Theme D: **Remediation Concepts & Technologies**

DIFFERENT STRATEGIES TO TEST THE FEASIBILITY OF "ENHANCED REDUCTIVE DECHLORINATION" REMEDIATION: ILLUSTRATION BY TWO CASE STUDIES

Koen Meskens[(1)], Karel Van Nieuwenhove[(1)] and Richard Lookman [(2)]

[(1)] Soresma, Britselei 23, 2000 Antwerpen, Belgium
+ 00 31/32215570, koen.meskens@soresma.be
[(2)] Vito, Boeretang 200, 2400 Mol, Belgium

Key words: CAHs, Enhanced Reductive Dechlorination, Microcosm, Pilot, PCR

Abstract
In case of a remediation of a groundwater contamination with chlorinated aliphatic hydrocarbons (CAHs) by "Enhanced Reductive Dechlorination", the feasibility needs to tested carefully. Executing both a microcosm test and a pilot test requires much time and expense and is not *always* justified. With the extra aid of PCR-analysis a more efficient approach is possible just performing one test. No general preference for either method should be claimed but site-specific criteria will decide. This is illustrated by the two case-studies. If the first screening of the assessment data strongly indicate that Enhanced Reductive Dechlorination is possible, an immediate field testing can be recommended (case-study A). Is the situation more complex, microcosm tests are preferred in combination with a limited injected test to be able to dimension a full-scale remediation (case-study B).

Introduction
Stimulated or enhanced (anaerobic) biodegradation is gaining more and more credibility as a (partial) solution for cleaning up groundwater contaminations with chlorinated aliphatic hydrocarbons (CAHs). The biological processes by which chlorine atoms of CAHs are replaced by hydrogen atoms - also called reductive dehalogenation - is stimulated by adding a carbon source (electron donor).
However, the efficacy of this technology appears to be site-specific. In order to be successful the following conditions need to be met: (i) strongly reducing conditions (methanogenic by preference), (ii) sufficient carbon source and nutrients and (iii) presence of bacteria which can catalyse a complete reduction. Therefore, the feasibility needs to be tested carefully.
After interpreting the site assessment results and - if necessary – an additional groundwater characterisation, a rating system can be used to evaluate the bioremediation potential of the site based on its contaminant, hydrogeological and geochemical profiles. If Enhanced Reductive Dechlorination remains a valid option and no regulatory objections or limitations in time are encountered, the treatability study can be continued. Regulators generally demand a successful microcosm test as well as a pilot test in order to approve the application of this technique.
Collected soil and groundwater samples are used to construct microcosms to test different electron donors for their ability to stimulate reductive dechlorination. As such, microcosms tests provide insight into the rate and the extent of reductive dechlorination and methods to further improve dechlorination such as the addition of nutrients and/or dehalogenating bacteria (bioaugmentation). Based on the microcosm results, an electron donor (with or without extra nutrients) can be selected for use in a field pilot test that is performed to determine if Enhanced Reductive Dechlorination can be applied in the field and to collect data required for the operation of a full-scale remediation.

A treatability study executed as described above requires much time and is costly. One can question wether this is *always* justified. The goal of this article is to demonstrate that microcosm *and* field testing are not always required both. With the extra aid of PCR-analysis - screening for the presence of genetic material by means of molecular Polymerase Chain Reaction detection for *Dehalococcoides* sp. and/or dechlorinases – a more efficient approach is possible.

Microcosm and field pilots both have their limitations and advantages so no general preference for either method should be claimed. Different strategies can be followed to test if the site can comply.

A pilot test can be immediately executed in the field, if the first screening of the assessment data strongly indicate that Enhanced Reductive Dechlorination seems possible. For example, when final

dehalogenation products ethene and/or ethane are already present in substantial quantities, a field pilot can be directly executed without a microcosm test previous to the pilot. An example of a pilot test is the repeated push-pull test illustrated in case-study A.
In more complex cases where one wants to test different conditions or when more than one type of pollutant is present (e.g. PCE and 1,1,1-TCA mixed pollutions), microcosm studies and PCR-analyses provide the necessary information as illustrated in case-study B. In that case, limited additional testing concerning the injection method of the carbon source needs to be executed in order to be able to extrapolate the test results to a full-scale remediation.

Case-study A

Background
A former fireworks and explosives manufactory operated at the subject site in Deurne, Belgium from 1890 until 1998. CAHs were stored and handled during operation. (Minor) spills of trichloroethene (TCE) resulted in impacts to the subsurface environment. The vertical and horizontal extent of the plume - TCE and its daughter products cis-1,2-dichloroethene (cDCE) and vinyl chloride (VC) – was delineated (Fig. 1). Horizontally, the dimensions of the plume are estimated to be 350 by 125 meters. Vertically, the boundary of TCE (above reference level) is at 25 meters below ground level (m bgl).

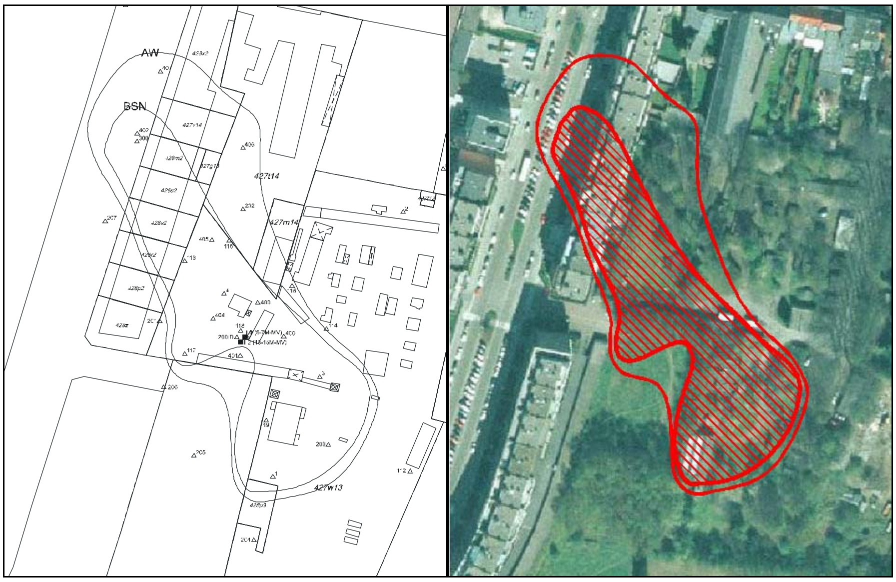

Figure 1.Horizontal extension of the TCE contamination and its daughter products

The maximum levels of CAHs observed in the groundwater are 24,000 µg/L for TCE, 13,000 µg/L for cDCE and 2,700 µg/L for VC. The local geology consists of quaternary or tertiary silty and clayey sands until 6 m bgl. Deeper, the quaternary sand becomes coarser and less silty until a depth of at least 25 m bgl. In the literature the tertiary clay of Boom is reported at a depth of appr. 37.5 m bls.
The groundwater table currently is at appr. 3 m bgl. The groundwater flow direction is north to northwest. The historical groundwater contamination plume has a more north western direction due to an extensive lowering of the groundwater table for construction purposes upwards this direction. The hydraulic conductivity varies from $0.5.10^{-5}$ m/s to 2.10^{-5} m/s, the higher values occur deeper in the aquifer.

Remediation strategy
Due to the large depth and extension of the CAH contamination - without an identified DNAPL source – an in-situ technique is recommended. Lab tests executed on a representative soil sample to determine the soil oxidation demand, showed that chemical oxidation should be excluded as a remediation option. Furthermore, the assessment results indicated that full biodegradation already occurred at the site. As illustrated by the presence of ethene in the plume, while the natural TOC-concentrations were very low. Sulphate concentrations however, were quite high.
As remediation strategy for both the source and the plume, Enhanced Reductive Dechlorination was proposed. It will be combined with a down-gradient groundwater extraction, to prevent further off-site plume migration as well as to speed up migration of the carbon source to be injected. The extracted (contaminated) groundwater will be re-infiltrated after enrichment with molasses and nutrients.

In order to test the feasibility of this concept, a pilot test was proposed (without a microcosm test previous to it).

Pilot design

The set-up of the pilot is a repeated push-pull test. In the centre of the groundwater contamination two infiltration filters were installed at different depths, two meters upgradient from the monitoring wells with the highest CAH concentrations of mother compounds (Fig. 2). The infiltration wells have the same screen interval as the corresponding monitoring wells: filter I1 from 5 to 7 m bgl and filter I2 from 13 to 15 m bgl. The pilot is conducted in the following steps.

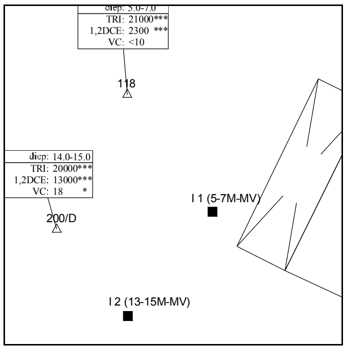

Figure 2. Pilot test set-up.

1. Pull-0: Consecutively 1 m³ of groundwater is extracted from each infiltration well and captured in a container. Molasses is added (to produce a 5% concentration) as well as nutrients (C:N:P proportion 100:10:5). Bromide (50 g/m³) is added as an inert tracer to follow the migration of the groundwater itself and to calculate dilution factors. Next, the groundwater and its additives are mixed thoroughly while being purged with nitrogen to maintain anoxic conditions. Finally, the mixture is re-infiltrated under constant nitrogen purging (push-1). Pictures are shown in figure 3.
2. Pull-1: after 1 to 2 months the container is refilled with extracted groundwater from the infiltration filters I1 en I2. Again molasses, nutrients and bromide are added and the mixture is re-infiltrated (push 2).
3. The previous step is repeated as pull-2 and push-3 after another 1 to 2 months if necessary.

Figure 3. Picturing during the pilot test. Left: groundwater is extracted and captured in a container (pull). Middle: Purging with nitrogen during mixing. Right: re-infiltration of mixture under constant nitrogen purging (push)

Both the extracted groundwater (from each pull-stage) and the groundwater from the downgradient monitoring wells Pb118 and Pb200 (monthly) are sampled and analyzed for the following parameters: pH, EC, T, ORP, oxygen, CAHs, DOC/TOC, chloride, nitrate, sulphate, iron (II), bromide and ethane/ethene/methane to follow-up biodegradation. Based on data for TOC and bromide and their difference, the migration of molasses can be quantified. The need for an additional groundwater extraction to stimulate the migration of the carbon source can be evaluated as well.

At the start, a groundwater sample is taken from both infiltration filters in order to conduct a PCR-analysis (detection of *Dehalococcoides ethenogenes* with primers from Löffler et al. (2000) and Hendrickson et al. (2002) as well as the detection of dechlorinases *tce*A and *pce*A (Regeard, 2004).

Results

The pilot is still in an early stage of execution. Baseline sampling was done on April 6, 2005. The first push-pull phase was conducted on April 13, 2005 and the first monitoring round on monitoring wells Pb118 and Pb200 was performed on May 9, 2005. Data will be shown on the poster presentation during Consoil 2005.

Case-study B

Background

A metalworks company has been operational at this site in Temse, Belgium since 1985. CAHs were stored and handled during operation. These activities resulted in a groundwater contamination with chromium (III), chromium (IV), TCE and its daughter products cDCE and VC. The vertical and horizontal extent of the CAH plume were delineated, neighbouring sites and the public domain are impacted as well (Fig. 4). Horizontally, the plume extends over an area of appr. 5.500 m². Vertically, the groundwater contamination is delineated at a depth of 4 m bgl, the depth of a confining layer of clay. This clay layer extends to a depth of appr. 17.5 m bgl. Above the clay the soil is described as silty sand or sandy silt.

The maximum levels of CAHs observed in the groundwater are 85,000 µg/L for TCE, 24,000 µg/L for cDCE and 7,300 µg/L for VC. Based on the concentrations of TCE, DNAPL (pure product) is likely to exist, although no elevated concentrations in the soil matrix have been measured.
The groundwater table varies from 0.25 to 1.4 m bgl. The groundwater flow direction is south-east. The hydraulic conductivity is estimated at 1.10^{-5} m/s.

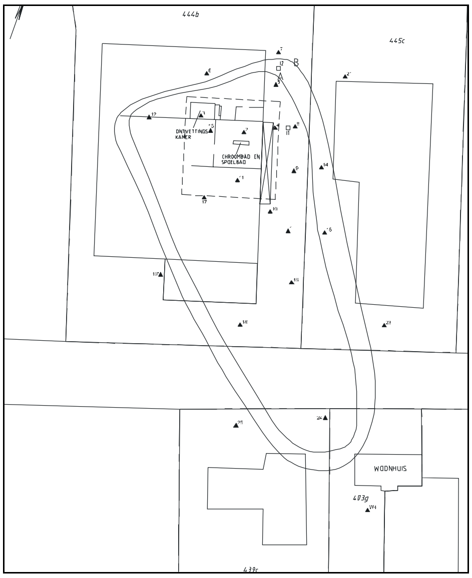

Figure 4. Horizontal extension of the CAH contamination at site

Remediation strategy

Due to the highly elevated CAH concentrations in the source area, a different approach for the source and plume would most likely be necessary. The assessment results indicated an ongoing biodegradation in the plume. Production of ethene (as high as 820 µg/L) and methane occurred on-site. Enhanced Reductive Dechlorination is proposed as a remediation technique for the plume with a down-gradient groundwater extraction as a temporary isolation measure to prevent further migration off-site. For the source area (appr. 700 m²), the initial stage will consist of a physical removal of the CAH contamination potentially followed by Enhanced Reductive Dechlorination as well. It will be part of the feasibility testing to answer this question.

Set-up of the feasibility test

Based on the data of earlier assessment activities, six monitoring wells in the core and the plume were selected for a further groundwater characterisation. This included analysis for the following parameters: pH, EC, ORP, oxygen, nitrate, sulphate, CAHs and – additionally on the earlier assessments - also for ethene/ethane and PCR for the presence of *Dehalococcoides ethenogenes*. Subsequently, anaerobic microcosm tests with Nutrolase (based on amylum, a detailed composition is shown in table 1) used as a carbon source were performed on soil and groundwater samples from both the source and the plume.

Table 1: Composition of Nutrolase (AVEBE, The Netherlands)

Component	mg/L	Component	mg/L
Totaal phosphor	6400	Oxalic acid	5200
Totaal sulphur	12.000	Malic acid	31.000
Totaal nitrogen	32.000	Citric acid	62.000
Raw proteins	199.000	Potassium	80.000
Amino acids	147.000	Magnesium	4.100
		Calcium	580
Fructose	31.000	Sodium	580
Glucose	31.000	Ammonium	3500
Sacharose	61.000		
		Chloride	4100
Reducing sugars	45.000	Nitrate	1740
Acetic acid	3500	Sulphate	18.000
Lactic acid	11.000	Fosphate	20.300

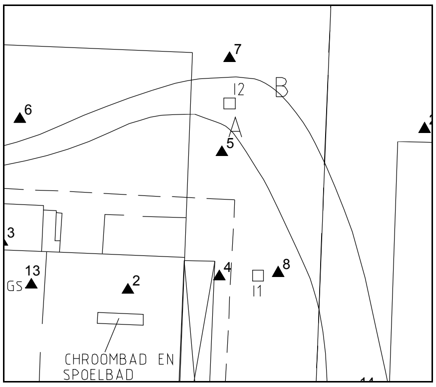

Figure 5. Detail of the injection test

Additional testing concerning the injection method of the carbon source is also executed in order to be able to extrapolate the tests to a full-scale remediation. The injection is performed with a mobile injection system. The carbon source enriched with nutrients consisted of a mixture of ammoniumchloride (8 kg), ethanol (2,5 L), sodium acetate (8 kg), sodium lactate (7,5 L, 60 %), molasses (7,5 L) and sodium hexametaphosphate (3 kg).
Firstly, an air bubble under groundwater level was created after lowering an air injection filter at the desired depth in the soil. Next, the solution with carbon source and nutrients was sprayed through the same injection filter along this air bubble. Then the depth of the injection filter was altered and the procedure was repeated. It is assumed that the brief injection of air during the test (1 day) has no long term negative effect on the anaerobic biostimulation. For the full-scale remediation, the use of nitrogen as carrier gas is proposed.
The test was performed at two locations I1 (between Pb4 and Pb8) and I2 (between Pb5 and Pb7) as illustrated in figure 5. The injection occurred at different depths (1.5; 2.0; 2.5; 3.0 and 3.5 m bgl.) in amounts of 20 L at each depth. Figure 6 shows an injection in progress.
With the aid of a portable spectrophotometer ortho-phosphate, ammonium, chloride and TOC were analyzed in the surrounding monitoring wells during the test in order to follow the migration.

Figure 6. Injection of carbon source and nutrients between Pb4 and Pb8 in progress

Results

Further groundwater characterisation

In the plume *Dehalococcoides ethenogenes* is present which is also illustrated by the presence of ethene and ethane (complete dechlorination). In the source *Dehalococcoides ethenogenes* is absent as well as ethene. Results are shown in table 2.

Table 2: Analytical results

Parameter	Pb 4	Pb 12	Pb 13	Pb 10	Pb 18	Pb 24
Location	Source	Source	Source	Plume	Plume	Plume
ORP	n.m.	n.m.	374	365	175	392
Oxygen	n.m.	n.m.	0.3	1.7	0.8	6.2
Nitrate	2.8	<1	65	12	<1	2.7
Sulphate	160	530	770	340	290	99
TCEl	6.000	8.8	< 0.2	5700	11	14
Cis-DCE	7.600	75	< 1	3000	23000	1800
VC	74	7.8	< 0.5	<200	7300	430
Ethene	1.1	< 0.1	< 0.1	3.1	200	0.8
Ethane	6.8	21	4.9	6.7	260	37
Dehalococcoides ethenogenes	-	-	?	?	+	+

n.m. not measured
\- not present
\+ present
? not analysed as no useful DNA could be extracted from the soil samples

The plume has the potential to completely reduce the CAH contamination to ethene. In the monitoring wells Pb18 and Pb24 *Dehalococcoides ethenogenes* is present. Complete reduction actually happened since the concentrations of ethene and ethane were raised very clearly in Pb18. In the groundwater of the source area no *Dehalococcoides ethenogenes* is found and therefore normally lacks the potential for a complete reduction. It needs to be noted that the results of a PCR-analysis are indicative as “false” positive or negative results might occur.

Both in the core and the plume, biodegradation is slowed down by the presence of high concentrations of sulphate and locally also of nitrate. In order to get a complete reduction from TCE to ethene, methanogenic conditions need to be obtained. These conditions are normally characterised by the absence of nitrate (<1 mg/L) and sulphate (<20 mg/L). However, it has also been reported that CAHs were reduced to ethene while sulphate concentrations were still high.

Microcosm tests

The results of the microcosm test (executed in triplo) are shown in Figure 7. Concentrations are expressed in µmol/kg.dm. In the batches with material from the core, TRI is degraded within 4 weeks into CIS and VC. After 8 weeks the process stagnates. Additional input of protamylasse did not help. Ethene or ethane were not encountered during the incubation period, probably due to the absence of *Dehalococcoides ethenogenes.*

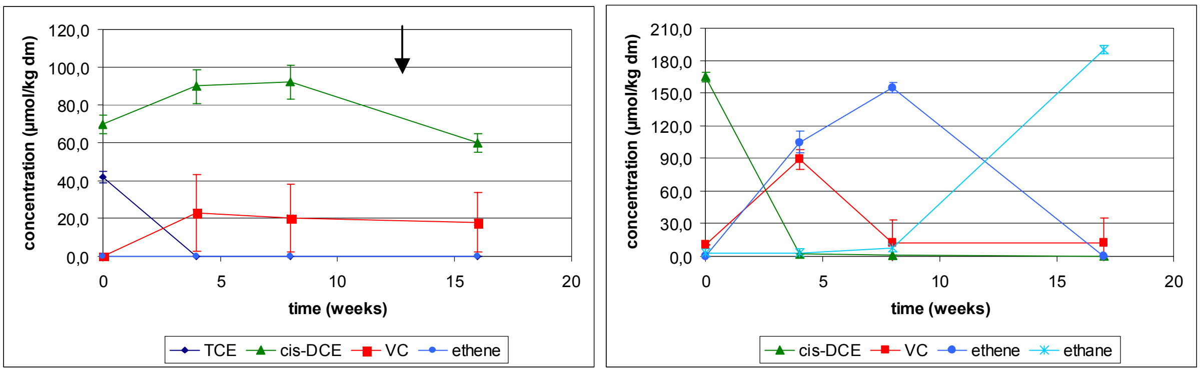

Figure 7. Biodegradation of CAHs in a microcosm test with material from the source (left) and the plume (right) (average of triplo test, the error bars represent the standard deviation). The arrow shows the moment of respiking with Nutrolase.

In the batches with material from the plume, complete reduction is obtained. After 8 weeks only ethene is still present. Ethene is even further degraded into ethane. These results correspond with the earlier groundwater characterisation.

In-situ injection test

The results of the injection of carbon source and nutrients at injection points I1 and I2 are shown in tables 3 and 4 respectively. In table 4, the situation of Pb 5 after the first injection was taken as start concentration.

Table 3.Analytical results of injection I1 between Pb 4 and Pb 8.

Monitoring well	Component	Unit	Start t = 10:30 Concentration	End t = 13:00 Concentration
Pb 4	DOC	mg/l	0.0	4.86
(4 m distance)	NH_4	mg/l	0.059	0.353
	ortho-PO_4	mg/l	0.408	1.77
	Cl	mg/l	33.9	38.4
Pb 8	DOC	mg/l	6.58	9.09
(2 m distance)	NH_4	mg/l	0.052	0.208
	ortho-PO_4	mg/l	0.281	1.15
	Cl	mg/l	24.2	42.8
Pb 5	DOC	mg/l	0.0	8.28
(7 m distance)	NH_4	mg/l	6.91	7.63
	ortho-PO_4	mg/l	1.57	2.23
	Cl	mg/l	134.6	139.8

The influence of the injection in I1 can be clearly observed in all three monitoring wells. Even at a distance of 7 meters a slight increase was noticed. By these measurements it is proven that the carbon source and nutrients adequately spread out and that the instantaneous radius of influence was appr. 7 meters.

Table 4: Analytical results of injection I2 between Pb 5 and Pb 7.

Monitoring well	Component	Unit	Start t = 13.00 Concentration	End t = 15:00 Concentration
Pb 5	DOC	mg/l	8.23	10.6
(4 m distance)	NH_4	mg/l	7.63	7.10
	ortho-PO_4	mg/l	2.23	2.28
	Cl	mg/l	139.8	140.6
Pb 7	DOC	mg/l	0.0	0.0
(3,5 m distance)	NH_4	mg/l	0.031	0.633
	ortho-PO_4	mg/l	0.12	3.22
	Cl	mg/l	59.8	73.6

The results of the injection in I2 are less clear, but support the above conclusions. During the execution of the tests it was also noticed that the radius of influence of the injection depended on the depth of injection. The results in the tables above show only the final result as it could be measured in the groundwater of the monitoring wells.

Conclusion for case-study B

The conclusion of the feasibility test was that a remediation by Enhanced Reductive Dechlorination is suitable for the plume in combination with a temporary isolation by groundwater extraction at the site boundary. For the source, the high concentrations first need to be reduced in a different way if possible. In a later stage, stimulation of the biodegradation in the source can still be an option if an inoculum of *Dehalococcoides ethenogenes* is added (e.g. by injecting groundwater from the plume into the source). Start of the full-scale remediation is scheduled for August, 2005.

Discussion

The advantage to go directly to a pilot test is that – in case of a positive result – it is more certain that Enhanced Reductive Dechlorination will be successful in-situ. As well as the fact that the costs made already have a positive effect on the test area. The limitation is that the initial costs are quite high and the outcome is uncertain. Furthermore, the selection of the carbon source needs to be made in advance, where as with microcosm studies it is possible to test a variety of carbon sources and other boundary conditions such as nutrient availability. We believe that the type of electron donor selected normally does not affect the reaction endpoint, but that it does impact the duration of the lag period and rate of dechlorination. Also, for aquifers with low pH-buffering potential, some carbon sources are less suited because they cause acidifiaction (e.g. molasses, lactic acid,…). Some 'plain' carbon sources such as methanol may need extra supplamentation of nutrients in order to stimulate dehalogenation effectively.

Microcosm tests in combination with PCR-analyses are normally less expensive then a pilot test. But in that case, limited additional testing concerning the injection method of the carbon source should be executed to provide essential information to scale-up to a full-scale remediation.

Even if a pilot test is performed, additional actions still may be required to determine the injection method and/or the migration characteristics of the carbon source. PCR-analysis during pilot tests can also provide important information. If the result of the PCR-test is negative, the addition of an inoculum can be recommended. A 'negative' field test result can in that way still be positively interpreted from the point of view of feasibility of bioremediation.

Final conclusion

The results of the full-scale remediation results at of both case study sites should be anticipated in order to be able to draw the correct conclusions about the treatability testing performed. Most often, microcosm tests and field testing need not both be conducted to prove that Enhanced Reductive Dechlorination is possible on-site. Which test method to select depends on site-specific criteria, no strict rules can be given.

Basically, if the first screening of the assessment data strongly indicate that Enhanced Reductive Dechlorination is possible, an immediate field testing can be recommended. Also when a pump-and-treat system is already present on-site, the choice for an immediate pilot testing is convenient. If the contamination or the geochemical situation is more complex or the selection of the carbon source is not straightforward, microcosm testing is proposed. In both methods, PCR-analyses are a valuable tool to support decisions.

Acknowledgements
We would like to thank the following parties for their support in the performance of the case-studies: Hak Milieutechniek, Bioclear, Hannover Milieu- en Veiligheidstechniek and VITO.

References

J. Gemoets; R. Lookman, B. Borremans, T. De Ceuster; **2003** *Consoil 2003 Conference Proceedings “Evaluation of anaerobic in-situ bioremediation of chlorinated hydrocarbons and BTEX with laboratory microcosm studies and PCR”*

Jeff J. Morse, Bruce C. Alleman, James M. Gosset, Stephen H. Zinder, Donna E. Fennel, Guy W. Sewell, Catherine M. Vogel; **1998** *Technical protocol “A Treatability Test for Evaluating the Potential Applicability of the Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) to Remediate Chloroethenes”*

James M. Gosset, Stephen H. Zinder, Donna E. Fennel, C. Morey, D.T. Adamson; **2003** *“Reductive Anaerobic Biological In Situ Treatment Technology (RABITT) Treatability Testing: Cost and performance Report”*

R. Lookman, T. De Ceuster, D. Van Houtven, J. Gemoets, J. Patyn, R. Smolders, L. Diels; **2004** *”Study regarding the remediation of urban CAH groundwater contaminations with dry cleaning facilities in Antwerp as example”*

Stephen S. Koenigsberg; **2003** *Consoil 2003 Conference Proceedings “Facilitated desorbtion and incomplete dechlorination: observation from 350 application of HRC”*