ABSTRACT BOOK

DehaloCon III 2021 SEPT 27- SEPT 30, ROME ITALY

Third International Conference on Anaerobic Biological Dehalogenation

Organized by

Water Research Institute, National Research Council - Italy
IRSA-CNR

Sponsored by

Environmental Biotechnology Section – European Federation of Biotechnology

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ABSTRACT BOOK

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ABSTRACT BOOK

PROGRAMME
DehaloCon III Virtual Conference
- All times in CEST (UTC +02:00) -

Monday, 27th September 2021

13:00 – 13:10 Welcome and opening

SESSION 1. ANAEROBIC DEHALOGENATION IN PRISTINE AND CONTAMINATED ENVIRONMENTS
Session 1.1 - Chair: Dr. Frank Loeffler

13:10 – 13:40 Keynote lecture – Dr. Jianzhong He "A journey to discover microorganisms that respire persistent organic pollutants"

Oral and flash-poster presentations
13:40 – 14.00 "Alleviation of N_2O-induced inhibition of organohalide respiration by clade I and clade II N_2O reducers" **Yin Y**, Kara Murdoch F, Xie Y, Sun Y, Löffler FE - *Networking Room #1*

14:00 – 14:20 "Organohalide-respiring bacteria in polluted urban rivers employ novel bifunctional reductive dehalogenases to dechlorinate polychlorinated biphenyls and perchloroethene" **Wang S**, Qiu L, Liang Y, Lu Q, Mai B - *Networking Room #2*

14:20 – 14:40 "*Dehalococcoides* enrichments reductively dechlorinate two chlorinated organophosphate esters" **Zhong Y**, Zhu X, Peng P - *Networking Room #3*

14:40 – 15:00 "The synergistic associations between methanogenesis and reductive dechlorination of chlorinated organic pollutants (COPs) in situ" **He Y**, Yuan J, Cheng J, Yang X, Xu J - *Networking Room #4*

15:00 – 15:04 "Complete reductive dechlorination of TCE with wood mulch-based amendment as electron donor in bench-scale microcosm studies" Masut E, Ferioli L, Legnani A, Battaglia A, **Tucci M**, Matturro B, Rossetti S, Aulenta F (Flash-poster) - *Networking Room #5*

15:05 – 15:20 Networking Session 1.1, Meet the authors

Session 1.2 - Chair: Dr. Ivonne Nijenhuis

15:20 – 15:50 Keynote lecture – Prof. Max M. Häggblom "Organohalide-respiring bacteria in the marine environment"

Oral and flash-poster presentations
15:50 – 16:10 "Reductive dechlorination sustained by microbial chain elongation" – **Robles A**, Yellowman TL, Rangan SM, Joshi S, Delgado AG - *Networking Room #6*

16:10 – 16:30 "Biogeography of organohalide-respiring bacteria and their roles in biotransforming halogenated persistent organic pollutants in wastewater treatment plants" – **Xu G**, He J - *Networking Room #7*

16:30 – 16:50 "Dual C-Cl isotope analysis for characterizing anaerobic transformation of hexachlorocyclohexanes by different consortia enriched from contaminated soil" – **Liu Y**, Zheng Y, Kümmel S, Nijenhuis I, Richnow H-H - *Networking Room #8*

16:50 – 17:10 "Effects of temperature on trichloroethene dechlorination potential, methanogenesis, and microbial community in contaminated sediment" - **Bin Hudari MS**, Deb S, Gargini A, Filippini M, Richnow HH, Vogt C, Nijenhuis I - *Networking Room #9*

17:10 – 17:14 "Dual element (C/Cl) isotope analysis and proteomics during trichloromethane degradation by a *Dehalobacter*-containing culture" **Soder-Walz JM**, Torrentó C, Wasmund K, Adrian L, Rosell M, Vicent T, Marco-Urrea E (Flash-poster) - *Networking Room #10*

17:15 – 17:30 Networking Session 1.2, Meet the authors

Tuesday, 28th September 2021

SESSION 2. ORGANOHALIDE RESPIRATION: BIOCHEMISTRY, ARCHITECTURE, REGULATION AND ECOPHYSIOLOGY
Chair: Dr. Shanquan Wang

13:00 – 13:30 Keynote lecture - Dr. Jun Yan "Corrinoid metabolism and utilization in organohalide-respiring bacteria"

Oral and flash-poster presentations
13:30 – 13:50 "Organohalide respiration with vinyl chloride by a novel anaerobic bacterium, '*Candidatus Dehalogenimonas etheniformans*'" **Chen G**, Kara Murdoch F, Xie Y, Yang Y, Yan J, Löffler FE - *Networking Room #1*

13:50 – 14:10 "Substrate-dependent competition and cooperation relationships between *Geobacter* and *Dehalococcoides* for their organohalide respiration" **Lu Q**, Liang Y, Wang S - *Networking Room #2*

14:10 – 14:30 "On the hot track of the membrane-bound organohalide respiration complex from *Dehalococcoides mccartyi* strain CBDB1" **Deobald D**, Budhraja R, Adrian L - *Networking Room #3*

14:30 – 14:50 "Absolute quantification of pceABCT gene products and characterization of the membrane protein complex responsible for the reduction of tetrachloroethene in *Dehalobacter restrictus*" **Cimmino L**, Duarte AG, Schmid A, Pereira IAC, Holliger C, Maillard J - *Networking Room #4*

14:50 – 15:10 "Complex I-like enzymes in organohalide-respiring bacteria" **Willemin M**, Armand F, Hamelin R, Holliger C, Maillard J - *Networking Room #5*

15:10 – 15:14 "Electron density is not an only factor governing microbial reductive dechlorination of polychlorinated biphenyls" **Zhang S**, Wang S (Flash-poster) - *Networking Room #6*

15:15 – 15:30 Networking Session 2, Meet the authors

SESSION 3. OMICS AND META-OMICS OF ORGANOHALIDE-RESPIRING AND NON-RESPIRING BACTERIA
Chair: Prof. Elisabeth Edwards

15:35 – 16:05 Keynote lecture – Prof. Lorenz Adrian "Metabolic integration of organohalide respiration in *Dehalococcoides*-related *Chloroflexi* (*Dehalococcoidia*)"

Oral and flash-poster presentations
16:05 – 16:25 "Genetic basis for anaerobic dichloromethane catabolism" **Murdoch RW**, Chen G, Kara Murdoch F, Mack EE, Villalobos Solis MI, Hettich RL, Löffler FE - *Networking Room #1*

16:25 – 16:45 "Ecology and evolution of organohalide-respiring *Dehalococcoidia*: a genomic perspective" **Yang Y**, Yan J, Löffler FE, Chen G, Li X, Cápiro NL - *Networking Room #2*

16:45 – 17:05 "Dehalogenation of lindane (γ-hexachlorocyclohexane) by *Dehalobacter* sp. HCH1" **Puentes Jácome LA**, Nesbø C, Wang PH, Picott K, Perera D, Lomheim L, Gaspard S, Tang X, Edwards EA - *Networking Room #3*

17:05 – 17:25 "Phage-inducible chromosomal islands identified in *Dehalococcoides mccartyi* and *Dehalogenimonas alkenigignens*" **Morson N**, Molenda O, Maxwell KL, Edwards EA - *Networking Room #4*

17:25 – 17:29 "Metagenomic analysis of dehalogenating microorganisms from a polychlorobiphenyl (PCB) contaminated sediment in Mar Piccolo (Taranto, Italy)" **Firrincieli A**, Zanaroli G, Cappelletti M (Flash-poster) - *Networking Room #5*

17:30 - 17:45 Networking Session 3, Meet the authors

Wednesday, 29th September 2021

SESSION 4. BIOELECTROCHEMICAL OHR: FUNDAMENTAL ASPECTS AND ENGINEERED SOLUTIONS
Session 4.1* - *Chairs: Dr. Aulenta Federico, Dr. Tobias Goris

13:00 – 13:30 Keynote lecture – Prof. Mauro Majone "Fundamentals and advances on bioelectrochemical dechlorination"

Oral and flash-poster presentations
13:30 – 13:50 "Exploring the microorganisms for achieving the bioelectrochemical dechlorination of *Dehalococcoides* via extracellular electron transfer" **Meng L**, Yoshida N - *Networking Room #1*

13:50 – 14:10 "Exogenous electronic regulation enhances the decomposition and transformation of TBBPA and the related microbial mechanism" **Li Z**, Lin X, Wang A - *Networking Room #2*

14:10 - 14:30 "Metagenomics of a bioelectroremediation system treating TCE and Cr(VI) co-contamination: revealing microbial diversity and interactions" **Matturro B**, Zeppilli M, Lai A, Majone M, Rossetti S - *Networking Room #3*

14:30 – 14:50 "Electrode material selection and voltage operation in a *Dehalogenimonas*-containing bioelectrochemical system degrading 1,2-dichloropropane" **Fernández-Verdejo D**, Cortés P, Blánquez P, Marco-Urrea E, Guisasola A - *Networking Room #4*

14:50 – 15:05 Networking Session 4.1, Meet the authors

Session 4.2 - Chairs: Dr. Aulenta Federico, Dr. Tobias Goris

Oral and flash-poster presentations
15:05 – 15:25 "A Sequential Reductive/Oxidative Bioelectrochemical process for chlorinated aliphatic hydrocarbons (CAHs) Removal: Evaluation of the process with a real contaminated Groundwater" **Dell'Armi E**, Zeppilli M, De Santis F, Majone M, Petrangeli Papini M - *Networking Room #5*

15:25 – 15:45 "Microbiome composition and dynamics in a reductive/oxidative bioelectrochemical system for perchloroethylene (PCE) removal: the effect of feeding composition. **Di Franca ML**, Matturro B, Zeppilli M, Dell'Armi E, Petrangeli Papini M, Majone M, Rossetti S - *Networking Room #6*

15:45 – 16:05 "Bioelectrochemical treatment of groundwater containing oxidable and reducible contaminants" Cruz Viggi C, **Tucci M**, Milani A, De Laurentiis C, Resitano M, Crognale S, Matturro B, Rossetti S, Aulenta F - *Networking Room #7*

16.05 – 16.20 *Networking Session 4.2 – Meet the authors*

16:20 – 16:50 e-POSTER SESSION

ePOSTER ROOM#1

- **"**Soil management plan to sustain PFAS degradation by novel *Acidimicrobia*" (Appeldoorn P, Brogioli F, **van Bemmel M**)
- "Expression and investigation of the reductive dehalogenase enzyme family" (**Picott KJ**, Edwards EA)
- "Cofator selectivitiy of a minimized cobamide-binding protein (**Petre E**, Kruse S, Schubert T)
- "Insights into the structure of the organohalide respiratory complex from *Dehalococcoides mccartyi* strain CBDB1 by using chemical modifications" (**Hellmold N**, Eberwein M, Deobald D, Adrian L)
- "RNA Sequencing of *Dehalococcoides mccartyi* Strain CBDB1 reveals differential expression of reductive dehalogenase genes with 1,2,4- and 1,2,3-trichlorobenzene" (**Greiner-Haas F**, Goris T, Förstner K, Türkowsky D, von Bergen M, Sawers G, Lechner U).
- "Metagenomic analysis of a novel *Dehalococcoides mccartyi* enrichment culture capable of PCE-to-ethene dechlorination" (**Matturro B**, Rossetti S)

ePOSTER ROOM#2

- "Dichloromethane degradation by an anaerobic microbial community used for chloroform bioremediation" (**Bulka O**, Webb J, Dworatzek S, Mahadevan R, Edwards EA)
- "The effect of humic acids on perchloroethene dechlorination by Dehalococcoides strains under anaerobic conditions" (**Wan J**, Shen C, Adrian L)
- "Colonization and growth of dehalorepiring biofilms on sportive amendments" (**Capozzi SL**, Payne RB, Sowers KR, Kjellerup BV).
- "Host specificity of sponge-associated dehalogenating bacteria" (**Hall LA**, Decker K, Scott K, Kerkhof L, Haggblom M)
- "Potential factors affecting dichloromethane biodegradation upon water table fluctuation" (**Lazaro Sanchez C**, Prieto M, Vuilleumier S, Imfeld G, Muller EELl)
- "Using cryo-EM to probe structures of hydroalkylation enzymes with potential use in bioremediation of crude-oil polluted regions" (**Andorfer MC**, King-Roberts DT, Levitz TS, Drennan CL)

ePOSTER ROOM#3

- "Meta-analysis and experimental studies on anaerobic biodegradation and biotransformation of simple and polyfluorinated compounds" (**Skinner J**, Robles A, Raderstorf A, Palar S, Allen C, Delgado A)
- "Reductive dehalogenation of tetrabromobisphenol a in an enrichment culture dominated by *Dehalobacter* spp." (**Liu G**, Qiao W, Li Z, Jiang J)
- "Effect of e- donors on the chloroethene dechlorination and pathogenic risk in groundwater augmented with *Dehalococcoides*" (**Tomita R**, Meng L, Yoshida N)
- "Molecular characterization of microbial communities in a peat-rich aquifer system contaminated with chlorinated aliphatic compound" (**Fedi S**, Filippini M, Cappelletti M, Firrincieli A, Gargini A, Ghezzi D)
- "Enhancement of perchloroethene dechlorination by a mixed dechlorinating culture via magnetic nanoparticle-mediated isolation method" (**Chen K**, Liu Z, Wang X, Yu C, Ye J, Yu C, Wang F, Shen C)
- "Combining a Groundwater Circulation Well (IEG-GCW) with nutrient distribution through Multilevel Injection Wells (MIW) for the remediation of a chlorinated solvent contaminated site" (**Ciampi P**, Bartsch E, Alesi EJ, Rehner G, Kneer A, Nestler B, Petrangeli Papini M)

Thursday, 30th September 2021

SESSION 5. OHR field-scale application
Session 5.1 - Chair: Prof. Marco Petrangeli Papini

13:00 – 13:30 Keynote Lecture – Prof. Birthe Veno Kjellerup "Biofilm based bioremediation of chlorinated contaminants"

Oral and flash-poster presentations
13:30 – 13:50 "PHA-based material from pure and mixed microbial culture as slow-release electron donor for sustainable in situ biological reductive dechlorination" **Amanat N**, Andreini F, Rossi MM, Matturro B, Rossetti S, Majone M, Petrangeli Papini M - *Networking Room #1*

13:50 – 14:10 "Cultivation of *Dehalococcoides mccartyi* strain CBDB1 in a continuous stirred tank reactor" Reino C, **Ding C**, Adrian L - *Networking Room #2*

14:10 - 14:30 "Using a biosolids-amended trench to enhance reductive dehalogenation of TCE contaminated groundwater in a biowall" **Saffari Ghandehari S**, Boyer J, Ronin D, Hapeman CJ, Schanzle D, Torrents A, Kjellerup BV - *Networking Room #3*

14:30 – 14:50 "Field application of a reagent for the ISC and ERD treatment of an aquifer contaminated with tetrachlorethylene, dichloropropane E R-130" **Leombruni A**, Mueller M, Collina L - *Networking Room #4*

14:50 – 14:54 "Natural-occurring microbial community in an organohalide polluted aquifer: preliminary evaluation of the effectiveness of permeable reactive bio-barriers for decontamination" **Bertolini M**, Zecchin S, Beretta G, Masetti M, Pietrangeli B, Cavalca L (Flash-poster) - *Networking Room #5*

14:55 – 15:10 Networking Session 5.1 – Meet the authors

Session 5.2 - Chair: Prof. Birthe Kjellerup

Oral and flash-poster presentations
15:10 – 15:30 "Lessons learned from the study of microbial communities in sediment for the design of bioremediation strategies" **Herrero J**, Puigserver D, Nijenhuis I, Carmona JM - *Networking Room #6*

15:30 – 15:50 "A Coupled Adsorption and Biodegradation (CAB) process employing PHB and Biochar as bio-based materials for TCE contaminated groundwater in-situ bioremediation" **Rossi MM**, Andreini F, Caruso P, Amanat N, Matturro B, Rossetti S, Petrangeli Papini M - *Networking Room #7*

15:50 – 16:10 "*Dehalococcoides*-mediated in situ remediation of vinyl chloride contaminated site" **Wu R**, Wang S - *Networking Room #8*

16:10 – 16:14 "Bioremediation of chlorinated compounds: examples of practical approaches using Next Generation Sequencing" Brogioli F, **van Bemmel M**, Appeldoorn P (Flash-poster) - *Networking Room #9*

16:15 – 16:30 Networking Session 5 – Meet the authors

16:30 – 16:45 Prizes for the best oral presentations and posters

16:45 – 17:00 Closure

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SESSION 1 (S1)

Anaerobic dehalogenation in pristine and contaminated environment

27th September 2021

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Session 1.1 (S1.1)

Chair: Dr. Frank Loeffler

KEYNOTE LECTURE - Dr. Jianzhong He

A journey to discover microorganisms that respire persistent organic pollutants

Zhao S, Xu G, ***He J***
Department of Civil and Environmental Engineering, National University of Singapore. Email: ceehj@nus.edu.sg

Urban watersheds, soil, and groundwater are important environments serving as sources of drinking water, often creating a great potential for migration of any aquatic contamination and resulting in wide distribution of contaminants such as persistent organic pollutants. Anaerobic bacteria play critical roles in environmental bioremediation of persistent halogenated compounds such as polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs). However, limited information is available on microbes which can tackle this group of persistent organics. Here we discuss a specialized microbial genus - *Dehalococcoides mccartyi* strains which can respire both chlorinated and brominated persistent organic pollutants in the environment. Especially we found that the functional reductive dehalogenase genes are not necessary substrate specific, indicating that one *Dehalococcoides mccartyi* strain could possibly respires an array of halogenated compounds via engaging one functional reductase genes. We also analyzed pangenomic information of available *Dehalococcoides* species and inferred the evolution of this special group of microorganisms. Thus this group of specialized microbes has the potential to remediate multiple halogenated compound contaminated sites via bioaugmentation strategy.

Biography

Dr. Jianzhong He is an Associate Professor in the Department of Civil and Environmental Engineering at the National University of Singapore. She received her Ph.D. degree at the Georgia Institute of Technology in 2003 from Professor Frank Löffler's lab. Prior to that, she obtained her M.S. and B.S. degrees from Tsinghua University and Harbin Institute of Technology in 1998 and 1995, respectively. She was a postdoctoral researcher in Professor Lisa Alvarez-Cohen's lab at the University of California Berkeley for two years before joining the National University of Singapore as an assistant professor in 2005. Dr. He's research focuses on discovering novel microorganisms to transform and detoxify environmental contaminants, enhancing biodegradation by optimizing the growth of functional microbes, biomass to bioenergy/biochemicals, nutrients removal from wastewater, and applying nucleic acid-based approach in laboratory cultures and *in situ*. During her tenure at NUS, Dr. He has generated more than 80 peer-reviewed publications while securing significant amount of external funding from diverse funding agencies (>17million in the past 10 years). So far, her publications have been cited for more than 5000 times (Google Scholar) and an H-Index of 31. She holds several patents and collaborates widely with industrial, governmental and scientific partners. She is also the editorial board member of the *Journal of Scientific Reports, the ISME Journal, Applied and Environmental Microbiology, and Associate Editor of the Frontiers in Microbiology*.

ABSTRACT BOOK

Oral and flash-poster presentations

S1.1. Alleviation of N_2O-induced inhibition of organohalide respiration by clade I and clade II N_2O reducers

Yin Yongchao[1,2]*, *Kara Murdoch F[1,2], Xie Y[2,3], Sun Y[2,3], Löffler FE[1,2,3,4,5]**
[1]Department of Microbiology, [2]Center for Environmental Biotechnology, [3]Department of Civil and Environmental Engineering; [4]Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, Tennessee 37996, USA; [5]Biosciences Division, Oak Ridge, Tennessee 37831, USA. *Emails: y.yin@northeastern.edu; frank.loeffler@utk.edu

Organohalide-respiring bacteria (OHRB), such as *Geobacter lovleyi* and *Dehalococcoides mccartyi* (*Dhc*), are keystone bacteria for *in situ* bioremediation of toxic and carcinogenic chlorinated ethenes. The activity of OHRB, however, is inhibited by micromolar levels of nitrous oxide (N2O), a common metabolite of microbial nitrogen turnover in groundwater aquifers, which causes incomplete biotransformation of chlorinated contaminants, including chlorinated ethenes. We demonstrate that N2O inhibition of OHRB is reversible and show that N2O removal by N2O reducers harboring N2O reductase (N2OR) alleviates N2O toxicity and restores dechlorination activity in cultures of *Geobacter lovleyi* strain SZ and *Dhc* strain BAV1. Elevated N2O concentrations in groundwater aquifers are not uncommon, and monitoring of N2O at sites where incomplete, stalled reductive dechlorination is observed should be considered to adjust site management decision-making and efficiently achieve clean-up goals.

S1.1. Organohalide-respiring bacteria in polluted urban rivers employ novel bifunctional reductive dehalogenases to dechlorinate polychlorinated biphenyls and perchloroethene

Wang Shanquan[1]*, *Qiu L[1], Liang Y[1], Lu Q[1], Mai B[2]*
[1]Environmental Microbiomics Research Center, School of Environmental Science and Engineering, Sun Yat-Sen University, Guangzhou, China 510275; [2]State Key Laboratory of Organic Geochemistry and Guangdong Key Laboratory of Environmental Protection and Resources Utilization, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China 510640.
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Surface sediments of polluted urban rivers could be a reservoir of hydrophobic POPs. Contamination assessment of PCBs and PBDEs in 174 nationwide polluted urban rivers showed their similar spatial distribution patterns but very different contamination levels (ΣPCBs, 10.73 ng/g dw; ΣPBDEs, 401.16 ng/g dw) in surface sediments. The tetra-/di-CBs and deca-BDE as major PCB and PBDE congener groups accounted for 59.11 and 95.11 mol% of ΣPCBs and ΣPBDEs, respectively. In contrast to the persistence of PBDEs, significant correlation between organohalide-respiring bacteria (OHRB) and PCBs ($p<0.01$) was observed. To further confirm the correlation, 174 microcosms were setup with the surface sediments, 135 of which were showed to dechlorinate both PCBs and PCE in different pathways. The 16S rRNA and RDase gene-based analyses, together with enantioselective dechlorination of chiral PCBs, suggested that *Dehalococcoides* and *Dehalogenimonas* in the 135 cultures largely employed distinctively varied novel bifunctional RDases to catalyse the PCB/PCE-dechlorination. Our results could expand our knowledge on organohalide respiration of PCBs and support future policy-making and technical development for management and pollution control of these organohalides in urban areas.

ABSTRACT BOOK

S1.1. *Dehalococcoides* enrichments reductively dechlorinate two chlorinated organophosphate esters

Zhong Yin[1]*, *Zhu X[1]*, Peng P[1]**
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In this study, we reported two *Dehalococcoides*-containing enrichment cultures with the ability to reductively dechlorinate tris(2-chloroethyl) phosphate (TCEP) and tris(1-chloro-2-propyl) phosphate (TCPP), respectively. TCEP was mainly dechlorinated via the cleavage of C-Cl bond followed by the cleavage of C-O bond to form bis(2-chloroethyl) phosphate (BCEP) and ethene and that ethene was further transformed to ethane. Similarly, TCPP was dechlorinated to bis(2-chloroisopropyl) phosphate (BCPP) and propene. The 16S rRNA gene sequencing, quantitative real-time PCR and metagenomic analysis demonstrated that *Dehalococcoides* strain was the predominant contributor to the reductive dechlorination of TCEP and TCPP.

S1.1. The synergistic associations between methanogenesis and reductive dechlorination of chlorinated organic pollutants (COPs) *in situ*

He Yan[1,2]*, *Yuan J[1,2], Cheng J[1,2], Yang X[1,2], Xu J[1,2]*
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Reductive dechlorination is the most efficient way for depletion of chlorinated organic pollutants (COPs) in anaerobic environment, where the methanogenesis are usually mass-produced. In this study, we applied meta-analyses, incubation experiment and quantum modelling to investigate the associations between reductive dechlorination and methanogenesis, as well as the potential role of methanogens during COP dechlorination *in situ*. Results indicated the accelerated methanogenesis were commonly synergistically coupled with the accelerated removal of COPs. Some methanogens were showed as the core taxa co-occurring with dechlorinators in the microbial networks of COP-polluted environments. Also, methanogenic species could promote some COP dechlorination by regulating cell metabolic functions, e.g., the coenzyme F430 could reduce the activation barrier of reductive dechlorination. Collectively, this work provides insight into a novel strategy to coordinate methanogenesis that promotes the anaerobic degradation of OCPs.

S1.1. Complete reductive dechlorination of TCE with wood mulch-based amendment as electron donor in bench-scale microcosm studies

Masut E[1], Ferioli L[1], Legnani A[1], Battaglia A[1], ***Tucci Matteo[2]****, Matturro B[2], Rossetti S[2], Aulenta F[2]*
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To evaluate the treatability of a saturated soil and groundwater contaminated by chlorinated ethenes, a wood mulch-based amendment was tested in a bench-scale microcosm experiment. In this framework, wood mulch was tested as an electron donor in order to assess its potential to sustain the biological reductive dechlorination (RD) of trichloroethene (TCE). The addition of millimetric iron filings, along with wood mulch, was also studied as a strategy to synergistically improve the RD process. Bioaugmentation with a dechlorinating inoculum was performed in all microcosms. All mulch-amended microcosms displayed the complete dechlorination of TCE to harmless ethene, although via transient accumulation of stoichiometric amounts of vinyl chloride (VC). Addition of iron filings accelerated the conversion of VC to ethene and also methane generation most likely by increasing H_2 availability.

Session 1.2 (S1.2)

Chair: Dr. Ivonne Nijenhuis

KEYNOTE LECTURE- Prof. Max M. Häggblom

Organohalide-respiring bacteria in the marine environment

Max M. Häggblom
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Organohalide compounds are widespread in the environment as a result of both anthropogenic activities and natural production. Organobromine compounds, including brominated flame retardants, have gained widespread use, and are now common anthropogenic contaminants of aquatic sediments. The marine environment is also a rich source of a great variety of natural organobromine compounds, such as those made by sponges. Reductive dehalogenation is thought to be an important process in the overall cycling of these compounds. Organohalide-respiring bacteria (OHRB), which utilize organohalides as electron acceptors for energy conservation, can readily be enriched and have been isolated from diverse marine environments, including organohalide-contaminated sediments, pristine sites, as well as marine sponges. Based on their metabolic versatility they can be classified into facultative vs. obligate OHRBs. The growth of obligate OHRBs is restricted to organohalide respiration, while facultative OHRBs are more versatile in their metabolism and can utilize diverse electron acceptors other than organohalides. Although an increasing number of OHRBs have been isolated, it is apparent that their diversity and distribution is even more extensive. For example, the capacity of organohalide respiration appears to be widely distributed in members of marine *Deltaproteobacteria*. A comprehensive survey of *Deltaproteobacteria* genomes revealed that approximately 10% contain reductive dehalogenase (RDase) genes, which are found within a common gene neighborhood, and their gene expression were experimentally verified. The tested *Deltaproteobacteria* strains were shown to reductively dehalogenate bromophenols and utilize them as terminal electron acceptors in organohalide respiration. Their debrominating activity was not inhibited by sulfate or elemental sulfur and these species are either sulfate or sulfur reducing bacteria. Organobromine-rich sponges provide a particular specialized niche for organohalide-respiring microbes. Using a combination of cultivation-based and molecular analyses, we have demonstrated that geographically and taxonomically disparate sponges harbor populations of dehalogenating bacteria that form stable populations within the sponge animal that function in the cycling of organohalide compounds. The dehalogenating strains enriched to date are closely related to *Deslufoluna spongiiphila*, suggesting a cosmopolitan association between *Desulfoluna* spp. and various marine sponges. The identification of reductive dehalogenase genes in diverse marine Deltaproteobacteria, and the confirmation of their dehalogenating activity through functional assays and transcript analysis extends our knowledge of the distribution and diversity of organohalide-respirating bacteria. *Deltaproteobacteria* may play an important role in natural organohalide cycling which may have a major impact on the fate of organohalide pollutants, such as brominated flame retardants, in the marine environment.

Biography

Dr. Max Häggblom is Distinguished Professor and Chair of the Department of Biochemistry and Microbiology. He earned his Ph.D. in General Microbiology from the University of Helsinki and after a Post-Doctoral Appointment at New York University joined the faculty of Rutgers University. Since 2011 he has served as Editor-in-Chief of FEMS Microbiology Ecology. His research interests are in microbial ecology, environmental biotechnology and in the bioexploration, cultivation and characterization of novel microbes. A common theme is the “unusual appetites” of bacteria in the Anthropocene, including the metabolism and detoxification of xenobiotic chemicals or natural products, such organohalides. His group has worked extensively on characterizing anaerobic bacteria involved in dechlorination and debromination and assessing their *in situ* metabolic activities

Oral and flash-poster presentations

S1.2. Reductive dechlorination sustained by microbial chain elongation

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In the field of *in-situ* groundwater and soil bioremediation of TCE, H2 for reductive dechloriation by *Dehalococoides mmcartyi* is typically supplied via fermentation of organic substrates. However, many pathways do not produce H2 and/or stimulate hydrogenotrophic methanogenesis via production of CO2. Microbial chain elongation, unexplored in bioremediation, converts acetate and ethanol into larger fatty acids, such as butyrate and caproate, while also reliably producing H2 as a by-product. In this study, we demonstrated microbial chain elongation as a novel approach for delivering the required H2 for complete reductive dechlorination of TCE. First, in soil microcosms containing aquifer materials from a contaminated site, acetate, ethanol, and 2 mmol L^{-1} TCE and subsequently in transfer cultures containing acetate and ethanol or ethanol only, and 0.5 mmol L^{-1} TCE.

S1.2. Biogeography of organohalide-respiring bacteria and their roles in biotransforming halogenated persistent organic pollutants in wastewater treatment plants

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WWTPs have been identified as the temporary sink and an emerging source of halogenated POPs to the environment.1,2 The widespread contamination of organohalides in WWTPs may has nurtured sewage sludge microbiota to develop microbial dehalogenation capability of organohalide pollutants. Although the first OHRB-*Desulfomonile tiedjei* was isolated from sewage sludge in 19903 and several studies also reported dehalogenation of other organohalide pollutants (e.g., PCBs and PBDEs) in sewage sludge,4, 5 it remains unclear whether the sewage sludge from geographically distant locations widely contains OHRB to dehalogenate organohalide pollutants. Moreover, the roles and diversity of OHRB and reductive dehalogenase genes in biotransforming halogenated POPs in sewage sludge are poorly investigated.
Here we reported the widespread distribution of organohalide-respiring bacteria (OHRB), the majority of which was the uncultured lineages of *Dehalococcoidia*, in WWTPs around the globe via meta-analyses of global sewage sludge microbiomes. Extensive microbial reductive dehalogenation of PCBs and PBDEs were also found in laboratory microcosms inoculated with 85 sewage sludge from 32 WWTPs in geographically distant locations. Analyses of obligate OHRB and known reductive dehalogenase genes indicated that *Dehalococcoides* and *Dehalogenimonas* played key roles in dehalogenating PCBs and PBDEs, but largely employing unidentified functional reductive dehalogenases. Our findings suggested that OHRB may largely contribute to the attenuation of halogenated POPs in WWTPs.

S1.2. Dual C-Cl isotope analysis for characterizing anaerobic transformation of hexachlorocyclohexanes by different consortia enriched from contaminated soil

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Hexachlorocyclohexanes (HCHs) are persistent environmental pollutants, which cause wide contamination in soil, sediment and groundwater. Anaerobic consortia enriched on α-,γ-, and δ-HCH, by inoculating soil from a contaminated area of a former pesticide factory, were named as consortium A, C and D, respectively. The consortia showed different activity for the four isomers, while the carbon and chlorine isotope enrichment factors (εC and εCl) were in the same range as previous studies. The correlation of 13C and 37Cl fractionation (Λ) was applied to characterize C-Cl bond cleavage. The different Λ value during α-HCH transformation by consortium A compared to other consortia indicates the different reaction model. The 16S rRNA gene sequencing showed quite different community compositions, which validates that different HCH isomers significantly affect the microbial community leading to variable isotope fractionation.

S1.2. Effects of temperature on trichloroethene dechlorination potential, methanogenesis, and microbial community in contaminated sediment

Mohammad Sufian Bin Hudari[1], *Deb S[1], Gargini A[2], Filippini M[2], Richnow HH[1], Vogt C[1], Nijenhuis I[1]*
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Bioremediation is one of the strategies employed to transform toxic chlorinated ethenes to harmless ethene. We consider the effects of temperature on this dechlorination process, which is in particular relevant in the context of aquifer thermal energy storage, where heat is stored and extracted in subsurfaces that may be contaminated with chlorinated solvents. Anaerobic microcosms were set up with sediments from a chlorinated ethenes contaminated site, supplemented with trichloroethylene (TCE) and lactate as the main electron acceptor and donor, respectively. These were incubated at six temperatures between 10 and 60°C to investigate temperature-influenced dechlorination potential, methanogenesis, and the underlying microbial community. Our results show that while methanogenesis was observed up to 40 degrees C, 30°C seems to be an upper limit for complete dehalogenation of TCE to ethene in these sediments.

S1.2. Dual element (C/Cl) isotope analysis and proteomics during trichloromethane degradation by a *Dehalobacter*-containing culture

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An anaerobic enrichment culture degrading trichloromethane (TCM) to dichloromethane was obtained from groundwater contaminated with a mixture of organochlorines in an industrial zone of Barcelona. The analysis of the microbial composition of the enrichment culture confirmed the presence of *Dehalobacter* together with two additional phylotypes belonging to *Desulfovibrio* and *Proteiniphilum.* This enrichment culture also completely degraded 1,1,2-trichloroethane. Carbon and chlorine isotope fractionation was assessed during TCM degradation in respiring cells, resting cells and cell free extracts. In addition, the proteome profile expressed during TCM and 1,1,2-trichloroethane dechlorination was analyzed and the involved reductive dehalogenases were identified using a combination of dehalogenation activity tests, blue native gel electrophoresis and the genome sequence of the *Dehalobacter* strain.

ABSTRACT BOOK

SESSION 2 (S2)
Organohalide respiration: Biochemistry, Architecture, Regulation and Ecophysiology

28th September 2021

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https://global.gotomeeting.com/join/360126621

Chair: Dr. Shanquan Wang

KEYNOTE LECTURE - Dr. Jun Yan

Corrinoid metabolism and utilization in organohalide-respiring bacteria

Jun Yan[1]*, *Huijuan Jin[1,2], Lisi Jiang[1,2], Jingjing Wang[1,2], Yiru Cui[1,2], Xiuying Li[1], Yi Yang[1], Frank E. Löffler[3,4,5,6,7]**

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Organohalide-respiring bacteria (OHRB) depend on corrinoids to utilize organohalogens for energy conservation. Physiological tests combined with genome analysis demonstrated that while metabolically versatile OHRB (e.g., *Sulfurospirillum*, *Desulfitobacterium*) generally are capable of *de novo* corrinoid biosynthesis, the majority of obligate OHRB (e.g., *Dehalococcoides mccartyi*, *Dehalogenimonas* spp., *Dehalobacter restrictus*) are corrinoid auxotrophs that strictly depend on external corrinoid supply. In recent years, novel cobamides (i.e., complete form of corrinoids) have been identified in several tetrachloroethene-dechlorinating *Sulfurospirillum* spp. and *Desulfitobacterium* spp., indicating that OHRB harbor a largely unexplored corrinoid biosynthetic capacity. Further, a number of studies have revealed that the naturally occurring cobamides are not functionally equivalent and taxonomically diverse OHRB exhibit distinct cobamide preferences. These findings emphasize a previously overlooked ecology between corrinoid prototrophs and corrinoid auxotrophs, with implications for the targeted manipulation of microbiome function, spanning applications from human health to environmental biotechnology, including enhanced anaerobic bioremediation at sites impacted by chlorinated contaminants.

Biography

Dr. Yan obtained the BS and MS degrees from Nanjing University (Nanjing, Jiangsu, China) and the Ph.D. degree from the Louisiana State University (Baton Rouge, LA, USA). He continued his research in the field of organohalide respiration as a postdoctoral researcher at the Georgia Institute of Technology (Atlanta, GA, USA) and the University of Tennessee (Knoxville, TN, USA). Dr. Yan worked as a research assistant professor in the Department of Microbiology at the University of Tennessee before started his tenure as a professor in the Institute of Applied Ecology at the Chinese Academy of Sciences. The research activities at Yan's lab focus on explore the fundamentals of relevant microbial processes (i.e. organohalide respiration, cobamide biosynthesis) involved in the biodegradation and biotransformation of hazardous contaminants. Yan's lab combine cultivation, molecular, biochemical and meta-omics approaches to advance understanding of essential metabolic pathways and the microbial ecology in the contaminated environments.

ABSTRACT BOOK

Oral and flash-poster presentations

S2. Organohalide respiration with vinyl chloride by a novel anaerobic bacterium, '*Candidatus Dehalogenimonas etheniformans*'

Chen Gao [1,2]*, *Kara Murdoch F*[1,5], *Xie Y*[1,2], *Yang Y*[6], *Yan J*[6], *Löffler FE*[1-5]*
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The microbial reductive dechlorination process can lead to the formation of vinyl chloride (VC), a human carcinogen, in groundwater aquifers and complicate cleanup efforts. *Dehalococcoides mccartyi* (*Dhc*) strains expressing the TceA, VcrA, or BvcA reductive dehalogenases convert VC to environmentally benign ethene and couple this dechlorination step with energy conservation (i.e., growth). *Dhc* are recognized as keystone bacteria for VC detoxification in anoxic groundwater and monitoring regime focus on *Dhc* biomarkers. We isolated a novel anaerobic bacterium '*Candidatus Dehalogenimonas* etheniformans' strain GP and characterized respiratory VC dechlorination to ethene by the non-*Dhc* bacterial isolate strain GP. The bacterium couples formate or hydrogen (H2) oxidation to the reduction of trichloroethane (TCE), dichloroethenes (i.e., 1,1-dichloroethene, *cis*-1,2-dichloroethene, and *trans*-1,2-dichloroethene) or VC. Acetate is a required carbon source. The results expand our understanding of the reductive dechlorination of dichloroethenes and VC and have direct implications for bioremediation at chlorinated solvent sites.

S2. Substrate-dependent competition and cooperation relationships between *Geobacter* and *Dehalococcoides* for their organohalide respiration

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Obligate and non-obligate organohalide-respiring bacteria (OHRB) play central roles in the geochemical cycling and environmental bioremediation of organohalides. Their coexistence and interactions may provide functional redundancy and community stability to assure organohalide respiration efficiency but, at the same time, complicate isolation and characterization of specific OHRB. Here, we employed a growth rate/yield tradeoff strategy to enrich and isolate a rare non-obligate tetrachloroethene (PCE)-respiring *Geobacter* from a *Dehalococcoides*-predominant microcosm, providing experimental evidence for the rate/yield tradeoff theory in population selection. Surprisingly, further physiological and genomic characterizations, together with co-culture experiments, revealed three unique interactions (i.e., free competition, conditional competition and syntrophic cooperation) between *Geobacter* and *Dehalococcoides* for their respiration of organohalides under difference condition.

S2. On the hot track of the membrane-bound organohalide respiration complex from *Dehalococcoides mccarty*i strain CBDB1

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Dehalococcoides mccartyi strain CBDB1 is a slow growing, obligately anaerobic bacterium of the phylum *Chloroflexi* utilizing hydrogen as sole electron donor and different persistent organohalides as terminal

electron acceptor in a process known as organohalide respiration (OHR) conducted by a multi-subunit membrane-bound OHR complex consisting of at least three modules composed of RdhA/RdhB, OmeA/OmeB/HupX and HupL/HupS. The knowledge on the OHR complex of *D. mccartyi* CBDB1 is limited due to low biomass production of the cells and the hydrophobic character of the membrane-bound protein subunits. Here we show how deeper insights into the architecture of the OHR complex is attained by *ex situ* experiments combining bottom-up mass spectrometry, inductively coupled plasma mass spectrometry, solubilization with different detergents, blue native-polyacrylamide gel electrophoresis, size-exclusion chromatography as well as introduction of chemical modifications.

S2. Absolute quantification of *pceABCT* gene products and characterization of the membrane protein complex responsible for the reduction of tetrachloroethene in *Dehalobacter restrictus*

Cimmino Lorenzo[1*], *Duarte AG[2], Schmid A[3], Pereira IAC[2], Holliger C[1], Maillard J[1*]*
[1]Laboratory for Environmental Biotechnology (LBE), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland; [2]Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal; [3]Proteomic Core Facility (PCF), Ecole Polytechnique Fédérale de Lausanne (EPFL), Lausanne, Switzerland
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The *pceABCT* gene cluster from *Dehalobacter restrictus* is one model system for tetrachloroethene (PCE) respiration. We aim at deciphering the stoichiometry of the *pceABCT* gene products and the composition of PceA-containing membrane-bound protein complexes in *D. restrictus*. Quantitative proteomics (qProt), membrane extraction, CN-PAGE, and an in-gel enzymatic assay were developed. A qProt analysis performed on the membrane fraction revealed an approximative 1.5:1.0 stoichiometry between PceA and PceB, while PceC was about 60-times less abundant than PceA. Data from CN-PAGE and a newly developed in-gel PceA enzymatic assay revealed several forms of active PceA-containing complexes with masses of approximately 140, 180, and 250 kDa, depending on the physiological state of *D. restrictus* cells.

S2. Complex I-like enzymes in organohalide-respiring bacteria

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Complex I (NADH:ubiquinone oxidoreductase, NUO) and complex I-like enzymes have been detected in several organohalide-respiring bacteria at genomic and proteomic levels. The question of the involvement of these enzymes in organohalide respiration remains to be elucidated. Comparative physiology and proteomics were applied to study the role of the 11-subunit complex I-like enzyme in *Desulfitobacterium hafniense* strain DCB-2, a model organism able to conserve energy via organohalide respiration (OHR). It appears that the complex I-like enzyme is generally used in the energy metabolism of strain DCB-2 (and not specifically during OHR), that it becomes essential when organic electron donors such as lactate are used, where it may act as the entry point in the membrane cytoplasmic side for the electron transfer chain.

S2. Electron density is not an only factor governing microbial reductive dechlorination of polychlorinated biphenyls

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Electron density of halogen substituents successfully rationalized *Dehalococcoides* mediated dehalogenation pathways of aromatics, where halogen substituents with higher Hirshfeld charges ($q_{Hirshfeld,X}$) were abstracted in experiments. However, the respective dechlorination pathways of polychlorinated biphenyls (PCBs) have not been targeted. Herein, we studied the electronic density of 27 PCB congeners metabolized by *Dehalococcoides mccartyi* strains 195, CBDB1, JNA, CG1, CG4, and CG5. It was found that higher $q_{Hirshfeld,Cl}$ fails to support the microbial reductive dechlorination pathways of 24 PCB congeners, suggesting that the electron density is not an only factor determining microbial reductive dechlorination. The low success rate of $q_{Hirshfeld,Cl}$ (11%) in pinpointing microbial reductive dechlorination pathways of PCBs would be raised by the steric effect of ortho-Cl, which has not been covered in the electron density calculations of substrates. With the steric effect of ortho-Cl in mind, the $q_{Hirshfeld,Cl}$ rationalized the dechlorination pathways of 24 PCB congeners, considering the difference of $q_{Hirshfeld,Cl}$ up to 0.003. The reason leading to three exceptions (PCB102, 133, and 187) deserves further study.

ABSTRACT BOOK

SESSION 3 (S3)

Omics and Meta-Omics of organohalide-respiring and non-respiring bacteria

28th September 2021

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https://global.gotomeeting.com/join/360126621

Chair: Prof. Elisabeth Edwards

KEYNOTE LECTURE - Dr. Lorenz Adrian

Metabolic integration of organohalide respiration in *Dehalococcoides*-related *Chloroflexi* (*Dehalococcoidia*)

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Bacteria of the class *Dehalococcoidia* catalyse many reductive dehalogenation reactions and link themto energy conservation in organohalide respiration. Most reductive dehalogenases (RdhA) from *Dehalococcoidia* form a phylogenetically homogeneous group separated from RdhA proteins of other phyla. Also the overall metabolic integration of organohalide respiration is distinct. Most important for the metabolism in *Dehalococcoides* strains is the lack of quinones as electron mediator in the membrane with wide implications on the intracellular redox equilibrium, architecture of the respiratory chain and organization of the respiratory complex. In addition, the physiology of the organisms, the electronic coupling within the respiratory complex, the electron transfer onto the halogenated substrates, and the metabolic integration of electron flows is impacted. We argue that some of the striking biochemical characteristics of RdhA protein activities in *Dehalococcoidia*, such as reductive dehalogenation of vinyl chloride, chlorinated benzenes, biphenyls and dioxins, but also dihalo elimination reactions of vicinally chlorinated alkanes, are consequences of the respiratory architecture. We use our genomic and proteomic data from *Dehalococcoides*, *Dehalogenimonas* and marine sediment *Dehalococcoidia* single cell genome assemblies to correlate the genes encoding the respiratory complex proteins with the biosynthesis of specific cofactors and the principles of the central metabolism. For example, we analyse the unidirectionality of gluconeogenesis in *Dehalococcoidia* and the presence of a unique complement of special core genes encoding a core methionine synthase, Re-specific citrate synthase, bifunctional fructose 1,6-bisphosphate aldolase/phosphatase and dapL (LL-diaminopimelate aminotransferase). All these genes are representatives of a streamlined, strongly reducing metabolism aligned with quinone-free complex-bound organohalide respiration. All this also argues against a strong role of inter-class horizontal gene transfer in the genes encoding the core metabolic functions in *Dehalococcoidia*.

Biography

Lorenz Adrian is co-head of the Department Environmental Biotechnology at Helmholtz Centre for Environmental Research -UFZ in Leipzig and Professor for Biotechnology at Technische Universität Berlin, Germany. He works since many years on the microbiology, ecology, genomics and biochemistry of Dehalococcoides strains and Organohalide Respiration. In other research projects he studies anaerobic ammonium oxidation (Anammox), anaerobic transformation of antibiotics and conducted a variety of protein mass spectrometric and stable isotope analyses mostly with anaerobic microbial cultures. He was co-speaker of a Research Unit on anaerobic dehalogenation 2013-2020 and hosted the Dehalocon II in Leipzig in 2017. ORCID 0000-0001-8205-0842, RID A-4443-2012, https://www.ufz.de/index.php?en=34233

ABSTRACT BOOK

Oral and flash-poster presentations

S3. Genetic basis for anaerobic dichloromethane catabolism

Murdoch Robert W [1*#], *Chen G*[1,2], *Kara Murdoch F*[1,7], *Mack EE*[5], *Villalobos Solis MI*[6], *Hettich RL*[6], *Löffler FE*[1,2,3,4,6,7*]

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A gene cluster (the *mec* cassette) responsible for anaerobic dichloromethane (DCM) metabolism was discovered by comparative genomics. This dehalogenating methyltransferase system is found in the genomes of each of the three known anaerobic DCM-degrading bacteria. Corresponding proteins are among the most abundant in the proteomes of two of these bacteria when grown with DCM. Homologous *mec* gene cassettes were identified in diverse natural environmental systems. Design and application of targeted qPCR assays demonstrated widespread presence of *mec* genes in marine systems (i.e., oxygen minimum zones), and DCM concentration-dependent gene and transcript abundances were observed across a groundwater DCM plume.

S3. Ecology and evolution of organohalide-respiring *Dehalococcoidia*: a genomic perspective

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Dehalococcoidia (*Dia*) class microorganisms are frequently found in various pristine and contaminated environments. The broad environmental distribution of *Dia*, as well as other organohalide-respiring bacteria, supports the concept of active halogen cycling and the natural formation of organohalogens in various ecosystems. Hydrogenotrophic organohalide-respiring bacteria, in particular *Dia*, consume hydrogen to low consumption threshold concentrations and enable syntrophic oxidation processes. These functional attributes and the broad distribution imply that *Dia* play relevant roles in carbon cycling in anoxic ecosystems. Metagenome-assembled genomes and single-cell amplified genomes studies have substantially improved the understanding of *Dia*'s microbial ecology and evolution. *Dia* microorganisms can be categorized into three groups, the terrestrial cluster that contains all *Dehalococcoides* and *Dehalogenimonas* strains, the marine cluster I, and the marine cluster II. The genomic differences between marine and terrestrial *Dia* may suggest distinct functions and roles in element cycling (e.g., carbon, sulfur, chlorine), which require interdisciplinary approaches to unravel the physiology and evolution of *Dia* in various environments.

S3. Dehalogenation of lindane (γ-hexachlorocyclohexane) by *Dehalobacter* sp. HCH1

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Lindane (γ-hexachlorocyclohexane, γ-HCH) is a carcinogenic persistent organic pollutant that was extensively used for agricultural pest control. Lindane and other HCH isomers can be biotransformed anaerobically. Reductive dehalogenation of HCH by *Dehalobacter* has been documented. *Dehalobacter* sp. HCH1, capable of dehalogenating lindane to monochlorobenzene and benzene, was identified in an enrichment culture derived from soils and sediments from Guadeloupe. Here, we discuss the high-quality metagenome-assembled genome of *Dehalobacter* sp. HCH1 and the identification of a novel reductive dehalogenase enzyme responsible for lindane (γ-HCH) dehalogenation.

S3. Phage-inducible chromosomal islands identified in *Dehalococcoides mccartyi* and *Dehalogenimonas alkenigignens*

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Over the last decade, there has been much speculation regarding horizontal gene transfer in *Dehalococcoides mccartyi*. Recently, extrachromosomal circular elements, called Integrative and Mobilizable Elements or IMEs, were identified in *D. mccartyi*. A sub-group of 15 IMEs, called IME1s, were further explored and discovered to have a highly conserved structural organization. We then identified 4 new IME1s in *D. mccartyi* and one in *Dehalogenimonas alkenigignens* strain BRE15M. The organization of all 21 IME1s was homologous to Phage-Inducible Chromosomal Islands (PICIs). PICIs hijack prophage packaging machinery to package their own genomic information into phage-like particles. We isolated the phage fraction of the *D. mccartyi*-containing culture, KB-1, and sequenced the metavirome. IME1s were found in very high abundance (100,000X read depth), suggesting that IME1 are packaged in phage-like particles. Therefore, IME1s are further categorized as PICIs.

S3. Metagenomic analysis of dehalogenating microorganisms from a polychlorobiphenyl (PCB) contaminated sediment in Mar Piccolo (Taranto, Italy)

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Metagenome sequencing studies have been increasingly employed to dissect the metabolic potential and ecological role of unculturable microorganisms in the degradation of halogenated hydrocarbons in natural and artificial ecosystems. Yet little is known about the diversity of organisms and more importantly enzymes catalyzing carbon-chlorine bond cleavage in the absence of oxygen. Recent studies have discovered putative new classes of reductive dehalogenases (*rdh*) having atypical features as compared to canonical *rdh* genes. In this study, we report the presence of putative new classes of *rdh* genes such as hybrid *rdh* and catabolic type-*rdh* genes in the Mar Piccolo microbial community inhabiting sediments highly contaminated by petroleum hydrocarbons and PCB. To our knowledge this is the first metagenomic study characterizing the functional diversity of microbial reductive dehalogenases in one of the most contaminated basins in the Mediterranean Sea.

ABSTRACT BOOK

SESSION 4 (S4)

Bioelectrochemical OHR: fundamental aspects and engineered solutions

29th September 2021

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Session 4.1 (S4.1)

Chairs: Dr. Aulenta Federico, Dr. Tobias Goris

KEYNOTE LECTURE - Prof. Mauro Majone

FUNDAMENTALS AND ADVANCES ON BIOELECTROCHEMICAL DECHLORINATION

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In last two decades, starting from the fascinating ability of several microbes to engage with solid phases to exchange electrons for their metabolisms, the research community showed a tremendous increase of interest in investigating and developing bioelectrochemical systems for a wide range of applications. Applications include low-voltage electricity generation from waste streams (microbial fuel cell), methane or hydrogen production (microbial electrolysis cells), production of added value building blocks (microbial electrosynthesis), and external control of redox condition for triggering preferred metabolic pathways (electrofermentation). Last but not least, bioelectrochemical systems have been investigated in the field of bioremediation of contaminated matrixes, especially sediments and groundwater for a range of reducible or oxidable contaminants, such as hydrocarbons, arsenic, chromiun, nitrate, sulphate and halogenated compounds. As for the latter, given that a bioelectrochemical system can create both reductive and oxidative environments in close proximity each other (but also fully separated), this approach can be particularly effective for highly chlorinated compounds (such as perchloroethylene, trichloroethylene and tetrachloroethane) where full reductive dechlorination is sometimes hindered or too slow. Moreover, given that the electrodic material, used to supply or catch electrons, can also offer the physical support for attached microbial growth, bioelectrochemical systems are a unique tool for activating microbialr eactions in a well-defined and controlled space. Fine tuning of electrochemical conditions, such as applied voltage, gives the system a highflexibility, which can be used to trigger wanted reactions against competing ones (e.g. methane formation or sulfate reduction).After a brief introduction on fundamentals of bioelectrochemical systems, this lecture will focus on their development for remediation of groundwater contaminated by aliphatic chlorinated compounds in last 15 years, moving from the proof of principles to most recent advances for filed application. The importance of combining process engineering aspects with microbiology and molecular biology will be highlighted

Biography

Professor of Chemical Engineering at the Department of Chemistry of the University of Rome “La Sapienza”- Head of the multidisciplinary Research Center for protection of Environment and Cultural Heritage (CIABC). -Research areas: environmental and Industrial biotechnologies for treatment and valorisation of waste and wastewater. Biopolymer (PHA) production. Remediation of polluted soils and groundwater. -Co-author of more than 200 papers on international scientific journals with peer review, which received more than 7300 citations (Scopus, HI=49). -Scientific coordinator of several research projects under public or private commitment, including principal investigator of Sapienza research units in several FP7 and H2020 Projects- Coordinator of the H2020 Project RES URBIS (GA 730349) “Resources from Urban BioWaste”.

ABSTRACT BOOK

Oral and flash-poster presentations

S4.1. Exploring the microorganisms for achieving the bioelectrochemical dechlorination of *Dehalococcoides* via extracellular electron transfer

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Bioelectrochemical remediation of groundwater employing *Dehalococcoides* (*Dhc.*) species become promising for the dechlorination of chlorinated ethylene (CE). However, the direct proof that the CE dechlorination by pure *Dhc.* culture, which gains electrons via extracellular electron transfer has never been demonstrated. This study explored the microorganisms that potentially play the role of transferring the electrons from electrodes to *Dhc.* As the results of 67 days incubation in a two-chamber bioelectrochemical system (BES), the dechlorination performance by inoculated with *Dhc.* and paddy soil was apparently enhanced, While the dechlorination could not be observed in the BES inoculated with pure *Dhc.* culture. Microbial community structure analysis indicating that *Desulfosporosinus* species was predominant in the BES inoculated with *Dhc.* and paddy soil, which potentially contributed to electron transfer from the electrode to *Dhc*. for the bioelectrochemical dechlorination.

S4.1. Exogenous electronic regulation enhances the decomposition and transformation of TBBPA and the related microbial mechanism

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Tetrabromobisphenol A (TBBPA), as one of representative brominated flame retardants, has posed great harm to human health and the ecological environment due to the persistence, bioaccumulation, biotoxicity and potential carcinogenicity. In aquatic ecosystems, TBBPA tends to be deposited in the sediment under anoxic/anaerobic condition. However, the microbial degradation of TBBPA under anaerobic condition always suffers from low efficiency and the accumulation of toxic metabolites (BPA). The bioelectrochemical systems and iron-based nanomaterials are two representatives biostimulation methods through applying electron donor/accepter which has been applied in the bioremediation of persistent pollutants in recent years. However, the feasibility of them in the enhanced biodegradation and detoxification of TBBPA, and the related mechanism of microbial succession and microbial ecological network associations are remained unknown. Herein, the influence of TBBPA degrading performances and routes under the conditions with bioelectrochemical anode system and nano Pd/Fe were investigated. And the microbial activation effect and the function mechanism of the function bacteria by these two biostimulation methods were revealed. Besides, the interacting mechanism among the core function bacteria were revealed. It will provide important basis for developing efficient and stabilized technology for the enhanced bioremediation of persistent organic pollutants.
With the inoculum of the sediment enrichment, the TBBPA degrading rate was obviously increased by the acclimation of bioanode (2.4 times higher than the open circuit), and the generation of toxic product BPA was depressed. In the bioanode, the first step of TBBPA degradation could be separated in two main pathways, one is the reductive debromination with bromophenol A (BPA) as final product, the other one is the hydrolytic debromination. And the products of hydrolytic debromination was further degraded through methylation and ring opening, finally the products of ring opened could be further degraded and possibly mineralized. The TBBPA degrading efficiency and routes were similar between opened bioanode and bioanode, while the transformation proportion of reductive debromination increased a little (from 5.1% to 8.3%), and the degradation efficiency of ring opened products decreased obviously. Indicating that the sufficient electron donor in bioanode could remove the barrier for the further mineralization of TBBPA metabolites. Cyclic voltammetry analysis showed that the reducing peak of TBBPA in bioanode shifted

positive (+220 mV) apparently, and meanwhile a weak oxidation peak arose, which indicated that the biofilm on the electrode distinctly increased the electrochemically catalytic activity. The reductive debromination and hydrolytic debromination of TBBPA were closely related to the lactate fermentation and carbon source transformation. Function bacteria in the plankton dominated in the reductive debromination and hydrolytic debromination. Besides, the generation of toxic product BPA was depressed due to the inactivation of reductive dehalogenators by the addition of electron accepter in bioanode. The activity of electroactive bacteria and the potential TBBPA degrading bacteria (e.g. *Geobacter*, *Acinetobacter* and *Holophaga*) was stimulated, and the relative abundance of them was distinctly increased and become predominated after the weak electrostimulation, they dominated in the TBBPA hydrolytic debromination, ring opening and further mineralization.
The addition of Pd/Fe nanoparticles distinctly increased the TBBPA degradation efficiency, and the toxic metabolites BPA was further degraded and possibly mineralized. After the nano Pd/Fe (optimized dosage 0.412 g/L, and Pd loading 0.5 wt%) was introduced into the sediment solution, 3.7 times of higher *k* (degradation rate constant) was observed in sediment fed with nano Pd/Fe when compared to the sediment solution. Reductive debromination was the only pathway in sediment solution with BPA as final product. But BPA could be further degraded through β scission and oxidative hydrolysis with the generation of 4-(allene)phenol and 2,2-bis(4-hydro-xyphenyl) propanoic acid, and they could be further degraded and mineralized in sediment fed with nano Pd/Fe. The addition of nano Pd/Fe distinctly improved the generation H2 and restricted the methanogenesis, providing sufficient electron donor for the reductive debromination of TBBPA. And it obviously stimulated the activity of potential reductive dehalogenators (e.g. *Dehalobacter*, *Desulfuromonas*). Meanwhile, the ferric oxide on the surface of Pd/Fe nanoparticles provided enough electron accepter for microbial degradation of BPA, activating the aromatics degraders (e.g. *Cryptanaerobacter*, *Citrobacter*, *Bacillus*). And the relative abundance of the function bacteria increased and became dominated in the system, which played an important role in the further degradation and mineralization of BPA.
The biostimulation methods through addition of electron accepter (bioanode) and electron donor (nano Pd/Fe) obviously optimized the microbial structure, they created livable environment (enough electron donor/accepter or appropriate redox potential, etc.) for the function bacteria, breaking the bottleneck during the TBBPA degradation. The biostimulation distinctly lowered the complexity of the microbial ecological network, higher the modularity and the niche differentiation. In the influence of enough electron accepter, the number of nodes which represented potential function bacteria (including reductive dehalogenators, hydrolytic dehalogenators and aromatics degraders) and electroactive bacteria (e.g. *Geobacter*, *Desulfovibrio*) increased distinctly in the core OTU of modules, and they shared more positive interaction. The number of nodes which represented potential reductive dehalogenators (e.g. *Desulfuromonas*) and BPA degraders (e.g. *Citrobacter*, *Bacillus* and *Cryptanaerobacter*) increased obviously in the core OTU of modules in the influence of electron donor, and the directly positive interactions were also increased. After the influence of the two biostimulation methods, the interactions among function bacteria were more tightened. The phenomenon of cross-feeding properties or shared similar niches was crucial for the enhanced TBBPA degradation and detoxification. Basing on the analysis of TBBPA degradation mechanism of the two biostimulation methods above, the related bioremediation mode was raised and the application potential was investigated.

S4.1. Metagenomics of a bioelectroremediation system treating TCE and Cr(VI) co-contamination: revealing microbial diversity and interactions

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Bioelectrochemical systems are attractive for the bioremediation of organic or inorganic pollutants, often found as co-contaminants in the environment. This study reports the microbial composition and interactions occurring at the biocathode of a BES where trichloroethylene (TCE) reductive dechlorination

(RD), Cr(VI) reduction, and hydrogenotrophic methanogenesis occurred. *Dehalococcoides mccartyi* established on the biofilm of the TCE/Cr(VI) biocathode (up to 3.2E+07 16S rRNA gene copies g-1 graphite). Metagenomics revealed a selected consortium on the TCE/Cr(VI) biocathode, including *D. mccartyi* which H2 uptake was the only electron supply mechanism suggesting that electroactivity is not a property of this microorganism. *Methanobrevibacter arboriphilus* and *Methanobacterium formicicum* were found as H2 consuming for CH4 production and cofactor suppliers for cobalamin biosynthesis to sustain *D. mccartyi* growth. *M. formicicum* also harbours gene-complexes involved in the Cr(VI) reduction.

S4.1. Electrode material selection and voltage operation in a *Dehalogenimonas*-containing bioelectrochemical system degrading 1,2-dichloropropane

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A *Dehalogenimonas*-containing culture stablished in a bioelectrochemical system was able to successfully degrade 1,2-dichloropropane (1,2-DCP) to propene. In order to optimize the hydrogen provided to the cathode vessel, which is required by *Dehalogenimonas* as electron donor, two electrode materials, a graphite brush and a carbon cloth, were tested. The degradation rates obtained when working with the graphite brush electrode at a cathode potential of -0.7 V vs Standard Hydrogen Electrode (SHE) were 16 times higher than the values obtained by the carbon cloth electrodes when operating under the same conditions. With the aim of reducing the required electric input of the degradation, a pulsed voltage operation was used, which increased the coulombic efficiency of the process up to 16%. The long-term operation of these bioelectrochemical reactors allowed to obtain cell densities of 108 cells of *Dehalogenimonas* per mL of culture at the end of the operation.

Session 4.2 (S4.2)

Chairs: Dr. Aulenta Federico, Dr. Tobias Goris

Oral and flash-poster presentations

S4.2. A sequential reductive/oxidative bioelectrochemical process for chlorinated aliphatic hydrocarbons (cahs) removal: evaluation of the process with a real contaminated groundwater

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Chlorinated aliphatic hydrocarbons (CAHs) are ubiquitous groundwaters and subsoils contaminants. To completely remove this unwanted species from groundwaters an innovative reductive/oxidative bioelectrochemical process has been developed by using of two tubular membrane-less microbial electrolysis cells (MECs) equipped with an internal graphite counterelectrode. The first MEC called reductive reactor was constituted by a granular graphite working electrode which had the role of electron donor (i.e. cathode) for the reductive dechlorination reaction. After the reductive MEC, a second oxidative MEC, aimed to the oxidative dechlorination of low chlorinated by-products using an MMO electrode which ensured the oxygen production through water oxidation. In the present study, the sequential reductive/oxidative bioelectrochemical process has been tested with a real groundwater coming from a

contaminated site located in northern Italy. The reductive reactor, operated at a flow rate of 4.5 L/d and polarized at -450 mV vs SHE allowed for the complete conversion of high chlorinated CAHs into vinyl chloride (VC) that was completely removed in the oxidative reactor operated under galvanostatic mode at +15 mA by increasing the hydraulic retention time (HRT) from 0.7 to with 1.7 days.

S4.2. Microbiome composition and dynamics in a reductive/oxidative bioelectrochemical system for perchloroethylene (PCE) removal: the effect of feeding composition

***Di Franca Maria Letizia*[1],** *Matturro B*[1]*, *Zeppilli M*[2], *Dell'Armi E*[2], *Petrangeli Papini M*[2], *Majone M*[2], *Rossetti S*[1]
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A bioelectrochemical system (BES) based on a reductive/oxidative sequential process for complete perchloroethylene (PCE) biodegradation has been recently proposed. In this study, we describe the microbiome composition of the BES operated under diverse feeding compositions (i.e., anaerobic mineral medium MM; synthetic groundwater SG; real groundwater RG) that differ for the presence of SO42-, NO3- or Fe3+. The medium composition affected the reductive dechlorination performances. Accordingly, *Dehalococcoides mccartyi* abundances in the BES fed with SG and RG were lower (5.09E+07 and 3.94E+08 16S rRNA gene copies/L, respectively) than those observed with the MM (1.03E+09 16S rRNA gene copies/L). In the cathodic compartment of the BES operated with SG and RG, *Sulfuricurvum* and *Thiobacillus* species established. The microbiome dynamics will be discussed, along with the main processes occurring in the BES under the tested conditions.

S4.2. Bioelectrochemical treatment of groundwater containing oxidable and reducible contaminants

Cruz Viggi C[1]*, ***Tucci Matteo*[1],** *Milani A*[1], *De Laurentiis C*[1], *Resitano M*[1], *Crognale S*[1], *Matturro B*[1], *Rossetti S*[1], *Aulenta F*[1]
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A lab-scale bioelectrochemical reactor named "bioelectric well" was continuously fed with artificial groundwater containing a mixture of oxidable (i.e., toluene) and reducible (i.e., chlorinated solvents) contaminants. The reactor consisted in a tubular glass cylinder where in a cylindrical graphite anode and a stainless-steel mesh cathode were concentrically placed. Throughout the experiment, the anode was polarized at +0.2 V vs. SHE. The performances of the system were evaluated in terms of toluene and chlorinated solvents removal and coulombic efficiency, and they were compared to an open circuit control. Moreover, the microbial composition of the biofilm and of the bulk liquid was analysed by NGS methods.

ABSTRACT BOOK

SESSION 5 (S5)
OHR field-scale application
30th September 2021

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Session 5.1 (S5.1)
Chair: Prof. Marco Petrangeli Papini

KEYNOTE LECTURE - Prof. Birthe Veno Kjellerup
Biofilm based bioremediation of chlorinated contaminants

Birthe Veno Kjellerup
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Stormwater runoff has been implicated as a major cause of (re)-contamination of sediment near stormwater and wastewater effluent discharge points in urban watersheds as well as Department of Defense sites. Removal of persistent organic pollutants (POPs), specifically polychlorinated biphenyls (PCBs) from stormwater, thus preventing or significantly reducing the discharge to urban discharge areas including aquatic sediments is a priority due to the ability of these contaminants to enter the food chain, where they can present potent toxic and carcinogenic properties. Commonly adop-ted sediment remedies include dredging and capping, which are associated with challenges including disruption of existing habitat and high cost. While in situ microbial degradation of PCBs represents an improvement, previous attempts have failed because of PCB stability, low bioavailability, low abundance and activity of indigenous PCB-degrading microorganisms. The high efficiency of activated carbon (AC) and other sorptive substrates to quickly adsorb PCBs from sediments has been demonstrated. Co-localizing PCB-degrading microbes onto surfaces of sorp-tive particles as biofilms and utilization as a delivery system pro-vi-des a novel approach to address PCB contamination. This approach can also be modified for treatment of contaminated stormwater and wastewater effluent prior to discharge. The effect of stormwater containing PCBs on the sediment quality was evaluated for multiple locations in Baltimore Harbor, where sediment core samples were evaluated and compared to historical PCB concentrations. Also, current strategies for bioremediation of PCBs in stormwater retention cells and well as in sediment were evaluated.

Biography

Dr. Birthe Kjellerup is an Associate Professor in the Department of Civil and Environmental Engineering at the University of Maryland with a secondary appointment in BioEngineering. Dr. Kjellerup began her training at Aalborg University, Denmark, in the Department of Life Sciences where she received her PhD in 2004 with her thesis titled "Monitoring, detection and control of bacteria involved in biocorrosion in district heating systems". As a part of her graduate studies she traveled to the international training center for biofilm research, the Center for Biofilm Engineering (CBE) in Montana, and has continued collaboration with CBE in her current position. Dr. Kjellerup then moved to Baltimore to become a postdoctoral fellow at the Center of Marine Biotechnology to continue her work on environmental biofilms and bioremediation. Dr. Kjellerup became an Assistant Professor in 2009 in the Biology Department at Goucher College, Baltimore, where she stayed until 2014. In January 2015 she accepted a position as Assistant Professor at University of Maryland at College Park. Sincearriving at University of Maryland, Dr. Kjellerup has continued her research and teaching interests in biofilms. Dr. Kjellerup has trained as an environmental engineer and microbiologist specializing in beneficial and detrimental aspects of biofilms for over 20 years. She has pioneered the application of biofilms on sorptive materials for bioremediation and energy recovery and used them, along with chemical analysis, to develop novel bioremediation strategies and approaches for groundwater and stormwater clean-up.Dr. Kjellerup has a strong background in organizing highly skilled colleagues in multidisciplinary research. She also has a strong working knowledge of budget development and has obtained nearly $4 million from local (DC Water at Blue Plains), state (Maryland StateHighway Administration) and national (SERDP, USDA) funding agencies in the past 7 years. Dr. Kjellerup has served on more than 20 graduate committees and is the primary advisor for 7 graduate students committees (5 PhD, 2 Masters). She has also mentored 5postdoctoral fellows Currently two in the research group), where one has progressed to an international faculty in addition to more than 25 undergraduate students with the majority at University of Maryland.

Oral and flash-poster presentations

S5.1. PHA-based material from pure and mixed microbial culture as slow-release electron donor for sustainable *in situ* biological reductive dechlorination

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The use of polyhydroxyalkanoates (PHA) as slow-release electron donors for environmental remediation represents a novel and appealing application that is attracting considerable attention in the scientific community. In this context, here, the use of PHA produced at pilot scale by mixed microbial cultures (MMC) using waste feedstock along with commercially available materials, produced from pure cultures, has been investigated for biological reductive dechlorination (BRD) process. As a main finding, a sustained, long-term production of organic acids production was observed with a low-purity MMC- deriving material, consisting of microbial cells containing 56% (*w/w)* of intracellular PHA. These results clearly suggest the possibility to directly use the PHA-rich cells deriving from the MMC production process, with no need of extraction and purification procedures, as a sustainable and effective carbon source bringing remarkable advantages from an economic and environmental point of view.

S5.1. Cultivation of *Dehalococcoides mccartyi* strain CBDB1 in a continuous stirred tank reactor

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Dehalococcoides mccartyi strain CBDB1 is a strictly anaerobic organohalide-respiring bacterium. To date, most of the cultivation was in bottles or batch-fed reactors. Challenges with such systems include low biomass yield and difficulties in controlling growth conditions. Here, we report the cultivation of planktonic *D. mccartyi* strain CBDB1 in a continuous stirring tank reactor (CSTR). With CSTR, cell densities as high as 1×109 cells mL-1 can be stably achieved, and growth conditions can be fine-tuned to allow intricate control of cell status. By monitoring hydrogen consumption using a pressure sensor, organohalide respiration rate can be followed in real time. After feeding the reactor with brominated phenolic compounds for about one month with a hydraulic retention time of 10 days, we observed a complete disappearance of the usually found reductive dehalogenases CbdbA0080 and CbdbA0084 in the proteome. Denaturing gradient gel electrophoresis (DGGE) showed that the majority of the population remained to be *Dehalococcoides* although the CSTR was operated at non-sterile conditions. In sum, cultivation of *Dehalococcoides* in CSTR gives us new hints to design and improve bioremediation strategies.

S5.1. Using a biosolids-amended trench to enhance reductive dehalogenation of TCE contaminated groundwater in a biowall

Shahrzad Saffari Ghandehari[1]*, *Boyer J[1]*, Ronin D[1]*, Hapeman CJ[2]*, Schanzle D[3]*, Torrents A[4]*, Kjellerup BV[4]**

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A biowall was installed downstream from a landfill in Maryland, USA to remediate groundwater contaminated with up to 600 ppb trichloroethene (TCE). Breakthrough of TCE degradation products required a trench to be installed upgradient from the biowall to promote reductive dehalogenation by increasing groundwater residence time, pH, and available organic carbon. Molecular biology techniques and batch reactor studies using the materials to be used in the trench were conducted to determine if biosolid use would affect overall TCE dehalogenation. *Dehalobacter* was detected in the biosolids, and methanogens were present and active, yet the increased amount of methane did not significantly affect the dehalogenation of TCE. Biosolids were used as a filling material for the trench, at this site, and a year later our preliminary results are encouraging.

S5.1. Field application of a reagent for the iscr and erd treatment of an aquifer contaminated with tetrachlorethylene, dichloropropane e r-130

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EHC® Liquid reagent is a combined product for In Situ Chemical Reduction (ISCR) and Enhanced Reductive Dechlorination (ERD) for the treatment of aquifers contaminated by chlorinated organic compounds and some heavy metals such as hexavalent chromium. Once in groundwater, EHC® Liquid rapidly generates enhanced reduction conditions, favouring both biotic and abiotic dechlorination reactions. The product has two components, a soluble organo-iron mix and ELS® Microemulsion, a lecithin- based substrate. These two components are designed to be easy to mix, dilute, and inject into the subsurface. This technology has been successfully applied in an abandoned industrial area of northern Italy, where the groundwater was historically contaminated by tetrachloroethylene (PCE) (> 0.1 ppb) and, to a lesser extent, by trichlorethylene (TCE), dichloropropane (DP) and 1.1, 2,2-tetrachloroethane (R-130). In less than 6 months after the injection of EHC® Liquid in the main source area, the concentrations of the contaminants have reached the site-specific remediation target values (CSC Legislative Decree 152/06) in the main monitoring piezometers present in the area, demonstrating the establishment of enhanced biotic and abiotic reducing conditions.

S5.1. Natural-occurring microbial community in an organohalide polluted aquifer: preliminary evaluation of the effectiveness of permeable reactive bio-barriers for decontamination

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Organohalides represent an issue for human health and are major contaminants around the world. At field scale along with chemical and physical treatments, often inefficient for organohalide degradation, biological approach based on bacterial anaerobic respiration can be applied. The aquifer under investigation is affected by multiple contaminations, *i.e.* organohalides, BTEX and petroleum

hydrocarbons. In order to propose a bioremediation treatment, a preliminary evaluation was conducted on indigenous bacterial communities. Environmental genomic analyses revealed the presence of naturally-occurring organohalide-respiring and of vinyl chloride-oxidizing bacteria. Preliminary batch and pilot tests indicated that the installation of anaerobic permeable reactive bio-barriers could be effective in organohalide decontamination at the site.

Session 5.2 (S5.2)

Chair: Prof. Birthe Kjellerup

Oral and flash-poster presentations

S5.2. Lessons learned from the study of microbial communities in sediment for the design of bioremediation strategies

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To illustrate the importance of the characterisation of microbial communities in the sediment for the design of the bioremediation strategy, two different studies are presented: 1) two boreholes located in the plume zone of a mega site polluted by carbon tetrachloride and chloroform among other pollutants; and 2) two boreholes located in the source area of a perchloroethene episode. From these two studies we highlight: 1) the relationship between microbial communities characterised in sediment at the centimetre level with microbial communities characterised in groundwater sampled in multilevel wells at the decimetre level; 2) the importance of porosity, as there is a porethroat diameter threshold that may result in exclusion of bacteria, and where there is high porethroat size, microbial communities are adapted to fluctuating hydrochemical and redox conditions; 3) the presence of ecotones at contact levels between fine and coarse materials; and 4) toxicity, determined for both microbial communities in sediment and groundwater.

S5.2. A Coupled Adsorption and Biodegradation (CAB) process employing PHB and Biochar as bio-based materials for TCE contaminated groundwater *in-situ* bioremediation

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The combination of chemical-physical and biological technologies is appealing to increase the effectiveness of remediation actions on chlorinated solvents contaminated groundwater. In this work, a Coupled Adsorption and Biodegradation (CAB) process has been proposed for trichloroethylene (TCE) removal by combining adsorption and anaerobic biological dichlorination by using bio-based materials. A cylindrical column system (150x10 cm) was realized with the biopolymer polyhydroxybutyrate (PHB) as a source of slow-release electron donor, and sequentially with a low-cost material biochar (BC), derived from the pinewood residues a TCE-to-Ethene dechlorinating culture as inoculum. The reactor operating with a 6 L day-1 flow rate, resulted in a 99.93 ± 0.29 of TCE removal. *Dehalococcoides mccartyi* enriched on the BC biofilm, suggesting the occurrence of biological dechlorination in addition to the TCE adsorption.

S5.2. *Dehalococcoides*-mediated *in situ* remediation of vinyl chloride contaminated site

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In this study, we have developed a simple, cost-effective, and highly efficient method for large-scale preparation of dehalogenation microcosm for *in situ* bioremediation applications. This method can be used for large-scale preparation of highly active organohalide-respiring bacteria (OHRB) that can completely dechlorinate PCE, TCE and/or *cis*-DCE to ethene. In addition, we also carried out pilot-scale *in situ* remediation experiments at an vinyl chloride (VC) contaminated site. Results shows that *Dehalococcoides* as the functional OHRB can effectively dechlorinate VC to ethene, and relative abundance of the *Dehalococcoides* significantly increase upon the VC dechlorination. This study provides new information on the large-scale production of OHRB and its application at contaminated sites.

S5.2. Bioremediation of chlorinated compounds: examples of practical approaches using Next Generation Sequencing

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A site contaminated with various chlorinated compounds and aromatics has been investigated with Next Generation Sequencing (NGS) for its biological degradation potential. The analysis performed consists in scanning and studying the whole biodiversity of the groundwater to identify bacterial species known to be able to degrade the harmful compounds. Results of the GPP analysis showed good correlation between concentration of a contaminant and species known to be involved in its degradation. Based on the results, it was suggested that it would be beneficial to apply a bio-stimulation approach to the site to speed up the degradation processes.

ePOSTER Session

ePOSTER room#1

#1 Soil management plan to sustain PFAS degradation by novel Acidimicrobia

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Poly- and perfluoroalkylated substances have many useful applications due to their properties of water and heat resistance. These substances are now found in soil, groundwater and even in people's blood. American researchers from Princeton University published a study in late 2019 on a soil bacterium called '*Acidimicrobium* A6', which can degrade PFAS by cutting the C-F bond. Based on this study, Geofoxx and Orvion conducted a study on this bacterium to detect it in soils from South Holland. Soil samples have been taken from various locations and analysed by qPCR. Related Acidimicrobia species were found in soils with favourable conditions. Further testing will show if these species can also degrade PFAS.

#2 Expression and investigation of the reductive dehalogenase enzyme family

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The characterization of the reductive dehalogenase (RdhA) family involved in organohalide respiration has lagged behind their gene discovery largely due to challenges in their heterologous expression. In this work we present a novel expression system in *Escherichia coli* that involves the co-expression of the RdhA with a vitamin B12 uptake (*Btu*) pathway using a strain of *E. coli* that is engineered for higher iron-sulfur cluster production. This system was optimized using the enzyme TmrA. We found that *Btu* co-expression and anaerobic conditions were required for TmrA activity, while the strain of *E. coli* increased activity. This expression system was applied to five other *Dehalobacter* RdhA to demonstrate that it can be generalized to dissimilar RdhAs.

#3 Cofator selectivitiy of a minimized cobamide-binding protein

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The structural basis for the selective binding of different cobamide cofactors was studied with a small cobamide-binding protein. The biologically produced structural variants of the cobalt-containing tetrapyrrole Vitamin B12, the so-called cobamide (Cba) cofactors, have been identified as essential components of reductive dehalogenase enzymes (RDases). In several studies, a preference in the utilization of structurally different Cbas by various RDases has been reported. Since respiratory RDases are the key enzymes in the energy metabolism of organohalide-respiring bacteria (OHRB), the understanding of Cba cofactor selectivity or promiscuity of these enzymes will substantially support all efforts in supporting the fitness of OHRB in natural or synthetic microbial communities. In this study, the capacity of binding a variety of structurally different Cba cofactors was examined by the use of a minimized Cba-binding protein that incorporates the metal cofactor in the base-off conformation, the binding mode also identified in RDases. The incorporation efficiency was analyzed by the use of a filtration-based binding assay applied to wild type and mutant proteins. Based on the results, conclusions were drawn on the structural basis for Cba selectivity in Cba-binding proteins in general and RDases in particular.

#4 Insights into the structure of the organohalide respiratory complex from *Dehalococcoides mccartyi* strain CBDB1 by using chemical modifications

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Dehalococcoides mccartyi strain CBDB1 is a strictly anaerobic microorganism respiring with halogenated organic compounds as electron acceptor. The process termed organohalide respiration (OHR) takes place in *Dehalococcoides* strains at a multi-subunit, membrane-bound, modular protein complex. Slow cell growth and low biomass production as well as the highly hydrophobic character of the transmembrane protein subunits hamper the analysis of the complex. Here we demonstrate how a combination of different biochemical methods like solubilization, complex enrichment and introduction of specific chemical modifications coupled to peptide mass spectrometry help to gain insights into the organization of the OHR complex giving a basis for functional investigations. By introducing additional positive charges through amidation of functional carboxyl groups, mass spectrometric detectability of the hydrophobic peptides of integral membrane proteins is improved. To attain more information about the topology of the OHR complex, tyrosine and tryptophan of solvent-accessible protein regions were modified.

#5 RNA Sequencing of *Dehalococcoides mccartyi* Strain CBDB1 reveals differential expression of reductive dehalogenase genes with 1,2,4- and 1,2,3-trichlorobenzene

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Dehalococcoides mccartyi strain CBDB1 was cultivated with either 1,2,3- or 1,2,4-trichlorobenzene (TCB) as electron acceptor and subjected to a global transcriptome analysis (RNA-seq). The analysis of transcripts within intergenic regions revealed nine putative small RNAs (sRNA), among which the putative 6S RNA was strongly expressed. Riboswitches, four transcripts encoding not-yet annotated small proteins and crRNA fragments of the CRISPR-Cas array were identified. Transcripts of the *rdhA*s *cbrA* and cbdbA80 were most abundant with 1,2,3-TCB, whereas the presence of 1,2,4-TCB, induced an additional strong transcription of *rdhA* cbdbA1588. Proteome analysis and dehalogenase activity assays indicate a role for RdhACbdbA1588 in the dechlorination of 1,2,4-TCB.

#6 Metagenomic analysis of a novel *Dehalococcoides mccartyi* enrichment culture capable of PCE-to-ethene dechlorination

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The development of novel and stable reductive dechlorinating cultures is fundamental for bioremediation studies, from investigating the biological processes to the field-scale applications. Here we present a novel *Dehalococcoides mccartyi (Dhc)*-enriched culture capable of PCE-to-ethene dechlorination with the maximum rate of 2.02 mmol Cl- L-1 d-1. Dhc represents 76% of total amplicon sequence variants (ASVs) observed by 16S rRNA amplicon sequencing. The *Dhc*-extracted genome from the assembled metagenome of the consortium revealed the presence of one gene encoding the catalytically active enzyme RdhA and nine distinct genes predicted to be reductive dehalogenases. The *Dhc*-extracted genome also harbours one CRISPR array with genes coding for the CRISPR-Cas system, recently found to be relevant for the reductive dehalogenase genes mobilization. Metagenomic features of the *Dhc* genome of the dechlorinating culture will be discussed, mainly regarding the crucial *Dhc* metabolism.

ePOSTER room#2

#7 Dichloromethane degradation by an anaerobic microbial community used for chloroform bioremediation

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Dichloromethane (DCM) degradation is a critical step in contaminated groundwater bioremediation around the world. A build-up of DCM in the system can prevent biodegradation of chloroform (CF), which subsequently halts degradation of other chloroalkanes and chloroalkenes. Of the limited number of DCM-degrading microbes identified to-date, most are very sensitive to CF, rendering their use for bioremediation impractical. The KB-1® Plus CF enrichment culture degrades CF to DCM, while concurrently degrading the DCM produced. This culture is relatively uncharacterized, and mechanism of DCM-degradation has not been studied. Here, a DCM-degrading sub-culture is enriched from KB-1® Plus CF and characterized in terms of composition and metabolism. Understanding this DCM-degrading

community will allow improvement of the CF bioremediation process, and benefit sites where the KB-1® Plus CF culture is currently applied for bioremediation through bioaugmentation.

#8 The effect of humic acids on perchloroethene dechlorination by *Dehalococcoides* strains under anaerobic conditions

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Perchloroethene (PCE) is a widely used chlorinated solvent. PCE is toxic to humans and has been identified as an environmental contaminant at thousands of sites worldwide. Several *Dehalococcoides mccartyi* strains can transform PCE to ethene, and thus contribute to bioremediation of contaminated sites. Humic acids (HA) are ubiquitous redox-active compounds of natural aquatic and soil systems and have been intensively studied because of the effect in electron transfer. We observed that the dechlorination of PCE was accelerated by HA in mixed cultures containing *Dehalococcoides* strains. However, anthraquinone-2,6-disulfonic acid (AQDS), a model compound for the quinone moieties in HA, inhibited PCE dechlorination and thus induced an opposite effect on PCE dehalogenation than HA. The same was observed with the pure culture of *Dehalococcoides mccartyi* strain CBDB1. Enzymatic activity tests confirmed that the dehalogenating activity of strain CBDB1 was increased by HA. Thus, we are now searching for another mechanism how HA support reductive dehalogenation than via shuttling of electrons.

#9 Colonization and growth of dehalorepiring biofilms on sportive amendments

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Removal of halogenated organic compounds (HOCs) from contaminated sediments is a priority because of their ability to enter the food chain and due to their toxicity. Commonly adopted remedies include dredging and capping which are associated with challenges including disruption of existing habitat and high cost. While *in situ* microbial degradation of polychlorinated biphenyls (PCBs) represents an improvement such as previous attempts have failed because of PCB stability, low bioavailability, low abundance and activity of indigenous PCB-degrading microorganisms. Recent success with reduction of PCBs bioavailability due to adsorption onto activated carbon led to the recognition of *in situ* treatment as a remediation approach. The high efficiency of activated carbon (AC) and other sorptive substrates to quickly sorb PCBs from sediments has been demonstrated. Co-localizing PCB-degrading microbes onto surfaces of sorptive particles as biofilms and utilization as a delivery system provides a novel approach to address PCB contamination. In this study, reduced bioavailability and subsequent break-down of halogenated compounds in dehalorespiring biofilms was investigated using *Dehalobium chlorocoercia* DF-1 and the WBC-2 mixed consortium. Biofilm colonization and growth on materials was also investigated.

#10 Host Specificity of sponge-associated dehalogenating bacteria

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Marine sponges produce numerous organobromine compounds of different structures and degrees of halogenation. These halogenated products bioaccumulate and have adverse health effects at low concentrations. Their production may create a selective environment in the sponge, enriching for bacteria that use these molecules for respiratory reductive debromination. This study aims to further our understanding of the natural cycling of organobromine compounds and the relationship between the sponge host and dehalogenating microbes. Debrominating enrichment cultures were established from homogenized sponge tissue. The data suggest that *Desulfoluna spongiiphila* is widespread geographically and found in several host sponges; however, strain-level differences exist among different host species and locations.

#11 Potential factors affecting dichloromethane biodegradation upon water table fluctuation

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Dichloromethane (DCM) is an industrial solvent frequently detected in multi- contaminated aquifers. Water table fluctuations were shown to influence DCM biodegradation. Such fluctuations may affect both redox conditions and humidity rate in sediments. Here, we present an experimental design and preliminary results of experiments aiming at deciphering the potential role of these two factors in DCM biodegradation.

#12 Using cryo-EM to probe structures of hydroalkylation enzymes with potential use in bioremediation of crude-oil polluted regions

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The “resolution revolution” of single particle cryo-electron microscopy (cryo-EM) has allowed structural biologists to probe the mechanism of enzymes and enzyme complexes in a solution state at atomic level detail. Enzymes within the glycyl radical enzyme (GRE) superfamily have been studied by X-ray crystallography, and resulting structures have elucidated molecular details of many different and unprecedented GRE mechanisms. One such GRE complex, which performs hydroalkylation chemistry to add a C–H bond of toluene across the alkene fumarate, is known as benzylsuccinate synthase (BSS). Toluene is a component of crude oil, and anaerobic crude-oil-polluted regions are especially recalcitrant to remediation. BSS and other succinate synthase enzymes are therefore prime candidates for bioremediation efforts of these difficult regions. Herein, we present high resolution cryo-EM structures of BSS and compare differences between these and crystal structures

ABSTRACT BOOK

ePOSTER room#3

#13 Meta-analysis and experimental studies on anaerobic biodegradation and biotransformation of simple and polyfluorinated compounds

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Per- and polyfluorinated compounds (PFAS) exhibit ubiquity and longevity in the environment, toxic effects upon organismal and environmental health and resistance to biodegradation Works focused on PFAS biodegradation showed that polyfluorinated compounds are more amenable to biodegradation than perfluoroalkyl compounds, less fluorinated structures in molecules are likely to experience defluorination first, shorter-chain PFAS are more likely to mineralize, and PFAS longer than 8 carbons typically ultimately degrade to perfluorooctanoic acid (PFOA) or perfluorooctanesulfonic acid (PFOS). The objectives of this project were 1) to conduct a meta-analysis based on peer-reviewed studies focused on PFAS anaerobic biodegradation and biotransformation and 2) to experimentally evaluate anaerobic biodegradation of fluorobenzene, trifluoroacetate, PFOA, and PFOS under various conditions in mixed microbial communities.

#14 Reductive dehalogenation of tetrabromobisphenol a in an enrichment culture dominated by *Dehalobacter* spp.

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In this study, we obtained an enrichment culture capable of reductively dehalogenating tetrabromobisphenol A (TBBPA) to bisphenol A (BPA), which can be readily biodegraded aerobically. The microbial dehalogenation activities halted in the absence of solid humin and was retrieved once the solid humin was supplemented. We hypothesize that the solid humin acts as the electron shuttle during the microbial dehalogenation. The reductive dehalogenation of TBBPA was carried out by the organohalide-respiring bacteria (OHRB) *Dehalobacter* spp. as revealed by quantitive PCR and 16S rRNA gene amplicon sequencing. A near complete (96.88 %) draft genome of *Dehalobacter* was successfully obtained through metagenomic sequencing. The genome draft harbored 34 putative reductive dehalogenase genes (*rdhA*). However, the phylogenetic alignments of reductive dehalogenases (RdhA) encoded in this genome showed a relatively low identity with all previously identified RdhA. Following studies are being conducted to illustrate the role of humin in the microbial dehalogenation of TBBPA and to functionally characterize the RdhA catalyzing the reductive dehalogenation of TBBPA.

#15 Effect of e- donors on the chloroethene dechlorination and pathogenic risk in groundwater augmented with *Dehalococcoides*

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Bioaugmentation of organohalide respirating bacteria has gained more attention for chloroethene dechlorination. To date, high molecular carbohydrates (e.g. lactate), has been used as e- donor due to the H2 productivity and long persistence. Despite the advantages mentioned above, the nonspecific growth of non-ORB potentially increases the pathogenic rick in groundwater. The concern motivated us to assay the applicability of low-carbon hydrates for the chloroethene dechlorination. As the results, complete dechlorination of 1 mM TCE within 80 d was observed when fed with formate and oxalate, which was shorter than lactate and citrate (140 d). 16S rRNA amplicon sequencing revealed that >21% and 47% of relative abundance for *Clostridium butyricum* and *Enterobacter kobei* when fed with lactate and

citrate, respectively. While no potentially pathogenic bacteria were detected when fed with formate and oxalate. This study showed the applicability of low-carbon hydrate for reducing pathogenic risk in bioaugmentation.

#16 Molecular characterization of microbial communities in a peat-rich aquifer system contaminated with chlorinated aliphatic compounds

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In an aquifer-aquitard system in the subsoil of the city of Ferrara (Italy) highly contaminated with chlorinated aliphatic toxic organics such as trichloroethylene (TCE) and tetrachloroethylene (PCE), a strong microbial-dependent dechlorination activity takes place during migration of contaminants through shallow organic-rich layers with peat intercalations. The *in-situ* microbial degradation community was assessed using Illumina sequencing of V4 hypervariable region of 16S rRNA gene and clone library analysis of dehalogenase metabolic genes. Taxon-specific investigation of the microbial communities catalyzing the chlorination process revealed the presence of dehalogenating bacteria such as *Dehalococcoides* and *Dehalobacter*, and non-dehalogenating bacteria and archaea able to accomplish hydrolysis and fermentation of complex organic matter, acidogenesis, acetogenesis, and methanogenesis, which can indirectly support the reductive dechlorination process.

#17 Enhancement of perchloroethene dechlorination by a mixed dechlorinating culture via magnetic nanoparticle-mediated isolation method

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This study attempted to enrich a rapid perchloroethene (PCE) dechlorinating culture via magnetic nanoparticle-mediated isolation (MMI). MMI is a novel method that can separate the fast- and slow-growing population in a mixed microbial community without labelling. In MMI process, PCE dechlorination was enhanced but the subsequent trichloroethene (TCE) dechlorination was inhibited. Meanwhile, fast-growing genera like *Dehalobacterium* and *Petrimonas* were enriched, while slow-growing *Methanosarcina* was eliminated. Several major genera including *Petrimonas* and *Methanosarcina* were positively related to TCE dechlorination and *Dehalococcoides*, while *Dehalobacterium* was negatively related to both above, indicating potential competition between *Dehalobacterium* and *Dehalococcoides*. Regrowth of *Methanosarcina* coupled well with recovery of TCE dechlorination, suggesting that methanogens may act as biomarkers for TCE dechlorination.

#18 Combining a Groundwater Circulation Well (IEG-GCW) with nutrient distribution through Multilevel Injection Wells (MIW) for the remediation of a chlorinated solvent contaminated site

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As part of the MicroBiome project, an *in situ* microbiological remediation process of groundwater contaminated by chlorinated solvents was developed at a polluted site located in Barcelona. At the site, TCE concentrations measured in groundwater locally reached a maximum value of 170 mg/L. The products of biological reductive dechlorination (DCE and VC) were detected at significantly lower concentrations. A new technology involving a recirculating well (IEG-GCW) combined with nutrient addiction was implemented at the site, with the goal of promoting biological dechlorinating activity and

abating concentrations of organochlorine compounds in the aquifer. Four multiple injection wells (MIWs) were realized to introduce nutrients into the subsurface. An IEG-GCW, 12 m deep and equipped with 2 fenestrated sections, was placed in the center of the MIWs. Groundwater extraction and re-injection at the two different filter sections of the multifenerstrate well induces the generation of ellipsoidal groundwater recirculation cells. Two multilevel monitoring wells (MLSW) allowed to collect undisturbed water samples to monitor the remediation process to capture hydrochemical peculiarities at different depth intervals along the vertical. The realization of the MIW, MLSW, and GCW was preceded by the realization of stratigraphic surveys, to adapt the construction characteristics of wells with different configuration to the site-specific geological peculiarities. A composite geodatabases and a multi-source model act as a tool to collect, merge and interpret multi-modality information during remediation remediation phasesl. The development of a 3D integrated hydrogeological model and the extraction of the geo-referenced information contained therein allowed to tailor the remediation technology to site-specific peculiarities. Hydrochemical monitoring of multilevel sampling wells reveals the mobilization of secondary contamination sources induced by recirculation with different configurations and the stimulation of biological activity following nutrient injection via MIWs. The multi-source model orients the location, configuration, and deployment of an advanced remediation strategy that is tailored to physicochemical conditions. Flow simulations provide valuable predictions of groundwater circulation flow. The IEG-GCW acts as a 3D distributor of nutrients in the aquifer. The findings demonstrate the stimulation of the dechlorinating biological activity and the abatement of chlorinated solvent concentrations in groundwater. The coupling of C-MIX and the GCW system exhibited a rapid decline of CAHs concentrations at different aquifer levels.

ABSTRACT BOOK

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