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Date and time:

3rd October 2024; 9:00 – 12:00

Location:

IHS, Institute for Housing and Urban Development Studies, Erasmus University Rotterdam

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9:00 - 9 :10 Welcome by IHS, EUR and Introduction (Dr. KE Qian and Prof. Timothy M. Vogel)

9:10 – 9:30 Aerobic metabolic TCE degradation - process characterization, scale up and molecular biological validation (Andreas Tiehm)

9:30 – 9:47 Electrokinetic-Bioremediation of Trichloroethylene and Co-contaminants in Low-Permeability Soils (Qizheng Cai)

9:47 – 10:05 Radius of influence of electrodes in soil microbial fuel cells for hydrocarbons degradation. (Azariel Ruiz Valencia)

10:05 – 10:25 Electrokinetic-Enhanced Bioremediation of Trichloroethylene-Contaminated Low-Permeability Aquifers: Mechanistic Insight into Bioaugmentation and Biostimulation (Songhu Yuan)

10:30 – 10:45 Break

10:45 – 11:02 Assessing Monitored Bioaugmentation Remediation Feasibility: A Case Study of TCE Degradation at the NAM Site, Belgium (Luca Trevisan)

11:02 – 11:20 Turning enemies into friends: sulfate-driven microbial collaboration for synergistic remediation of chloroethene-heavy metal pollution (Zhengtao Li)

11:20 – 11:37 Simultaneous immobilisation of arsenic and degradation of PAH in contaminated soil by electrokinetics (Kim Johansson)

11:37 – 11:57 The Enhancement of Persistent Organic Pollutants (POPs) Microbial Degradation by Plants (Chunling Luo)

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Aerobic metabolic TCE degradation
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process characterization, scale up and molecular biological validation

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As an alternative to reductive dechlorination, TCE and the lower chlorinated ethenes can be degraded in aerobic processes. These processes can be of co-metabolic as well as metabolic nature. While co-metabolic processes rely on the presence of auxiliary substrates, metabolic processes utilize the contaminants as growth substrate as well as energy-source. The chloroethenes are mineralized during the aerobic metabolic processes with CO_2 and Cl^- as the end products of the metabolic pathways. The potential formation and accumulation of cDCE and VC, as during the reductive dechlorination, is avoided. The degradation of the contaminants is targeted and the oxygen usage efficiency high, resulting in a remediated and oxygenated aquifer after treatment is completed.

In lab studies, the effect of co-contaminants (chloroethenes and heavy metals) on the aerobic metabolic chloroethene degradation was investigated. To accommodate the dependance on oxygen as terminal electron acceptor, the viability of electrolysis of water and the usage of ORCs as oxygen source were explored.

While bioaugmentation with the TCE degrading bacteria for was previously demonstrated in batch experiments, the transfer from closed 2 L batches to 400 L flow through systems was a crucial step in the technology scaling to a pilot field site.

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Additionally, the aerobic metabolic TCE degradation was successfully established on a site in southern Germany. To provide a reliable tool to monitor the aerobic metabolic TCE degradation, a molecular biological approach has been taken. Based on a metagenomic analysis of the TCE degrading culture, qPCR primers targeting the 16S rRNA of the TCE assimilating bacteria as well as the associated functional genes have been established.

Keywords:

Bioaugmentation, Scale-Up; aerobic chloroethene degradation, TCE-degradation, qPCR;

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Electrokinetic-Bioremediation of Trichloroethylene and Co-contaminants in Low-Permeability Soils

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Co-contaminants and complex subsurface conditions pose a significant challenge for soil remediation. This study investigates the feasibility and reveals the underlying mechanisms of electrokinetic bioremediation (EK-BIO) for treating co-contaminants of trichloroethylene (TCE), chromium (Cr), and arsenic (As) in low-permeability soils with high sulfate content. For 199 days, an EK-BIO experiment was conducted in columns packed with low-permeability soil obtained from a field. The experimental setup included the introduction of an electrolyte containing 20 mg/L TCE, 250 µM Cr(VI), 25 µM As(III), 10 mM lactate, and/or sulfate, along with periodic injections of a dechlorinating consortium containing *Dehalococcoides* (*Dhc*). A voltage gradient of 1 V/cm was applied throughout the experiment. The findings indicate a sequential process of sulfate reduction, Cr/As immobilization, and complete TCE biodechlorination. The EK-BIO approach effectively facilitated lactate delivery, establishing reductive conditions that promoted contaminant removal. Supplementary batch experiments highlighted the pivotal role of sulfate reduction in immobilizing and detoxifying Cr and As through the generation of sulfide species, thereby enhancing TCE biodechlorination. Metagenomic and transcriptomic analyses shed light on the high As(III) tolerance of the dechlorinating community and the crucial role of *Dhc* in TCE biodechlorination, even in the presence of toxic Cr(VI). Metagenomic binning analysis further revealed the potential for synergistic interactions among diverse microbial species, which collectively contributing to the mitigation of heavy metal toxicity. This research provides critical mechanistic insights into EK-BIO treatment, demonstrating its feasibility for complex co-contaminant scenarios and offering valuable guidance for optimizing remediation strategies.

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Radius of influence of electrodes in soil microbial fuel cells for hydrocarbons degradation.

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Radius of influence of electrodes in soil microbial fuel cells for hydrocarbons degradation.

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The bioelectrochemical remediation (BER) is an emerging technology for the remediation of polluted soils. BER is based on the capability of microbial communities to metabolize the pollutants at the anode and to transfer the electrons to the cathode, where the oxygen reduction occurs. This overcomes the lack of strong electron acceptors under anaerobic conditions. However, the electrochemical stimulation of the microbial community becomes weaker at distance from the anode. The objective of this work was to study the distance of stimulation of the microbiological communities in soil microbial fuel cells.

Two horizontal acrylic cylinders (DI=44 mm, L=60 cm) were used as BERs. The anode was assembled by several layers of carbon-fiber tissue held by a titanium wire, and it was placed at one extreme of the reactor. The cathode was placed 60 cm from the anode and it consisted of a cylinder mesh filled with activated carbon granules with a titanium wire as current collector. A 1000Ω resistor closed the circuit and the voltage production was recorded each hour. Another reactor was launched in open circuit as negative control. Both reactors were filled with diesel-polluted soil (10 mg/g soil) and it was saturated with synthetic groundwater. The sampling ports were located at 5, 15, 30, and 45 cm from the anode and samples were taken after 3 months of experiments.

The BERs showed a voltage production as of the first week, reaching a maximum after 10 days. At the same time, the microbiome composition was influenced differently along the different sampling ports.

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Electrokinetic-Enhanced Bioremediation of Trichloroethylene-Contaminated Low-Permeability Aquifers: Mechanistic Insight into Bioaugmentation and Biostimulation

Chongwen Shi [1)], Qizheng Cai [1)], Zixuan Cao [1)], Man Tong [1)], Zhengtao Li [2)], Ping Li [1)], Yuxi Lu [1)], Hui Liu [1)], He-Ping Zhao [2)], Songhu Yuan [1)]

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Electrokinetic-enhanced bioremediation (EK-Bio) offers the most environmentally friendly and cost-effective solution for remediating chlorinated ethenes pollution, which globally infiltrate and accumulate in low-permeability aquifers. However, the implantation of exogenous agents into indigenous microbial populations to enhance bio-degradation activity under a DC-electric field remains unclear. To address this gap, we investigated the response of biodehalogenation activity to implantation under EK-Bio through column and batch experiments. Six EK-Bio columns were operated at a constant 0.66 V/cm voltage for varying periods, while five additional columns were subjected to increasing voltages from 0 to 1.33 V/cm over a 28-day period. A 60-day microcosm study was employed to assess the spatio-temporal variations in biodehalogenation activity in soils due to EK enhancement. We demonstrated that the biodehalogenation activity of trichloroethylene (TCE) to *cis*-1,2-dichloroethylene (cDCE) near the anode can be accelerated by a higher direct current (DC) field, where the supply of electron donors mainly controls the enhancement. Conversely, near the cathode, the biodehalogenation activity of cDCE to ethylene (ETH) is higher at a low DC field, where the supply of electron acceptors mainly controls the enhancement. By combining high-throughput sequencing and numerical modeling, we reveal that the microbial cleanup process is primarily controlled by 4 core populations under EK-Bio: fermentation populations, versatile organohalide-respiring bacteria (OHRB), obligate OHRB, and special OHRB. Essentially, oxidative stress, substrate transportation, dynamics of microbial populations, and interactions together control the pollutant transformation. This finding is significant for guiding and promoting the development of EK-Bio operations.

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Assessing Monitored Bioaugmentation Remediation Feasibility: A Case Study of TCE Degradation at the NAM Site, Belgium

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This study presents a reactive transport model developed using FEFLOW to simulate the aerobic microbial degradation of trichloroethylene (TCE) at the NAM brownfield site in the Walloon Region, Belgium. The model incorporates key species including dissolved oxygen, trichloroethylene (TCE), cis-1,2-dichloroethylene (cis-DCE), and chloride to capture the dynamics of contaminant degradation. Physico-chemical and biological parameters essential for the model were gathered from laboratory experiments conducted at TZW and VEGAS, ensuring robust and site-specific input data.

The site, characterized by complex hydrogeological conditions, presents significant challenges for stakeholders, particularly regarding the feasibility and implementation of innovative remediation strategies. The primary objective of this work is to evaluate the feasibility of Monitored Bioaugmentation (MBR) strategy under field conditions, specifically within the context of inherent uncertainties in hydrogeological settings. The model provides insights into the spatial and temporal evolution of contaminant plumes and the effectiveness of remediation strategies in reducing concentrations of hazardous compounds.

Simulation results demonstrate the potential of MBR to enhance microbial degradation processes, thereby reducing TCE concentrations in groundwater. The model also explores various implementation scenarios, providing valuable data for decision-making processes. The findings suggest this strategy shows promise, its success is highly dependent on the accurate characterization of site-specific conditions, particularly with respect to oxygen delivery and microbial activity distribution.

This study underscores the importance of comprehensive modeling efforts in the planning and execution of bioremediation projects, offering a framework that can be adapted to other contaminated sites facing similar challenges.

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Turning enemies into friends: sulfate-driven microbial collaboration for synergistic remediation of chloroethene-heavy metal pollution

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The treatment of heavy metal(loids) (HM) composite pollution has long been an intricate challenge for in-situ bioremediation of organochlorine-contaminated sites. Given the prevalent co-habitation of sulfate-reducing bacteria (SRB) with organohalide-respiring bacteria (OHRB), we proposed a sulfate-amendment strategy to achieve synergistic bioremediation of trichloroethene and diverse HM [As(III), Ni(II), Cu(II), Pb(II)]. Dechlorination kinetics and MATLAB modeling indicated that sulfate amendment comprehensively improved the reductive dechlorination performance in the presence of As(III), Ni(II), Pb(II), and mixed HM. Additionally, sulfate introduction accelerated Ni(II), Pb(II), Cu(II), and As(III) detoxification, with removal efficiencies of 76.87%, 64.01%, 86.37%, and 95.50% within the initial three days, respectively. 16S rRNA gene sequencing and metagenomic analysis revealed despite the inherent disadvantages of obligate OHRBs in HM resistance, the HM detoxification was accomplished jointly by SRB and HM-resistant bacteria via extracellular precipitation (metal sulfide) and intracellular sequestration, while their contribution depended on the specific coexisting HM species present. This study, for the first time, deciphers the interspecific collaboration mechanisms between OHRB and SRB confronting HM exposures and provides guidance for a cost-effective in-situ bioremediation strategy against composite organochlorine contaminations.

Keywords:

Organohalide respiring bacteria, Groundwater, Composite pollution, Reductive dechlorination, Chloroethene, Heavy metal.

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Simulateous immobilisation of arsenic and degradation of PAH in contaminated soil by electrokinetics

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The possibilities of optimizing electrokinetic treatment (EK) to remediate mixed contaminants *in situ*, simultaneously, were investigated. In addition to this, effects on microbial communities and bioavailability (reed canary grass) were also observed. The experiment was performed in two boxes that each contained arsenic (As) contaminated sandy soil and polycyclic aromatic hydrocarbons (PAH) contaminated organic soil (peat). Application of a low, pulsating voltage current aimed at purposely corroding the iron (Fe) electrodes, and thereby amending the soil with Fe for As immobilisation, as well as inducing oxidative degradation of PAH. At the end of the experiment a 77-86% decrease in the PAH concentration in soil solution was observed, while PAH concentrations in soil simultaneously decreased. Results also showed that As concentration in porewater and plants decreased over time. By the end of the experiment, a small increase in dissolved As concentration was observed, probably related to the simultaneous drop in redox potential in the soil. The Gammaproteobacteria phyla were the most abundant microorganism, despite treatment. Differences in remediation effect between sand and peat was probably related to their differences in organic matter content and experimental design. This study showed that EK could be a suitable remediation technology for a simultaneous remediation of mixed organic and inorganic contaminants, although further research should be focused on how to control redox potential to optimize EK as a suitable remediation technology.

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The Enhancement of Persistent Organic Pollutants (POPs) Microbial Degradation by Plants

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Soil acts as a major reservoir for persistent organic pollutants (POPs), with plants playing a pivotal role in enhancing the microbial degradation of these contaminants. Understanding the mechanisms of plants regulate rhizosphere microbial degradation during plant life cycle is essential for understanding the POPs fate in soil and advancing soil rhizoremediation techniques. Here, the critical genetic and metabolic insights into uncultivable POPs-degrading bacteria in the rhizosphere were revealed by using stable isotope probing and metagenomic sequencing. The results showed that POPs and its metabolites are not major factors influencing the active degraders. Plant can release carbohydrate to relieve the carbohydrate metabolism pressure and improving the survival ability of r-strategy microbes. Rhizosphere effect on POPs degradation efficiency depends on the existence of active degraders that have competitive advantages in carbohydrate metabolism. Additionally, the role of root decomposition on POPs microbial after plant harvest were also investigated. Similar with rhizosphere effect, root decomposition also reduced the competition between microbes and allow them to survival better. Metabolites associated with the root decomposition process critically affected both the whole microbial community and active POPs degraders. Compounds related to carbohydrate and lipid metabolisms were enriched during the root decomposition process and might be important to the active POPs-degrading community assembly. Besides, some compounds such as short peptides identified as key regulators in this microbial degradation process. Our results confirmed that not only living plant, but also root decomposition after plant harvest can promote the microbial degradation of POPs.

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