

Supplementary Information

Effectiveness of Permeable Reactive Bio-Barriers for Bioremediation of an Organohalide-Polluted Aquifer by Natural-Occurring Microbial Community

Martina Bertolini¹, Sarah Zecchin¹, Giovanni Pietro Beretta², Patrizia De Nisi³, Laura Ferrari⁴ and Lucia Cavalca^{1,*}

¹ Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS), Università degli Studi di Milano Via Celoria 2, I-20133 Milano, Italy; martina.bertolini@unimi.it (M.B.); sarah.zecchin@unimi.it (S.Z.)

² Dipartimento di Scienze della Terra “Ardito Desio”, Università degli Studi di Milano Via Mangiagalli 34, I-20133 Milano, Italy; giovanni.beretta@unimi.it

³ Dipartimento di Scienze Agrarie e Ambientali (DISAA), Università degli Studi di Milano Via Celoria 2, I-20133 Milano, Italy; patrizia.denisi@unimi.it

⁴ TAUW Italia, I-20133 Milano, Italy; l.ferrari@tauw.com

* Correspondence: lucia.cavalca@unimi.it

1. Supplementary materials and methods

Amplification of bacterial 16S rRNA genes

Full-length bacterial 16S rRNA gene PCR amplification was carried out in a 25 µL reaction mixture with primers 27F-1492R (Table S1) 0.3 µM and 1x Taq PCR Master Mix (QIAGEN, Hilden, Germany) with the following thermal protocol: 95°C for 5 min followed by 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 40s, and elongation at 72°C for 1 min and 40s; the final elongation was performed at 72°C for 10 min.

Table S1. List of primers used in this study.

Target gene	Primers	Sequences	Product size (bp)	References
Bacterial 16S rRNA	Eub338f	ACT CCT ACG GGA GGC AGC AG	180	[1]
	Eub518r	ATT ACC GCG GCT GCT GG		
	27f	AGAGTTTGATCMTCTGCTTC	1,465	[2,3]
Archaeal 16S rRNA	1492r	TACGGYTACCTTGTAGGCTT		
	Arc787F	ATT AGA TAC CCS BGT AGT CC	272	[4]
Geobacteraceae 16S rRNA	Arc1059R	GCC ATG CAC CWC CTC T		
	Geo546F	AAGCGTTGTCGGAWTTAT	294	[5,6]
	Geo840R	GGCACTGCAGGGGTCAATA		
Dehalococcoides 16S rRNA	Dhc1154f	CAC ACA CGC TAC AAT GGA CAG AAC		
	Dhc1286r	GAT ATG CGG TTA CTA GCA ACT CCA AC	132	[7]
<i>tceA</i>	TceA1270F	ATC CAG ATT ATG ACC CTG GTG AA		
	TceA1336R	GCG GCA TAT ATT AGG GCA TCT T	66	[8]
<i>vcrA</i>	Vcr1022F	CGG GCG GAT GCA CTA TTT T		
	Vcr1093R	GAA TAG TCC GTG CCC TTC CTC	71	[9]

In order to obtain a standard DNA for qPCR quantification of Eubacteria, 16S rRNA genes amplified with primer 27F-1492R (Table S1) were cloned in pCR™2.1-TOPO® vector using the TOPO®TA Cloning® Kit (Invitrogen, Massachusetts, US) according to manufacturer's protocol. The transformed plasmids were isolated with UltraClean™ 6 min Mini Plasmid Prep Kit™ (MO BIO, Laboratories, Inc., Carlsbad, CA, United States) and quantified spectrophotometrically at 260/280 nm wavelength.

Table S2. Plasmids used to set up standard curves for quantification of genetic target through qPCR.

Source organism	Name of the clone	Vector (plasmid)	Target	Reference
<i>Dehalococcoides ribotype BTF08</i>	BTF08-1492 a.		Dhc	[10]
<i>Geobacter lovleyi strain SZ</i>	16S-Geo a.		Geo	[11]
<i>Methanobacterium formicum</i>	b.		Arch	[12]
<i>Dehalococcoides ribotype BTF08</i>	vcrA-BTF08 a.	pGEM-T Easy	vcrA	[13]
<i>Dehalococcoides ethenogenes</i> 195	tceA-195 a.		tceA	[10]
				[11]
				[13]

Clone provided by:

a.Dr. Ivonne Nijenhuis, Department of Isotope Biogeochemistry of the Helmholtz Center of Environmental Research (UFZ) in Leipzig, Germany

b.Rago et al., 2015

2. Supplementary Results

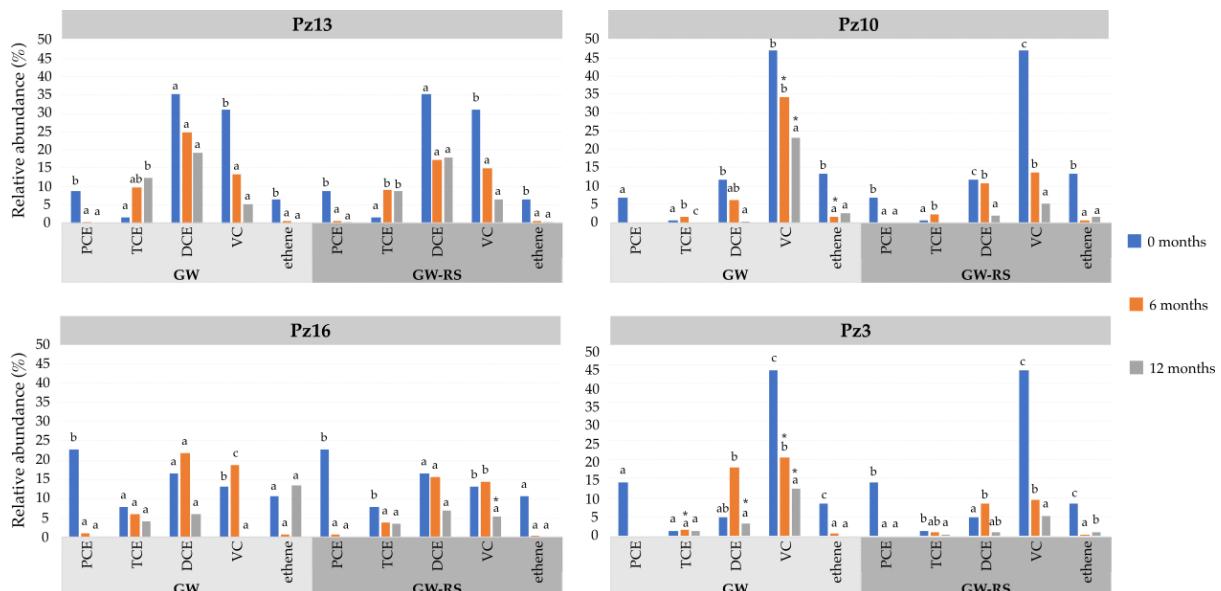


Figure S1. Relative concentrations of CE measured in the microcosms without (GW) and with (GW-RS) substrate addition over time. Lowercase letters indicate statistically significant differences over time (ANOVA and Tukey's test, $p \leq 0.05$). Stars indicate statistically significant differences between different treatments (GW vs GW-RS).

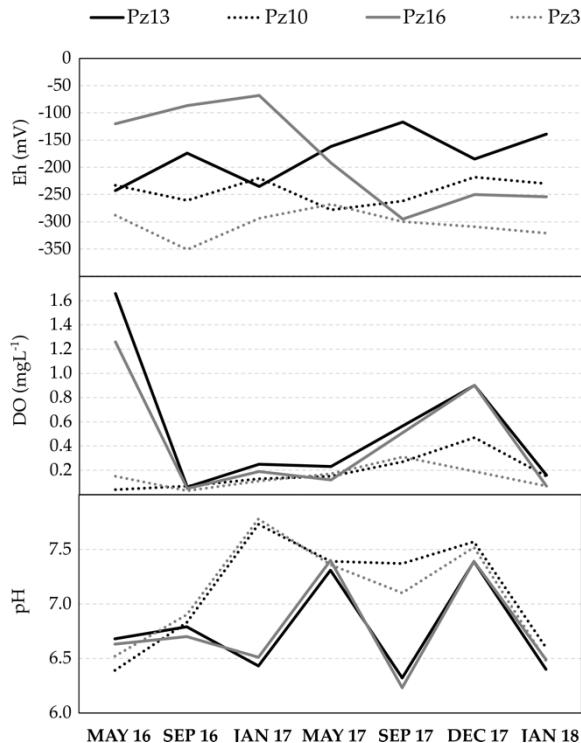


Figure S2. Variation of Eh, DO and pH over the in situ pilot scale experiment.

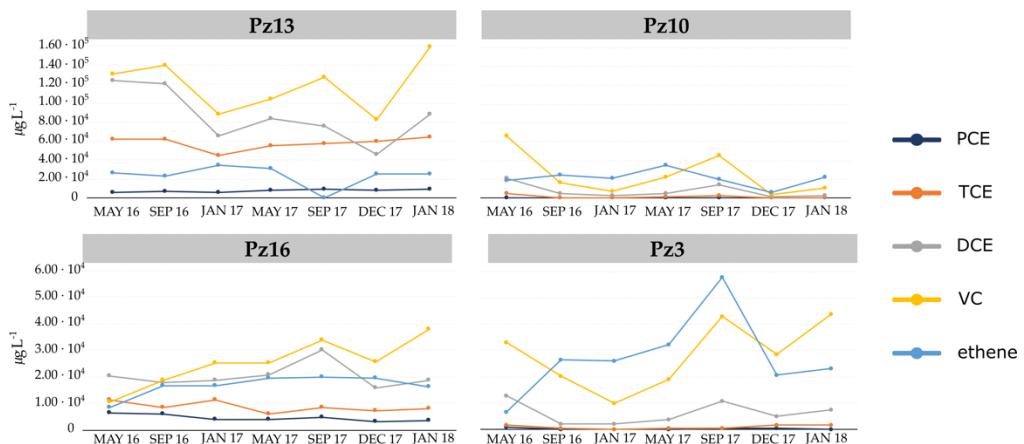


Figure S3. Chloroethenes concentrations over the pilot scale experiment in the piezometers Pz13, Pz10, Pz16 and Pz3.

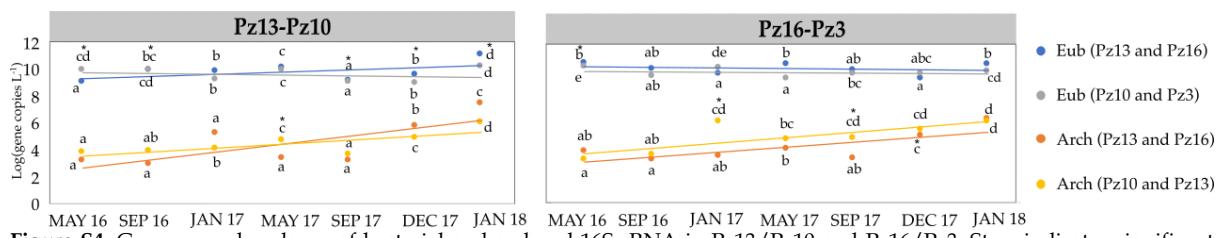


Figure S4. Gene copy abundance of bacterial and archaeal 16S rRNA in Pz13/Pz10 and Pz16/Pz3. Stars indicates significant difference between piezometers of transect up-stream and downstream injection wells of anaerobic bio-barrier (t test, $\rho \leq 0.05$). Lowercase letters indicate significant or not significant difference between different time (Tukey's test, $\rho \leq 0.05$)

References

1. Battelle Memorial Institute. *Permeable Reactive Barrier Cost and Performance Report*; NAVFAC: Washington, DC, USA, 2012.
2. Edwards, U.; Rogall, T.; Blöcker, H.; Emde, M.; Böttger, E.C. Isolation and direct complete nucleotide determination of entire genes. Characterization of a gene coding for 16S ribosomal RNA. *Nucleic Acids Res.* **1989**, *17*, 7843–7853.
3. Lane, D.J. 16S/23S rRNA sequencing. In *Nucleic Acid Techniques in Bacterial Systematics*; Stackebrandt, E., Goodfellow, M., Ed.; John Wiley & Sons: New York, NY, USA, 1991; p. 115–175.
4. Yu, Y.; Lee, C.; Kim, J.; Hwang, S. Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. *Biotechnol. Bioeng.* **2005**, *89*, 670.
5. Cummings, D.; Snoeyenbos-West, O.; Newby, D.; Niggemeyer, A.; Lovley, D.; Achenbach, L.; Rosenzwieg, R. Diversity of Geobacteraceae species inhabiting metal-polluted freshwater lake sediments ascertained by 16S rDNA analyses. *Microb. Ecol.* **2003**, *46*, 257–269.
6. Sandford, R.; Wu, Q.; Sung, Y.; Thomas, S.; Amos, B.; Prince, E.; Löffler, F. Hexavalent uranium supports growth of *Anaeromyxobacter dehalogenans* and *Geobacter* spp. with lower than predicted biomass yields. *Env. Microbiol.* **2007**, *9*, 2885–2893.
7. Krzmarzick, M.; Crary, B.; Harding, J.; Oyerinde, O.; Leri, A.; Myneni, S.; Novak, P. Natural niche for organohalide-respiring Chloroflexi. *Appl. Environ. Microbiol.* **2012**, *78*, 393–401.
8. Johnson, D.; Lee, P.; Holmes, V.; Fortin, A.; Alvarez-Cohen, L. Transcriptional expression of the tceA gene in a *Dehalococcoides* containing microbial enrichment. *Appl. Environ. Microbiol.* **2005**, *71*, 7145–7151.
9. Ritalahti, K.; Amos, B.; Sung, Y.; Wu, Q.; Koenigsberg, S.; Löffler, F. Quantitative PCR targeting 16S rRNA and reductive dehalogenase genes simultaneously monitors multiple *Dehalococcoides* strains. *Appl. Env. Microbiol.* **2006**, *72*, 2765–2774.
10. Maymo-Gallett, X.; Chien, Y.; Gossett, J.M.; Zinder, S.H. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. *Science* **1997**, *276*, 1568–1571.
11. Pöritz, M.; Goris, T.; Wubet, T.; Tarkka, M.T.; Buscot, F.; Nijenhuis, I.; Lechner, U.; Adrian, L. Genome sequences of two dehalogenation specialists—*Dehalococcoides mccartyi* strains BTF08 and DCMB5 enriched from the highly polluted Bitterfeld region. *Fems Microbiol. Lett.* **2013**, *343*, 101–104, doi:10.1111/1574–6968.12160.
12. Kaufhold, T.; Schmidt, M.; Cichocka, D.; Nikolausz, M.; Nijenhuis, I. Dehalogenation of diverse halogenated substrates by a highly enriched *Dehalococcoides*-containing culture derived from the contaminated mega-site in Bitterfeld. *FEMS Microbiol. Ecol.* **2012**, *83*, 176–188.
13. Rago, L.; Ruiz, Y.; Baeza, J.A.; Guisasola, A.; Cortés, P. Microbial community analysis in a long-term membrane-less microbial electrolysis cellwith hydrogen and methane production. *Bioelectrochemistry* **2015**, *106*, 359–368.