CL:AIRE

EiCLaR 02
(December 2024)

EiCLaR bulletin

CL:AIRE's EiCLaR bulletins describe *in situ* bioremediation technology developments and tools created within the EiCLaR project. This bulletin describes the development of the groundwater remediation technology of electro-nanobioremediation for the treatment of chlorinated solvents.

Electro-nanobioremediation

1. INTRODUCTION

This bulletin describes the sequential groundwater remediation technology of electro-nanobioremediation (ENB) for the treatment of chlorinated solvents, its development, benefits and potential applications. Within the EiCLaR project, this technology has been developed from technology readiness level (TRL) 6 to TRL 9. ENB combines the application of zero-valent iron nanoparticles (nZVI, **nano**) with microbial degradation (**bio**) using an electric field (**electro**) to tackle predominantly chlorinated hydrocarbons (CHC). First, the pollutant load is reduced by nZVI and in a second step, the residual pollutants and metabolites are biologically remediated. This first step aims to reduce the contaminant load to create a less toxic environment for bioremediation. In the second step, microbial degradation is stimulated by the injection of a carbon substrate. Both steps can be electrokinetically enhanced with the application of low-voltage direct current across the section of the contaminated aquifer (see Figure 1).

The TRL of this technology was advanced by conducting experiments at batch-, medium- and large-scale under controlled conditions at the Technical University of Liberec (TUL) and at VEGAS University of Stuttgart (USTUTT). The technology was further explored at a pilot site by Photon Water Europe (PWEU) and TUL. Results show that the electric field prolongs the effectiveness and remediation efficiency of ZVI particles and fosters favourable conditions for microbial degradation.

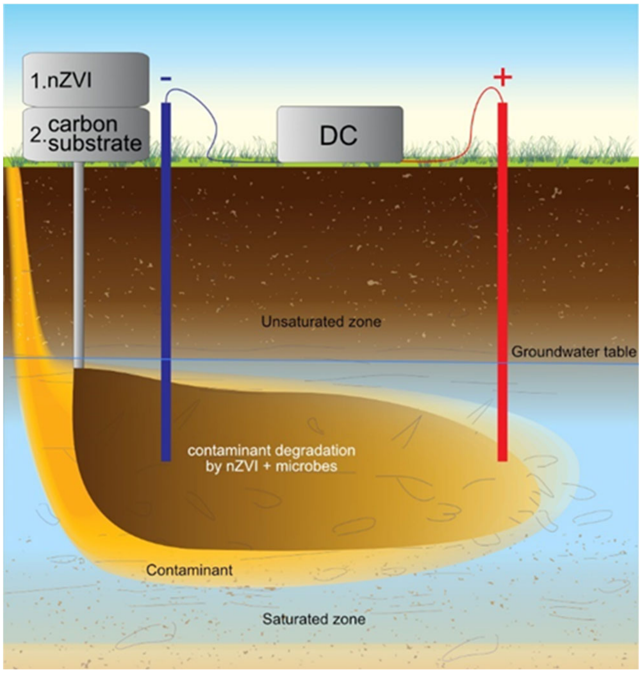

Figure 1. Principle of electro-nanobioremediation technology developed within EiCLaR, showing injection of nano-scale zero-valent iron (nZVI) particles for main pollutant reduction, injection of carbon substrate to enhance microbial activity, and the application of direct current (DC) to electrokinetically support the remediation process.

2. BACKGROUND OF THE TECHNOLOGY

The concept of electro-nanoremediation (EN) was first created in the Czech Republic in 2009. The work was a collaboration between TUL and the remediation company MEGA a.s. The goal of this technology was to increase the remedial effectiveness of nZVI, a research and deployment initiative that started in the Czech Republic as early as 2005. The aim of the direct-current (DC) electric field was to increase the reactivity and extend the longevity of the nZVI, resulting in significantly improved operational economics and a higher remediation effect. From 2011 through 2021, TUL implemented several national R&D projects with selected remediation companies (including PWEU) in the Czech Republic. These projects focused on laboratory and field research of EN technology. During this period, approximately ten pilot field tests and three full-scale remediation operations where EN technology was deployed were also carried out as part of R&D activities. These resulted in several patents. The method has become a widely accepted technology in the Czech Republic for remediation of subsurface environments, demonstrating very good efficiency and minimal negative effects on the environment (Pavelková *et al.*, 2020, 2021).

Enhanced and Innovative *In Situ* Biotechnologies
for Contaminated Land Remediation

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°965945. This output reflects only the authors' views and the European Commission is not responsible for any use that may be made of the information it contains.

2.1 Concept of electro-nanoremediation (EN)

Chemically supported reductive dechlorination of chlorinated ethenes involves substituting chloride atoms with protons, a process that consumes electrons. To achieve effective dechlorination, it is essential to generate a substantial surplus of protons and electrons within the geochemical system. This can be achieved practically, for example, through the reaction of Fe^0 with water. Additionally, applying a DC electric field at an appropriate current density can enhance the process by ensuring an electron-rich environment while minimizing excessive Fe^0 consumption due to side reactions with water. This approach establishes optimal, long-term conditions for the reductive dechlorination of chlorinated ethenes.

Beta-elimination involves the removal of a halogen atom and a proton from adjacent carbons, leading to the formation of less chlorinated alkenes and eventually ethene via acetylene. In contrast, hydrogenolysis replaces halogen atoms on the carbon chain with hydrogen atoms, progressively dechlorinating to less harmful compounds like ethene or ethane. Arnold and Roberts (2009) investigated reaction pathways for chlorinated ethenes during reduction by Fe^0 and hypothesized that the major pathway is beta-elimination, and thus is most relevant for EN.

Figure 2 shows the Eh-pH diagram of iron, which shows the transformation of nZVI in different conditions. The diagram illustrates the synergistic effect of nZVI combined with DC. The red area corresponds to the anodic area (low pH, fast oxidation of Fe^0, Eh about 0.2 V), orange area represents optimal conditions for effective reductive reactions with contaminants (8-10 pH, Eh about -0.5 V), and blue area shows cathodic reactions characterized by overly reductive conditions, leading to Fe^0 being converted to the stable, nonreactive form, $Fe(OH)_4^-$.

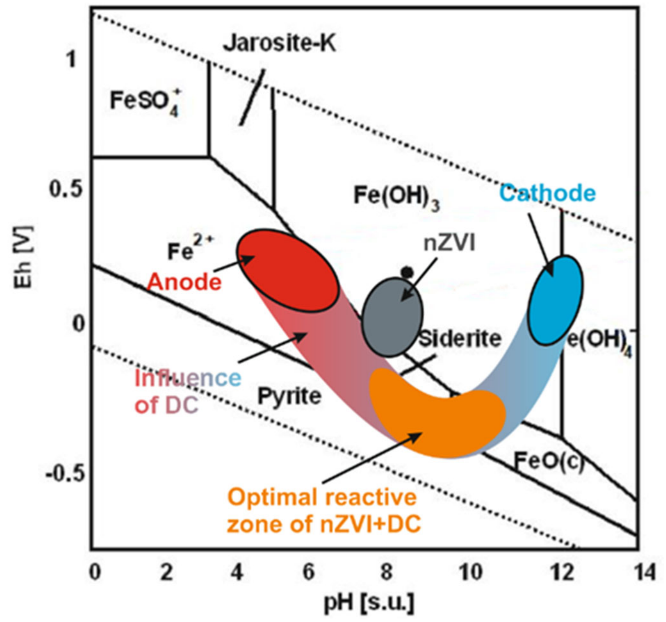

Figure 2. Iron Eh-pH (Pourbaix) diagram - model interpretation of electrode processes and optimal zone of reactivity of nZVI + DC.

2.2 Concept of electro-nanobioremediation (ENB)

The concept validated in the EiClaR project represents the next logical step in the evolution of the EN technology. In the EN phase, an initial decrease in CHC concentrations by nZVI reduces toxicity, thereby facilitating the implementation of biological remediation. The subsequent bioremediation phase involves the stimulation of microorganisms already present within the soil with a carbon substrate, which provides the necessary energy and nutrients for microbial metabolic processes. Thus, microorganisms can further reduce contaminant concentrations by direct reductive dechlorination dominated by hydrogenolysis (e.g. *Dehalococcoides sp.*; Tiehm *et al.*, 2011) or through reactivation of passivated aged nZVI (*Geobacter sp.* and *Shewanella sp.*; Shin *et al.*, 2007).

3. DEVELOPMENT OF THE TECHNOLOGY AT LAB-SCALE

ENB technology was developed through laboratory experiments conducted at various scales. These studies primarily focused on tetrachloroethene (PCE), a solvent that is one of the most significant subsurface contaminants. PCE often resides as a non-aqueous phase liquid (NAPL) in the subsurface, where it slowly dissolves into groundwater. Its maximum solubility is approximately 160 mg/L, making it a common source zone contaminant in hotspot areas.

Subsurface contamination can generally be categorized into two zones:

1. **Source zone** (typically above 50 mg/L): This zone represents the point of contamination release, typically containing residual NAPL. It has the highest contaminant concentrations and serves as the primary source of contamination spread via rainwater infiltration and groundwater flow.
2. **Contamination plume** (typically below 50 mg/L): This zone surrounds the source zone. While the free phase is no longer present, the groundwater can remain heavily contaminated. This zone is usually characterized by lower contaminant concentrations, which can be more readily addressed by microbial degradation.

The upscaling concept allowed for the successive increase of complexity under controlled conditions to deepen the process understanding. Both standard experimental methodologies and completely new approaches were used to develop ENB (Figure 3). The experimental methodologies were selected with the purpose of studying and optimizing the synergistic processes in the application of DC current. The combination of experimental methodologies and new approaches enabled experimental conditions to closely match field conditions at the pilot sites.

A batch arrangement was used to verify the reactivity of selected nanomaterials and to study the influence of biological substrates on bioremediation processes.

Reactor tests were used to study the effect of the application of the DC system in an aqueous environment. These experiments were conducted in closed, stirred systems with a volume of 2.5 L, equipped with electrodes for applying a DC field. By connecting two reactors separated by an ionic membrane, the processes in the vicinity of the electrodes could be studied separately (R4, R5).

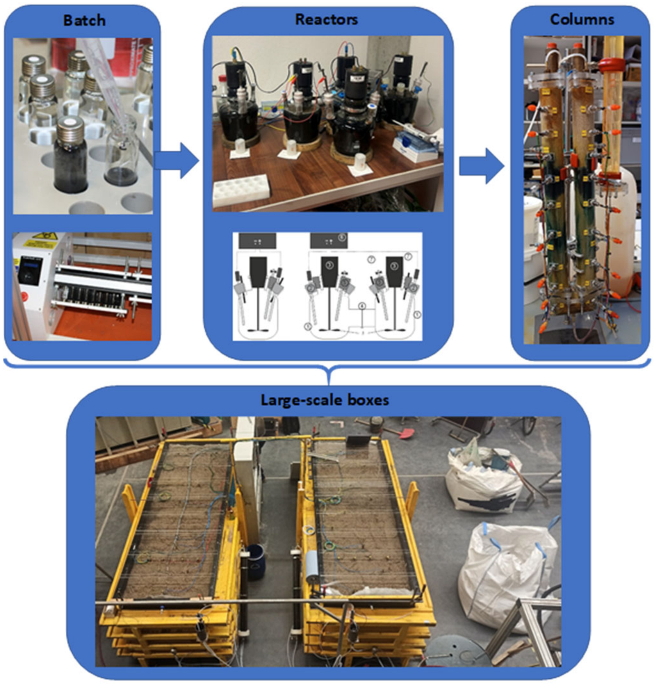

Figure 3. Laboratory testing methodologies used for ENB technology development.

Both batch and reactor tests were used to explore new applications of ENB technology using real groundwater contaminated by chlorinated methane (CLM) from a local site. During the batch tests, it was determined that the optimum concentration of nZVI for degrading CLM (up to 0.6 g/L of CLM with 60% dichloromethane) is 20 g/L. Six reactors were designed: R1 was the control, R2 applied only nZVI, R3 and R6 applied different nZVI and DC, and R4 and R5 applied nZVI and DC but separated anode from cathode. In reactor tests with an nZVI concentration of 20 g/L (identified as optimal in batch tests), up to 80% of CLM was removed in reactors R4 and R5 (see Figure 4).

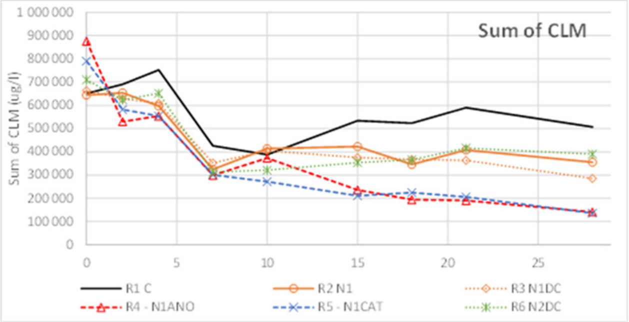

Figure 4. Concentration evolution of chlorinated methane in reactor tests.

Column tests allowed the study of processes in flow-through soil matrix systems. The soil used was a natural uncontaminated sand from the Rhine valley in Germany. Within the EiClaR project, this methodology has been further optimized for the application of DC systems to study the effect of DC field, electro-biostimulation, and EN. This setup consisted of two parallel columns connected at the outlet which allowed for monitoring the influence of cathode and anode processes without mutual interference. Figure 5 shows the concentrations of the sum of gaseous degradation products of CHCs (acetylene, ethene, ethane) for each system studied, with higher values representing higher efficiency (reactivity). Within the electro-biostimulation phase (blue shaded area), the application of carbon substrate (glycerol based) combined with the DC field promoted microbial dechlorination. Low current densities resulted in 8 times higher biodegradation at the cathode compared to the anode. Further increase of DC potentials then negatively affect biodegradation. Thus, a 12-fold increase in degradation efficiency was achieved during the EN phase (nZVI combined with a DC field, indicated by the green-shaded area), particularly near the cathode and under moderate applied potentials.

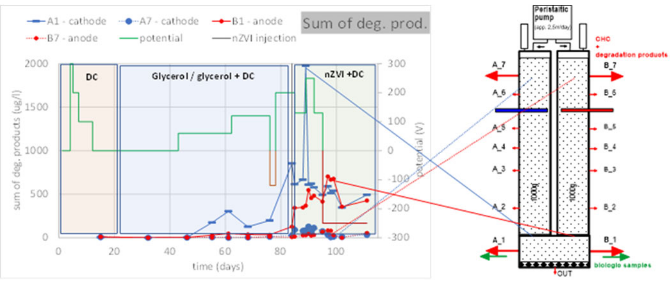

Figure 5. Concentration evolution of gaseous degradation products in column tests.

These laboratory methodologies were used to study selected processes separately to deepen their understanding.

Large-scale box tests were conducted to bridge the gap between lab and field and aimed at quantifying the efficiency of ENB compared to state-of-the-art nanoremediation. This approach enabled testing under more complex conditions while still allowing for thorough evaluation and control of boundary conditions. It also represented the most realistic system model of the actual technology deployment on site. Per design, the experiment was conducted in two subsequent phases, (1) EN and (2) bioremediation which included the substrate addition.

Two identical boxes (L/W/H = 3.1 m/1 m/1 m) were designed and set up at USTUTT in cooperation with PWEU and TUL (see Figure 6). The experiment detailed in Box 1 was conducted without the application of a DC electrical field, whereas in Box 2, a DC electrical field was applied. This allowed for direct comparison of results. The unconfined aquifer was achieved using the same natural sand (0-4 mm) from the Rhine valley which formed two aquifer zones either side of the reactive zone. The reactive zone (L/W/H = 1 m/1 m/1 m) consisted of a composite material consisting of nano- and micro-ZVI which was mixed with the sand before filling. Ten sampling wells (W1-W10) and 8 steel electrodes were installed within the aquifer zones. The boxes were sealed with lids to prevent air venting. PCE was used as a contaminant and was continuously injected into both boxes in degassed tap water at saturation concentration (plume treatment at maximum solubility, concentration = 160 mg/L, flow rate 1.5 L/h, residence time 35 days in box and 12 days in the reactive zone). A DC system ensured a potential of 60 V in Box 2 between cathodes (inflow side) and anodes (outflow side). Physicochemical parameters (pH, temperature, redox potential (ORP), electrical conductivity (EC)) were measured automatically in each well every three days. PCE and degradation products (trichloroethene (TCE), dichloroethane (DCE), vinyl chloride (VC), chloride, hydrogen, methane, ethane, ethene, acetylene) were monitored on a weekly basis in the inflow and outflow as well as in all sampling wells.

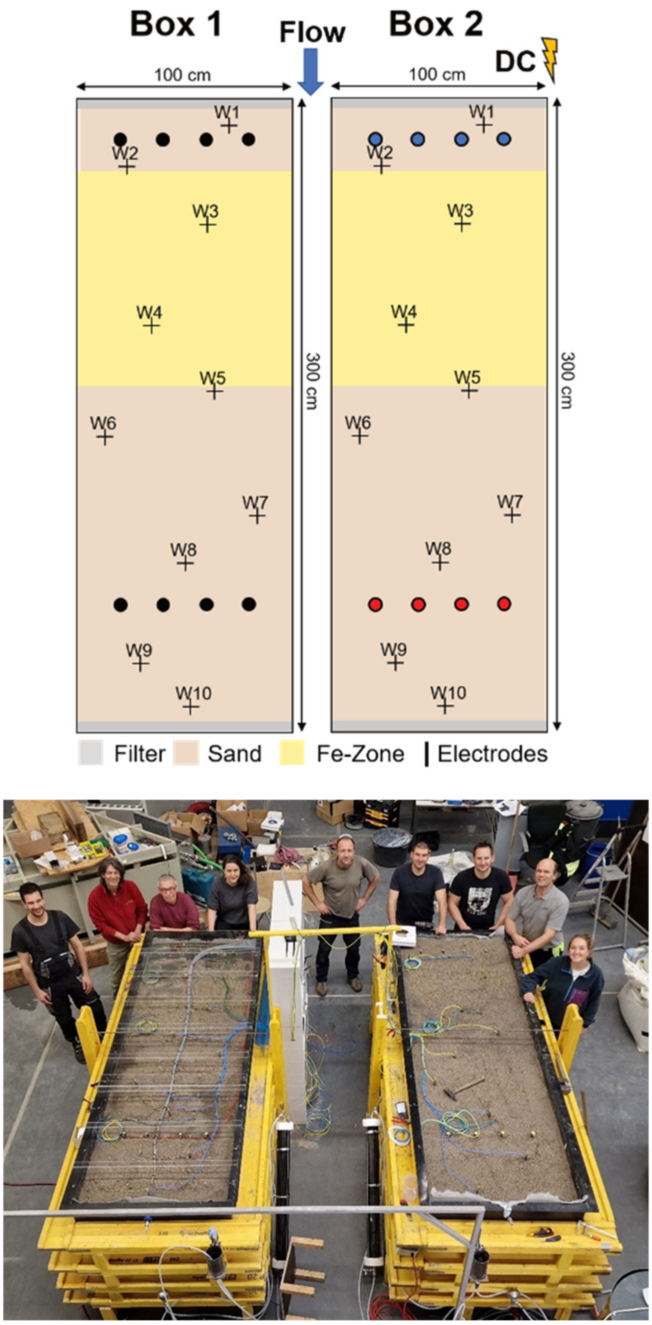

Figure 6. Top view of large-scale box experiments (top, schematic diagram; bottom, team photo in lab).

Results of the EN phase show that downstream of the cathode of Box 2, the pH increased as high as pH 12 and the ORP decreased as low as -500 mV in the reactive zone. In contrast, Box 1 showed pH values between 7.5 to 8.0 throughout the box and a minimum ORP of -300 mV. The increase of pH reduced the corrosion of the nZVI particles (reaction of Fe^0 with water), thus improving their longevity. Figure 7 shows the evolution of gaseous degradation products in both boxes on a weekly basis. Results show that a two times higher amount of acetylene, an indicator for the beta-elimination pathway, formed in Box 2 with DC compared to Box 1, suggesting a higher reaction rate and thus better degradation efficiency. Other reductive dechlorination products TCE, DCE, and VC were not detected at concentrations relevant to the mass balance. This highlights the critical role of reductive beta-elimination in the degradation pathway (Brown *et al.*, 2009; Arnold and Roberts, 2000).

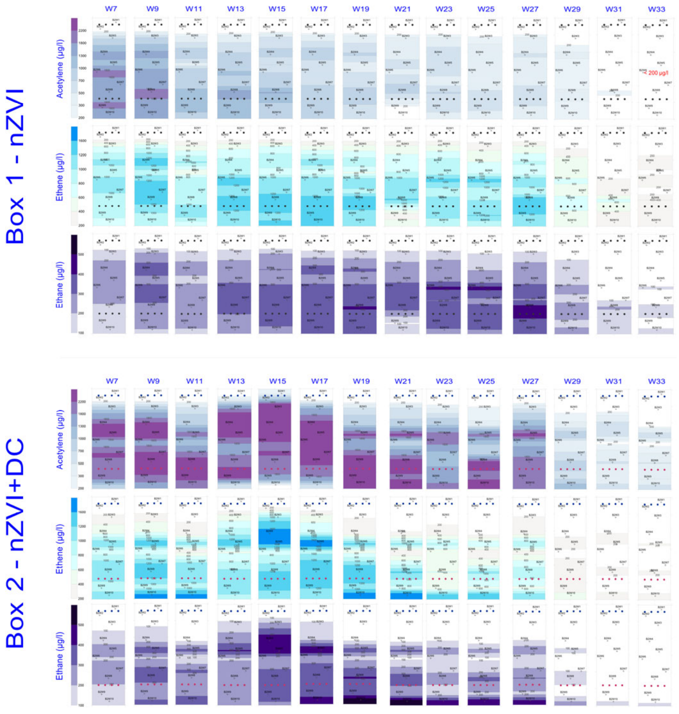

Figure 7. Qualitative bi-weekly evolution of gaseous degradation products (acetylene, ethene, and ethane) in Box 1 and Box 2 (with DC) based on sampling well data. Each rectangle represents a top view of the box at a specific point in time. Each column of plots represents one week starting at week 7 until week 33 of the experiment.

The mass balance was calculated on the concentrations in the inflow and outflow of both boxes after an operation of 6 pore volumes (215 days) and included all substances relevant for PCE degradation in chloride equivalents. Usually, chloride can be considered a robust parameter to assess the degradation of chlorinated solvents due to its non-volatility and, thus, accurate quantification. Complete degradation of one mole of PCE results in four moles of chloride. The chloride recovery from the outflows was quantified as 59.3% in Box 1 and 53.2% in Box 2, respectively, with PCE being the only chloride source. A low recovery might have been caused by volatilization losses of PCE or storage of chloride ions in the soil of Box 2 due to the application of the DC electric field. Regarding chloride formation due to PCE degradation, a total of 5.07 moles (corresponding to 210.0 g of degraded PCE) were detected in the outflow of Box 1 after 6 pore volumes, compared to 6.60 moles (corresponding to 273.3 g of degraded PCE) in Box 2. Thus, 1.4 times more chloride was formed in Box 2 by PCE degradation, which directly reflects the increase in degradation efficiency due to the application of the DC electric field, supporting the results of the gaseous degradation products. However, chloride formation in Box 2 collected in the outflow may be underrepresented due to the formation and storage of iron-chloride complexes (e.g. chloride green rust) as well as the formation of chlorine gas (electrolysis) because of the DC field. Conclusively, the combined system of nZVI + DC (Box 2) was more effective compared to the application of nZVI only. The degradation of PCE in Box 2 compared to Box 1 generated approximately 2 times the amount of acetylene and more than 1.4 times the amount of chloride. These values are still underestimated for the reasons stated above.

During the second phase, which focused on bioremediation, the DC system was turned off while groundwater flow continued until the pH stabilized. The DC electric field can be employed to alter pH to create more favourable conditions for microbial growth. However, this was not necessary in this experiment, since degassed tap water with a neutral pH was used. Only Box 2 was continued in this phase, since the soil of Box 1 was used for column experiments to investigate iron regeneration. Soil samples were taken prior to the start and analyzed at Technologiezentrum Wasser (DVGW) confirming the presence of iron-reducing bacteria *Geobacter* in the natural sand of Box 2, which could help microbial degradation and regenerate passivated nZVI. Subsequently, 620 g of glycerol was injected weekly as a microbial substrate into the inlets of Box 2. After 18 days of injection, the degradation product cis-1,2-DCE began to appear in the outflow, increasing from 0.5 mg/L (maximum during EN phase) to 15.2 mg/L over the next 35 days. The chloride recovery after 3.5 pore volumes (120 days) was quantified as 71.2% and a total of 2.47 moles (corresponding to 112.4 g of degraded PCE) were collected in the outflow. The rapid increase in cis-1,2-DCE demonstrated effective stimulation of dehalorespiring bacteria by glycerol addition, suggesting potential for biotic contaminant degradation of low contamination areas after abiotic nZVI + DC treatment in a field setting.

4. DEMONSTRATION OF THE TECHNOLOGY AT FIELD-SCALE

The pilot site, shown in Figure 8, is located within the Solvay production site in Spain. The site is characterized by complex geological conditions: the geology comprises sands, clays and sandy clays, gravels and sandy/clay gravels; the coefficient of permeability is around 10^{-5} m/s; water flow velocity is 0.05-0.15 cm/s; water level measured 1-5 m below ground surface; and the phreatic aquifer was found 1-20 m below ground surface.

Figure 8. Pilot site of ENB showing contamination, ENB treatment area as well as treatment and monitoring wells.

The site is contaminated with CHCs, primarily chloroethenes (PCE, TCE, cis-DCE, VC), chloromethanes (PCM, TCM), and hexachlorobutadiene (HCBu) with observed concentrations of total CHCs in the hotspot >100 mg/L.

At this site, 12 wells were drilled in the application zone, and 20 wells were drilled across the entire area of interest (well depths 6-20 m). A DC control system and a groundwater real-time monitoring system (GW-RTMS) were installed. The DC control system included the installation of 100 monitoring (steel bars for monitoring voltage) and power electrodes (permanently installed in wells connected into the DC circuit) to create and monitor the DC field and potential. The nZVI composite NANOFER STAR DC (200 kg) was applied directly into the wells and temporarily sealed with packers, to define the injection position. This setup allowed for the application of several bars of pressure, facilitating the migration of nZVI into the soil and increasing the reaction surface between contaminants and the agent.

In the first treatment phase, EN was implemented to reduce pollutant concentrations and therefore reduce toxicity in the aquifer prior to the start of the second phase stimulating bioremediation.

The DC electric field maintained the reactivity (monitored by contaminant reduction) of the injected nZVI for over 12 months, and allowed for effectively controlling pH / ORP in the aquifer. Real-time physicochemical monitoring documented changes in the vicinity of the electrodes; the results showed high pH levels near the anodes which dropped from 10 to around 6 (within several days), and pH levels of 11 near the cathodes. ORP values near the cathodes decreased to between -500 and -200 mV.

Regular monitoring at the end of the EN phase, illustrated in Figure 9, showed an average decrease of >80% in CHCs (over 20 CHC compounds analyzed) around the cathodes and >47% around the anodes, compared to pre-treatment conditions. Concentrations of degradation products (e.g. acetylene, ethene, ethane, methane) increased by approximately 216% around the anodes and approximately 426% around the cathodes.

Fourteen months after the start of the EN phase, a glycerol-based substrate was applied to stimulate microbial activity. A total of 8 tonnes of substrate was dosed in two rounds for this pilot application. Using a DC system, the pH was remotely maintained in the range of 6-8. Real-time monitoring of on-site parameters (pH, ORP, conductivity and temperature) was carried out for this purpose.

The enhanced microbial activity lasted for about 5 months. The basic strains required for CHC dehalogenation (*Eubacteria*, *Dehalococcoides*, *Desulfomonas*, *Dehalobacter*, *Desulfitobacterium*) were measured in water samples. At the same time, the sequential transformation PCE -> TCE -> DCE -> VC -> ethene/ethane was observed. Figure 10 shows the degradation (decrease) of PCE in the first EN phase without intermediates, and the increase in PCE concentrations (release of residual contamination) after substrate application which was and is being subsequently microbially degraded to end gas products, according to the degradation pathway mentioned above. This successfully initiated process was still ongoing at the time of this report. As a result it was decided to continue and replicate the use of ENB technology to treat the second hot spot at the same site in the proximity of the EiCLaR pilot test zone.

Figure 9. Concentration evolution of sum CHCs (upper) and sum of degradation products (permanent gases; lower) in the nZVI application area measured in wells.

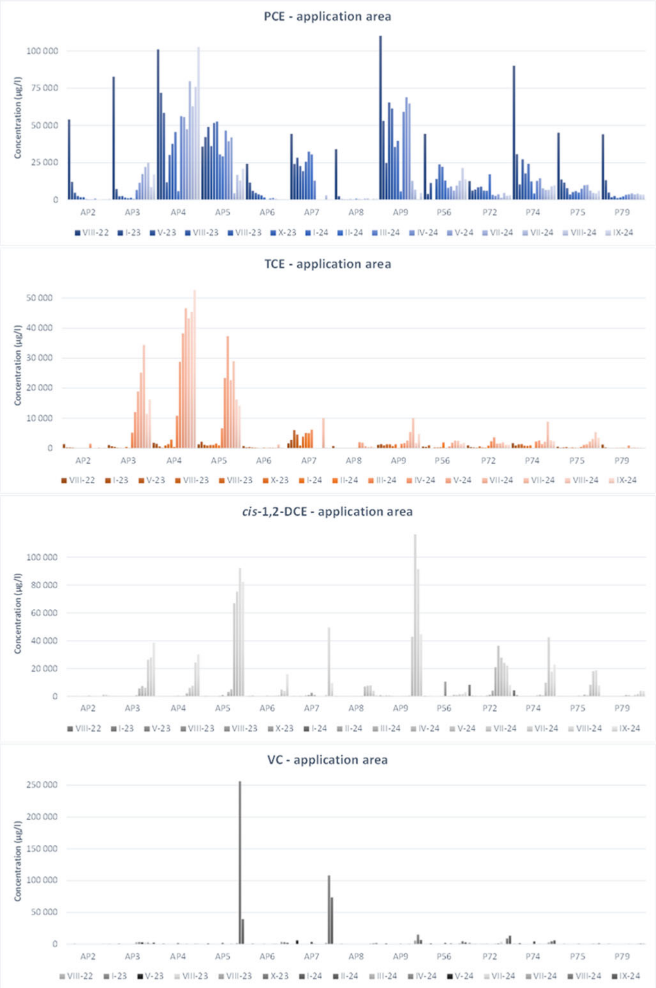

Figure 10. Evolution of contaminant concentrations during the biostimulation phase of ENB application.

5. APPLICATION OF THE TECHNOLOGY

Site considerations

ENB technology is a combined process, which applies the simultaneous operation and running of equipment for electrochemical, physical and biological processes. The technology involves continuous operation and generation of electric fields. The electric field itself modifies the conditions for chemical and biological reduction of the contamination using nano-scale iron and a biodegradable substrate. The application therefore involves the supply, installation, and operation of the following components:

- Boreholes comprising electrodes, nZVI and substrate delivery and piezometers.
- Power source (from the grid or from a renewable source).
- Systems to operate the electrodes and DC electric field.
- Groundwater real-time monitoring system.
- Nano-scale iron or iron nano-composite (nZVI), in one or more rounds.
- Bio substrate, in one or more rounds.
- Control point to monitor and manage groundwater levels and flow, concentrations of contaminants, intermediates and their degradation products, and physicochemical parameters and "natural" groundwater chemistry.

Regulatory requirements

The authorization of ENB technology and the conditions for its operation are similar to other *in situ* remediation methods. However, specific differences exist between countries, particularly due to potential restrictions on nanomaterials. No EU country currently has a moratorium on the use of iron nanoparticles for water decontamination and environmental remediation. However, their application may require permits or reporting and monitoring and it is good practice to adopt early regulator liaison.

The application of DC electric current is applied within a safe voltage range of 0-60 V, eliminating the need for special permits.

Potential future developments

ENB technology is already in full-scale industrial use for CHCs and is relatively well described and studied in terms of critical applicability parameters and limits of its effectiveness. However, for different conditions and more complex and less known contaminants, further development and advancement is expected at least in the following directions:

- Improvements in nanoparticle properties and cost.
- Optimization of the timing of the chemical and biological reduction steps (partly site specific).
- Adaptation of the technology for other persistent contaminants and substances (e.g. PFAS).
- Detailed study of the benefits and conditions of electric current application to promote bio reduction.

6. CONCLUSIONS

The ENB technology has demonstrated its applicability and effectiveness in treating chlorinated solvents in groundwater, achieving a significant advancement from TRL 6 to TRL 9 under the EiCLaR project. ENB combines nZVI with an electric field and microbial degradation to create a synergistic system for contaminant reduction. Laboratory tests and the pilot study revealed that the electric field extends the longevity and enhances the remediation efficiency of nZVI particles while optimizing conditions for microbial degradation. Microorganisms further degrade contaminants through reductive dechlorination and reactivation of passivated iron, thus enhancing efficiency, cost-effectiveness, and sustainability.

The key benefits of ENB include efficient contaminant reduction through nZVI particles, prolonged reactivity due to the DC electric field, and thus reduced mass requirements for iron particles for full scale applications, yielding economic advantages. The DC field also activates iron minerals in the soil matrix, adjusts pH levels to facilitate microbial growth, and reduces toxicity. Providing carbon substrates stimulates metabolic processes. Hence, ENB supports the degradation of low concentration areas by stimulating the growth of resident microorganisms and could biologically reactivate iron, increasing its reducing capacity and reactivity.

These results demonstrate the potential of ENB to be a leading technology for groundwater remediation.

Acknowledgements

The work presented in this bulletin was accomplished through a collaboration between partners TUL, PWEU, USTUTT, and DVGW.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement N°965945. This project is also co-funded by the National Natural Science Foundation of China (NSFC).

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