*Proceedings of the 4th International Conference on Environmental Pollution and Remediation*
*Prague, Czech Republic, August 11-13, 2014*
*Paper No. 172*

# Remediation of Chlorinated Ethenes Using Reactive Iron Barrier and Its Impact on Indigenous Bacteria

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***Abstract*** - Chlorinated ethenes are among the most often detected organic contaminants in groundwater. Under anaerobic conditions, tetrachloroethene (PCE) and trichloroethene (TCE) can be degraded by the sequence of reductive dechlorination steps through the intermediate products *cis*-dichloroethene (*cis*-DCE) and vinyl chloride (VC) to ethene. This process is driven either by specific microorganisms or by reductive agents, such as nano or microscale zero-valent iron particles (nZVI or mZVI). A permeable reactive barrier was prepared by applying a mixture of nZVI and mZVI to the series of wells downstream from the source of the contamination. Polymerase chain reaction (PCR) and real-time PCR analysis of microbial composition of groundwater upstream and downstream of the barrier revealed strong influence of iron-driven dechlorination on the recovery of microbial colonization of groundwater including bacteria capable of degrading chlorinated ethenes.

***Keywords*:** Chlorinated ethenes, nZVI, Bioremediation, *Dehalococcoides, Dehalobacter, Sulfurospirillium.*

## 1. Introduction

Chlorinated ethenes, such as PCE and TCE, are widely used in industry as cleaning and degreasing agents, solvents etc. and are often released into the environment as a result of improper handling or storage. Degradation of these compounds is limited under aerobic conditions, and therefore they can easily seep into the groundwater and travel with the flow. Degradation of chlorinated ethenes is much more effective under anaerobic conditions and is mostly achieved by the process of reductive dehalogenation (Aulenta et al., 2006). PCE and TCE are dechlorinated abiotically or by microorganisms - they undergo sequential reductive dechlorination to *cis*-DCE, VC and ethene as a final product. However, the process is sometimes incomplete, resulting in accumulation of *cis*-DCE or VC. VC is more toxic and carcinogenic than its parent compounds, therefore stimulation of VC degradation and careful monitoring of the site is necessary (Tobiszewski and Namieśnik, 2012). Several bacteria, such as *Dehalobacter, Desulfuromonas, Sulfurospirillum, Desulfitobacterium* or *Geobacter*, have been described to be able to dechlorinate PCE and TCE to *cis*-DCE as the end product. However, only bacteria from the *Dehalococcoides* group have been shown to degrade *cis*-DCE or VC completely to ethene (Tiehm and Schmidt, 2011). Two vinyl chloride reductases, with corresponding genes *vcrA* and *bvcA*, have been described to be directly involved in reductive dechlorination of VC to ethene (Krajmalnik-Brown et al., 2004; Muller et al., 2004).

In comparison to biodegradation of chlorinated ethenes, abiotic degradation is usually slower but complete and can be speed up by reductive agents, such as nZVI or mZVI. The nZVI is very effective due

to its high specific surface area and high reactivity, and it is also advantageous for its ability to dechlorinate chlorinated ethenes to nontoxic ethene (Lacinová et al., 2012).

This study describes a field experiment carried out at Spolchemie site in the Czech Republic that is highly contaminated with chlorinated ethenes. The main purpose was to determine the influence of combined nZVI and mZVI treatment on the reduction of contaminants together with the effect on indigenous bacterial groups capable of reductive dehalogenation.

## 2. Results and Discussion

Permeable reactive barrier was prepared by nZVI and mZVI application to the series of wells downstream from the source of contamination. Three months after the application, concentration of chlorinated ethenes and indigenous microbial communities were analysed in the wells upstream and downstream from the barrier and one well in the barrier. The sum of all chlorinated ethenes was 15 121,4 µg/L in the upstream well, dropped to 2 195,0 µg/L after reaction with nZVI and mZVI in the reactive barrier, and then increased to 8 474,0 µg/L in the downstream well, probably due to rebounding. The presence of *Dehalococcoides*, *Sulfurospirillium* and also of two vinyl chloride reductase genes, *vcrA* and *bvcA*, were proved downstream from the reactive barrier (tab. 1).

Table 1. Total DNA yields from 1 L of groundwater sample and detection of specific bacterial groups and enzymes.

| sampled well | DNA yield | *Dehalococcoides* | *Dehalobacter* | *Sulfurospirillium* | *vcrA* | *bvcA* |
|---|---|---|---|---|---|---|
| upstream | 4,975 µg | ndt | ndt | * | ndt | ndt |
| in the barrier | 0 µg | ndt | ndt | ndt | ndt | ndt |
| downstream | 19,675 µg | * | ndt | * | * | * |

ndt: not detected, * present

Reactive iron barrier had strong impact on the microbial community. Total DNA yield increased five times in the sample downstream compared to the sample upstream from the barrier. This DNA can be of prokaryotic but also of eukaryotic origin. It is in accordance with real-time PCR analysis of total 16S rDNA which showed 3,45 times higher abundance of bacteria in the well downstream from the barrier. No DNA could be extracted from the water sample directly in the barrier. Possible explanation is that iron particles were applied in high concentration toxic to bacteria or they might interfere with the DNA isolation itself.

The real-time PCR and PCR analysis for the presence of various bacterial genera and degradation enzymes showed significant impact of the reactive iron barrier (fig. 1). In the sample downstream from the barrier, vinyl chloride reductase genes, *Sulfurospirillium* sp. and *Dehalococcoides* sp. were detected, while in the untreated water only noticeably smaller amount of *Sulfurospirillium* sp. of all the tested genes and species were present. *Dehalobacter* sp. was not detected in any sample.

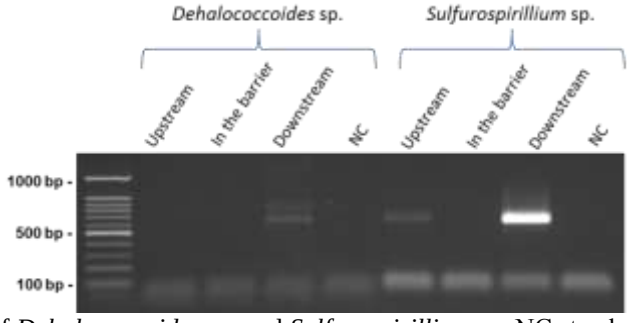


Fig. 1. PCR detection of *Dehalococcoides* sp. and *Sulfurospirillium* sp. NC stands for „negative control".

The large differences in microbial colonization between the samples can be explained by the influence of the applied nZVI and mZVI. Upstream from the reactive barrier the concentration of pollutants was so high it inhibited the growth of any bacteria, including specific degraders. Directly in the reactive barrier, the applied iron might wipe out all the bacteria present, but it is not very probable. As results of Němeček et al. (2013) indicate, the nZVI injection in soil does not negatively affect the viability of indigenous bacterial populations. More probable explanation is that iron particles interfered with DNA extraction and thereby distorted the yield. Downstream from the barrier the concentration of chlorinated ethenes dropped two times causing recovery of microbial community including bacterial groups capable to degrade chlorinated ethenes.

## 3. Materials and Methods

### 3. 1. Application of nZVI and mZVI

Combination of 300 kg of mZVI (Carbonyl Iron Powder HQ, BASF, Germany) and 400 kg of nZVI in suspension (Nanofer STAR, NANO IRON s.r.o., Czech Republic) was applied to the series of 10 infiltration wells to form a permeable reactive barrier. Nanofer STAR is an air-stable powder, consisting of Fe(0) surface-stabilized nanoparticles with average particle size of 50 nm and an average surface area of 20-25 $m^2/g$. Carbonyl Iron Powder HQ is a powder with iron content up to 97.8% and average particle size of 2 µm. Samples for chemical and biological analysis were taken 3 months after the application from one well 12 m upstream, one well in the barrier and one well 30 m downstream from the barrier.

### 3. 2. Quantification of Chlorinated Ethenes

Concentration of chlorinated ethenes was determined in each sample using a CP-3800 gas chromatograph with a Saturn 2200 ion trap mass spectrometry detector (Varian, CA, USA).

### 3. 3. Molecular Analysis

Microbial biomass was sampled from each well (0,4 L) and the water samples were concentrated by filtration (filter pore size 0,22 µm). DNA was extracted from the filter using FastDNA Spin Kit for Soil (MP Biomedicals, CA, USA) according to manufacturer's protocol. Homogenizing device Bead Blaster 24 (Benchmark Scientific, NJ, USA) was used for cell lysis. Extracted DNA was then quantified with Qubit 2.0 fluorometer (Life Technologies, MA, USA).

Reactions for PCR were prepared as follows: per 25 µl reaction volume, 12.5 µl 2x MyTaq Mix (Bioline, UK), 0.75 µl of 20 µM forward and reverse primer mixture and 10.75 µl of ultra-pure water were used. 1 µl of extracted DNA was added to the reaction. PCR was performed in a Veriti 96-well thermocycler (Applied Bioscience, MA, USA). The presence of *Dehalococcoides* and *Sulfurospirillium* sp. was determined by horizontal gel electrophoresis on 1% agarose gel.Vinyl chloride reductase genes *vcrA* and *bvcA*, *Dehalobacter* sp. and total bacterial biomass were detected by real-time PCR method. Reactions for real-time PCR were prepared as follows: per 10 µl reaction volume, 5 µl LightCycler® 480 SYBR Green I Master (Roche, Switzerland), 0.4 µl of 20 µM forward and reverse primer mixture and 3.6 µl of ultra-pure water were used. 1 µl of extracted DNA was added to the reaction. Samples were analysed in duplicate. Real-time PCR was performed in LightCycler® 480 instrument (Roche, Switzerland). Cp values were obtained using Fit Points method available in LightCycler® 480 Software.

Total bacterial biomass was detected using universal 16S rDNA primer set (Clifford et al., 2012). Specific primer sets were used for detection of *Dehalobacter* sp. (Smits et al., 2004), *Dehalococcoides* sp. (Hendrickson et al., 2002), *Sulfurospirillium* sp. (Ebersole and Hendrickson, 2002) and vinyl chloride reductase genes *vcrA* and *bvcA* (Behrens et al., 2008).

## 4. Conclusion

To conclude, our field experiment proved high remediation potential of combined nZVI and mZVI application as a permeable reactive barrier. Remediation using ZVI particles promoted rapid abiotic degradation of chlorinated ethenes and thereby supported growth of indigenous bacteria including key

dehalogenating bacteria, and thus enhanced natural bioremediation. Detection of specific bacterial species and vinyl chloride reductase genes helps to evaluate actual site potential for biological dechlorination.


### Acknowledgements

This study was supported through the Technology Agency of the Czech Republic, project no. TA02020534 and TE01020218. The work of Marie Czinnerová was supported by the Ministry of Education of the Czech Republic within the SGS project no. 21066/115 on the Technical University of Liberec.


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