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ABSTRACT BOOK

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VOCs potentially migrating from beneath the former facility. A total of 4900 kg of ELS concentrate was emulsified and injected under pressure thought 51 fixed wells in the swallow contaminated aquifer. Subsequent field monitoring showed PCE and TCE below detection limits at all wells after 6 months. A 99.8% reduction of PCE and TCE was observed in the source and plume areas along with the reduction of the recognized catabolites, such as DCE or VC. Moreover, complete reductive dechlorination of 1,2-dichloropropane has also been observed in all the monitoring wells.

TU251

Cheese whey effects on microbial communities in contaminated groundwater of an urban area

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Chlorinated ethenes (CE) are the second most ubiquitous contaminants worldwide. Herein we describe an urban locality Novy Bydzov (Czech Republic) where groundwater pollution was identified in private wells in 2007. The source of CE was machinery, metal cutting, and chemical industry, now out of order. The improper handling of hazardous compounds (e.g. chlorinated hydrocarbons, mineral oils etc.) caused uncontrolled contamination of Quaternary aquifer which is about 4-5 meters thick, composed of sandy gravel and delimited by impermeable 400 meters thick Mesozoic strata. Application of different carbon sources (lactate, glycerol, cheese whey and polyhydroxybutyrate) on the CE-contaminated groundwater was previously tested in the bench-scale studies and based on these experiments, cheese whey was chosen for the *in situ* application. The effect of three consecutive cheese whey applications (first was in October 2017) on indigenous microbes was described using qPCR Due to the techniques after sampling time the DNA extraction was performed using a FastDNA Spin Kit for Soil according to manufactures' protocol. Extracted DNA was quantified using Qubit 2.0 fluorometer. Isolated samples were tested using qPCR method. An universal marker, 16S rDNA gene (total bacteria marker) was used as a control. Other monitored specific markers were focused on presence of *Dehalococcoides*, *Dehalobacter*, *Sulfurospirillium* and vinyl chloride (VC) reductases vcrA and bvcA. In addition denitrifying bacteria were monitored by *nirK* marker and sulfate reducing bacteria by *dsrA2* marker. All data are counted in relative values. Higher bacterial abundance was detected based on all tested markers after the first cheese whey application. This application will be repeated two more times. Generally, application was successful and bacterial biomass and specific markers for organohalide respiration increased and prevailed in higher concentrations. Moreover, higher bacterial abundance triggered efficient sequential dehalogenation of the CE contaminants. Specific markers are still being monitored in the treated groundwaters and will be discussed together with physico-chemical results.

TU252

The Influence of Nanoscale Zero-valent Iron (nZVI) in Combination with Various Organic Compounds (Modifiers) on Dehalorespiring Microflora

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Among all the groundwater contaminants chlorinated ethylenes (CE) such as trichloroethylene (TCE) can be transformed by combination of abiotic and biotic methods under anaerobic conditions. Currently, nanoscale zero-valent iron (nZVI) is used for the treatment of chlorinated compounds via its strong reducing property. Biological reductive dechlorination of CE is contributed by dehalorespiration. The influence of nZVI in combination with carboxymethyl cellulose (CMC), molasses and detergent (anionic surfactant) on the specific dehalorespiring microflora was tested within this study. Groundwater contaminated with CE (1,2-cis-DCE and TCE) was collected from the chemical factory Spolchemie a.s. Batch tests with iron composite and various concentrations of CMC (0,25, 0,5 and 1 g/l), detergent (5, 10 and 20 g/l) and molasses (5, 10 and 20 g/l) were performed for periods ranging from 6 to 26 days. DNA was extracted after filtration of the tested water and used as a template for a real-time PCR amplification. 16S rDNA gene was used as a total bacterial community marker. Specific genes were used for detection of ongoing reductive dehalogenation (vcrA, bvcA, Dre DHC-RT and Dsb) and to monitor denitrifying and sulphate reducing bacteria (nirK and apsA). CMC bacteria protecting effect when nZVI is applied was observed. Positive effect was exhibited in total bacteria amount (16S rDNA), denitrifying (nirK) and sulphate reducing bacteria (apsA). CMC as the substrate for dehalorespiring bacteria was not confirmed. Detergent enhances nZVI subsurface migration parameters. Direct positive effect on bacterial populations only in denitrifying bacteria was observed. Detergent had even inhibiting influence on dehalorespiring bacteria. Molasses as carbon and electron source had positive effect on all studied groups of bacteria. Interestingly, in combination with nZVI molasses enhanced growth of dehalorespiring but not denitrifying and sulphate reducing bacteria. Molasses is suggested to serve as the substrate for fermentation which produces electrons utilised by dehalorespiration. Molasses as the substrate and nZVI with its pH buffering capacity presented the best conditions for dehalorespiring bacteria. The authors acknowledge the assistance provided by the project No. TF02000064 supported by TACR.

TU253

Mechanistic insight into microbial reductive dehalogenation

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Microbially mediated reductive dehalogenation provides a promising approach to remediate and detoxify halogenated aromatics. Despite extensive respective studies, the mechanistic understanding of the underlying chemical reactions is still limited. Interestingly, *Dehalococcoides mccartyi* strain CBDB1 and *Dehalobacter* strain 14DCB1 share a common substrate spectrum but yield different dehalogenation patterns, suggesting different sites of primary attack (aromatically bound halogen vs. H) by the nucleophile cob(I)alamin (vitamin B12).[1] The latter was unraveled through quantum chemical analyses of respective electronic structure characteristics. Building on these recent results, a perturbational molecular orbital (MO) approach has been developed for a more detailed analysis of the molecular initiating event triggering the reductive dehalogenation. Application to 93 aryl halides covering chlorinated benzenes, phenols, anilines, biphenyls, dibenzo-p-dioxins, and brominated benzenes reveals that the lowest symmetry-compatible σ* orbital located at the carbon-halogen bond mediates the dehalogenation step, and enables discriminating CBDB1-active from non-active substrates to 92%.[2] In the present communication, these findings are discussed including applications of the MO approach for predicting dehalogenation pathways and regioselectivity. Overall, our approach supports the view that the reductive aromatic dehalogenation proceeds through an inner-sphere electron transfer. [1] Zhang, S.; Wondrousch, D.; Cooper, M.; Zinder, S. H.; Schüürmann, G.; Adrian, L. 2017. Anaerobic Dehalogenation of Chloroanilines by *Dehalococcoides mccartyi* Strain CBDB1 and *Dehalobacter* Strain 14DCB1 via Different Pathways as Related to Molecular Electronic Structure. Environ. Sci. Technol. 51, (7), 3714–3724. [2] Zhang, S.; Adrian, L.; Schüürmann, G; submitted 2017.

TU254

Bacterial biosorption of PFOS from contaminated waters

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Per- and polyfluorinated alkyl substances (PFASs) have been extensively used for commercial and industrial products since the mid-1900, and are still in use although they have been classified as bioaccumulative hazardous organic compounds (Stockholm convention 2009). Perfluorooctane sulfonate (PFOS) is highlighted as the most abundant PFAS reported to contaminate the environment, animals and humans. The most frequently applied method for PFOS remediation of water is by passing it through activated granular carbon filters. Currently, there are increasing efforts to find new strategies for equally efficient and cost-effective methods for PFOS remediation of contaminated waters. This study investigated the possibility of removing PFOS by microbial binding. We tested the binding capacity of live and dead *Escherichia coli* OP50 in different PFOS concentrations. The exposed bacterial pellets were subsequently analyzed for PFOS by UPLC-MS/MS. The deadbacteria were found to have high adsorption (286-3324 μg/g of bacterial pellet) whereas live *E. coli* cells showed 5 – 7 fold lower binding capacity (38-675 μg/g of bacterial pellet). Importantly, the data also revealed that dead bacteria have at least equal affinity for PFOS isomers as the linear compound; which defines the applicability of PFOS bioremediation with dead bacteria as a promising alternative approach. We propose that microbial binding of PFOS can be applied as a novel, less costly technique for PFOS environmental elimination.

TU255

Hexavalent chromium reduction in a biocathodic microbial electrolysis cell

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Groundwater is the environmental matrix most frequently affected by anthropogenic hexavalent chromium contamination. Due to its cancerogenicity, Cr(VI) has to be removed, hopefully using environmental-friendly and economically sustainable remediation technologies. To overcome the limits of the currently applied bioremediation technologies, an alternative strategy is the use of BioElectrochemical Systems (BESs) to stimulate bioreduction of Cr(VI). BESs include a set of technologies based on biological reactors where an electrode (anode) can function as the final electron acceptor for the oxidation of organic compounds; then electrons flow through the circuit and reach the cathode that acts as the electron donor for the bioreduction of oxidized species. In the present study, we have assessed if Cr(VI) can act as an efficient terminal electron acceptor for an anaerobic biocathode in a Microbial Electrolysis Cell (MEC). The cathode was first inserted into the cathodic compartment of a dual-chamber Microbial Fuel Cell, and inoculated with autotrophic colture origenate from anaerobic digester sludge. After 30 days of acclimation, the electrode was transferred into the cathodic chamber to work at -300 mV (vs. SHE) as the biocathode in a Cr(VI)-reducing MEC. An

abiotic control and an open circuit (OC) control were also operated in parallel. Hexavalent chromium dissolved concentration was analyzed at the initial, during the experiment and final time by spectrophotometric method, while the dissolved total chromium was analyzed by ICP-MS. During the whole test, the current intensity was monitored. At the end of the experiment, the microbial characterization of the communities enriched on the biocathode and in the cathodic solution was performed by 16S rRNA gene sequencing. The acclimation phase in the MFC allowed the formation of an electroactive biofilm on the electrode. A decrease in Cr(VI) concentration was observed at the end of the tests, both in the polarized reactor and in the OC reactor. However, the BES ensured higher removal efficiency than the pure chemical process. In addition, higher current values were measured in the BES compared to the abiotic control, thanks to the biofilm interaction with the electrode. The results from microbial characterization showed that the bacterial community on the surface of the electrode was affected by the cathodic polarization, and it was different from the biomass on graphite in the open circuit system.

TU256

Enhancing Reductive Dechlorination Combined with In-Situ Chemical Reduction for the Remediation of a Heavy Contaminated Chlorinated Solvents Source Zone in South of Italy

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The present site comprises an urban site where a historical Chlorinated Compounds-CHC (mostly PCE) contamination has been released in aquifer before eighties, and characterized by a long-term monitoring activity. Contamination is present in shallow aquifer and was higher than 10 mg/L. The efficiency of the remediation is currently about 99.9%, removed more than 300 Kg PCE. The site characterization integrated with a MIP investigation to identify the plume. The plume has been addressed into four areas then a combination of In-Situ Enhanced Reductive Dehalogenation and In-Situ Chemical Reduction was selected to secure contaminant removal due to biodegradation, approaching the electron donors for PCE. This combination allows to have a reducing ambient due to producing hydrogen which helps groundwater to reach an anaerobic environment which is favorable for the microorganisms to degrade the PCE into the end product, ethylene. The first injection applied in a pilot scale (Phase1) to calibrate the injection for the site conditions. Based on the successful results of this phase, the full-scale planned for phase two and applied in two steps. First step covered the northern part of the plume (area A) in the upgradient and main source zone (area B) which is the most contaminated area. In Area B also the vadose zone has been treated. After a year (step 2), the injection took place in area C near to the site boundary and in area D downgradient of the site. Due to PCE bioremediation we have production of daughter products to prevent the accumulation of these by product an air sparging and soil vapor extraction plants have been installed in the site boundary to remove them from the soil vapor and aquifer. During the ERD we have observed methane production because of methanogenesis reaction, CH2M has decided to install a biofiltration plant, to prevent any dangers for the residential areas nearby. The challenge this complex geology has been solved by using fixed injection points with non-return valves corresponding to the depth of treatment in each aquifer. This allowed for accurate and tailored dosage application of the product without any risk of cross-contamination. Due to the rapid effect of injection, it has been possible to observe very good reduction rates within only few months from the application. PCE, has already shown reduction of three orders of magnitude and in some points, we reached the target, with daughter compounds appearing without accumulation.

TU257

Bioelectrochemical sulfide scavenging from hydrocarbon contaminated marine sediments

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Thermodynamically favorable electron acceptors (e.g. oxygen) are quickly consumed in hydrocarbon contaminated marine sediments. In this environment the biodegradation of contaminants often occurs via anaerobic pathways. Due to the high abundance of sulfate in marine water, hydrocarbons are often degraded by sulfate reduction and toxic sulfide is produced. Several studies have showed the possibility to couple anaerobic hydrocarbons degradation to current production in Bioelectrochemical Systems (BES), in which an electrode (anode) can be used as a solid electron acceptor by several groups of microorganisms. Anodic sulfide oxidation is another important process that can scavenge sulfide from the environment through biological and abiotic processes. The aim of this work was to assess if the bioelectrochemial stimulation is an effective strategy to promote both hydrocarbons and sulfide removal from marine contaminated sediments. A reactor (POL) was built by connecting a bioelectrochemical cell to three holders containing artificially contaminated sediment. The anode (polarized at 0 mV *vs* Ag/AgCl) was made by a graphite plate and the cathode was made using a stainless steel mesh. Weathered North Sea crude oil was used to contaminate the sediment. Artificial marine water was continuously recirculated into the system (flow rate 0.69 L/day). An abiotic control (ABI) and an open circuit control (OC, disconnected electrodes) were also set up. Total petroleum hydrocarbons (TPH) in the sediment, sulfur species, and current production were monitored over time. Samples of the sediment and of the anodic biofilm were collected to characterize the microbial communities by high-throughput sequencing of the 16S rRNA gene. TPH removal was observed in all the tested conditions. Contaminants removal was linked to current production up to around 5 mA (POL) and negligible current was observed in ABI. Sulfate reduction was also observed indicating the involvement of the sulfur cycle in the process. Members of the families *Desulfuromonadaceae* and *Prolixibacteraceae* dominated the anodic community.

TU258

Freshwater sediment enrichments to improve MFCs performance for in situ remediation application: a phylogenetic microbial characterization

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One of the possible application for Microbial Fuel Cell (MFCs) is the in situ remediation of contaminated sites. MFCs operation links the removal of pollutants from contaminated sites to the production of current by means of the activity of electrochemically active microorganisms (EAMs), able to degrade substrate producing a flow of electrons. EAMs have potential applications in bioenergy production, green chemical synthesis, bioremediation, bio-corrosion mitigation, and biosensor development. The aim of this work was to investigate the effect of two enrichments, a general (Gen) and a ferric citrate (FeC) ones, to increase the percentage of EAM in order to improve the MFCs performances. A freshwater sediment (Fw) sample was chosen as inoculum source. The effect of the enrichment procedures was compared in term of both electrochemical performance and biological characterization. The microbial community was subjected to three sequential enrichments and then used as inoculum for the MFCs. Anodic potential and voltage were continuously monitored. DGGE, sequencing and rt-qPCR techniques were used to investigate the EAM community. Moreover microbial α-diversity was calculated. The enrichment effect was evaluated both for the precultures and for the three components of MFCs (planktonic, biofilm and rod). Results showed that the MFC inoculated by Gen enrichment preculture had better performance than the FeC one (shorter start-up time, lower anode potential, higher current and power density). The main source of variability resulted to be the kind of enrichment, both in the preculture and in the MFCs. Proteobacteria, Bacteroidetes e Firmcutes resulted as the main Phyla in our samples. *Geobacteraceae spp.* and *Pseudomonas spp.* decreased more during the FeC enrichments and their DNA concentration was higher in the Gen-MFCs and FeC-MFCs, respectively. Microbial population enriched with FeC showed a lower Shannon diversity index, both in the preculture and at the MFCs level ($p< 0.05$). Enrichment with FeC decrease the relative abundance of EAM and the microbial diversity. Previous studies show the need of a heterogeneous community dominated by EAM to improve the remove of contaminants and to increase the performance of the MFCs. The present work indicates that Gen enrichment promoting the development of a self-balancing community seems to be a preferential approach to be implemented in *in situ* application.

TU259

Integration of molecular and isotopic analyses to investigate the potential of aerobic biodegradation at a site contaminated by Monochlorobenzene

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Bacterial communities associated with contaminated sites represent a great opportunity for environmental bioremediation considering that bacteria are able to use a wide number of chemical compounds as a source of carbon and energy. The use of an integrated approach based on different methodologies to gather more information about site-specific potential for bioremediation is gaining a wider acceptance from public authorities. The main objective of our work was to define quantitative indicators to assess the intrinsic degradation potential of a monochlorobenzene (MCB)-contaminated aquifer by the use of a “toolbox” based on isotopic and molecular biology analyses. Microcosms with groundwater collected from a MCB-contaminated site were set up under aerobic and anaerobic