Biobarrier

Generic guideline

Target audience: Scientists, Consultancies, contractors, authorities, feasibility test labs

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Project website address: aquarehab.vito.be

Table of Contents

1 INTRODUCTION

Permeable reactive biobarriers (biobarriers) are an innovative in-situ remediation technology for contaminated groundwater. This document intends to provide information about the biobarrier technology and its application area and boundary conditions for consultants, authorities, and feasibility testing labs. The aim is to offer support when evaluating the feasibility and the impact of the biobarrier technology to rehabilitate degraded waters, as well as when designing, implementing and monitoring biobarriers.

This document was composed in the frame of the FP7 project AQUAREHAB (GA 226565), and comprises outcomes and lessons learned during this project.

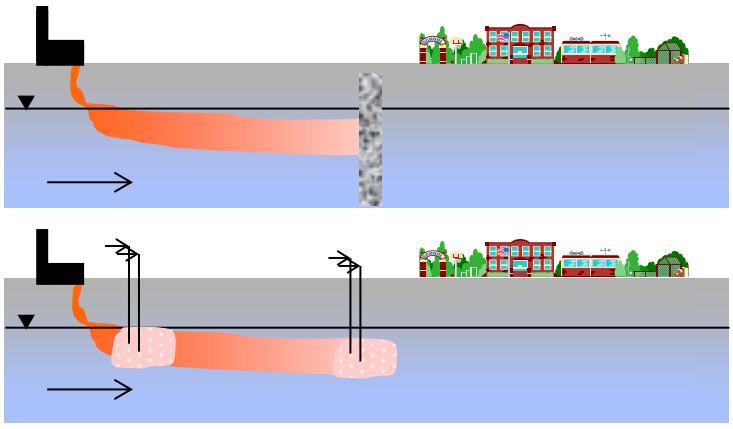
DISCLAIMER: Although the information described in this document is believed to be reliable and accurate, the guideline does not offer warranties of any kind.

2 BACKGROUND INFORMATION ON THE BIOBARRIER TECHNOLOGY

2.1 GENERAL EXPLANATION OF THE TECHNOLOGY PRINCIPLE

Permeable reactive barriers (PRBs) are installed in the subsurface downstream of a contamination source. In the barrier, pollutant removal processes are activated, which degrade the pollutants in the groundwater while it flows through the barrier. Generally, no pumping is involved and the natural hydraulic gradient is the driving force to move the groundwater through the barrier. Therefore, the PRB technology is a semi-passive to passive technology.

A permeable reactive biobarrier (biobarrier), is a kind of PRB where locally microbial processes are induced to prevent further spreading of the pollutants. The terminology used here, includes biobarriers sensu stricto (excavation & refilling of trench) as well as bioreactive zone (injection of substances that stimulate biodegradation without excavation) as indicated in Figure 1.



Biobarrier (sensu stricto)

Bioreactive zone (near source zone or in the contamination plume)

Figure 1 Schematic representation of two types of biobarriers.

A more detailed representation of one biobarrier type is given in Figure 2. After installation, the system can remain reactive for years when maintained well.

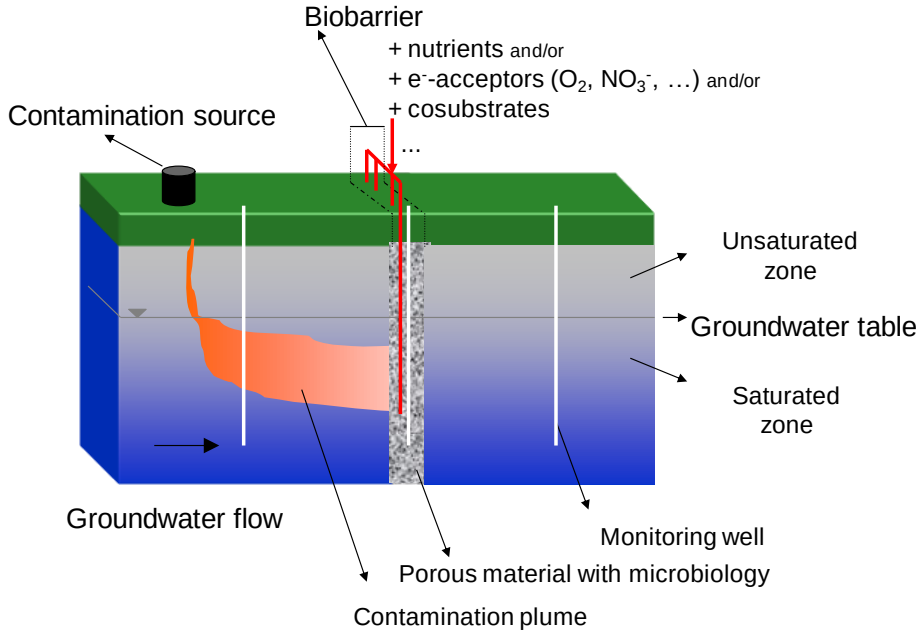


Figure 2 Detailed representation of a trenched biobarrier.

Sediment biobarriers can be considered as naturally existing biobarriers (Hamonts et al., 2009). The sediments which exist at the interphase of groundwater and surface water may typically possess unique environmental and microbial conditions that allow for the microbial removal of groundwater contaminants before the groundwater reaches the surface water. Active sediment capping to improve the biodegradation in the groundwater-surface water interphase is explained more in detail in a separate guideline.

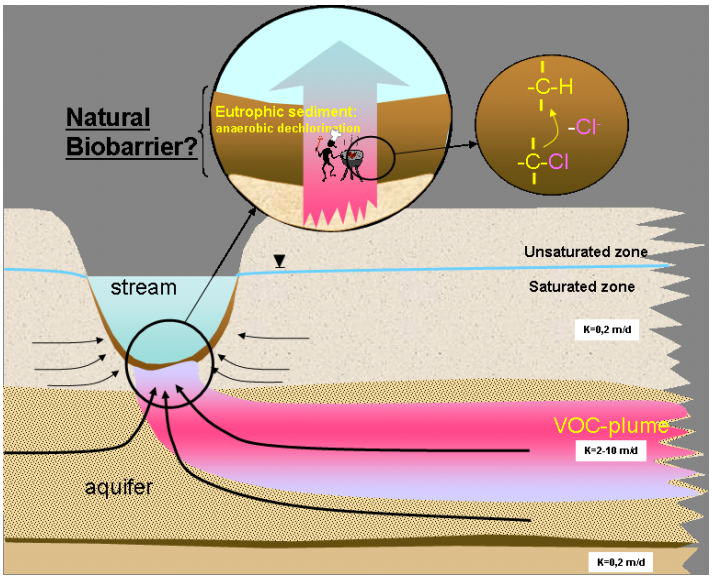


Figure 3 Schematic representation of sediment biobarrier.

2.2 TARGETED SUBSTANCES

Substances that can be targeted by the biobarrier technology are given in Table 1 as examples, along with their potential emission sources.

Table 1 Overview of substances that can be tackled by the biobarrier technology.

Targeted substances		Aerobic biodegradation	Anaerobic biodegradation
Class	Specific substance		
CAHs (chlorinated aliphatic hydrocarbons) *Emission source: Drycleaner activities, degreasing activities, ...* *Degradation products of other chlorinated compounds +* *Degradation products of PCE and TCE*	Tetrachloroethylene (PCE)	-	++
	Trichloroethylene (TCE)	-	++
	Cis-dichloroethylene (cDCE)	++	++
	Trans-dichloroethyele (tDCE)	++	++
	Vinylchloride (VC)	++	+
	1,1,1-trichloroethane (TCA)	-	++
	1,1-dichloroethane (DCA)	-	++
	chloroethane	+	+/-
	1,2-dichloroethane	+/-	+
	Tetrachloromethane (PCM)	-	+
	Trichloromethane (TCM) (chloroform)	-	+
	Dichloromethane (DCM)	+/-	+
Aromatic compounds *Emission source: Petrol gas station & storage places*	BTEX (benzene, toluene, ethylbenzen, xylenes)	+++	+ (benzene very difficult)
Fuel oxygenates *Emission source: oil industry & petrol gas station & story places*	Methyl ter-buty ether (MTBE)	++	-
	tertiary-butyl alcohol (TBA)	++	-
Metals *Emission source: chemical industry, nonferrous industry, metal coating and processing,*	Zn, Cu, Cr, Pb, Cd, ...	-	In-situ bioprecipitation under sulphate reducing conditions

+++: Very easy; ++: biodegradable; +: more difficult to biodegrade; +/-: rarely biodegradable; -: not or very slowly biodegradable. References see Figure 5, Figure 6 and Figure 7.

2.3 REACTION MECHANISMS AND PATHWAYS

Biodegradation/biotransformation is based on electron transport facilitated by the micro-organisms (bacteria) from an electron donor to an electron accepting component, whereby pollutants are degraded/transformed via oxidation or reduction reactions. Several elements, including carbon, nitrogen, oxygen, sulphur, iron and/or manganese are key components involved in these reactions. The pollutant can act as:

1. **Electron donor**, as in the case of BTEX compounds, where the electron acceptor is oxygen under anaerobic conditions. Under anaerobic conditions a set of potential electron acceptors exists comprising nitrate, ferric iron, manganese, sulphate (Figure 4).

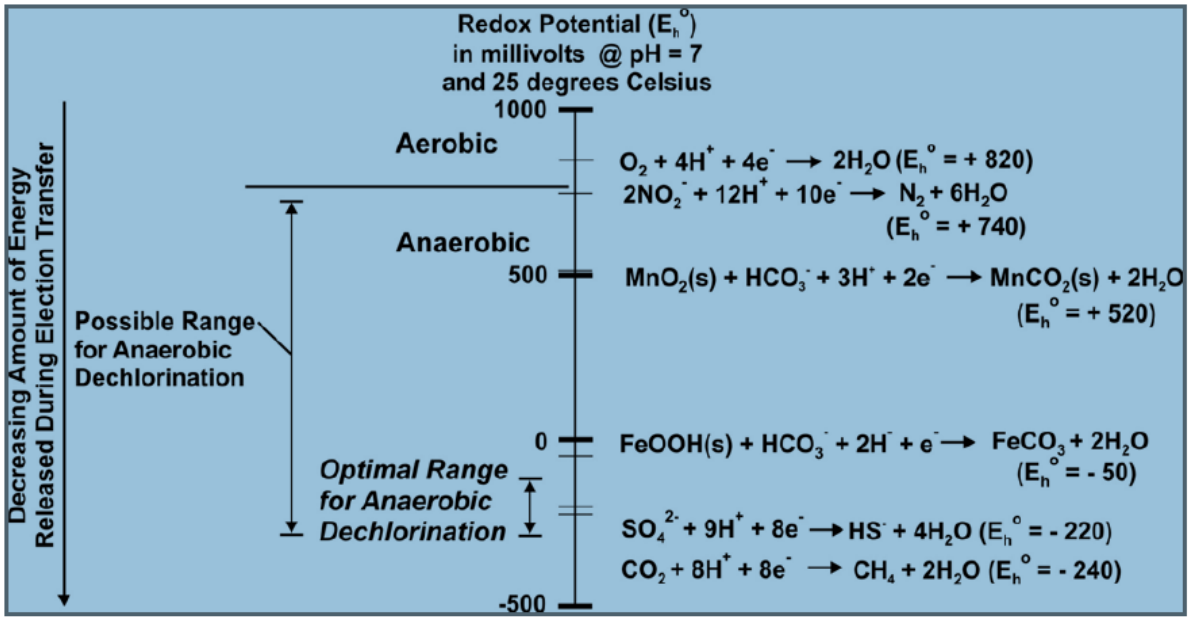


Figure 4 Overview of different electron-accepting processes (ITRC, 2011; based on Bouwer, 1992).

2. **Electron acceptor**, as in the case of chlorinated ethenes, nitrate and sulphate under anaerobic conditions. The electron donor can be organic matter in the soil or groundwater, but it is often a limiting factor in groundwater contamination plumes. Therefore, addition of electron donors (carbon source, molecular hydrogen, ...) is often needed to activate the anaerobic biodegradation process.

Table 2: Overview of common electron donors.

Type	Compound	Application	Remarks
Pure chemicals	Lactate (polylactate)	Injectable	Pure substance, food grade
	Acetate, butyrate, methanol,	Injectable	Pure substance
	Molecular hydrogen	injectable	Added as a gas, short lifetime
Industrial process residues (mixture of compounds	Molasses	Injectable	Residue from sugar refining
	Nutrolase	Injectable	Residue from potato processing
	Glycerol (from biodiesel)	Injectable	Potential high sulphate content
	Cheese whey	injectable	Residue from cheese

Type	Compound	Application	Remarks
			production
Natural materials	Mulch and compost	Biobarrier trench	Mix with coarse sand or pea gravel
	Tree bark or wood chips	Biobarrier trench	Combined with other ED
	Emulsified vegetable oils	Injectable	long lifetime, combine with tree bark
	Crustacea shells (chitin)	Biobarrier trench	

3. **Electron donor nor electron acceptor**. Some metals are removed from groundwater by a secondary reaction, being precipitation with for instance sulphide, that was produced by microbial reduction of sulphate (in-situ bioprecipitation).

Biodegradation reactions are part of the cell metabolism to survive and to multiply. Therefore, also other element (N, P, …) and vitamins are needed in trace amount to facilitate the biodegradation reaction. Most of the trace elements are by nature present in the subsurface. Nitrogen and phosphor, also called nutrients, are needed in a ratio of C:N:P = 100:10:1. In highly polluted areas, (high carbon concentration) addition of nutrients may be needed.

Reaction pathways

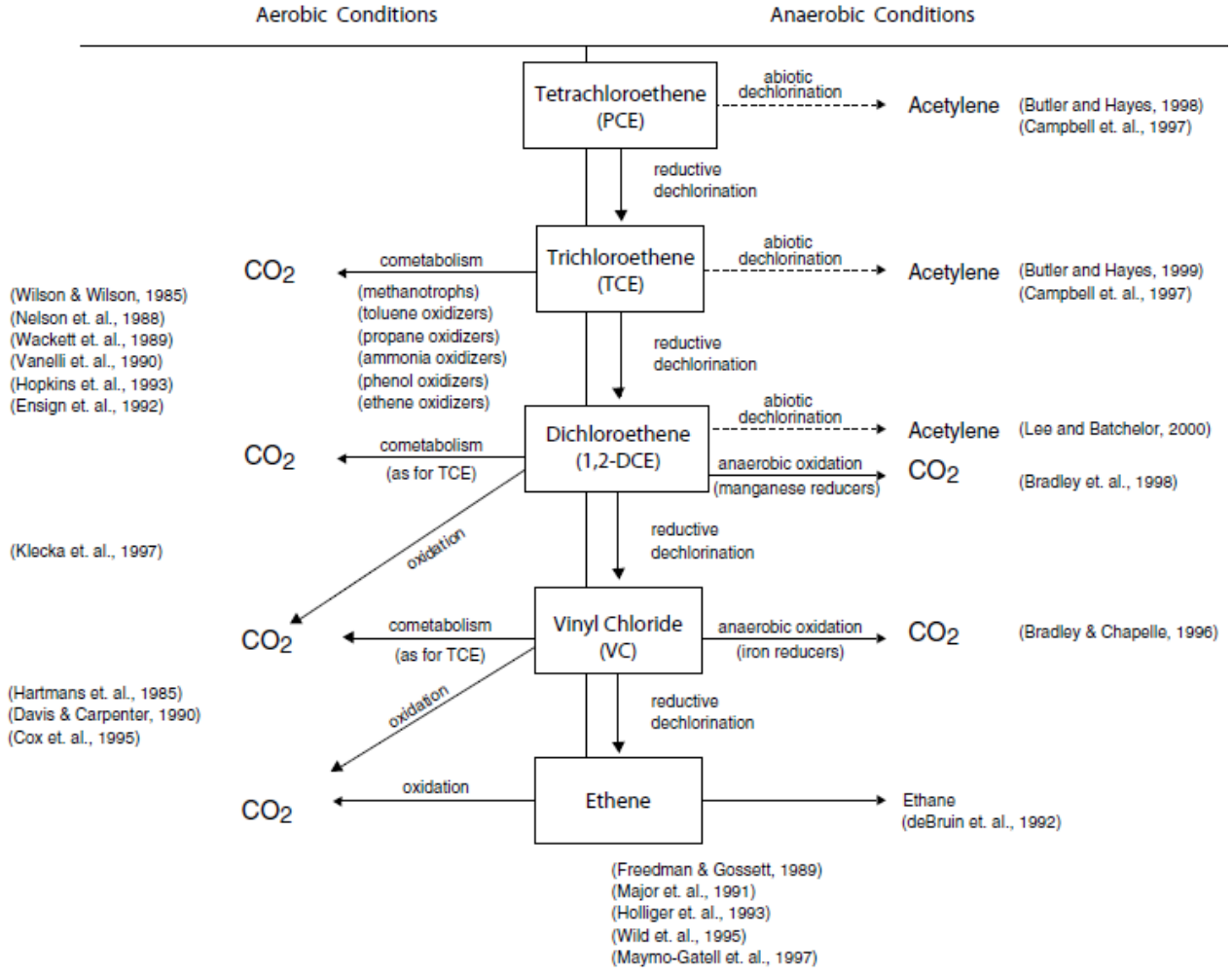


Figure 5 Pathways for biodegradation (→) of chlorinated ethenes (AFCEE, 2008).

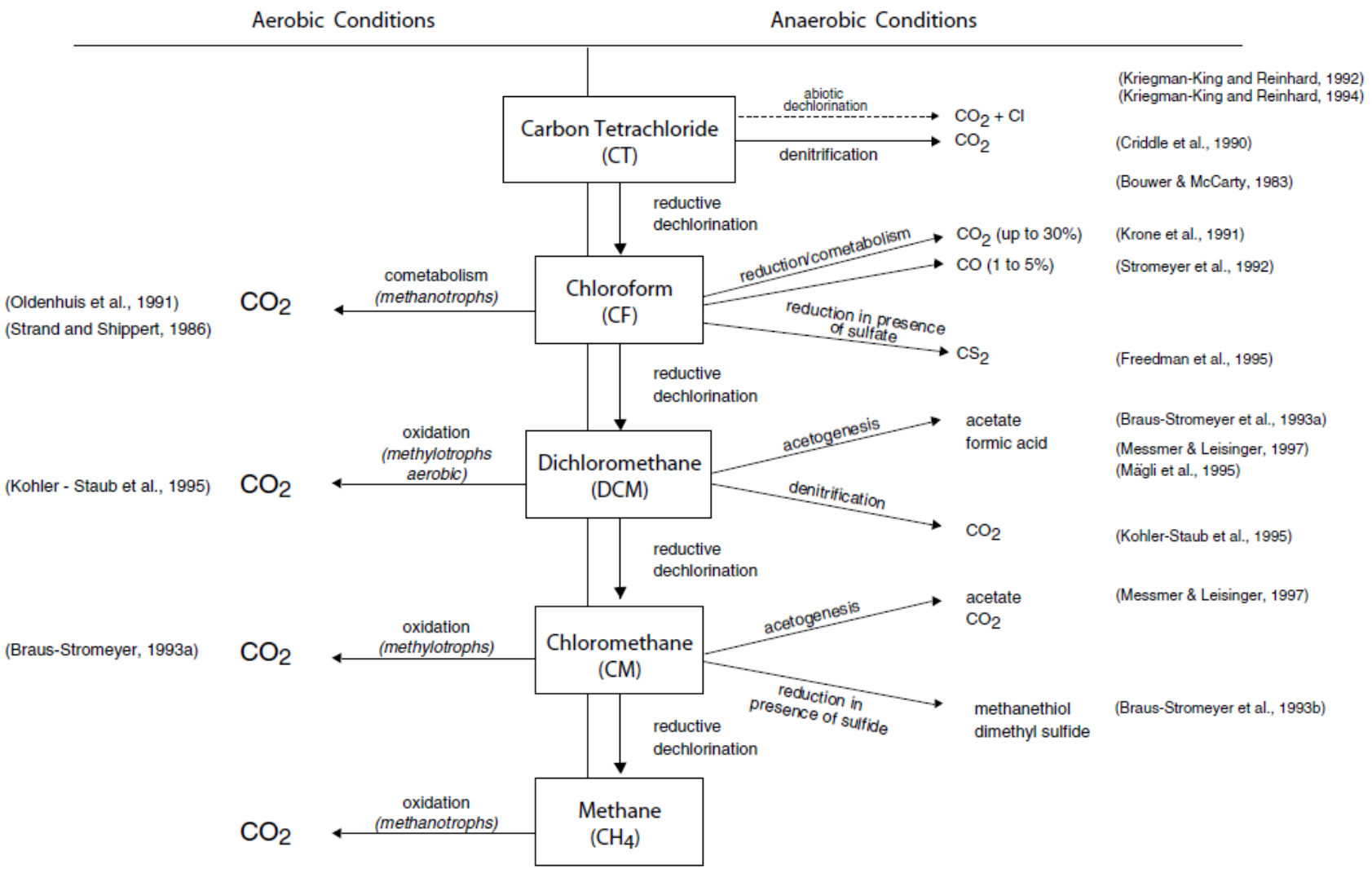


Figure 6 Pathways for biodegradation (→) of chlorinated methanes (AFCEE, 2008).

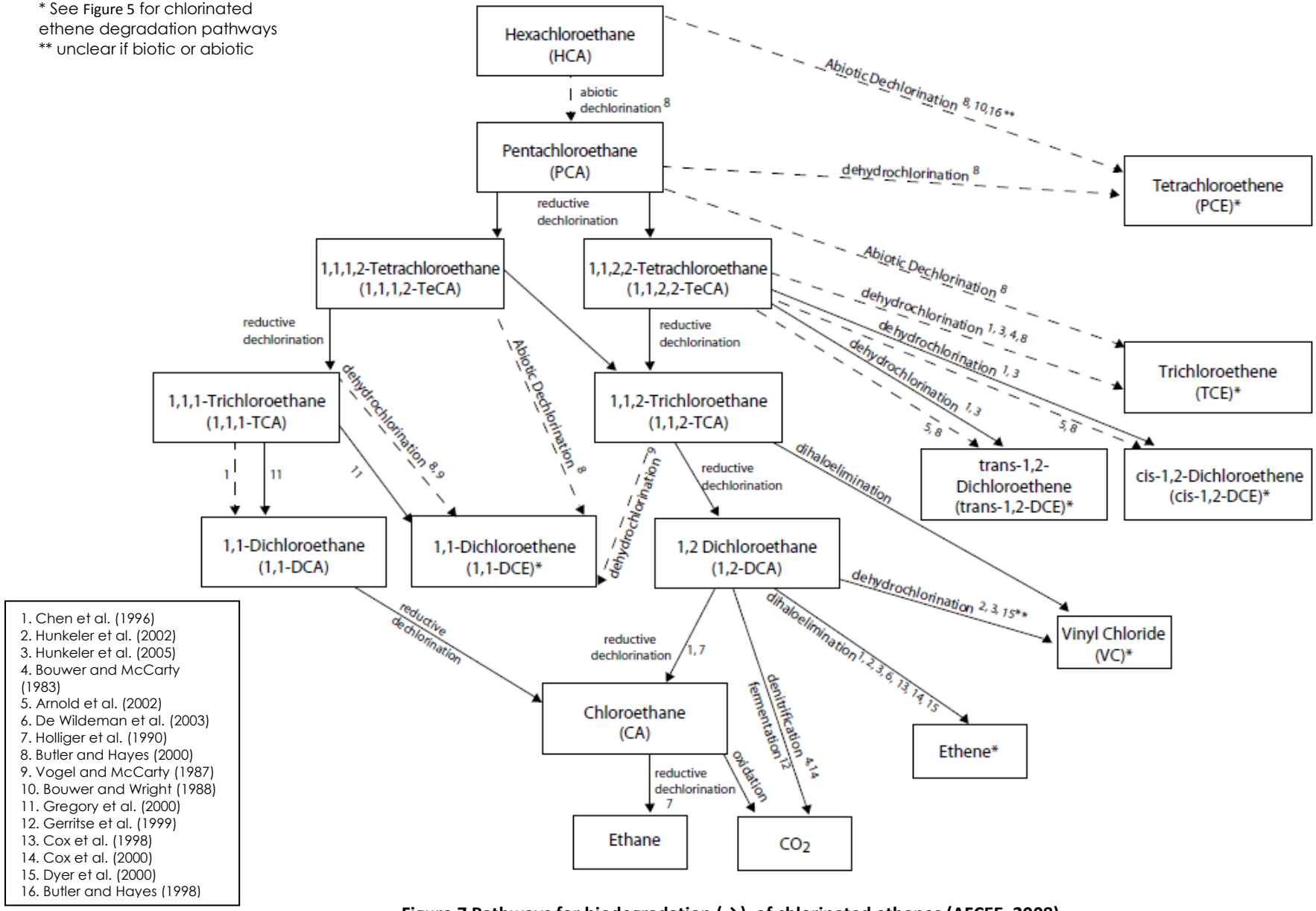


Figure 7 Pathways for biodegradation (→) of chlorinated ethanes (AFCEE, 2008).

2.4 REACTION RATES

Table 3 and Table 4 give an overview of first order degradation rates in function of different redox conditions for selected contaminants. These data provide a first indication of degradation rates that may be expected to verify the feasibility of a biobarrier. However, the final design of a biobarrier should be based on laboratory feasibility column tests which are performed with site specific materials and/or on pilot scale field tests.

2.5 DEVELOPMENT STAGE OF THE TECHNOLOGY

Within technology development, the following stages can be defined:

- A technology is very emerging when it is at the research stage (not even implemented in other sectors).
- It is emerging when it is implemented in another sector and is being developed in the concerned sector (but it is not at the pilot plant trial stage yet).
- It is becoming transferable when it is at the pilot plant trial stage in the concerned sector.
- It is transferable when it is at the full scale trial stage in the concerned sector.
- It is available when it is commercially available and in use in the concerned sector.

The biobarrier technology is **available** and **well accepted** for a number of pollutants in many European countries. Numerous labscale studies have been performed (Careghini et al., 2013) and biobarriers in the field at pilot scale and full scale have been described in literature from before 1995. Some details for a number of biobarriers are given in Table 5.

Table 3: First order degradation rates [day^{-1}] for some selected hydrocarbons in function of different test conditions and redox processes.

Substance	System characteristics	Carbon added	Redox process						Reference
			Aerobic respiration	Nitrate reduction	Iron reduction	Sulfate reduction	Methanogenesis	Mixed	
Benzene	n.d.	n.d.	0.335	0.008	0.009	0.008	0.010	0.009	Suarez and Rifai, 1999
	In situ	N.A.	-	0.007	0.005	0.014	0.011	0.003	Lawrence, 2006
	In situ	N.A.	0.096	-	-	-	-	-	Lawrence, 2006
Toluene	n.d.	n.d.	0.262	0.459	0.012	0.062	0.037	0.302	Suarez and Rifai, 1999
	Column test	N.A.	0.080	-	-	-	-	-	Saponaro et al., 2009
	In situ	N.A.	-	0.053	0.001	0.011	0.014	0.058	Lawrence, 2006
	In situ	N.A.	0.200	-	-	-	-	-	Lawrence, 2006
	Batch test	N.A.	-	-	-	-	0.000	-	Upsoil (EU FP7)
Ehtylbenzene	n.d.	n.d.	n.d.	0.270	0.003	0.002	0.010	0.010	Suarez and Rifai, 1999
	In situ	N.A.	-	0.007	0.000	0.004	0.003	0.015	Lawrence, 2006
	In situ	N.A.	0.113	-	-	-	-	-	Lawrence, 2006
	Batch test	N.A.	-	-	-	-	0.000	-	Upsoil (EU FP7)
m-Xylene	n.d.	n.d.	0.163	0.089	0.010	0.081	0.019	0.004	Suarez and Rifai, 1999
	In situ	N.A.	-	0.006	0.000	0.005	0.002	0.016	Lawrence, 2006
	In situ	N.A.	0.054	-	-	-	-	-	Lawrence, 2006
o-xylene	n.d.	n.d.	0.086	0.012	0.003	0.027	0.026	0.009	Suarez and Rifai, 1999
	In situ	N.A.	-	0.006	0.000	0.006	0.002	0.021	Lawrence, 2006
	In situ	N.A.	0.054	-	-	-	-	-	Lawrence, 2006
p-xylene	n.d.	n.d.	0.207	0.068	0.010	0.011	0.018	0.006	Suarez and Rifai, 1999
	In situ	N.A.	-	0.006	0.000	0.004	0.002	0.015	Lawrence, 2006
	In situ	N.A.	0.054	-	-	-	-	-	Lawrence, 2006
MTBE	In situ	N.A.	0.107	-	-	-	-	-	Wilson et al., 2002
	Column test	N.A.	0.031	-	-	-	-	-	Saponaro et al., 2009
	Batch test	N.A.	-	-	-	-	-	0.032	Wilson et al., 2005
	In situ	N.A.	-	-	-	-	0.010	-	Lawrence, 2006
	In situ	N.A.	0.004						Lawrence, 2006

Substance	System characteristics	Carbon added	Redox process						Reference
			Aerobic respiration	Nitrate reduction	Iron reduction	Sulfate reduction	Methanogenesis	Mixed	
TBA	Batch test	N.A.	-	-	-	-	-	0.000	Wilson et al., 2005
	Hyporheic zone	N.A.	0.009	-	-	-	-	-	Greenwood et al., 2007
	In situ	N.A.	-	-	-	-	0.026	-	Lawrence, 2006

n.d.: no data
N.A.: Natural Attenuation

Table 4: First order degradation rates [day^{-1}] for some selected chlorinated hydrocarbons in function of different test conditions and redox processes.

Substance	System characteristics	Carbon added	Redox process						Reference
			Aerobic respiration	Nitrate reduction	Iron reduction	Sulfate reduction	Methanogenesis	Mixed	
CF	Column test	Compost	-	-	-	-	-	2.310	Lorah et al., 2008
CT	n.d.	n.d.	n.d.	0.078	0.117	n.d.	0.320	n.d.	Suarez and Rifai, 1999
	Column test	Compost	-	-	-	-	-	2.830	Lorah et al., 2008
	In situ	N.A.	-	-	-	-	-	0.016	Lawrence, 2006
DCM	Batch test	N.A.	-	-	-	-	0.016	-	Upsoil (EU FP7)
	Batch test	Lactate	-	-	-	-	0.028	-	Upsoil (EU FP7)
TeCa	Column test	Compost	-	-	-	-	-	1.870	Lorah et al., 2008
	Batch test	Lactate	-	-	-	-	0.8*	-	Jones et al., 2006
TCA	n.d.	n.d.	0.002	0.000	n.d.	0.010	0.498	n.d.	Suarez and Rifai, 1999
	In situ	N.A.	-	-	-	-	-	0.015	Lawrence, 2006
	Batch test	Lactate	-	-	-	-	0.3*	-	Jones et al., 2006
DCA (all isomers)	n.d.	n.d.	0.000	n.d.	n.d.	0.003	0.006	n.d.	Suarez and Rifai, 1999
	In situ	N.A.	-	-	-	-	-	0.006	Lawrence, 2006
PCE	n.d.	n.d.	0.001	0.000	0.004	n.d.	0.100	n.d.	Suarez and Rifai, 1999
	Column test	Chitin	-	-	-	-	-	6.900	Brennan et al., 2006
	Column test	Chitin	-	-	-	-	-	1.400	Lorah et al., 2008
	In situ	n.d.	-	-	-	-	-	0.018	Schaerlaekens et al., 1999
	In situ	N.A.	-	-	-	-	-	0.001	Lawrence, 2006
	Batch test	N.A.	-	-	-	-	0.191	-	Upsoil (EU FP7)
	Batch test	Lactate	-	-	-	-	0.046	-	Upsoil (EU FP7)
	Batch test	N.A.	-	-	-	-	-	0.012	Aquarehab (EU FP7)
	Batch test	Lactate	-	-	-	-	0.147	-	Aquarehab (EU FP7)
TCE	n.d.	n.d.	0.005	n.d.	0.003	0.011	0.015	0.001	Suarez and Rifai, 1999
	Column test	Chitin	-	-	-	-	-	6.600	Brennan et al., 2006
	Column test	Compost	-	-	-	-	-	3.000	Lorah et al., 2008

Substance	System characteristics	Carbon added	Redox process						Reference
			Aerobic respiration	Nitrate reduction	Iron reduction	Sulfate reduction	Methanogenesis	Mixed	
	Mixed	n.d.	-	-	-	-	-	0.004	Schaerlaekens et al., 1999
	Batch test	#	-	-	-	-	0.170	-	Aquarehab (EU FP7)
	In situ	N.A.	-	-	-	-	-	0.001	Lawrence, 2006
	Batch test	N.A.	-	-	-	-	-	0.131	Aquarehab (EU FP7)
	Batch test	N.A.	-	-	-	-	0.016	-	Upsoil (EU FP7)
	Batch test	Lactate	-	-	-	-	0.018	-	Upsoil (EU FP7)
DCE (all isomers)	n.d.	n.d.	n.d.	n.d.	0.002	0.045	0.047	0.001	Suarez and Rifai, 1999
	Column test	Chitin	-	-	-	-	-	52.900	Brennan et al., 2006
	mixed	n.d.	-	-	-	-	-	0.004	Schaerlaekens et al., 1999
	Batch test	#	-	-	-	-	0.144	-	Aquarehab (EU FP7)
	Batch test	N.A.	-	-	-	-	-	0.131	Aquarehab (EU FP7)
	Batch test	N.A.	-	-	-	-	0.056	-	Upsoil (EU FP7)
	Batch test	Lactate	-	-	-	-	0.056	-	Upsoil (EU FP7)
VC	n.d.	n.d.	0.087	n.d.	0.260	n.d.	0.230	n.d.	Suarez and Rifai, 1999
	In situ	n.d.	-	-	-	-	-	0.001	Schaerlaekens et al., 1999
	Batch test	#	-	-	-	-	0.132	-	Aquarehab (EU FP7)
	In situ	N.A.	-	-	-	-	-	54.794	Lawrence, 2006
	Batch test	N.A.	-	-	-	-	0.006	-	Upsoil (EU FP7)
	Batch test	Lactate	-	-	-	-	0.066	-	Upsoil (EU FP7)

n.d.: no data
N.A.: Natural Attenuation
#: a range of carbon sources was tested with lactate, molasses and sediment extracts.
* data were recalculated to obtain a first order degradation rate

Table 5: Examples of biobarriers.

Site	Offutt Air Force Base Nebraska (US)	Naval Basr Ventura County California (US)	The DOW Chemical Company California (US)	Nickle Rim Ontario (US)	Naval Base Ventura County California (US)
Scale & biobarrier type	Oilot & full scale Continuous biobarrier	full-scale, biobarrier	full scale & 3 bioreactive zones	full-scale continuous biobarrier	full-scale, 2-biobarrier (phase 2) –mid-plume and toe barrier
Installation date	1999; 2001	2000	2000; 2002	1995	2003
Pollutants	TCE	MTBE, TBA (> 10 mg/L) 1.5 km by 152 m plume	PCM, MCM, PCE, TCE	2400-3800 ml/L sulphate, 10 mg/ L Ni	MTBE, TBA (> 10 mg/L) 1.5 km by 152 m plume
Pollutant removal mechanisms	reductive dehalogenation	aerobic biodegradation	reductive dehalogenation	bioprecipiation of metals	aerobic biodegration
reactive media	Sand Wood mulch	oxygen gas, specialised MTBE-degrading bacteria	propylene glycol bio-augmentation	compost, wood chips, pea gravel	oxygen gas, specialised MTBE-degrading bacteria
Dimensions of the barrier (L: length; T: Thickness; D: depth; H: height)	L: 152 m; T: 45 cm; D: 1-7.5 m	L: 152 m; D: 3-6 m;	L: 400 m; T: 6 M; D: 41 m	L: 15m; T: 3.6 m; D: 4.2 m	L: 274 m
Installation method	Continuous trencher	252 gas injection wells – direct push	39 subsurface circulation Wells aligned in three distinct linear segments		direct push
Location monitoring wells		174 monitoring wells		multilevel monitoring wells	
Installation costs		435.000 $		30.000 $	500.000$
Annual monitoring cost		75.000 $ (40 years expected to be needed)			125.000 $
Total costs		34 million			
Information source	ITRC, 2005	ITRC, 2005 ITRC, 2011	ITRC, 2005	ITRC, 2005	ITRC, 2005 ITRC? 2011

NA = Not available;

Table 5: Examples of biobarriers. – Cont.

Site	Altus AFB Oklahoma (US)	Oklahoma Pork Facility (US)	BG05 South Dakota (US)	Ash Landfill site Seneca Army Depot, NY	Industrial site in Lier, (Belgium)
Scale & biobarrier type	full-scale Continuous biobarrier	full-scale Continuous biobarrier	Full scale Continuous biobarrier	Full-scale Dual continuous biobarrier	Pilot-scale reactive zone
Installation date	2002	2002	2005	2005	2006
Pollutants	TCE, c-DCE	nitrate	perchlorate	TCE, c-DCE, VC	PCE, TCE, DCE, VC
Pollutant removal mechanisms	reductive dehalogenation	denitrification by heteratrophic bacteria		reductive dehalogenation	Reductive dehalogenation
reactive media	shredded mulch (300 cy), cotton gin compost (60cy), sand (265 cy)	wheat straw	30.000$ Mulch	shredded tree mulch and sand coated with soy bean oil	EHC (Adventus), 8500 kg (1% of mass of soil)
Dimensions of the barrier (L: length; T: Thickness; D: depth; H: height)	L: 138 m; T: 45 cm; D: 7.3 m	L: 243 m; T: 3.6 m; D: 3 m	L: 177 m; T: 60 cm; D: 9.7 m	L: 2x150 ft, T: 3ft, D: 11ft Two parallel biowalls 15ft apart Scaled up to 3 sets of dual biowalls 2720 ft long	L : 50 m; T: 2,5 m; D: 5-10 m
Installation method	continuous one-pass trencher	vertical trencher	continuous one-pass trencher	Excavated trench	Direct push injection 40 injection points over 2 lines
Location monitoring wells	2 transects perpendicular to the barrier	2 transects perpendicular to the barrier		11 monitoring wells (upgradient, within biowalls, between biowalls and downgradient)	1 within the bioscreen and 3 downgradient at distances of 3 m
Installation costs	165.000 $ (360$/linear foot)		293.200 $		
Annual monitoring cost	27.000$		42.000 $		
Total costs					
Information source	ITRC, 2011	ITRC, 2011	ITRC, 2011	USAF, 2008	confidential

NA = Not available;

Table 5: Examples of biobarriers. – Cont.

Site	Shilbottle, UK	N. Ireland SEREBAR	Offenbach, D Gaswork	Wageningen Walstraat (NL)	Industrial site in Ghent (Belgium)
Scale & biobarrier type	Full scale, Filled trench	Full scale sequential biobarrier funnel & gate	Full-scale Funnel & gate	Full scale biobarrier Injection	Full scale aerobic biobarrier
Installation date	2002		2007	2004	2008
Pollutants	Acid mine drainage metals (Zn, Ni, Mn, Fe) & sulfate	BTEX,	BTEX, PAH, heterocyclics	Chloro-ethenes (PER, TRI, DCE)	Vinyl chloride
Pollutant removal mechanisms	Bioprecipitation, sorption, biodegradation	Anaerobic and aerobic biodegradation and adsorption on GAC	Aerobic biodegradation	Anaerobic biodegradation	Aerobic biodegradation
reactive media	50% aggregate (calcite limestone, blast furnace slag) , 25% horse manure and 25% green waste compost	Air sparging to create aerobic conditions	Injection of hydrogen peroxide	Soluble electron donor “Percol”	Air introduced by in-situ air sparging (biosparging)
Dimensions of the barrier (L: length; T: Thickness; D: depth; H: height)	L= 170 m, T = 2m, D = 3m Retention time 48 hrs	Funnel= L = 175 + 70 m, D = 6-8 m		L = 80 m, D = 28 m	L = 300 m, D = 20 m
Installation method	Excavated trench	6 reactive vessels in series (2 anaerobic, 2 aerobic, 2 GAC)	3 in-situ reactive vessels	Injection wells at 4 depths, 4x9 wells spaced 7,5 m apart	2x23 nested microbubble spargepoints at 15 + 20 m-bgl spaced 15 m apart
Location monitoring wells	Two sets of multilevel piezomers within biobarrier	Influent, effluent, between and within each vessel		5 wells at 10 m downgradient (5 depths) and one nested well at 80 m downgradient (3 depths) 2 wells 20 and 40 m upgradient	Upgradient : 6 nested wells (12 + 16 + 20 m-bgl); downgradient : 6 nested wells (12 + 16 + 20 m-bgl); Plume downgradient : 6 nested wells (15/ 20/25 mbg)
Installation costs	?			60000 EUR installation 30000 EUR/year maintenance	115.000 EUR installation 20.000 EUR/year maintenance
Annual monitoring cost	?				30.000 EUR
Total costs	?				230.000 EUR
Information source	IMWA 2005 (Bowden)	ES&T, 2007	Water Science & Technology 58(7)	SKB report PT4111 (2006)	Contractor (anonymous)

NA = Not available;

3 APPLICABILITY AND BOUNDARY CONDITIONS OF THE TECHNOLOGY

The applicability area of the biobarrier technology is determined by different aspects.

Microbial aspects:

- The pollutants present in the groundwater are biodegradable, and do not result in accumulation of non-degradable harmful metabolites.
- Pollutants are present in the dissolved phase.
- Environmental conditions (pH, temperature, redox conditions, dissolved oxygen concentration, …) at the site could allow biological processes, which exist under natural conditions or which can be created.

Site specific aspects :

- The depth of the groundwater contaminant plume is preferably not located deeper than 40-50 m below ground surface (bgs), more preferably below 20 m bgs. For deeper plumes, the installation cost will increase significantly and biobarriers at depths greater than approximately 10 m bgs would be restricted to reactive zones (injection of reagents).
- The groundwater flow direction needs to be known and should be relatively stable in time.
- The presence of a shallow impermeable layer sealing the bottom of contamination plume is an advantage for the biobarrier technology as it prevents contaminants passing underneath the biobarrier. Also when no low permeability layer is present, biobarriers can be applicable when this aspect is taken into account during the feasibility and design phase (especially for LNAPL sites).
- In principle, the biobarrier technology is applicable for a wide range of groundwater flow velocities. For higher flow velocity, larger dimensions of the biobarrier are generally needed (to ensure sufficient contact time) resulting in higher costs. The required quantities of amendments such as electron donor or electron acceptor would also be greater.
- The hydraulic conductivity of the barrier should be equal or higher than the permeability of the surrounding aquifer to avoid mounding and by-passing of the groundwater.
- The site is accessible for the installation of the barrier, which may imply the excavation of soil and refilling the trench with reactive media (for barrier sensu stricto) or the installation of injection wells and equipment (for bioreactive zones). After the installation, there may be injection filters that need to remain accessible for repeated injection, or continuous dosing systems. Also monitoring filters which remain accessible are required.

The use of biobarriers is not recommended:

- For pollutants that have not been shown to be biodegradable, or that are likely to be transformed in harmful reaction products that may accumulate.
- For sites where free product is expected to migrate into the barrier.
- For sites with groundwater contaminations situated in deep subsurface (> 50 m bgs), due to technical and budget issues.
- When substances (co-pollutants) are present at the site that can inhibit bacteria.

Positive side effects linked to the biobarrier technology:

- Micro-organisms and soluble reactive substances can migrate outside the barrier, predominantly in the downstream direction, and enlarge as such the dimensions of the biobarrier.

Negative side effects linked to the biobarrier technology:

- Changes in redox condition or pH may lead to precipitation of inorganics in the biobarrier, reducing the permeability of the system.
- Stimulation of biodegradation processes implies stimulation of bacterial growth. Over time, the biomass or the accumulation of metabolic gases such as methane may reduce the permeability of the system, especially of infiltration filter/areas.

More details on the criteria for application of anaerobic, reducing microbial reactive zones are summarised in Table 6.

Table 6 Suitability of site screening characteristics for an anaerobic, reducing, microbial *in situ* reactive zone (IRZ) implementation.

Site characteristics	Suitable for IRZ	Marginally suitable for IRZ	Unsuitable for IRZ
Hydraulic conductivity	$10^{-1} > K \geq 10^{-4}$ cm s^{-1}	$10^{-6} \leq K \leq 10^{-4}$ cm s^{-1}	$K < 10^{-6}$ cm s^{-1}
Groundwater velocity	> 9 m $year^{-1}$ and < 278 m $year^{-1}$, TOC demand is reasonable to high	278 to 334 m $year^{-1}$, TOC demand is very high	< 6 m $year^{-1}$, hard to deliver organic carbon > 445 m $year^{-1}$, TOC demand is excessive
pH	5.5 < pH < 8.0	4.0 < pH < 5.5 8.0 < pH < 9.0	pH < 3.0 pH > 9.5
Natural degradation prior to injection of organic substrate	slow, complete or partial degradation of parent compound(s)	no or very little degradation but the aquifer is mildly anaerobic or transient anaerobic	no degradation at all and the system is highly aerobic; TOC demand is excessive and injection costs are high
DNAPL presence	presence of dissolved, sorbed and emulsified contaminants	most of the mass is still in emulsified NAPL form	recoverable separate phase contaminant within the targeted zone of remediation
Presence of electron acceptors O_2, NO_3^-, SO_4^{2-}	low enough to enable reasonable consumption of TOC to create sufficiently reducing conditions (SO_4^{2-} < 700 mg/L)	moderate flux of incoming electron acceptors demand continuous injection of high levels of organic carbon (SO_4^{2-} > 700 mg/L)	excessively high flux of incoming electron acceptors make it difficult for the target biogeochemical regime(s) to be established
Contaminant level	100s of µg L^{-1} to 100s of mg L^{-1} dissolved concentrations within contaminated plumes	dissolved contaminants with a large fraction of adsorbed or emulsified mass of LNAPL or DNAPL	very large volumes of NAPL mass, either floating or sinking, present within a large area
Presence of metals	metal concentrations at nontoxic levels, < 100s of mg L^{-1}	metal concentrations at inhibitory levels	metal concentrations at toxic levels, 1000s of mg L^{-1}
Oxidation state of contaminants	oxidized	neutral	highly reduced

Source: Suthersan and Payne, 2005

4 PERFORMANCE OF THE TECHNOLOGY

4.1 PERFORMANCE

The **abatement rate** can be defined as the pollutant concentration after the technology implementation divided by the pollutant concentration before implementation of the technology. Biobarriers aim at a reduction of the pollutant below regulatory limits in the downstream area, implying an abatement rate close to 95-100%.

Efficiency drivers: The performance of a biobarrier is for a large part determined by the biodegradation or fixation rates that are achieved within or downstream of the barrier. These depend on multiple factors such as the redox conditions, pH, the concentration of the electron donor or acceptor, the microbial community etc. These effects are generally lumped using first order kinetics to describe the degradation rate *in situ*. The biodegradation rates may change over time as concentrations of electron donor or acceptor and environmental conditions may change.

Examples of case studies for biobarriers can be found in the reports on biobarriers by ITCR, 2011 and AFCEE, 2008.

4.2 LONGEVITY

The **longevity of the technology** is influenced predominantly by (1) the evolution of the permeability of the system, and the (2) the maintenance of good biodegradation conditions.
The evolution of the permeability over time is determined by (1) the initial permeability of the system, (2) the composition of the groundwater, (3) the processes induced in the biobarrier (impacting pH, ORP, bacterial growth, precipitation of metals such as iron hydroxide, ...), (4) the groundwater flow velocity, and (5) potential biofouling controlling actions.
Good biodegradation conditions imply (1) the presence of sufficient electron donor, or electron acceptors, nutrients, ... and (2) the absence of inhibiting substances, comprising degradation products.
The lifetime of biobarriers can be in the order of years to decades. Biobarriers which are based on trenches filled with solid reactive materials will become exhausted in terms of availability of electron donor at some time. Periodic replacement of the barrier filling may be needed in these cases, or one may shift to additional injections of soluble substrates. Some reported longevity spans are listed in Table 7.

Table 7 Overview of reported longevity spans of biobarriers.

Barrier type/ filling materail	Longevity span	Reference
Mulch and compost biobarriers	5 to 10 years	Careghini et al., 2013
biowalls	5 to over 8 years 7 to 15 years for cellulose based biobarriers	ITRC, 2011

The most useful geochemical parameters to determine when a biowall may need to be replenished are as follows (ITRC 2011):

- contaminant concentrations

- bioavailable organic carbon or electron donor supply (TOC or DOC, dissolved hydrogen, or humic and fulvic acids)
- indicators of predominant TEAPs (ferrous iron, sulfate, and methane)
- indicators of redox state and chemical equilibrium (ORP/Eh and pH)

For instance, at an industrial site near Antwerp (Belgium) a pilot test has been conducted for a biobarrier to contain a contamination with TCE. The biobarrier was created by injection of EHC at 1% loading (m/m relative to soil mass) over an area of 50 m long and 2,5 m wide. EHC (Adventus) is based on a core of zerovalent iron that is coated with an organic slow release substrate. An effective reduction of concentrations of TCE and DCE, with formation of small amounts of ethane and no significant accumulation of VC, was achieved. However, the effective lifetime of the reactive material was found to be limited to less than 1 year. Injection of higher doses of EHC was recommended, but eventually the site owner did not implement this approach on full scale. Instead, a regular permeable reactive barrier (a filled trench) has been installed.

5 COST OF THE TECHNOLOGY

Cost drivers for biobarriers comprise (1) the dimensions of the barrier (depth, length and thickness), (2) the price of the reactive material, (3) the local situation on the site (accessibility, surroundings buildings, underground constructions, type of subsurface ...), (4) the local labour costs (country dependent), and (5) amount of maintenance that is needed to keep the biobarrier active and permeable.

The **investment costs** of biobarriers cover a wide range (22-321 keuro based on Table 5 and Table 8) depending on the barrier concept, but they are usually higher than the investment costs for pump&treat systems. The maintenance cost is generally significantly lower for biobarriers (20-70 keuro/year) in comparison with pump&treat.

A comparison of the costs associated with biobarriers versus pump & treat systems has been performed for two sites under a Dutch SKB-project. The biobarriers are based on the injection of soluble electron donors in the groundwater for enhancing anaerobic biodegradation of chlorinated hydrocarbons (chloroethenes and chloroethanes). The findings are summarized below. The biobarriers were more economical over a 10 year period, which was to a large extent the result of the absence of above ground water treatment systems.

Table 8. Cost comparison of biobarriers and pump&treat remediation for 2 specific sites (source: SKB).

	investment installation €	maintenance €/year	costs 10 year period €
Wageningen - screen Walstraat: width 80 m, depth 28 m-gl			
bioscreen	60.000	30.000	360.000
'pump en treat'	90.000	70.000	790.000
Eindhoven - screen DAF: width 80 m, depth 20 m-gl			
bioscreen	140.000	70.000	840.000
'pump en treat'	160.000	80.000	960.000

More examples of costs of biobarriers can be found in Table 5.

6 GENERIC APPROACH TO DETERMINE APPLICABILITY OF A BIOBARRIER FOR A SPECIFIC SITE OR AREA

For a successful application of the biobarrier technologies, the following stepped approach is recommended:

Step 1: Site characterisation

A site characterisation is required for checking the application and boundary conditions associated with the technology (see section 4)

Step 2: Select removal pathway for the pollutants

Step 1 results in a list of pollutants that needs be reduced in concentration. For each of these pollutants a biodegradation pathway needs to be selected. The oxidation state of the contaminants should be evaluated to determine whether an oxidative or reductive pathway is the right strategy to follow. For oxidized compounds like PCE or carbon tetrachloride, a reductive strategy must be chosen. For reduced compounds like benzene or vinyl chloride, an oxidative strategy should be chosen. However, vinyl chloride can also be reduced to ethene. Details on potential pathways for chlorinated aliphatic are given in figures 9, 10 and 11.

Step 3: Feasibility test at lab scale

Lab scale tests may be required (1) to verify the presence of suitable pollutant degrading micro-organisms a specific site, (2) to verify the degradability of the target components, (3) to select suitable reactive substance (Carbon source, electron-acceptor, micro-organisms, nutrients, ...) for the biodegradation process, or more general, to determine the required environmental conditions. Further, for biobarrier design, degradation rates of the pollutants and other needed input parameters need to be deduced from labscale test, preferably column tests. Based on this information, minimal required contact times of the groundwater within the biobarriers to meet the regulatory limits can be calculated. A time period of at least 2 and 6 months should be taken into account for aerobic and anaerobic tests, respectively.

Step 4: Design & dimensioning of pilot/full scale biobarriers

Biobarriers can be installed (A) as continuous barriers or funnel-and-gate PRB systems, or (B) as reactive zones where biodegradation enhancing substance are injected into the subsurface. For an envisioned installation location at the site and the selected barrier type, the required length and depth of the barrier to catch the groundwater contamination plume are determine based on the collected field information. Based on the expected concentrations of contaminants in the influent of the barrier, the groundwater flow velocity, the design parameters deduced from the laboratory feasibility test and the regulatory limits, a minimal thickness of the biobarrier or width of the reactive zone is deduced.

Step 5: Implementation of the biobarriers

This step comprises the installation of the biobarrier conform to the design parameters. Different implementation methods do exist and a selection is to be made based on the barrier type and dimensions and site characteristics.

Step 6: Monitoring of the biobarrier

A post installation monitoring aims at following the performance of the barrier, where reduced pollutant concentrations downstream of the biobarrier are envisioned. Generally, permanent groundwater monitoring wells are installed upstream and downstream of the biobarrier at different depths and are sampled during the whole operation time. They may also be installed within a biobarrier trench, in-situ vessel or in a reactive zone to monitor the operational conditions. Beside chemical contaminant parameters, process parameters such as the groundwater level, groundwater velocity and direction, pH and redox conditions are to be followed. Further, specific actions towards monitoring of biological processes and adjustment of condition in the biobarrier to keep the biology active, are recommended.

Step 7: Closing the site

Generally, biobarriers are expected to remain in the subsurface once the site is closed.

Safety considerations

Health and safety regulations are important to keep in mind during all steps listed above.

- Toxicity and safety of reagents
- methods of transport, storage, mixing and application
- materials of construction should be chemically compatible with selected reagents
- vapour migration: e.g. methane during enhanced reductive dechlorination, oxygen gas for aerobic processes based on infusion of oxygen gas, Hydrogen gas when molecular hydrogen gas would be infused, ...

Step 1 and steps 3 to 6 are elaborated in more detail in the next sections.

Interesting overview documents comprise:

- Sutherson, 1997
- EPA/600/R-98/125 (1998)
- Vidic, 2001
- Carey et al., 2002
- ITRC, 2005
- ITRC, 2011
- Careghini et al., 2013

7 SITE CHARACTERISATION (STEP 1)

Site characterisation comprises:

- Identification of the type and concentration of contaminants that are present
- Determination of the location of the pollution (soil, groundwater, depth, ...)
- Collection of information on the geology (types of soil layers, permeability across the entire depth profile of the contamination , ..)
- Collection of hydrological data (groundwater flow direction, flow velocity, fluctuations in elevation, ...)
- Evaluation of the accessibility of the site.

Suthersan & Payne (2005) emphases the following general aspects of site characterisation:

7.1 HYDROGEOLOGIC MODEL

The tools that are used to develop a hydrogeologic model are: texture analysis, bore logs, geologic cross-sections, interpretation of pumping or slug tests,...
Important parameters are particle size distribution, hydraulic conductivity, groundwater velocity, groundwater flow direction,...

7.2 CONTAMINANT DISTRIBUTION

The type of contaminant and level of contamination can be determined by analysis of soil and groundwater samples. The location of source and plume zones should be identified. Contaminants can be present in the groundwater, adsorbed to the soil and/or in the non aqueous-phase liquid (NAPL). There are two types of NAPLs: DNAPL (dense NAPL) or denser than water and LNAPL (light NAPL) or less dense than water. In general, LNAPLs like BTEX accumulate near the groundwater table, while DNAPLs like chlorinated ethenes can penetrate the water table and sink to deeper geologic layers.
A useful tool to visualize contaminant distribution is horizontal contaminant maps for a certain depth.

7.3 BIOGEOCHEMICAL CHARACTERIZATION

Biogeochemical characterization is intended to evaluate the applicability of a certain remediation technology. Some examples are given below:

- High ORP and dissolved oxygen concentrations indicate that higher amounts of electron donor are required to create an anaerobic biobarrier.
- Existing reducing conditions and the presence of high concentrations of natural organic carbon will create a high demand for oxidation reagents.
- Low pH is a disadvantage for an anaerobic biobarrier.

8 LAB FEASIBILITY TESTING (STEP 3)

8.1 INTRODUCTION

The benefit of a lab feasibility study for a biobarrier is to be evaluated for each case. For example, it can be argued that a degradation test may not be necessary for a PCE-contaminated site where degradation products like vinyl chloride and ethane were found, and where a reactive zone is envisioned. The direct execution of a field study in that case may be more appropriate. However, for biobarriers, less obvious sites, complex contaminations like a mixed pollution of chlorinated ethenes and ethanes, or for exotic contaminants like some pesticides, chlorinated methanes, perchlorate etc., lab feasibility tests are very useful.

To increase the success of a biobarrier, feasibility tests at lab scale are considered to be valuable prior to the installation of the system in the subsurface. Different types of feasibility tests exist.

- Degradation test (batch) to screen the presence at the envisioned site of a biodegradation potential towards the envisioned pollutant.
- Degradation tests (batch) to evaluate and optimise barrier materials & operational conditions. The selection of a suitable organic substrate can also be part of these tests.
- Column test: For each bio-barrier implementation, the performance of a lab scale column test under in-situ conditions is recommended for deriving design parameters (degradation rates, minimal required retention times, ...). Also information on the longevity of filling materials, biofouling, … can be evaluated.
- Pilot test in the field: especially for evaluating the injection characteristics (pressure, radius of influence, required time, … for bioreactive zones) , some work in the field can be every useful.

It is recommended to perform the lab scale tests with groundwater (and aquifer material) from the site.

8.2 EVALUATING THE PRESENCE OF A BIODEGRADATION POTENTIAL (BATCH TEST)

Batch biodegradation experiments are set-up with groundwater and aquifer material from the site to evaluate the biodegradation potential present at the site with and without addition of extra electron donor. The tests also give a first idea of the biodegradation rate.

Typically, aquifer material and groundwater form the site are incubated in vials (glass) and are incubated at a temperature which is representative for the subsurface at the site (for instance 12°C for central Europe). In function of time, the remaining pollutant concentrations are monitored and compared with the concentrations measured in a poisoned control.

As an example for evaluating an anaerobic CAH-biodegradation potential, the following anaerobic test conditions can considered in 160 glass vials:

- **Control:** 30 g aquifer (dry) + 70 ml groundwater + poison;
- **Natural attenuation:** 30 g aquifer (dry) + 70 ml groundwater;
- **Stimulation:** 30 g aquifer (dry) + 70 ml groundwater + electron donor

It is advised to perform each test condition at least in triplicates. The evolution of CAHs, breakdown products and ethane/ethane can be followed by performing direct GC-measurement in the headspace of the vials. Other parameters of concern are redox potential (ORP), pH and remaining carbon source. Typically, it takes 6 to 12 months to evaluate the biodegradation potential. A biodegradation potential is considered to be suitable for a bioreactive zone if (1) a removal up to formation of ethane/ethane is observed, and (2) the removal rates is significant.

It is recommended to sample the aquifer material and groundwater near the place where the biobarrier is envisioned. Spatial heterogeneity of the biodegradation characteristics at a site has been reported. For instance, within AQUAREHAB degradation tests were performed with material from 6 distinct spots within a same site. The CAH-biodegradation characteristics (lag time, biodegradation capacity & removal rates) were found to vary between the spots (Table 9).

Table 9: Overview half lives (days) and TOC value for different location of site A as observed in lab scale biodegradation experiments after 11-12 months of incubation. Observed lag-phase (months) is indicated between brackets.

Substrate	**PB305 (deep)**	**PB603 (shallow)**	**PB402**	**PB404**	**PB504**
Half lives (lag phase) - Lactate stimulated condition					
PCE	4,37 ± 0,28 (0-1)	5,83 ± 1,03 (4-6)	6,83 ± 0,89 (5-10)	2,82 ± 0,06 (2-3)	3,80 ± 0,95 (3-4)
TCE	1,09 ± 0,08 (0-1)	6,59 ± 0,61 (4-8)	9,58 ± 6,98 (5-10)	12,35 ± 0,72 (1-2)	2,68 ± 0,51 (0-1)
cDCE	2,86 ± 0,22 (0-1)	245,11 ± 13,01 (2-3)	22,30 ± 26,73 (12)	2,89 ± 0,32 (2-3)	2,52 ± 0,99 (0-1)
VC	2,17 ± 0,75 (0-1)	/**	+*	4,29 ± 2,37 (0-1)	2,38 ± 1,16 (0-1)
Half lives (lag phase) - Natural attenuation					
PCE	-	-	-	-	60,15 ± 15,99 (3)
TCE	-	-	-	-	5,29 ± 0,85 (0-1)
cDCE	-	-	-	-	5,61 ± 0,45 (1-2)
VC	-	ND	ND	ND	146,99 ± 62,08 (2-3)
TOC value of used aquifer (% DW)					
	< 0,2	< 0,2	0,2	0,3	5,4

- : no degradation
ND : not determined
* : degradation, but not enough points to calculate degradation
** : no VC/ ethene or ethane measured, only a lot of methane

In case no (or an insufficient) biodegradation potential is detected at a site, use of specialised bacteria cultivated in the lab and supplied to the biobarrier can be considered. Injection of specialized cultures in biobarriers (bioaugmentation) has become a common practice (Careghini et al., 2013).

8.3 SELECTION OF SOLUBLE ELECTRON DONORS FOR REACTIVE ZONES

There are a variety of reagents that can be used for the creation of a bioreactive zone. Some examples are sodium lactate and food-grade carbon sources like molasses, cheese whey and vegetable oils. Other examples are given in Table 2.

Reagent selection is based on (1) physical state, (2) speed of utilization and (3) cost. Complexity in composition is desirable because it stimulates a more diverse microbial community. In systems that are naturally aerobic, a soluble, rapidly acting carbon source is preferred to quickly decrease the redox potential and to overcome the microbial lag phase. Once reducing conditions are achieved, the substrate can be injected at low dosages and frequencies.

8.4 SELECTION OF BARRIER FILLING MATERIAL

8.4.1 Barrier fillings

Barrier filling materials must ensure optimal growth conditions for microorganisms and exhibit a higher hydraulic conductivity as compared to the surrounding aquifer material to avoid groundwater by-pass (Careghini et al., 2013). Other aspects of concern are (1) longevity of the filling material in terms of stability; (2) longevity of the material to release growth supporting substances; (3) no release of hazardous compounds; as well as (4) availability & costs. Some examples of barrier filling materials are given in Table 10.

Table 10 Overview of various biobarrier filling materials tested for removal of specific pollutants.

Barrier filling material	Pollutant	Remarks	Reference
Plant mulch	CAHs	Releases growth supporting compounds	ITRC, 2005 - Table 5
Hard wood bark mulch	PAHs	Releases growth supporting compounds	ITRC, 2005 - Table 5
Compost	CAHs	Releases growth supporting compounds	ITRC, 2005 - Table 5
Wheat straw	nitrate	Releases growth supporting compounds	ITRC, 2005 - Table 5
Pumice granulates (0.4-0.6 mm)	Toluene	Good microbial binding capacity	Di Lorenzo et al., 2005
Expanded perlite (2-3 mm)	MTBE	High porosity, poor reactivity, low cost	Liu et al., 2006
Quartz sand (< 1mm)	MTBE, BTEX	Inert, homogeneous, good biofilm formation, but limited hydraulic conductivity	Saponare et al., 2009 Yeh et al., 2010
Sand (1-2 mm)	BTEX, MTBE, CAHs Ammonium, nitrate	Good hydraulic conductivity, compatible with bioprocesses, Relatively inert	Dries et al., 2003 Van Nooten et al., 2007 Bastiaens et al., 2007 Bastiaens et al.,2008 Van Nooten et al., 2008 Van Nooten et al., 2010
Stainless steel pieces (0.6 cm) + peat moss granulated (4-7 mm)	Gasoline pollutants	Sorption by peat moss, compatible with biodegradation	Yerushalmi et al., 1999
Peat	Petroleum hydrocarbons	High sorption capcity	Guerin et al., 2002
Activated carbon	Fuel oil, PAHs	Very high sorption capacity, good support for biomass,	Leglize et al., 2006
Pozzolan	Fuel oil, PAHs	High sorption capacity, but recued	Leglize et al., 2006

Barrier filling material	Pollutant	Remarks	Reference
		mineralisation rates	
Mixture: mulch + Zerovalent iron	CAHs	Releases growth supporting compounds & lowers redox potential	
Mixture: calcite, furnace, horse manure, green wast compost	Metals, sulphate	Releases growth supporting compounds	IMWA 2005 (Bowden) - Table 5
Zeolites (clinoptilolite)	Ammonium	Support for biofilm & ion exchange of ammonium as back-up removal mechanism	Van Nooten et al., 2011

PAH = polycyclic aromatic hydrocarbons

For pollutants that are present in trace concentrations (micropollutants) or that are slowly degraded, carrier materials with a certain sorption characteristic can be used to concentrate the pollutants and/or to provide a longer reaction time.

8.4.2 Feasibility test

A bench-scale test may give information on the suitability of a barrier material (1) to allow biological processes, and (2) to support/stimulate the envisioned process in the biobarrier.

Compatibility with biological processes: The aim of the test is to reveal whether the envisioned filling materials do not create conditions with an adverse effect on the biodegradation activity. A low buffering capacity of the material or induced reaction may lead to undesirable evolutions of the pH.
Support/stimulate biodegradation: Filling materials may be selected for their release of biodegradation supporting compounds like for instance organics serving as electron donor or nutrients (N, P, ..). Test may have the aim to reveal the leached compounds and the duration of the leaching. The latter is related with the longevity of the biobarrier.
Feasibility test: The following section describes feasibility tests for the evaluation of a biobarrier concept which is based on an excavated trench which would be filled with a solid organic substrate.

Samples of a selected barrier material and site groundwater are mixed for a batch test to determine the pH and geochemical properties after an appropriate incubation period. The incubation period may last several weeks to allow the mixture to generate anaerobic conditions. After this, the water is drained and sampled, and replaced with fresh groundwater. The batch mixture may then be allowed to incubate for a period corresponding to the anticipated residence time in the biowall or bioreactor (typically 1 to 2 weeks). This water is sampled and analysed for pH, ORP and contaminants of concern. Optional parameters may include nitrate, ferrous iron, manganese, sulphate, carbon dioxide, methane, sulphide. Optionally, the batch mixture could be allowed to incubate for another 4 to 6 weeks and the sampling repeated. Conducting multiple incubations and sampling events may provide insight into how the geochemistry of the mulch mixture may change over time.

Selected barrier filling materials are contacted with the pollutants of concern and micro-organisms that are able to degrade the pollutants.

Generally, these tests are performed in closed system, preferably triplicates. In time the evolution of pollutants & breakdowns products is monitored and compared with the results of abiotic controls.

Figure 8 A batch test bottle with mulch, soil and groundwater.

8.5 SIMULATION OF BIOBARRIER TO DERIVE DESIGN PARAMETERS (COLUMN TESTS)

In many cases a batch test may be sufficient for selection of materials that are local to the site. Column studies which are more expensive and time consuming may be warranted in situations where degradation pathways or kinetics are poorly understood, or where co-contamination exists. In column test a biobarrier can be simulated under more realistic continuous conditions, and may be conducted

- to determine the evolution of the geochemistry of the barrier filling material and its hydrogeologic properties (*e.g.*, hydraulic conductivity) over a longer time.
- To determine degradation rates
- To evaluate the hydrological properties of the biobarrier.

For the test, the column is packed with barrier filling material and representative water (preferably representative groundwater from the site) is pumped in an upflow manner through the column (Figure 9). The pollutant degrading bacteria can be introduced by mixing the barrier filling material with aquifer from the site. Alternatively, specialised bacteria can be cultivated in the lab and be bio-augmented in the column. The hydraulic retention time of the water in the biobarrier is function of the pumping rate, dimensions of the column and porosity of the biobarrier. By analysing water samples taken at the influent, effluent and intermediate spots along the column, information on degradation rates and the residence time required for treatment. Is is advised to measure also the evolution of the pH, redox potential and growth supporting compounds like electron donor/accepter, nutrients, … . The column should be allowed to acclimate over a prolonged period (typically several months). To make a distinction between biodegradation and abiotic processes (sorption, volatilisation, …) it is recommended to include a poisoned control column in the test in parallel to the test column.

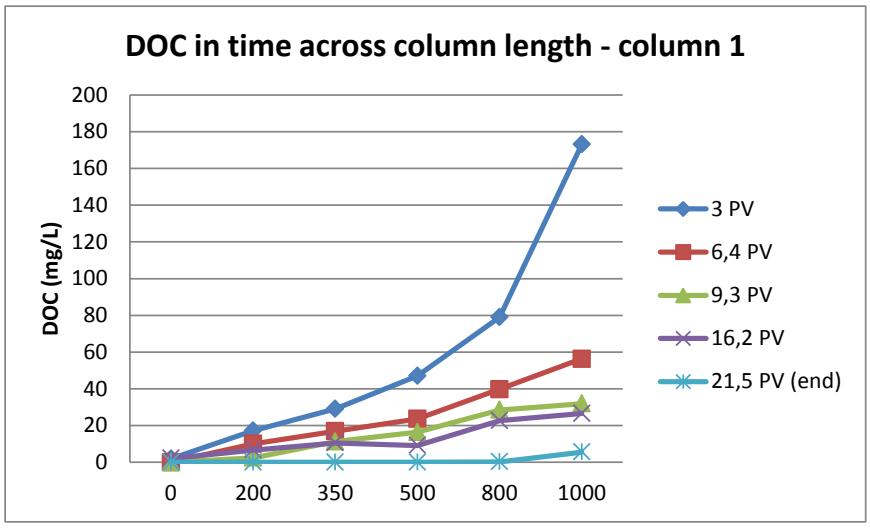


Figure 9 Example of a column test setup with mulch (50 vol%), soil (50 vol%) and groundwater.

Figure 13 shows a picture of a column test setup for a biobarier in which the mixtures of soil and mulch in the test columns have turned black. The profiles of concentrations of dissolved organic carbon across the column length show that the release of soluble organic carbon from the mulch has declined with time. That indicates that the mulch at some time may need to be supplemented with injection of a soluble organic substrate.

8.6 FIELD TESTING – BIOREACTIVE ZONE

A pilot test in the field has two goals:

- Injection test to obtain critical design data: injection radius of influence, injection pressure, injection volume, injection frequency, reagent concentration, reagent volume,...
- To evaluate in-situ the pollutant removal process (reductive dechlorination, bioprecipitation of metals,...) of the technology

The location of the pilot test has to be a good representative for the site (contaminant concentration, hydrogeology) and needs to have good access (not disturbing site activities).

The field test has a minimum number of injection wells or points (e.g. 3) to properly deliver the reagent and a minimum number of monitoring wells (e.g. 6) to properly monitor the distribution of reagent and degradation of contaminant. It is preferable for monitoring wells to be located at variable distances from the injection wells, both parallel to the direction of groundwater flow and perpendicular to the direction of groundwater flow. The wells are typically spaced at one, two and three months groundwater flow time downgradient. When possible, existing monitoring wells are used to control costs and to use historical monitoring data. Also an upstream monitoring well is useful as baseline.

The duration of a bioreactive zone pilot test is typically six to twelve months. The frequency of injections varies with hydrogeologic and biogeochemical conditions of each site. Initially, monthly injections are typical, followed by less-frequent injections after bioreactive zone has formed. The testing of a bioreactive zone is complete when (1) redox conditions downgradient of the injection wells are significantly reduced, (2) the contaminant is degrading and (3) the amount of final end product (e.g. ethene) has increased.

9 GENERIC APPROACH TO DESIGN A BIOBARRIER (STEP 4)

9.1 INTRODUCTION

During the design phase, based on site information collected in step 1 and the biodegradation process information (biodegradation potential, degradation rates, ...) from step 2 and 3, a biobarrier concept is selected and dimensioned.

For continuous barriers and funnel and gate biobarrier concepts, part of the aquifer is removed and replaced by coarse reactive material. Here biodegradation promoting substances are preferable added as slowly releasing solids (like mulch). Ideally after installation no active addition of substances is needed, but often it may be needed to sustain microbial activity over a long time.

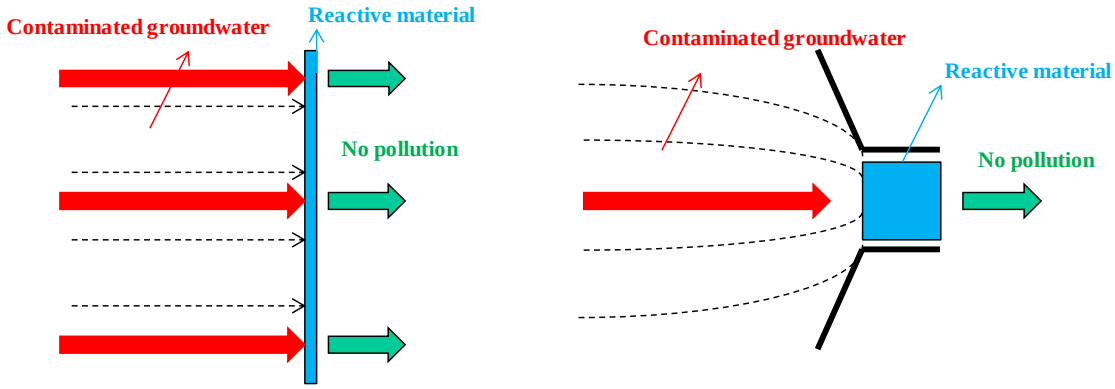


Figure 10 Schematic representation of a continuous (left) and funnel & gate (right) PRB concept.

In the case of bioreactive zones (Figure 11), the injected substances that promote biodegradation are preferably liquids, but they can also be suspensions of small (lower µm rage) particles or gasses (air, technical oxygen gas, hydrogen gas, ...). To maintain the required concentrations, repeated injections over time, or even continuous dosing may be necessary.

When the feasibility tests revealed that no (or an insufficient) suitable biodegradation potential is present at the site, addition of specialised bacteria cultivated in large amount in the lab, can be considered (= Bioaugmentation).

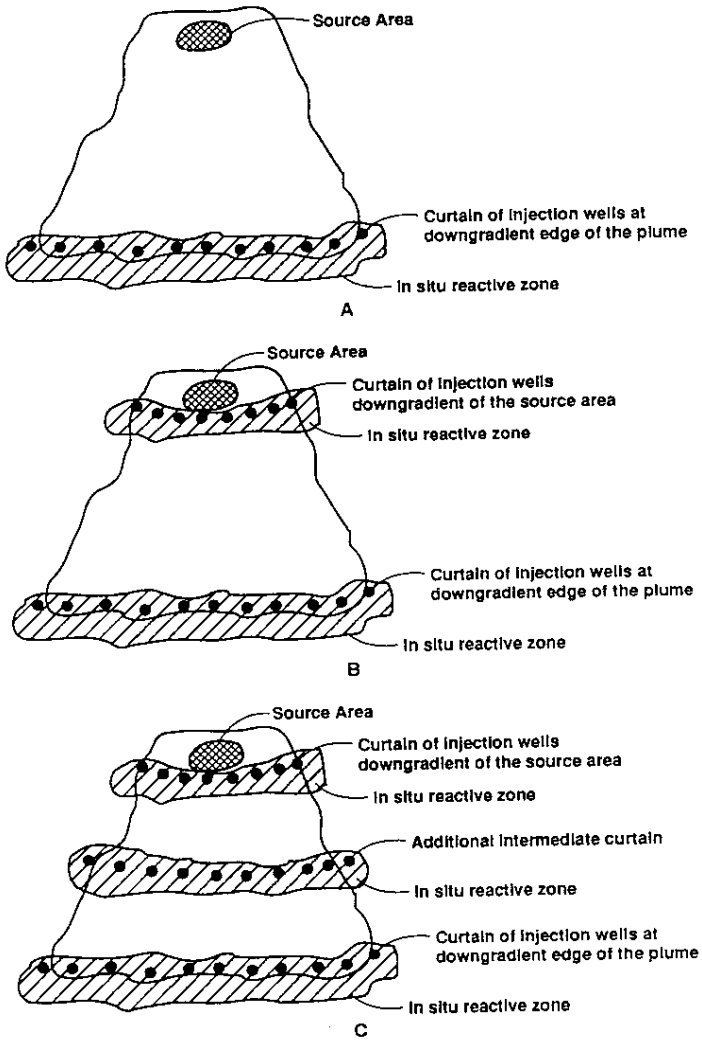


Figure 11 Schematic representation of a reactive zone concept.

9.2 SYSTEM DESIGN FOR BIOREACTIVE ZONES

9.2.1 Introduction

The two largest cost factors for the implementation on a bioreactive zone are the injection well(s) installation and reagent delivery. Three site-specific factors that contribute to the cost of a bioreactive zone are:

- Plume size to be treated: the larger the plume, the more wells are needed and the more time is needed for each reagent injection.
- Depth of target zone: the deeper the contaminant, the higher the installation costs.
- Groundwater flux through zone of treatment: At sites with a high groundwater flux, more reagent injections are required.

9.2.2 Hydrogeology

Hydrogeologic data are important to design the delivery of the reagents at the desired concentrations and the distribution to the target zone. A complex lithology is problematic for most

remediation technologies. A pumping test for determining the hydraulic conductivity is useful to evaluate the suitability of a site for injections in the bioreactive zone application and can predict the performance of a full-scale delivery system.

The hydraulic conductivity is used, together with the hydraulic gradient, to determine the groundwater velocity and the amount of reagent to be injected. As the hydraulic conductivity increases, the distribution of reagent from a single injection point along the flow direction increases. This is also important to evaluate the full-scale or pilot-scale impact in a certain timeframe.

Groundwater flow characteristics (velocity, direction, horizontal and vertical gradients) impact the effectiveness of reagent injections. A low velocity system requires a lower reagent mass feed rate. The depth to groundwater and saturated thickness will define well design and installation cost. An overview of specific (hydro)geologic parameters for IRZ design is given in Table 11.

Table 11. Specific (hydro)geologic parameters for IRZ design.

(Hydro)geologic parameter	Design impact
Depth to impacted groundwater	Injection well depth and screen locations
Width of contaminant plume	Number of injection wells
Thickness of contaminant plume	Number of injection points within a well cluster Pressure injection vs. gravity feed
Groundwater velocity	Injection volume and frequency, residence time for the target reactions, dilution of end-products
Hydraulic conductivity	Mixing zones of reagents, extent of reactive zone Number of injection points within a well cluster
Geologic variations, layering of various soil sediments	Location of well screens at injection points
Soil porosity and grain size distribution	Removal of end-products resulting from immobilization reactions (such as heavy metals precipitation)

9.2.3 Bioreactive zone concepts

Reactive zones are created by installing injection well or performing direct push injection. Reactive zones can be implemented using different configurations and approaches. There are three basic layouts:

- **A cut-off barrier** is a series of reagent injection wells/points typically in a row perpendicular to the groundwater flow direction at a critical boundary e.g. a property line. However, the entire plume is not being remediated and this can have a negative impact on remediation time.

- **Plume-wide reactive zones** are a few reactive zones across the entire plume, leading to higher costs but faster site closure.
- **Hot spot reactive zones** target the source area and speed up the remediation process. In a number of cases, the remediation strategy includes a source treatment, combined with one or more bioreactive zones downgradient of the source area. The thickness of a bioreactive zone is generally 100 days of groundwater travel time from the point of injection at most sites.

9.2.4 Delivery system design

For a bioreactive zone, the cost of the reagent is relatively insignificant. The majority of costs related to reagent injection are labor cost and installation of injection wells. The most used delivery systems are injection wells and direct-push well points. During the design, the injection approach is to be selected taking into account the injection depth, the required injection pressure and expected frequency of injection. Permanent injection wells allow for multiple injections to establish and maintain the reactive zone. They are necessary in situations where depths or soil strata make direct-push techniques impractical. Direct-push delivery is limited to shallow, unconsolidated formations at depths typically limited to 15 m. The use of direct-push is recommended only when the groundwater flow is relatively slow (less than 11 to 22 m $year^{-1}$) and direct-push can be made at intervals that make sense economically (6 to 12 months).
The geology and groundwater velocity will control the radius of influence. For example, in a tight geologic unit, groundwater moves slowly. The reactive zone from the injection point will have a limited impact laterally and in the direction of groundwater flow. Therefore, many points are needed with closer spacing.
Within AQUAREHAB it was found essential to evaluate in advance the injection modus, being permeation or preferential flow (see also DL5.5-guideline injectable ZVI). When permeation is envisioned, a low injection pressure is needed which can be most easily applied via injection wells. On the other hand, when high injection pressures are required, direct push injections may be more suitable.

9.2.5 Scale up issues

The main scale up issue after a pilot test is the addition of more injection wells to create a larger bioreactive zone. If pilot testing indicates that the effective area of influence of a given injection well is small, a large number of injection wells are required. If drilling costs are high, this leads to high remediation costs.
In many cases with a large number of injection wells, the frequency and volume of the injection is such that manual batch injections are used. However, in some cases, scale up to a full-scale system will require the implementation of an automatic reagent feed system.

Remediation failure is often caused by inadequate reagent distribution. Data collection from full-scale monitoring is used to check the conceptual site model and to update the model if needed. Predetermined remediation design and implementation need to be updated if necessary. Treatment optimization requires aquifer tuning (e.g. injection volume, well spacing, recirculation, injection frequency, reagent selection and dosing, injection strategy,...) (Suthersan et al, 2010).

9.3 SYSTEM DESIGN FOR BIOBARRIERS (SENSU STRICTO)

9.3.1 General considerations

Based on site information collected in step 1 a conceptual model of the pollution at the site needs to be prepared and used as a basis for the biobarrier design.

- At first, it is advised to check the conceptual model with the application area and boundary conditions of biobarrier technology (see section 3).
- If the site conditions are still within the application area, a next action is to list potential locations for implementing the biobarrier to control further migration of the contamination.

Biobarriers typically take advantage of the naturally present hydraulic gradient. Once installed, this passive regime is difficult to alter. Therefore the design and dimensioning of biobarriers needs to be made with care taking into account site hydrology, contaminant type & extent, and minimal required hydraulic retention times.

- The groundwater water flow direction determines the direction of the biobarrier. Barriers are preferentially installed perpendicular the groundwater contamination plume. It is important to take seasonal variations of groundwater flow direction into account in the design phase. Flow directions may be altered in time as a result of off-site groundwater extraction.
- The length of the biobarrier is determined by the width of the contamination plume. Hydrological studies are advised to be performed to make sure that the barrier is able to capture the entire plume.
- The thickness of the biobarrier is function of a number of parameters, comprising:
    - The minimal required hydraulic retention time (HRT) of the water in the biobarrier (function of influent concentration and required effluent concentration)
    - The groundwater flow velocity: the higher the flow, the thicker the biobarrier needs to be to ensure a certain HRT. Groundwater flow velocity may vary significantly as a function of depth, depending on the permeability of different soil layers.
    - Porosity of the barrier: the porosity and permeability of the barrier should be at least equal to surrounding aquifer. As stimulation of the microbiology results in biomass formation, a higher porosity in the biobarrier is advised.
    - The barrier concept: for a funnel and gate concepts (see Figure 10) all water is funnelled through the gate which needs to be wider than in comparison with a continuous biobarrier concept.

9.3.2 Dimensioning

The transport of a non-reactive sorbing contaminant through the biobarrier can be estimated from the Darcy equation with retardation where the linear transport velocity is determined by:

$$u = \frac{\frac{K_s \nabla h}{\theta}}{\left(1 + \frac{K_d \rho}{\theta}\right)}$$

With u the contaminant velocity (m/d), K_s the hydraulic conductivity (m/d), ∇h the hydraulic gradient (-), θ the porosity (-), K_d the water-solid partitioning coefficient (m^3/kg) and ρ the bulk

density (kg/m^3). The value for K_d can be determined from the carbon-water partitioning coefficient (K_{oc}) and the organic carbon fraction (f_{oc}) by $\boldsymbol{K_d = K_{oc} \times f_{oc}}$.

A worst-case scenario can subsequently be developed for a rough estimation of the technology's performance depending on the local hydrogeology and the applied technique (fraction organic carbon, porosity and thickness). This should be performed taking into account multi-species reactive transport with sorption in the barrier. However, this requires specific software (e.g. Hydrus, BIOCHLOR etc.). A preliminary estimation can be made assuming first order degradation for the slowest degrading compound and for the compound with smallest sorption constant. The earliest breakthrough will be observed for these compounds if eventual previous degradation steps are not rate-limiting. As such, the worst-case scenario can be developed for these compounds. The technique should be dimensioned so that degradation proceeds faster than the transport through the biobarrier, e.g. for vinylchloride (VC):

$$\left.\frac{dVC}{dt}\right/_{\boldsymbol{u}} \geq \frac{VC_{in}}{\delta} \text{ or } \boldsymbol{\delta \geq \frac{u}{k_{vc}}}$$

With $\left.\boldsymbol{dVC}\right/_{\boldsymbol{dt}} = \boldsymbol{k_{vc} \times VC_{in}}$ the reaction rate of VC (µM/d), VC_{in} the concentration (µM) at the bottom of the barrier and δ the thickness of the biobarrier (m). As such, the abatement rate of 100% is a function of $\boldsymbol{\nabla h}$, K_s, θ, ρ, K_{oc}, f_{oc}, k_{vc} and δ. Table 12 indicates the desired characteristics of the biobarrier for different boundary conditions with θ, ρ, K_{oc} and k_{vc} fixed at 0.4, 1.1 kg/m^3, 8.2×10^{-3}, respectively 0.11 d^{-1}. In effect, θ and ρ depend partly on f_{oc} but this is neglected for simplicity. Scenario 3, 4, 7 and 8 indicate that the technology should be treated with special care in areas where large hydraulic gradients can be expected or where a large heterogeneity creates zones with high hydraulic conductivities. This would require a thicker biobarrier for 100% abatement.

Table 12: a rough estimation of the effect of different boundary conditions on the desired thickness of the biobarrier. ∇h the hydraulic gradient; K_s the hydraulic conductivity; f_{oc} the organic carbon fraction; δ the thickness of the biobarrier.

Scenario	∇h (-)	K_s (m/d)	f_{oc} (-)	**δ (cm)**
1	1.E-03	1	0.1	**0.2**
2	1.E-03	1	0.3	**0.1**
3	1.E-03	10	0.1	**2.1**
4	1.E-03	10	0.3	**0.9**
5	1.E-02	1	0.1	**2.1**
6	1.E-02	1	0.3	**0.9**
7	1.E-02	10	0.1	**21.3**
8	1.E-02	10	0.3	**8.9**

9.4 STRATEGIES TO LIMIT FOULING IN BIOBARRIERS

Stimulation of the biomass in a bioreactive zone or biobarrier to degrade pollutants, is associated with biomass growth that can reduce the permeability. This may lead to clogging of the system or preferential flow paths. Especially near injection points where growth supporting substances like electron donor (organics, hydrogen, ..), electron accept (oxygen, …), additives are added, biofouling is realistic. This is a point of attention during the design phase, especially for aerobic systems.

These strategies may comprise the following measures:

- For biobarriers that are based on particulate organic substrates it is recommended to mix sufficient coarse inert materials (coarse sand or pea gravel) with the organic substrate(s) to maintain porosity within the biobarrier. The organic substrates may be a blend of materials with varying physical rigidity. Compost may be used as a source of nutrients which is blended with tree mulch that has a longer life time and higher rigidity.
- For bioreactive zones it is recommended to do proper periodic maintenance of the injection wells for soluble organic substrates and to use an appropriate injection scheme that minimizes biofouling of the injection wells and the soil that surrounds them.

10 GENERIC APPROACH TO IMPLEMENT THE BIOBARRIER TECHNOLOGY (STEP 5)

10.1 BIOREACTIVE ZONES

Bioreactive zones can be created by injecting reagents in the subsurface via a number of possible systems. These methods comprise (1) injection via permanent vertical wells, (2) Direct push injections, (3) horizontal drains and (4) recirculation wells. The methods are illustrated in Figure 13. The first two approaches are the most commonly used.

Figure 12 Injection of substrate via permanent vertical wells to create a reactive zone.

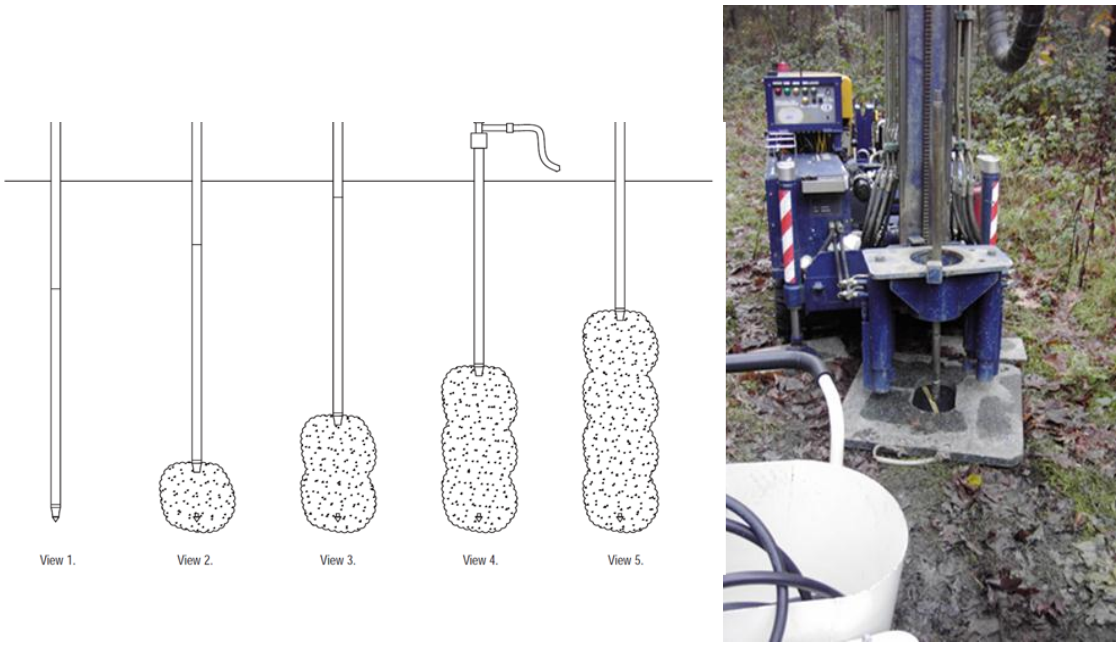


Figure 13 Direct push approach for supplying substrates into the subsurface to create a reactive zone.

More information on direct push injection:
http://www.coreprobe.com/PDF/Materials%20Injection.pdf

The injection of amendments can be performed on a continuous basis or with periodic injections. Fouling of injections wells needs to be considered. The time intervals would depend on the lifetime of the reagents that are injected (as determined by the quantity injected and the dissolution rate in the groundwater) and the influx of contaminants and natural electron acceptors or donors that compete with the contaminants. Table 13 displays an overview of substrates, their typical delivery techniques, form of application and frequency of injection.

Table 13. Substrates used for enhanced anaerobic bioremediation (USEPA 2013, modified from ITRC 2008, AFCEE 2004).

	Substrate	Typical delivery techniques	Form of application	Frequency of injection
Soluble substrates	Lactate and butyrate	Injection wells or circulation systems	Acids or salts diluted in water	Continuous to monthly
	Methanol and ethanol	Injection wells or circulation systems	Diluted in water	Continuous to monthly
	Sodium benzoate	Injection wells or circulation systems	Dissolved in water	Continuous to monthly
	Molasses, high- fructose corn syrup	Injection wells	Dissolved in water	Continuous to monthly
	Whey (soluble)	Direct injection or injection wells	Dissolved in water or slurry	Monthly to annually
Slow-release substrates	HRC® or HRC-X®	Direct injection	Straight injection	Annually to biennially for HRC (typical), every 3–4 years for HRC-X, potential for one-time application
	Vegetable oils	Direct injection or injection wells	Straight oil injection with water push or high oil/water content (>20% oil) emulsions	One-time application (typical)
	Vegetable oil emulsions	Direct injection or injection wells	Low oil content (<10%) microemulsions suspended in water	Every 2 to 3 years (typical)
Solid substrates (barrier wall applications)	Mulch and compost	Trenching or excavation	Trenches, excavations, or surface amendments	One-time application (typical)
	Chitin (solid)	Trenching or injection of a chitin slurry	Solid or slurry	Annually to biennially, potential for one-time application

10.2 BIOBARRIER SENSU STRICTO

Biobarriers can be installed via (1) continuous trenching, (2) refilling of a stabilised (sheet piles, or guar gum) and non-stabilised trenches and (3) soil mixing.

A funnel & gate system with permeable gates is most suitable for bioreactive material that may need to be replaced periodically. Injection wells may also be installed within a biobarrier trench to inject amendments that can sustain microbial activity for a longer time (see Table 13).

Figure 14: Implementation of biobarriers via continuous trenching (left) and refill of a trench (right).

11 GENERIC APPROACH TO MONITOR THE BIOBARRIER TECHNOLOGY (STEP 6)

The goal of monitoring a biobarrier is (1) to collect data on the pollutant-degrading processes in the biobarrier or reactive zone (process monitoring), and (2) to evaluate the performance of the biobarrier as technology to remediate the site (performance monitoring). The monitoring approach can be slightly different for bioreactive zones compared to biobarriers.

11.1 PROCESS MONITORING

11.1.1 Bioreactive zones

The intent is to provide real-time feedback to control the development reactive zone. The typical frequency is weekly to biweekly during the first month of injections, biweekly or monthly during the next two to three months and bimonthly to quarterly for the remainder of the active treatment period.
Parameters to include in the monitoring are field parameters like pH, electrical conductivity, dissolved oxygen, ORP, temperature. Other parameters are linked to the type of reagent: for example dissolved organic carbon (DOC) for injection of carbon source, sulphate for injection of persulphate, bromide for injection of bromide tracer,...

11.1.2 Biobarrier

The main aim of process monitoring in biobarriers is to verify (1) if the conditions in the system remain as needed for biodegradation and (2) if the biological processes leading to pollutant removal do occur in the biobarriers system.
Parameters that may be included in the monitoring are field parameters (pH, electrical conductivity, dissolved oxygen, ORP, temperature), growth supporting substance (electron donor, electron acceptor, nutrients, …), presence of breakdown products, microbial assessment. Occasionally, biobarrier filling material can be sampled to evaluate biofilm formation, stability of the material, porosity, …)

11.1.3 Microbial assessments

Microbial characterization of a site can be useful, but the required extend may differ from site to site. Different molecular microbial techniques based on DNA- and RNA-analyses can be used to collect information on the presence and activity of micro-organisms, respectively.

- DGGE (denaturing gradient gel electrophoresis) is a DNA-based screening technique to detect the microbial diversity in a soil or groundwater sample
- PCR (polymerase chain reaction) represents a technique to amplify specific DNA or RNA fragments from for instance environmental samples. During the last decade, the method develop extensively and allows to detect the presence of specific types of bacteria or specific genes. For example, PCR is frequently used for proving the presence of *Dehalococcoides*

species, a well-known degrader of chlorinated ethenes, at a site. Some genes involved in reductive dehalogenation of CAHs are given in Figure 15.

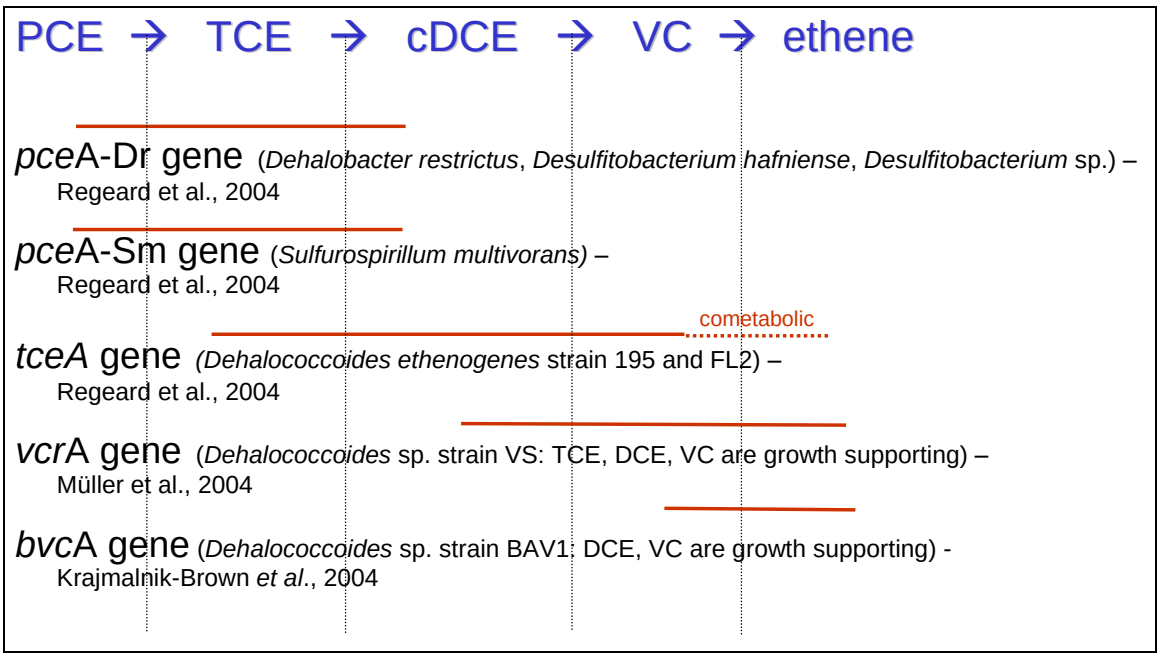


Figure 15 Examples of catabolic genes involved in the degradation.

- Q-PCR (Quantitative PCR) is a more advanced method as compared to PCR, and allows to quantify DNA-fragments. As such a semi-quantification of specific biomass/genes is possible. A large variety of primers have been developed to amplify specific species and genes.

Table 14 Examples of bacterial species and genes that can be detected with q-PCR.

Specific species	Specific genes
Sulfurospirillum species	*Sulfurospirillum* tetrachloroethene reductive dehalogenase
Dehalococcoides ethenogenes	Trichloroethene reductive dehalogenase
Dehalococcoides CBDB1	Chlorobenzene reductase *Dehalococcoides CBDB1*
Desulfitobacterium species	*Dehalococcoides pceA*
Dehalobacter species	Vinyl chloride reductive dehalogenase
Desulfuromonas species	Dee. BAV1 Vinyl chloride reductive dehalogenase
Geobacter species	Chlorophenol reductive dehalogenase A1 from *Desulfitobacterium*
	Chlorophenol reductive dehalogenase A1 from *Desulfitobacterium*
	Desulfitobacterium pceA
	Dichloroethane reductive dehalogenase *Desulfitobacterium*

11.2 PERFORMANCE MONITORING

Performance monitoring is required to determine the effectiveness of the treatment. The frequency is less frequent compared to process monitoring. Generally, monitoring wells associated with the reactive zones will be measured more frequently than the site-wide monitoring wells. The list of parameters measured includes the concentration of contaminants and degradation products.

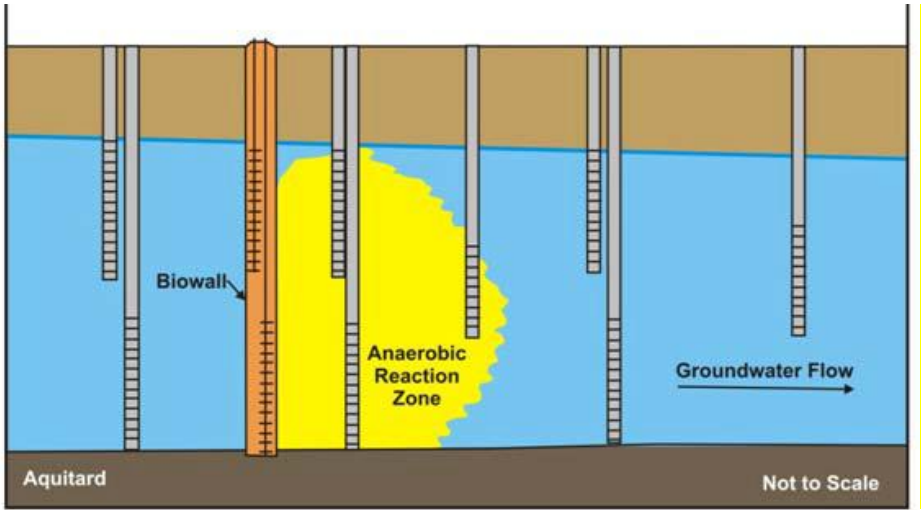


Figure 16: Cross-section of a monitoring well transect for a biobarrier (ITRC, 2011).

11.2.1 Monitoring well placement

It is advised to use one or more previously existing monitoring wells since they often have useful historical data regarding contamination trends. New monitoring wells are placed within a reactive zone at monthly intervals of groundwater travel time (e.g. at one, two and three months travel time from the injection location).
For more details, the reader is referred to DL4.3A as the principles are comparable for ZVI-barriers and biobarriers, even reactive zones.

11.2.2 Groundwater sampling and analysis

Groundwater sampling should be done with extreme care, utilizing low-flow pumping, micropurge procedures, peristaltic pumps or passive diffusion bags. For different monitoring events on the same groundwater well, efforts should be made to use the same pump and purging method.

The typical list of field parameters includes the following:

- pH
- temperature
- redox potential
- dissolved oxygen
- conductivity

Other biogeochemical parameters are:

- dissolved organic carbon
- nitrate, nitrite
- sulphate, sulphide

- iron (total and dissolved)
- manganese (total and dissolved)
- carbonate
- alkalinity
- metals

11.2.3 Compound specific isotope analyses

Isotopes of an element have the same amount of protons, but differ in the number of neutrons, and therefore, they have different masses. For instance, carbon exhibits two stable isotopes; one with the mass 12 (^{12}C: 6 protons and 6 neutrons) and one with the mass 13 (^{13}C: 6 protons and 7 neutrons). The quotient between the amounts of the heavy and the light isotopes is called isotope ratio ($R = {}^{13}C/{}^{12}C$). For better comparability, this ratio is noted as isotope signature, which is relative to the ratio of a worldwide defined reference substance (e.g. Vienna Pee Dee Belemnite for $^{13}C/^{12}C$) according to

$$\delta^{13}C_{sample}\ [‰] = ((R_{sample} - R_{reference})/R_{reference}) \times 1000.$$

Non-degraded contaminants generally have a carbon isotope signature between -22 δ‰ and -35 δ‰, i.e. they have a lower amount of heavy isotopes than the reference substance. However, the so-called primary isotope value increases through biodegradation, since the turn-over of molecules with heavy isotopes is slower than those with light isotopes. This process is called isotope fractionation or isotope enrichment.

Biodegradation within contaminated aquifers can be assessed because of substance-specific proportionality of isotope fractionation and contaminant decrease, which is expressed by the isotope enrichment factor ε. More details of this monitoring approach and examples from the AQUAREHAB project are elaborated in Annex 1 of this document.

11.2.4 Numerical modellering

Based on degradation rates determined via labscale test and hydrological parameters of the site, evolution of the contaminant in time and space can be modelled. More details are given in DL4.3A, DL4.3C and DL7.5.

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14 ANNEX 1: COMPOUND SPECIFIC STABLE ISOTOPE ANALYSES (CSIA)

14.1 INTRODUCTION

Isotopes of an element have the same amount of protons, but differ in the number of neutrons, and therefore, they have different masses. For instance, carbon exhibits two stable isotopes; one with the mass 12 (^{12}C: 6 protons and 6 neutrons) and one with the mass 13 (^{13}C: 6 protons and 7 neutrons). The quotient between the amounts of the heavy and the light isotopes is called isotope ratio ($R = {}^{13}C/{}^{12}C$). For better comparability, this ratio is noted as isotope signature, which is relative to the ratio of a worldwide defined reference substance (e.g. Vienna Pee Dee Belemnite for $^{13}C/^{12}C$) according to

$$\delta^{13}C_{sample}\ [‰] = ((R_{sample} - R_{reference})/R_{reference}) \times 1000.$$

Non-degraded contaminants generally have a carbon isotope signature between -22 δ‰ and -35 δ‰, i.e. they have a lower amount of heavy isotopes than the reference substance. However, the so-called primary isotope value increases through biodegradation, since the turn-over of molecules with heavy isotopes is slower than those with light isotopes. This process is called isotope fractionation or isotope enrichment.

14.2 APPLICATION OF CSIA FOR BIOBARRIERS

14.2.1 Isotope Enrichment Factors and Quantitative Evaluation of Biodegradation

Biodegradation within contaminated aquifers can be assessed because of substance-specific proportionality of isotope fractionation and contaminant decrease, which is expressed by the isotope enrichment factor ε *(*see data base at *www.isodetect.de/isofracDB.php*).

The commonly used mathematical description of microbial isotope fractionation processes is the Rayleigh equation, where δ_x is the isotope signature of the substrate at a downstream monitoring well, δ_0 is the isotope signature of the substrate in an upstream well , C_{Bx}/C_0 is the fraction of substrate remaining during biodegradation along the flow path, and ε is the isotope enrichment factor given in ‰. The amount of contaminants degraded is then given by

$$B[\%] = \left(1 - \frac{C_{Bx}}{C_0}\right) \cdot 100 = \left[1 - \left(\frac{\delta_x + 1000}{\delta_0 + 1000}\right)^{\left(\frac{1000}{\varepsilon}\right)}\right] \cdot 100$$

Thus quantitative conclusions from isotope enrichment require only three analytical parameters, i.e. isotope values upstream and downstream from a flow path, and the compound-specific isotope enrichment factor.

Based on percentual biodegradation along a flow path, *in situ* zero- and first-order biodegradation rate constants can be calculated regarding the distance between monitoring wells (which delivers halflife distance) and the flow velocity (which delivers travel time and finally halflife time).

Equations to calculate these parameters are noted in various guidelines on isotope monitoring at contaminated sites (*US EPA 2005, US EPA 2008, Eisenmann & Fischer 2010*).

On the compound-specific level, the fraction of heavy isotopes increases in the primary CAH contaminants (PCE or TCE) during hydrogenolysis (Figure 1, left). Thus the metabolites DCE and VC are depleted in ^{13}C, particularly in the initial period of their formation. Incipient dechlorination of metabolites then leads to additional enrichment of ^{13}C. Assuming a complete hydrgenolysis and no further biodegradation of ethene, the accumulated ethene should have the same isotope ratio than the initial CAH.

14.2.2 The importance of integrated isotope values and lumped enrichment factors

Evidence for total dechlorination - i.e. either elimination of VC via hydrogenolysis or direct dichloroelimination of PCE, TCE or cDCE/tDCE - can be derived from the integrated value of bulk chlorinated ethenes. This value merges isotope signatures of single CAH compounds to an aggregate compound ($\delta^{13}C_{\Sigma CAH}$) by weighting the individual isotope values ($\delta^{13}C_i$) with their respective concentrations ($conc_i$ and $conc_{CAH}$):

$$\delta^{13}C_{\Sigma CAH}\,[‰] = \frac{\Sigma(conc_i * \delta^{13}C_i)}{conc_{CAH}}$$

The bulk isotope value will be enriched only, when the pool of chlorinated ethenes is diminshed (Figure 1). Through degradation by ZVI this can occur by two different pathways: Exclusive hydrogenolysis implies delayed enrichment of the ΣCAH isotope value via VC degradation. Exclusive dichloroelimination, however, leads to immediate enrichment, since PCE is degraded directly to chloroacetylenes.

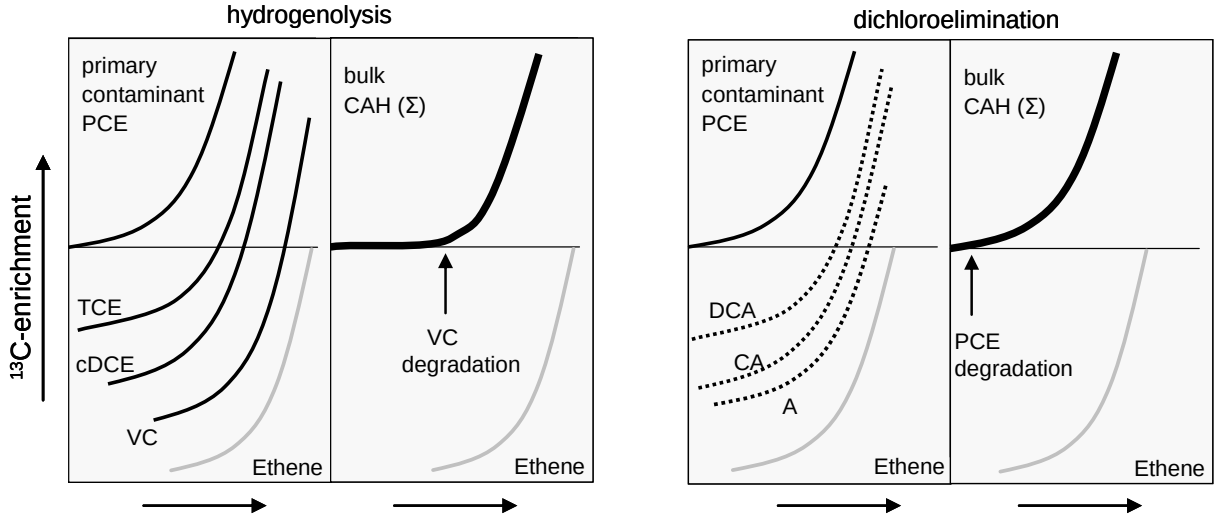

Figure 1: Temoral evolution of isotope signatures of single CAH and bulk CAH during potential hydrogenolyses or dichloroelimination triggered by ZVI (DCA = dichloroethane, CA = chloroethane, A ethane; dotted lines = generally not relevant because of rapid turnover).

The complexity of potential PCE degradation and thus isotope enrichment processes that can be triggered by zerovalent iron stimulated an empirical determination of an enrichment factor that is representative for the entity of final dechlorination. Parallel laboratory assays performed in AQUAREHAB WP4 retrieved a range for this lumped isotope enrichment factor from $\varepsilon_{\Sigma CAH}$ = -11,1

‰ to $\varepsilon_{\Sigma CAH}$ = -24,8 ‰ depending on occasional domination of specific reactions. Compared to recent theoretical concepts for quantitative isotope monitoring of bulk CAH via reductive dehalogenation this range is relatively close (*Aeppli et al. 2010*). We recommend to use the mean value ($\varepsilon_{\Sigma CAH}$ = -18,0 ‰) to quantify total dechlorination by ZVI through Rayleigh calculations.

14.2.3 Benchmarks for Qualitative and Quantitative Evaluation of Biodegradation by CSIA

At contaminated sites, the concentration of pollutants can be diminished by several processes such as biodegradation, dispersion, dilution, volatilization or adsorption. Isotope enrichment occurs exclusively during degradation and thus allows conclusions on attenuation processes independently from contaminant concentrations. Generally, carbon isotope values of CAH more positive than -20 ‰ are a significant qualitative indication for biodegradation, because the values of primary contaminants are more negative than -22 ‰ (*Eisenmann & Fischer 2010*). The range of 2 ‰ includes fourfold the overall analytical uncertainty of carbon isotope measurements (*Sherwood-Lollar et al. 2007*), and is therefore the benchmark for significant isotope enrichment (i.e. biodegradation) along a flow path (*US EPA 2008*). Minor enrichments >0,5 ‰ are assessed as indicative values.

The isotope enrichment factor is a critical value for the precision of quantification, because it varies with environmental conditions, microbial communities and the complexity of parallel pathways of degradation (see *www.isodetect.de/isofracDB.php)*. From the range of potential isotope enrichment factors, the median or mean value will deliver the most probable scenario for the intensity of biodegradation and is therefore recommended for application. Isotope enrichment factors close to zero retrieve a progressive result (i.e. probable overstimation of biodegradation), while the most negative enrichment factor ends up in a conservative estimation (i.e. underestimation).

Finally, several simplifications on hydrogeological processes are generally made in quantitative isotope monitoring: 1) linear and direct groundwater flow between considered monitoring wells; 2) constant flow velocity; 3) no influence of additional contaminant sources; 4) continuous and constant isotope enrichment.

Clearly, all simplifications imply a substantial uncertainty in the quantitative conversion of isotope enrichment to biodegradation. Nevertheless, the remarkable advantages of the method are more than a compensation: 1) only 3 parameters necessary, 2) discrimination of dilution/dispersion from biodegradation, 3) *in situ* data directly retrieved from contaminants, 4) extensive information on compound-specific percentual degradation and degradation rates at 5) clearly defined zones of biodegradation within a plume, and 6) discrimination of aerobic and anaerobic biodegradation. At a glance, these features make isotope monitoring a powerful tool in the exploration of hyporheic biobarriers.

14.2.4 Benefit of repeated isotope monitoring at field site

Evidence for natural attenuation is particulary necessary for sites with extended contamination. Many technologies for contaminant removal are limited to the source zone or to a barrier function (such as ZVI). Hence monitored natural attenuation is the option for spacious downstream regions

of plumes. Isotope monitoring is going to be a routine method to assess the feasibility of MNA in these regions. However, the necessity of repeated isotope surveys might exist depending on specific environmental conditions of the area.

Table 1: *Weighted average isotope values ($\delta^{13}C$‰; upper table) and concentrations (µM/L; lower table) of bulk chlorinated ethenes during six monitoring campaigns (single values, means, standard deviations). Colours are related to qualitative evaluation (see below).*

characteristics	well	Feb. 10	Jul. 10	Nov. 10	Apr. 11	Jul. 11	Nov. 11	mean	sd
primary source	PB104	-24.9	-24.6	-23.8	-24.1	-23.7	-23.7	-24.1	0.5
biodegradation	B4	-20.3	-21.9	-21.7	-21.4	-22.4	-23.3	-21.8	1.0
neglectable contamination	B6		-19.0				-18.5	-18.8	0.4
neglectable secondary source	PB505	-25.6						-25.6	
minor secondary source	PB603	-26.2	-23.9			-24.8	-23.0	-24.5	1.4
biodegradation	PB305	-17.6	-17.9	-17.1	-15.4	-16.9	-15.4	-16.7	1.1
variable flow, biodegradation	PB401	-13.5	-18.1	-5.5	-10.0	-21.3	-18.9	-14.6	6.0
close to major source	PB402	-20.5	-24.9	-24.1	-24.5	-24.9	-25.0	-24.0	1.7
major source, biodegraded	PB405	-22.3	-21.1	-19.9	-18.9	-21.5	-19.4	-20.5	1.3
minor biodegradation	PB501	-22.4		-18.8		-23.1	-23.0	-21.8	2.0
variable biodegradation	PB404	-15.9	-15.8	-20.2	-11.8	-21.9	-21.7	-17.9	4.0
major/variable biodegradation	PB504	-16.8	-9.9	-13.8	-10.3	-5.7	-8.7	-10.9	3.9

upstream ZVI barrier	PB104	34.4	12.5	30.9	25.0	15.1	27.7	24.3	8.7
just downstream	B4	5.5	8.5	4.9	3.9	12.0	9.5	7.4	3.1
just below ZVI barrier	B6		3.8				2.5	3.1	0.9
close downstream ZVI	PB505	0.2	0.1						
close downstream ZVI	PB603	0.1	0.5			0.1	0.3	0.2	0.2
downstream	PB305	10.1	16.1	29.3	10.0	9.9	9.4	14.1	7.8
more downstream east	PB401	0.2	1.3	0.2	0.2	5.1	5.0	2.0	2.4
more downstream west	PB402	9.2	6.9	6.5	2.2	7.5	10.4	7.1	2.8
far downstream east	PB405	20.9	50.9	57.8	27.8	31.1	74.1	43.7	20.5
far downstream east	PB501	0.2	0.1	0.3		0.2	0.7	0.3	0.2
farer downstream west	PB404	6.0	6.9	14.1	8.5	33.5	23.1	15.4	10.9
farest downstream west	PB504	48.7	33.8	47.3	25.5	16.9	44.2	36.1	12.9

red = contaminant sources (i.e. most negative isotope signatures)
green = evidence for biodegradation
blue = high variation of values
yellow = highest concentrations

Within AQUAREHAB, six monitoring campaigns for carbon isotopes of CAH (Feb 2010, Jul 2010, Nov 2010, Apr 2011, Jul 2011, Nov 2011) were performed at an industrial field site in Belgium (site A), where chlorinated ethenes had been detected in quaternary sediments close to a lowland river. A zerovalent iron (ZVI) barrier had been installed downstream from a major contaminant source (PB104). Groundwater flow velocity is slow, but variable within one order of magnitude (around 2-20 m/year). At least 12 wells were monitored for carbon isotopes of CAH in parallel with a survey of contaminant concentrations and environmental parameters (Table 1).

The *temporal dynamics* of isotope and concentration values were clearly different within the time frame of 2 years. Bulk isotope values exhibited very constant levels in most of the wells (sd ≤ 2 ‰),

while bulk concentration of CAH was variable to a large extent. Obviously, the isotope values were clearly less affected by hydraulic dynamics that had occured wthin the investigation period than concentration values. A trend for temporal shifts of isotope signatures not discernable. Generally, an increase of isotope values at the same spot would indicate plume retardation (because of major biodegradation), while a decrease would suggest plume extension (because of minor biodegradation). Probably, the time frame of the study was too short to reflect such long-term processes.

Only three wells showed a high variability in isotope values. Thus it can be concluded that a single isotopic survey already can provide pronounced evidence to quantify total dechlorination at the investigated site with sufficient precision. However, this conclusion should be generalized only for sites with similar hydrogeological properties. If significant quantification of biodegradation is required for specific flow paths, or if the state of retardation/expansion of the plume is in the focus of exploration, repeated isotope surveys are recommended.

Two secondary sources of contamination were identified due to very negative and non-enriched isotope signatures (PB603, PB402; Figure 2). Calculated from weighted average isotope enrichment of bulk chloroethenes and an average lumped fractionation factor for reductive dehalogenation (*Aeppli et al. 2010*), the percentual degradation of total CAH along potential flow paths was in the range of 5 to 50 %. Accordant halflife distance was inbetween 70 m and 700 m, which is typical for most contaminated sites (*US EPA 2002*). On the other hand, the halflife period was generally much longer than 100 years (as a consequence of low flow velocity). Thus isotope investigations indicated that complete dechlorination of CAH at the site is prevalent, but apparently too weak for notable retardation of the contaminant plume. Therefore, active remediation technologies such as ZVI barriers were adequate to achieve a more effective removal of contaminants.

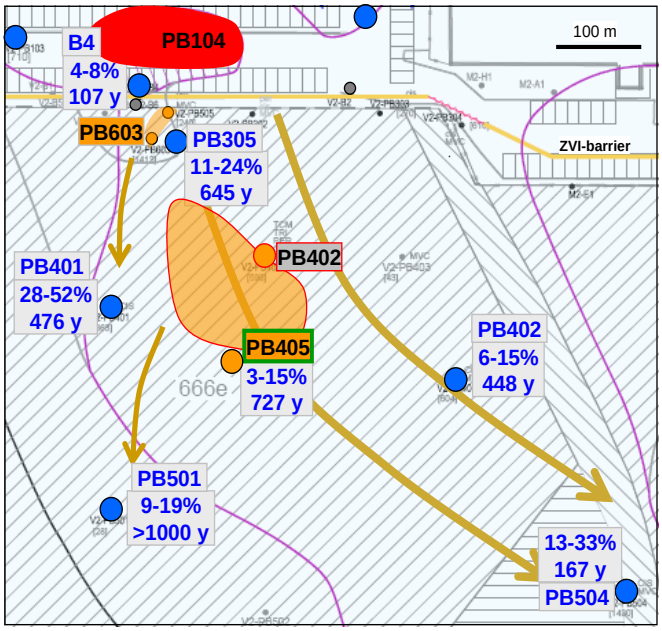

Figure 2: Conceptual site model derived from isotope values and concentrations of CAH showing primary (red) and secondary source (amber), zones exhibiting complete dehalogenation (blue), and major direction of groundwater flow. Moreover, percentual biodegradation and halflife periods of degradation were calculated for selected flow paths downstream from the primary source (PB104).

14.3 Requirements and Logistics of Isotope Monitoring

Analytical requirements for isotope monitoring are contaminant concentrations from 2 to 20 µg/L, which is the determination limit for chlorinated ethenes. Additionally, the selection of monitoring wells should regard defined transects or center lines of the plume. Furthermore, it is very important to know the primary isotope value (or at least the most negative isotope value) of contaminants, which generally can be found at the contaminant source.

Once monitoring wells have been selected, sampling (250 to 1000 mL groundwater in glas bottles; preserved with NaOH pellets) and shipment of samples are easy to handle. For isotope measurements, which require 1 - 3 months, it is necessary to provide contaminant concentrations. Including expertise, which should be compiled by experts, the costs for 10 monitoring wells are in the range of 4500 € to 6000 € depending on the analytical effort and the complexity of the site.

14.4 Citations

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