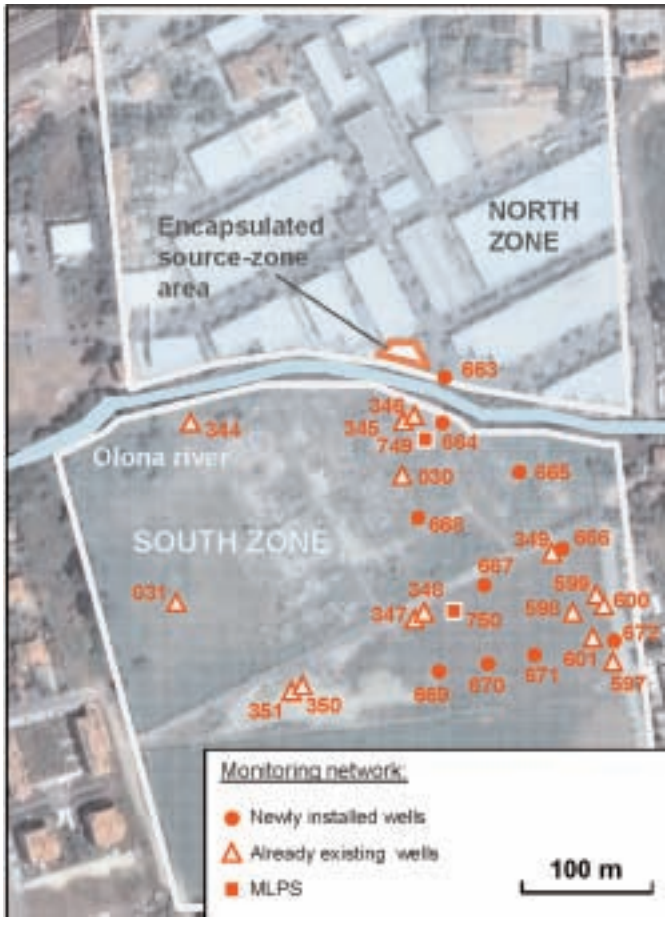

Fig. 2 - Aerial view of the test site and of the monitoring network

where spilled contaminants migrated from the underground tank downward through the deeper aquifer where they are still occurring as DNAPL pools acting as long-term sources of contamination (Fig.3).

However, the shape and fate of the dissolved plume could hardly be defined, based solely on the results of the hydro-chemical profiles, since suspected pools of residual DNAPL might be present at some unknown spots within the deep aquifer matrix.

Preliminary evidence of this was obtained by the reconstruction of the actual contaminants' distribution along the aquifer depth accomplished by drilling two additional wells equipped with Multi Level Packer Systems for depth-oriented GW sampling. A pressurized air-driven device was used for low-flow GW extraction at ten fixed depth (one every 2 m from 10 to 30 mbgs) within the deeper aquifer in the downgradient area (wells 749 and 750 in Figure 2). Typical profiles for CAHs and anions recorded at well 750 are presented in Fig. 4.

Denser than water contaminants' concentration (i.e. CAHs) show an increasing trend with depth down to about 20 mbgs whereas anions profiles appear to have more stable values; this finding con-

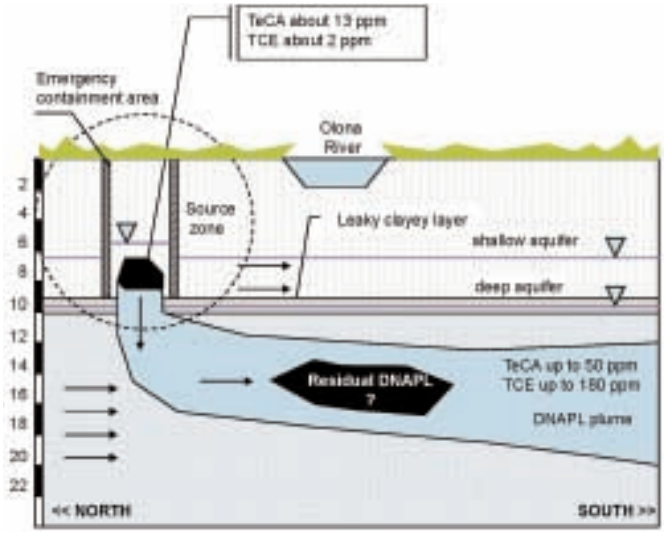

Fig. 3 - Site conceptual model and contamination

firms that a stratification in the concentration of CAHs is occurring apparently indicating that DNAPL pools might be present.

In order to better understand the contamination scenario dowgradient the source area further investigations were planned to localize and possibly quantify such residual DNAPL pools by cross-checking whether low-conductivity lenses could locally be detected where higher dissolved phase CAHs concentrations were observed. In order to reduce possible mobilization of the DNAPL it was decided that the investigations rely only on non- or less-invasive techniques; therefore geophysical surveys and direct-push logs, properly combined with further GW sampling campaigns, were selected as a feasible strategy.

Such measurements were done in cooperation with the Helmholtz-Centre for Environmental Research (UFZ), in the frame of the SAFIRA2 project and TRANS-IT bilateral activities; results from November 2006 campaign are presented in the paper by WERBAN *et alii* (2007) also published in this issue.

Besides this focused hydro-geochemical study research investigations were addressed to the treatability issue; following the hypothesized contamination scenario it was decided that experimental activities be all carried out within the presumed source zone, where higher concentrations of CAHs persist. This is because the proposed procedure appears to be better suited for DNAPLs source zone treatment rather than for plume decontamination. Furthermore the emergency containment action, formerly undertaken in such area, provides quite safe operation allowing for easier regulatory approval. Therefore GW and soil samples for preliminary lab studies as well as installations for the field testing were taken from or set in such area.

LAB SCALE TREATABILITY STUDY

Microcosm studies

In the attempt to stimulate microorganisms to consume CAHs as electron acceptors, an electron donor is required. Several laboratory

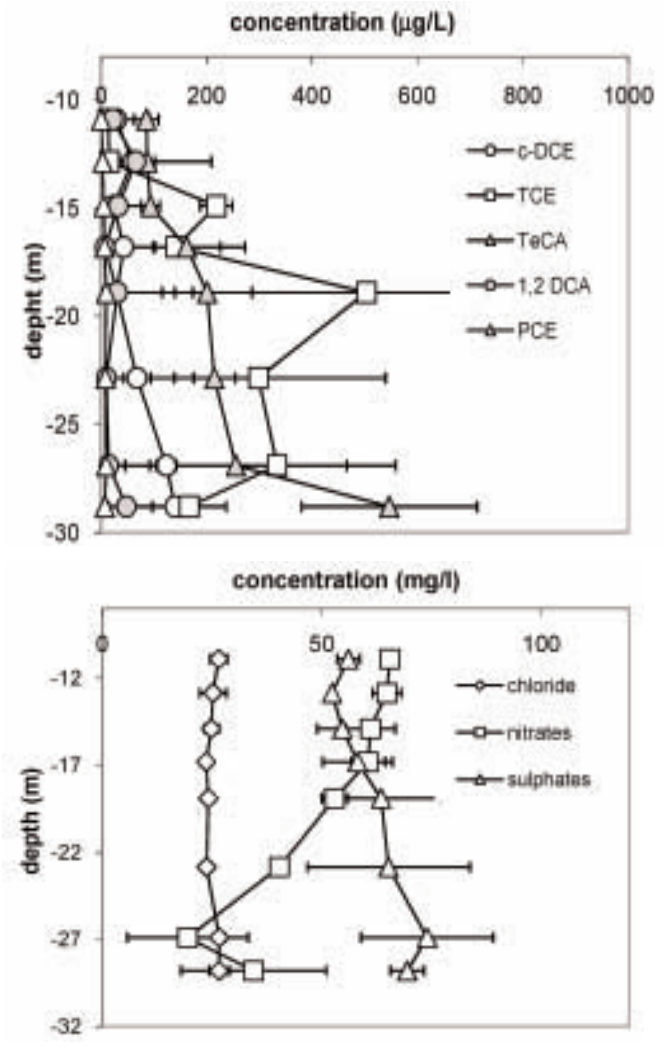

Fig. 4 - Mean values and standard deviations of three samples for CAHs (a) and anions (b) depth-determined concentration profile at well 750

studies indicate that molecular hydrogen is the primary electron donor for declhorinating biomass (DI STEFANO *et alii*, 1992); this can be either added directly or rather derived from the fermentation of organic substrates. In the latter case several fermentable organic substrates may be used for the production of H_2 and each one may result in different reaction pathways and rates depending on the site specific conditions; therefore laboratory microcosm studies are a necessary step of a screening protocol to assess the potential of enhancing the biological reductive dechlorination (RD) at a contaminated site as a feasible remediation strategy. In the present study microcosm tests were designed as to compare the effects of the addition of different substrates (i.e. yeast extract, lactate, butyrate, hydrogen) and growth factors (i.e. yeast extract, vitamin B12) on soil and water samples taken from the site. Also the effects of bioaugmentation with a

Dehalococcoides spp. containing inoculums were tested. A summary of the experimental conditions and the obtained results for each bottle microcosm after 384 days of incubation are shown in Table 1.

Treatment	***Soil and groundwater added with:***	***Cumulative released chloride[1] (µmol/l)***
1	None (abiotic control)	56.3
2	None (biotic control)	60.7
3	None + g.f.[2]	34.2
4	Yeast Extract (200 mg/l) + g.f.	338
5	Lactate (3 mmol/l)	147.8
6	Lactate (3 mmol/l) + g.f.	170.1
7	Butyrate (3 mmol/l)	84.2
8	Butyrate (3 mmol/l)+ g.f	264.2
9	Hydrogen (3 mmol/l)	183.4
10	Hydrogen (3 mmol/l) + g.f.	178.3
	Groundwater added with:	
11	Hydrogen (3 mmol/l) + Inoculum[3] + g.f.	273.6

[1] the chloride cumulative release is the amount of chloride released by the dechlorination processes as calculated (at any time) from the sum of all the measured (by gas-chromatography) dechlorination intermediates, by taking into account their initial and residual chlorination degree

[2] g.f. (growth factors): yeast extract (20 mg/l) and vitamin B_{12} (0.05 mg/l)

[3] the inoculum is a H2-utilizing PCE dechlorinating culture containing *Dehalococcoides* spp.

Tab. 1 - Bottle microcosms eperimental conditions and dechlorinating activity observed after 384 days of incubation

All tested electron donors proved to enhance dechlorination, but the rates and type of the final by-products differ significantly depending on the specific added electron donor. Indeed the most rapid dechlorination was observed in the microcosms amended with lactate where reducing condition favourable to dechlorination where rapidly established, whereas the full conversion of highly chlorinated ethanes (TeCA) and ethenes (PCE and TCE) into ethene (ETH) could only be obtained with yeast extract (Aulenta *et al*., 2006).

A fast dechlorination of the considered contaminants (TeCA, PCE, and TCE) was also accomplished in the microcosm with GW bioaugmented with an H_2-utilizing *Dehalococcoides* spp.-containing culture.

Results from the microcosm study indicate that full dechlorination of GW contaminants at the considered site to harmless non-chlorinated end-products might successfully be obtained by means of a combined strategy consisting in the primary addition of lactate to rapidly establish optimal reducing condition and achieve fast reduction of highly chlorinated compounds (mainly to DCEs and VC) followed by the injection of yeast extract to promote their further transformation to ETH.

A detailed description of microcosms experimental conditions and results is reported in Aulenta *et alii*., 2006.

Microbial ecology studies

Dehalococcoides spp. has been identified as the only microbial strain capable of fully dechlorinating most of the CAHs commonly encountered as GW pollutants (Maymò-Gattell *et alii,* 1999). Considering that bioaugmention is hardly accepted as a remediation strategy, the possibility of qualitatively determining the presence of *Dehalococcoides* spp. in GW and soil samples represents a critical issue in the preliminary screening phase; in fact, should native microbial population not include declorinating microorganisms the site might not be suited for implementing RD as a feasible decontamination technique. Furthermore quantitative microbial assays might help to have a more sound interpretation of microcosm results. However such type of bacteria is hardly isolated by traditional microbiological methods (i.e. MPN) requiring more sophisticated detection techniques such as molecular-based approaches (Aulenta *et al*., 2004). In this work fluorescent in situ hybridisation (FISH) and polymerase chain reaction (PCR) methods were both considered. Their application to GW and soil samples taken from the source area resulted in *Dehalococcoides* being successfully detected.

FIELD TEST

Based on the satisfactory results of the laboratory investigations, indicating that RD might successfully be enhanced at the considered site, the design and execution of a field test was performed as the final step of the evaluation process. This was necessary because aspects related to amendant delivery to the GW can only be investigated at the field scale, and lack of effective distribution of such substances may result in the process being not completely enhanced or controlled. Moreover, possible competitive and/or inhibitor effects could appear different at the field scale in an open system with respect to those observed in closed microcosm tests. Indeed the first task of performing an in-situ field test was to carry out a focused hydrogeological study in order to gain information about GW flow and contaminant and amendant transport. Such information may then be used to conduct a correct field study aimed at finally obtaining kinetic data required for the full-scale implementation of the investigated process.

Testing facility design and implementation

The field test was realized in the encapsulated source-zone, where higher concentrations of CAHs persist and safer testing might be performed. Due the absence of a naturally occurring hydraulic gradient in such area an active system able to deliver the chosen amendant under hydraulically controlled conditions was to be implemented; this was done by means of a closed external-loop circulation system (i.e. extraction-reinjection).

VISUALMODFLOW-aided simulations were carried out to define the optimal system configuration. The resulting facility extends over an area 11mx4m and consists of one injection well (PM2), one extraction well (PM7) and a network of monitoring wells. All wells have a PVC casing, an inner diameter either 50 or 100mm and were screened (with laser cutting) from 3 to 9 mbgs, with

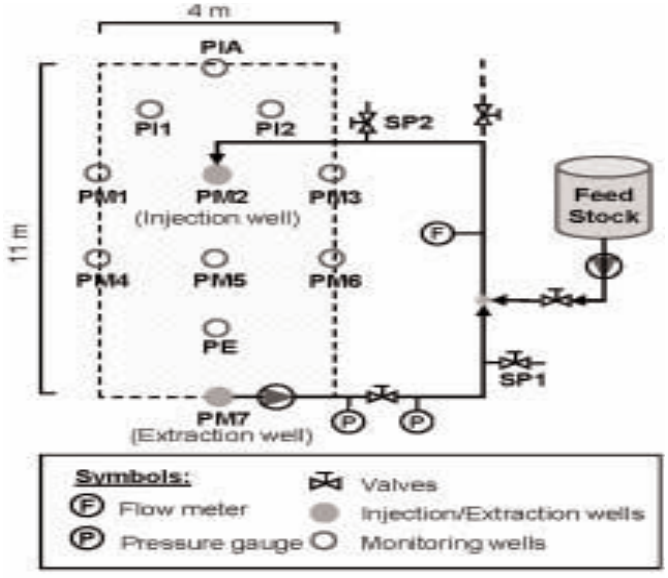

Fig. 5 - Schematic of the field test installation

Fig. 6 - Overview of the aboveground equipment

the exception of PE (screened from 6 to 9 mbgs), PI1, PI2, and PIA (screened from 7.5 to 9.0 mbgs).

A schematic of the system is presented in Figure 5, while Figure 6 shows an overview of the aboveground equipment.

Once the system was realized a conservative tracer test (CTT) was conducted in order to verify that an hydraulically controlled reaction volume could actually be established in the aquifer and that effective mixing of contaminated GW with the amendants was provided.

Tracer testing was performed by injecting three pulse slugs of a concentrated sodium chloride solution (final concentration of the re-injected GW of about 10 g/l) at different flowrates and subsequently tracking its dispersion in the specific geosystem by collecting GW samples. Samples were taken at a fixed depth (7.5 mbgs) using a tubing network installed at each monitoring well endowed with mini-pressure pneumatic pumps which allow for simultaneous low-flow water samples collection at the monitoring locations. Samples were transported to the laboratory and analyzed by ion chromatography for chloride concentration.

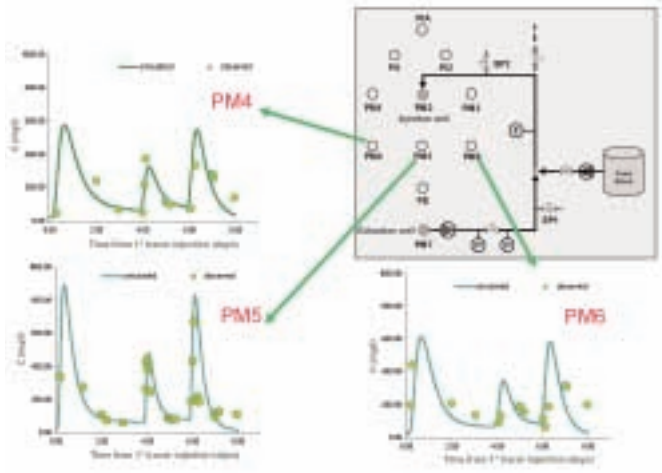

Fig. 7 - Assumed K distribution for the computer-aided CTT interpretation

Quantifiable concentrations of chloride appeared at all monitoring locations in a rather short period of time. Under the specific field conditions the tracer Breakthrough Curves (BTCs) could not be observed with a data collection frequency high enough to provide the amount of data needed for a reliable computation of the temporal moments (Ptak and Teutsch, 1994). Therefore a curve fitting procedure, based on VISUALMODFLOW simulations, for each of the monitoring wells was believed to be a more promising approach. Furthermore it allowed for eventual heterogeneities to be considered providing a more realistic estimate of actual aquifer parameter values. Indeed the experimentally derived tracer BTCs could only be satisfactory reproduced under a non-homogeneous hydraulic conductivity field (Fig. 7). Heterogeneity was also introduced in hydraulic conductivity-related parameters (i.e. porosity and storage), whose range of variation is reported in Table 2.

	Conductivity		Porosity	Storage	Dispersivity		
	$K_x=K_y$ (m/s)	K_z (m/s)	n (%)	S_s (m^{-1})	α_L (m)	α_T/α_L (m)	α_z/α_L (m)
Shallow aquifer	1 x 10^{-6}	1 x 10-6	1223	1.852.7 x 10^{-2}	0.33-0.5	0.02	0.001
Deep aquifer	1 x 10^{-6}	1 x 10^{-8}	45	1.0 x 10^{-4}	0.33-0.5	0.02	0.001

Tab. 2 - Hydrodynamics and dispersive properties of the aquifers derived from the CTT

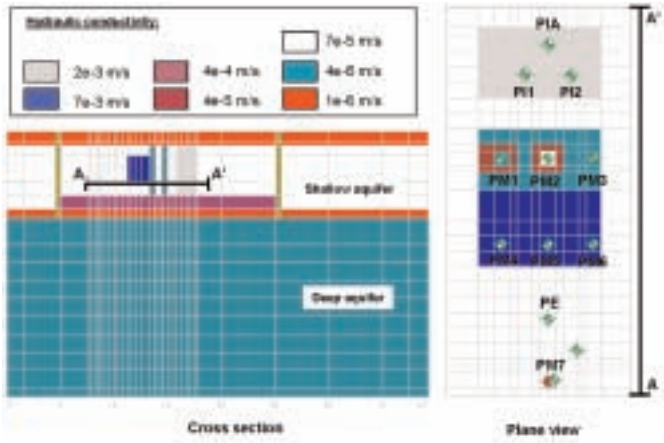

Fig. 8 - Comparison between observed and simulated BTCs at monitoring wells PM4, PM5 and PM6

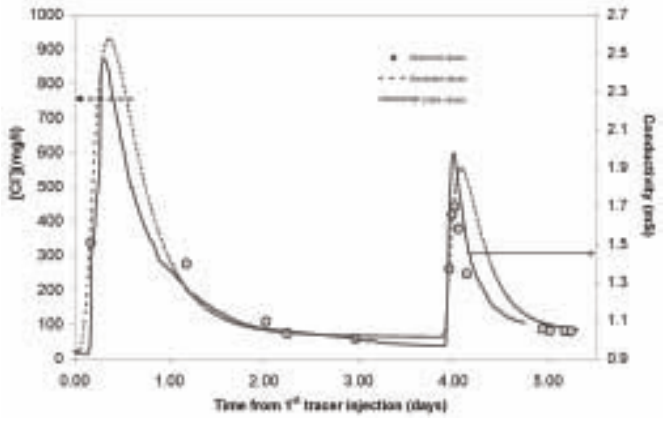

Fig. 9 - Simulated profiles vs probe records and observed values at monitoring well PM5

A comparison between observed and simulated tracer concentrations at three monitoring wells is presented in Fig. 8.

As a control on the quality of the interpolation a multi-parametric submersible probe (Aquamaster model 345, AMEL) has been permanently installed in the monitoring well (PM5) immediately downgradient the injection well, in order to allow for continuous monitoring of environmental parameters (pH, Temperature, DO, Eh) while tracing chloride concentration variations by recording electrical conductivity time-profiles. As shown in Errore. L'origine riferimento non è stata trovata. the conductivity profile, properly scaled, compares favourably with the simulated tracer concentration curve fitted on the discrete field observations.

Modelling results pointed out that, despite the small dimension of the investigated area, local heterogeneities in the aquifer matrix are to play a significant role in the tracer actual dispersion.

Tracer test results confirmed that developed site model is capable of reliably simulating the forced circulation system and its dispersive properties.

As a result of tracer test interpretation more detailed information on the actual hydraulic conductivity and dispersion of the geosystem at the field test location could be gained and subsequently used to correctly address further investigation activities consisting of the actual test performance.

Preliminary field test

The very last experimental phase consisted in the injection of the selected amendants in the field facility in order to stimulate microbial activity of native populations and verify if the microcosm study can be advantageously adopted in the proposed strategy (by comparing field and laboratory results). The test was accomplished in three subsequent stages by recirculating water at an almost constant flowrate of about 0.54 m^3 h^{-1}over a period of 100 days.

During the first two days (phase I) GW was extracted from PM7 and reinjected in PM2 in order to activate the internal circulation loop. Once steady state conditions were achieved extracted GW started to be continuously added with an electron donor solution prior to injection in the GW for almost 30 days (phase II). A mixture of lactic acid and yeast extract to a final concentration in the reinjected GW of 400 mg/l was used, based on the results of the microcosm study previously done. This phase was aimed at rapidly consuming alternative electron acceptors and establishing anaerobic reducing conditions, by supplying an excess of lactate.

During phase III extracted GW was pulse added with the electron-donor solution once every two weeks with each pulse having a duration of 8 h. The aim of phase III was to verify the possibility to sustain continued reductive dechlorination with a lower electron donor loading rate.

During and after the amendants' injection the fate of the added electron donor and of the naturally occurring electron acceptors was observed by collecting GW samples at discrete time intervals using the system described above. Chlorinated and non chlorinated ethanes and ethenes were analyzed by headspace gas chromatography (GC) with a flame ionization detector (FID). Volatile fatty acids (lactate, acetate, and propionate) were analyzed as liquid also by GC-FID. Anions (nitrate, nitrite, sulphate and chloride) were analyzed using ion chromatography. Other parameters such as dissolved oxygen, redox potential, pH, and conductivity were measured in situ (PM5) and on-line, using a multiparametric probe (Aquamaster model 345, AMEL).

As shown in Figure 10 continuous addition of lactate resulted in its rapid accumulation within the test zone; upon depletion of available oxygen lactate (Figure 11) is fermented to produce acetate and propionate.

As expected, alternative electron acceptors, naturally occurring in the GW environment, are rapidly consumed (Figure 11) with the exception of sulphate, whose concentration remains almost stable indicating that sulphate reduction is not occurring. The lack of sulphate consumption observed in the field differs significantly from the

results of the microcosms, where such compound was almost completely consumed in about 70 days of incubation. The reason for such discrepancy is currently unclear but can possibly be explained by considering a potential toxicity effect exerted on the sulphate-reducing microorganisms by the higher chlorinated solvent concentrations (up to 70 mg/l of TeCA) obtained in the field with respect to those present in the soil and GW samples used for the microcosms (about 13 mg/l).

Figure 12 shows the time profile for both chlorinated ethanes and ethenes observed during the operational phase of the filed test. A sudden increase in the concentration of all chlorinated parent compounds (TeCA, PCE and TCE) was detected; such a behaviour was not at all detected in the microcosms and therefore seems likely to be explained by considering the different fluid dynamic conditions Indeed the forced GW recirculation might have enhanced DNAPL dissolution by possibly increasing the liquid-phase mass transfer coefficient. Upon establishment of anaerobic conditions (Figure 11) and consumption of alternative electron acceptors partial dechlorination was observed (TCA, DCA and c-DCE are formed) indicating that under the specific field condition RD can effectively be stimulated. However due to the limited duration of the undertaken experimental phase full dechlorination could not be achieved.

A more detailed description of the procedures and results of the field treatability study can be found in AULENTA *et alii*., (2007), where such results are also duly compared with microcosms.

FINAL EVALUATION

The development and application of a procedure for assessing the bioremediation potential at a chlorinated solvents contaminated site in the presence of DNAPL has been herewith presented.

Based on the results of the field test the proposed approach proved that effective reduction of the strength of DNAPL at the source area can be obtained. However residual concentrations as low as those imposed by stringent regulatory limits for CAHs can hardly be achieved making a further downgradient plume decontamination necessary. Therefore when properly combined with strategies for dissolved phase post-treatment (i.e. P&T or PRB) enhanced in-situ bioremediation by anaerobic RD appears to be a valid decontamination strategy at sites where chlorinated DNAPLs are occurring.

Beyond the considered specific case the following general considerations may be drawn:

- accurate characterization is necessary to both localize DNAPLs source zones and possibly define the shape and fate of the downgradient dissolved-phase plume; this should be done by cross-checking and comparing the information resulting from different available investigation techniques which need to be selected in order to reduce possible mobility of the contaminants;
- microcosm studies and field tests provide complementary information and appear both necessary for implementing a source treatment strategy, in that lab studies are required to investigate such

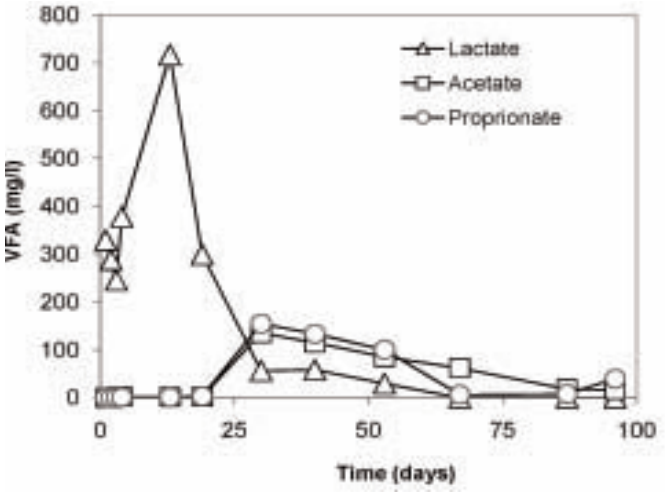

Fig. 10 -Volatile Fatty Acid (VFA) time profile observed at PM5

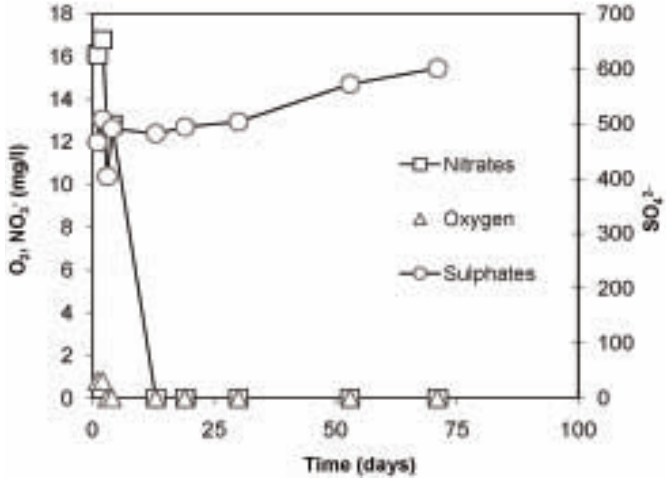

Fig. 11 -Naturally occurring alternative electron acceptor time profile observed at PM5

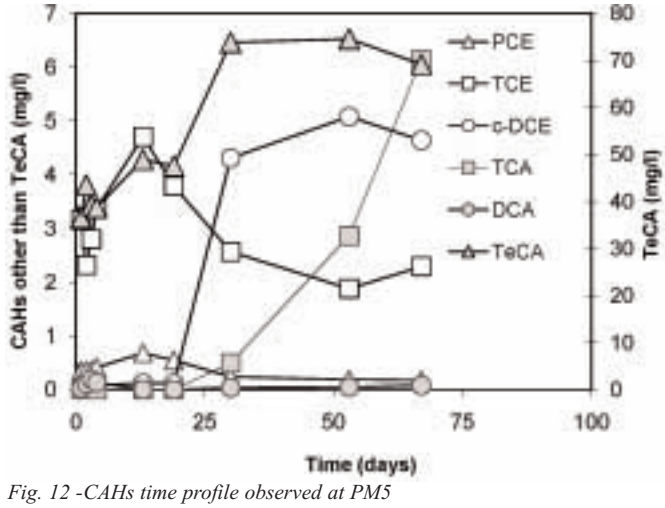

Fig. 12 -CAHs time profile observed at PM5

aspects associated to the choice of the optimal electron donor which is not straightforward since it should consider possible stimulation of undesired metabolisms which may result, for instance, in the accumulation of high levels of dissolved metals, fermentation byproducts, with resulting deterioration of groundwater quality.

- field experiments account for investigation of those aspects related to the selection of the most appropriate electron donor delivery system which is affected by local heterogeneity and might depend upon the type of electron donor to be added. Furthermore they also allow for investigation of those strongly scale-dependant effects, such as the enhanced DNAPL dissolution resulting from the induced GW recirculation observed in our case;
- further implementation of molecular techniques at field scale and definition of ecotoxicity bioassays should be included in the design approach.

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REFERENCES

Abriola L. M., Drummond C. D., Hahn, E. J., Hayes K. F., Kibbey T. C., Lemke L. D., Pennell K. D., Petrovskis E. A., Ramsburg C. A., Rathfelder K. M. (2005) - *Pilot-scale demonstration of surfactant-enhanced PCE solubilization at the Bachman Road site. 1. Site characterization and test design*. Environmental Science & Technology **39** (6): 1778-1790.

Adamson D. T.; McDade J. M.; Hughes J. B. (2003) - *Inoculation of a DNAPL source zone to initiate reductive dechlorination of PCE*. Environmental Science & Technology **37**(11): 2525- 2533.

Aulenta F., Canosa A., Leccese M., Petrangeli Papini M., Majone M., Viotti P. (2007) - *Field Study of In Situ Anaerobic Bioremediation of a Chlorinated Solvent Source Zone*. Industrial & Engineering Chemistry Research, **41** (21): 6812-6819.

Aulenta F., Potalivo M., Majone M., Petrangeli Papini M. and Tandoi V. (2006) - *Anaerobic Bioremediation of Groundwater Containing a Mixture of 1,1,2,2-Tetrachloroethane and Chloroethenes*, Biodegradation, **17** (3): 193-206

Aulenta F., Rossetti S., Majone M., Tandoi V. (2004) – *Detection and quantitative estimation of Dehaloccoccoides spp. in a dechlorinating bioreactor by a combination of fluorescent in situ hybridisation (FISH) and kinetic analysis*. Applied Microbiology and Biotechnology **64** (2): 206-212

Costanza J.; Davis E. L.; Mulholland J. A.; Pennell K. D. (2005) - *Abiotic degradation of trichloroethylene under thermal remediation conditions*. Environmental Science & Technology **39** (17): 6825- 6830

di stefano T. D.; Gossett, J. M.; Zinder S. H. (1992)- *Hydrogen as an electron donor for dechlorination of tetrachloroethene by an anaerobic mixedculture*. Applied Environmental Microbiology **58**: 3622-3629

Fennell D.E. and Gossett J.M. (2003): *Microcosms for site specific evaluation of enhanced biological reductive dehalogenation*. In: Häggblom, M.H. and Bossert I.D. (Ed.) Dehalogenation: Microbial processes and environmental applications, Kluwer Academic Publisher, 1-11

Friis A. K.; Albrechtsen H. J.; Heron G.; Bjerg P. L. (2005) - *Redox processes and release of organic matter after thermal treatment of a TCE-contaminated aquifer*. Environmental Science & Technology **39** (15): 5787- 5795

Johnson R. L. and Pankow, J. F. (1992) - *Dissolution of dense chlorinated solvents into groundwater. 2. Source functions for pools of solvent*. Environmental Science & Technology, **26** (5): 896-901

Li X. D. and Schwartz F. W. (2004)- *DNAPL remediation with in situ chemical oxidation using potassium permanganate. Part I. Mineralogy of Mn oxide and its dissolution in organic acids*, Journal of Contaminant Hydrology **68** (1-2): 39-53

Maymò-Gatell X; Anguish T and Zinder SH (1999) - *Reductive dechlorination of chlorinated ethenes and 1,2-dichloroethane by "Dehalococcoides ethenogenes 195*". Applied Environmental Microbiology **65**: 3108–3113

Morse J. J.; Alleman B.C.; Gossett J.M.; Zinder S.H.; Fennell D.E.,; Sewell G.W.; Vogel C.M.. (1998) *Draft Technical Protocol – A Treatability Test for Evaluating the Potential Applicability of the Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) to Remediate Chloroethenes*. US DoD Environmental Security Technology Certification Program. (www.estcp.org).

Ptak T., G. Teutsch (1994) *Forced and natural gradient tracer tests in a highly heterogeneous porous aquifer: instrumentation and measurements,* Journal of Hydrology **159**: 79-104

Ramsburg C. A.; Pennell K. D.; Abriola L. M.; Daniels, G.; Drummond C. D.; Gamache M.; Hsu, H. L.; Petrovskis E. A.; Rathfelder K. M.; Ryder J. L.; Yavaraski,T. P. (2005) - *Pilot-scale demonstration of surfactant enhanced PCE solubilization at the Bachman Road site. 2. System operation and evaluation*. Environmental Science & Technology **39** (6) 1791-1801

Smith B. A.;, Teel A. L., .; Watts, R. J. (2006) - *Mechanism for the destruction of carbon tetrachloride and chloroform DNAPLs by modified Fenton's reagent*, Journal of Contaminant Hydrology **85** (3-4): 229-246

Werban U.; Leven C.; Reboulet E.; Dietrich P. (2007) *Technologies for a fast characterization of subsurface structures – An example from the Milano-Rho site.* In this issue.