EFFECT OF ELECTRIC CURRENT ON ORGANOHALIDE RESPIRING BACTERIA FROM CONTAMINATED LOCALITY TREATED BY nZVI

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Abstract

Chlorinated ethenes (CEs) are the second most common contaminant worldwide. Remediation procedures combining treatment with nanoscale zero-valent iron (nZVI) and anaerobic microbial degradation supported by electric current belongs to one of the most successful approaches. Here we describe an effect of electric current on indigenous microbial degraders of CE during a field study. Groundwater samples were taken from anode well, four cathode wells and three control wells and isolated DNA was analyzed using real-time PCR. Total bacterial biomass was quantified using 16S rDNA gene. Markers of organohalide respiration included Dehalococcoides sp., Desulfitobacterium spp. and vinyl chloride (VC) reductases *vcrA* and *bvcA*. Denitrifying bacteria were determined by nirK marker and sulfate reducing bacteria by apsA2. Representative samples were analyzed using NGS method targeted to the V4 region of 16S rDNA gene. qPCR analysis of the anode well reveal significant decrease in all tested markers connected to eminent decrease in pH to strongly acidic values. Cathode wells maintained high level of bacterial growth even if there were slight changes in pH. Composition of bacterial communities in the anode and cathode wells strongly differed and changed over time. In the control wells, a long-term stability in composition of bacterial community was observed. To conclude, electric current caused decrease in organohalide bacteria and other markers in the anode well, all other wells showed ongoing organohalide respiration important for CEs degradation.

Keywords: Electric current, NGS, nZVI, organohalide respiration, qPCR

1. INTRODUCTION

Chlorinated ethenes (CEs) are widely used chemical substance for many industrial applications [1], thus CEs belong to the most common detected organic contaminants in groundwater. Their microbial degradation takes place mostly through anaerobic organohalide respiration [2]. For chlorinated solvents, indicators of biodegradation include: depletion of electron acceptors and donors; increasing metabolic byproduct concentrations; decreasing parent compound tetra- (PCE) and trichloroethene (TCE) concentrations and increasing daughter product cis-dichloroethene (cis-DCE) and vinyl chloride (VC) concentrations [3]. *Dehalococcoides* sp. and *Desulfitobacterium* spp. belongs to the most common PCE and TCE degraders. VC is degraded by *bvcA* and *vcrA* reductases [4].

Application of electric current is one of the most promising approaches within microbial remediation processes [5]. Delivering remediation reagents into heterogeneous deposits is improved by electrokinetic injection. It is an alternative method to enhance ion migration and electroosmosis in soil and groundwater [6].

Abiotic CEs degradation e.g. using nanoscale zero valent iron (nZVI) is faster compared to biodegradation. The main advantage of nZVI is in its high reactivity and its great usability if the CEs concentration is too high and inhibitory for microorganisms. Combination of nZVI and electric current might thus improve biodegradation potential of indigenous microorganism.

The main aim of this study was to describe direct effect of electric current on organohalide-respiring bacteria and VC reductases on CE-contaminated locality.

2. EXPERIMENTAL

2.1. Sampling site and arrangement of electrodes

The nZVI pilot application on a CE-contaminated locality in the north of Bohemia started in 2009. At the end of 2010 a pilot test of nZVI application together with electric field on two test polygons was taken. This electric system was expanded to 9 polygons at the end of 2013. On these polygons a full-service pilot application combining nZVI and electric current (DC) was applied until 2014. Moreover from the end of 2013 one polygon test area has been focused on an electric field influence on an indigenous microbial community. An area of 30 m^2 has 14 narrow-profile wells and 1 well with 120 mm in diameter.

Five electrodes (1 anode, 4 cathodes) were placed on a polygon. These electrodes were applying DC into the rock environment (24V until December 2015, then 48V until September 2016 when the source has been disconnected). The electrodes were designed to form a cross arrangement whereas the anode IS-10 was installed in the center (steel rod with the 20 mm in diameter). The cathodes were made by steel lining to edges of the cross (I2, I4, I5 and I7).

2.2. Physico-chemical methods

Groundwater samples from the locality were taken in 8 sampling rounds (2015 - 2017) from all 15 wells in the static mode by manual peristaltic pump (PluNoTech s.r.o.) through a Teflon tube from horizon under the ground. The physico-chemical parameters (conductivity, pH and oxidation reduction potential) were measured immediately by WTW 3430 Multimeter (WTW, Germany) equipped with SenTix pH electrodes (TMultiLine® Multi 3430 IDS). Sterile groundwater samples were also taken for further analyses: complete chemical analysis, CE concentration (GC/MS method) and molecular genetic analysis.

2.3. Molecular-genetic methods

All groundwater samples earmarked for the molecular genetic analysis were immediately cooled and stored at 4°C until following steps. Groundwater samples (0.13 - 0.54 L) were filtrated through a 0.22 µm membrane filters (Merck Millipore, Germany). DNA from the filters was extracted using a FastDNA Spin Kit for Soil (MP Biomedicals, CA, USA) according to manufacture's protocol. Extracted DNA was quantified using a Qubit 2.0 fluorometer (Life Technologies, MA, USA).

Quantitative polymerase chain reactions (qPCR) were focused on the relative abundance of *Dehalococcoides* spp., *Desulfitobacterium* sp., as well as the relative abundance of the VC reductase genes *bvcA* and *vcrA*. In addition, we also performed analyses of the presence of sulfate-reducing bacteria by detecting levels of genes for the adenosine-5'-phosphosulphate reductase *apsA2* and denitrifying bacteria by detecting nitrite reductase enzymes *nirK*. Relative abundance of 16S rRNA gene (the total bacteria marker) was determined as a control marker. All tested markers were analyzed similarly as described in Dolinová et al. [2].

For the next-generation sequencing (NGS) we have chosen representative samples which characterize microbial changes on the locality. NGS analysis was targeted at the 16S rRNA gene. Amplification of the region V4 of eubacterial 16S rRNA gene was performed with barcode primers 515F (5´-TGCCAGCMGCNGCGG-3´) [7] and 802R (5´-TACNVGGGTATCTAATCC-3´) [8]. Subsequently, an in-house prepared mock community was sequenced to verify the experimental conditions.

The 16S rDNA sequences were processed using Mothur, including quality filtering and chimera check using chimera UCHIME. UCHIME improves sensitivity and speed of chimera detection [9]. The sequences were aligned against SILVA database (version 128). Mitochondrial and chloroplast sequences were removed prior

to OTU construction. Alignments were constructed using Needleman-Wunsch pairwise alignment method implemented as align seqs command in Mothur. Cut-off value of 97% was used for clustering of sequences into operational taxonomic units (OTUs). The number of sequences in each library was adjusted to 5 038 by random subsampling. For further results analysis was used the Vegan package in the R statistical package. Phyla/families with greater than 5% abundance were visualized as heat maps and the remaining visualized in graphs as others and unclassified [10].

3. RESULTS AND DISCUSSION

3.1. Physico-chemical analysis

pH values of the anode well fluctuated slightly over pH 7 (**Figure 1a**) in the beginning and decreased to strongly acidic conditions at the end of DC application (**Figure 1b**). When the DC influence disappeared, the pH changed back to normal. pH values of cathode wells were stabilized all the time in alkaline scale and monitoring wells showed stabilized neutral pH.

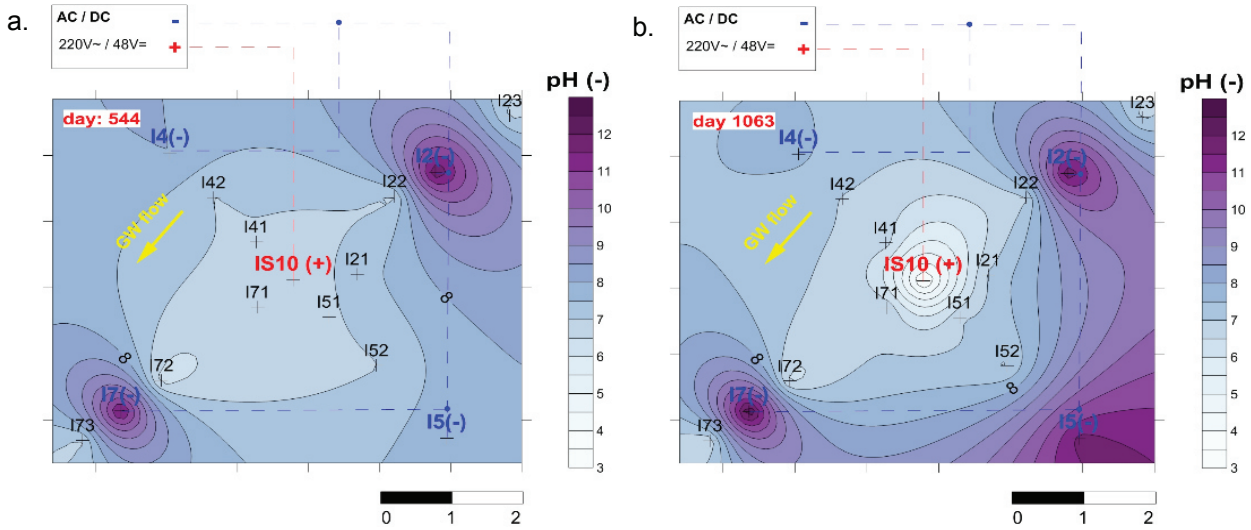

Figure 1 pH development at CE-contaminated area - a. pH at the beginning of DC application; b. situation at the end of DC application. Positions of the wells with anode (red - IS10), cathodes (blue - I2, I4, I5 and I7) and monitoring wells (black). Yellow arrow - direction of groundwater flow. Scale bar - m.

Initial conductivity in anode well (app. 470 $\mu S \cdot cm^{-1}$) decreased after the DC application (117 $\mu S \cdot cm^{-1}$). Cathodes I2 and I7 showed the same fluctuating trend as I4 and I5, but with higher conductivity values (I2 and I7 in range 791 - 2 050 $\mu S \cdot cm^{-1}$, I4 and I5 in range 225 - 778 $\mu S \cdot cm^{-1}$). The anode well changed from reductive conditions (-110 mV) to oxidative (400 mV) after the DC application and back to reductive when DC was turned off. Biggest influence on total CEs concentration after the DC application was observed in anode well (from 27 709 $\mu g \cdot L^{-1}$ to 8 791 $\mu g \cdot L^{-1}$ at the end).

3.2. Effect of electric current on organohalide-respiring bacteria in groundwater

Lowest levels of total bacterial biomass were observed in the anode groundwater (**Figure 2).** Low relative abundance of *bvcA*, *vcrA*, *Dehalococcoides mccartyi* and *Desulfitobacterium* spp. was also detected. Only two samples from 17th August 2015 and 8th March 2017 showed higher amount of bacteria, whereas the second date was after switch off the DC application and pH recovery from strong acidic to almost neutral values.

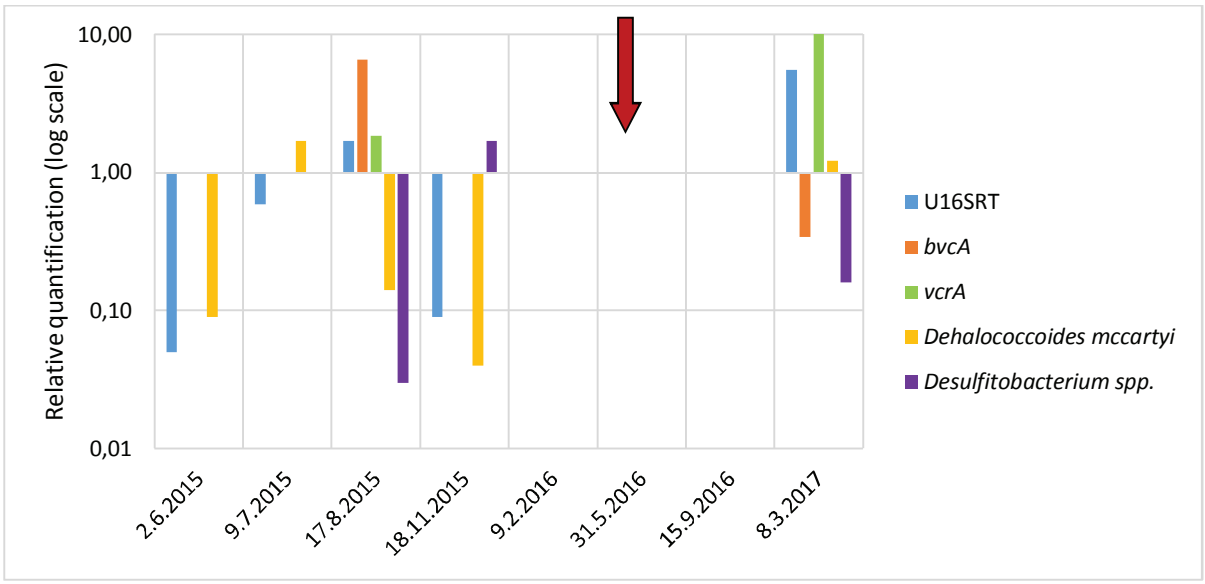

Figure 2 Relative quantification of total bacterial biomass (U16SRT), *bvcA* and *vcrA* genes and organohalide respiring bacteria *Dehalococcoides mccartyi* and *Desulfitobacterium* spp. of anode well IS10. Red arrow - DC source disconnection

Total bacterial biomass in all cathode wells reached highest levels and behaved similar to each other after one year of DC application (**Figure 3**). *bvcA* and *vcrA* genes together with *Dehalococcoides mccartyi* and *Desulfitobacterium* spp. were almost always detected as it is usual at other CE-contaminated localities [10]. The termination of DC application caused strong decrease in all markers on 15th September 2016 and more than five months later these increased again.

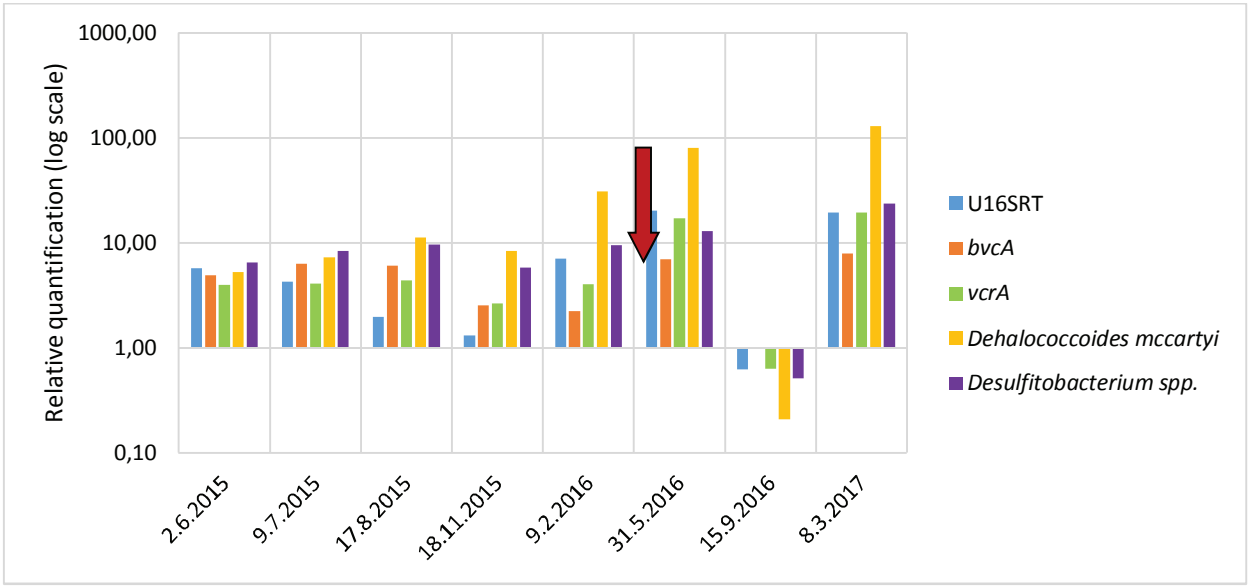

Figure 3 Relative quantification of total bacteria biomass (U16SRT), *bvcA* and *vcrA* genes and organohalide respiring bacteria *Dehalococcoides mccartyi* and *Desulfitobacterium* spp. of anode well I4. Red arrow - switch off DC source

All monitoring wells demonstrated the most stabilized conditions in all tested markers. Total biomass showed high level of bacterial abundance. Organohalide respiring bacteria and also genes *bvcA* and *vcrA* were almost

always detected. Gene *bvcA* was rather scarce and in the I23 well it even disappeared for more than 18 months (from 9. 2. 2015 up to 15. 9. 2016). Generally in that period all tested markers were slightly reduced.

3.3. Next-generation sequencing (NGS)

NGS was used to characterize bacterial communities in groundwater of the anode (IS10), one cathode (I4) and one monitoring well (I23) before (15. 4. 2015), during (15. 9. 2016) and after termination of the DC application (8. 3. 2017).

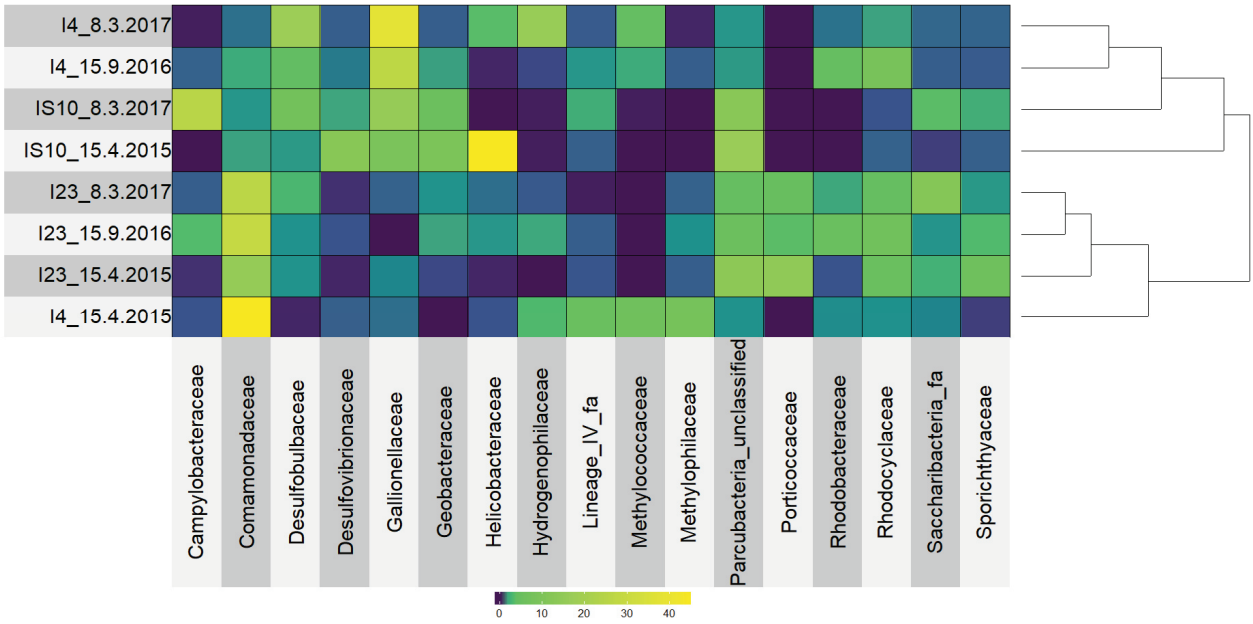

Figure 4 Analysis at family level showing composition of bacterial communities. Dark blue - low abundance, yellow - high abundance

Bacterial composition of the anode well IS10 significantly changed over time (**Figures 4, 5a**). There we have found the lowest diversity and different composition of bacterial community not typical for contaminated locality. After remediation, there were 50% of new OTUs. The *Helicobacteraceae* family was the most abundant in the first sampling round. The end of electric current application and subsequent pH recovery into neutral values caused proliferation of *Campylobacteraceae*, *Gallionellaceae* and *Parcubacteria* families contrary to disappearance of *Helicobacteraceae* (**Figure 4**). This can be explained by the fact that *Helicobacteraceae* can survive low pH conditions [10].

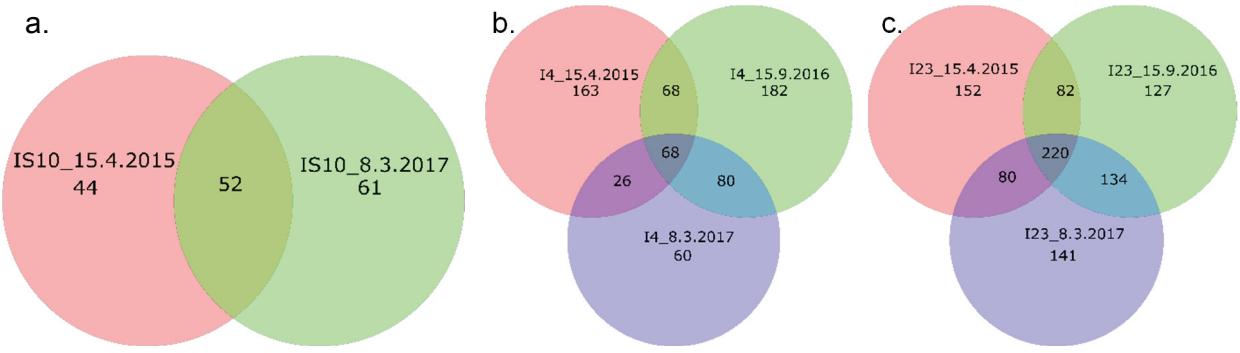

Figure 5 Venn diagrams of bacterial community (OTUs) in different wells - a. anode, b. cathode, c. monitoring well

First sampling in the cathode I4 showed clustering similar to the monitoring well (**Figures 4, 5b**). We have found decreasing *Comamonadaceae* and increasing abundance of *Gallionellaceae*, *Desulfobulbaceae* and *Hydrogenophylaceae*. Although *Comamonadaceae* were previously recorded in CE contaminated environments, *Gallionellaceae* are related to presence of higher iron concentrations, probably generated by the nZVI application in the past.

Monitoring well showed long-term stability in the bacterial composition with the highest diversity (**Figures 4, 5c**).

4. CONCLUSIONS

CE-contaminated area was first treated with nZVI and subsequently by electric current to enhance clean-up processes including microbial biodegradation. Crucial players in CE biodegradation, organohalide-respiring bacteria were monitored together with VC-reductases. In the cathode wells, ongoing organohalide respiration with all important markers (*bvcA*, *vcrA*, *Dehalococcoides mccartyi*, *Desulfitobacterium* spp.) was clearly proven. The electric current caused decrease in pH in the anode well triggering undesired decline of all organohalide respiration markers. When the electric current application was stopped, sulfate-reducing (*apsA2* marker) and denitrifying (*nirK* marker) bacteria were observed in larger abundances in the anode well. Generally recovery of all tested markers was detected few months after the electric current disappeared in the anode well. Significant changes were also displayed by NGS analysis where the bacterial composition of the anode IS10 and cathode well I4 dynamically changed during whole monitoring period and was also dissimilar to the control monitoring well I23.

Bacterial abundance and composition in the cathode and monitoring wells was similar to the typical composition described on a contaminated locality [4, 10]. However the anode well displayed low heterogeneity with prevalent taxons not present in other wells most probably connected to extreme conditions (low pH, *Helicobacteraceae*). After the DC influence disappeared and the conditions returned back to pre-treatment values the bacterial composition changed again to the taxons commonly detected (e.g. *Campylobacteraceae*) on CE-contaminated sites.

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REFERENCES

[1] PAUL, Laiby a Erik SMOLDERS. Inhibition of iron (III) minerals and acidification on the reductive dechlorination of trichloroethylene. *Chemosphere* [online]. 2014, **111**, 471-477. ISSN 00456535. Accessible from: doi:10.1016/j.chemosphere.2014.04.057.

[2] DOLINOVÁ, Iva, Marie CZINNEROVÁ, Lukáš DVOŘÁK, Vojtěch STEJSKAL, Alena ŠEVCŮ a Miroslav ČERNÍK. Dynamics of organohalide-respiring bacteria and their genes following in-situ chemical oxidation of chlorinated ethenes and biostimulation. *Chemosphere* [online]. 2016, **157**, 276-285. ISSN 00456535. Accessible from: doi:10.1016/j.chemosphere.2016.05.030.

[3] CHEREMISINOFF, Nicholas P. *Groundwater remediation: a practical guide for environmental engineers and scientists* [online]. 2017 [vid. 2017-09-04]. ISBN 978-1-119-40773-7. Accessible from: http://search.ebscohost.com/login.aspx?direct=true&scope=site&db=nlebk&db=nlabk&AN=1538696.

[4] DOLINOVÁ, Iva, Martina ŠTROJSOVÁ, Miroslav ČERNÍK, Jan NĚMEČEK, Jiřina MACHÁČKOVÁ a Alena ŠEVCŮ. Microbial degradation of chloroethenes: a review. *Environmental Science and Pollution Research* [online]. 2017, **24**(15), 13262-13283. ISSN 0944-1344, 1614-7499. Accessible from: doi:10.1007/s11356-017-8867-y.

[5] LAI, Agnese, Federico AULENTA, Marina MINGAZZINI, Maria Teresa PALUMBO, Marco Petrangeli PAPINI, Roberta VERDINI a Mauro MAJONE. Bioelectrochemical approach for reductive and oxidative dechlorination of chlorinated aliphatic hydrocarbons (CAHs). *Chemosphere* [online]. 2017, **169**, 351-360. ISSN 00456535. Accessible from: doi:10.1016/j.chemosphere.2016.11.072.

[6] MAO, Xuhui, James WANG, Ali CIBLAK, Evan E. COX, Charlotte RIIS, Mads TERKELSEN, David B. GENT a Akram N. ALSHAWABKEH. Electrokinetic-enhanced bioaugmentation for remediation of chlorinated solvents contaminated clay. *Journal of Hazardous Materials* [online]. 2012, **213**-**214**, 311-317. ISSN 03043894. Accessible from: doi:10.1016/j.jhazmat.2012.02.001.

[7] DOWD, Scot E, Todd R CALLAWAY, Randall D WOLCOTT, Yan SUN, Trevor MCKEEHAN, Robert G HAGEVOORT a Thomas S EDRINGTON. Evaluation of the bacterial diversity in the feces of cattle using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). *BMC Microbiology* [online]. 2008, **8**(1), 125. ISSN 1471-2180. Accessible from: doi:10.1186/1471-2180-8-125.

[8] CLAESSON, Marcus J., Qiong WANG, Orla O'SULLIVAN, Rachel GREENE-DINIZ, James R. COLE, R. Paul ROSS a Paul W. O'TOOLE. Comparison of two next-generation sequencing technologies for resolving highly complex microbiota composition using tandem variable 16S rRNA gene regions. *Nucleic Acids Research* [online]. 2010, **38**(22), e200-e200. ISSN 0305-1048, 1362-4962. Accessible from: doi:10.1093/nar/gkq873.

[9] EDGAR, Robert C., Brian J. HAAS, Jose C. CLEMENTE, Christopher QUINCE a Rob KNIGHT. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* [online]. 2011, **27**(16), 2194-2200. ISSN 1460-2059, 1367-4803. Accessible from: doi:10.1093/bioinformatics/btr381.

[10] NĚMEČEK, Jan, Iva DOLINOVÁ, Jiřina MACHÁČKOVÁ, Roman ŠPÁNEK, Alena ŠEVCŮ, Tomáš LEDERER a Miroslav ČERNÍK. Stratification of chlorinated ethenes natural attenuation in an alluvial aquifer assessed by hydrochemical and biomolecular tools. *Chemosphere* [online]. 2017, **184**, 1157-1167. ISSN 00456535. Accessible from: doi:10.1016/j.chemosphere.2017.06.100.