Organohalide respiration potential in marine sediments from Aarhus Bay

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One sentence summary: Eco-physiological characterization of organohalide respiration from Aarhus Bay marine sediments.

Abstract

Organohalide respiration (OHR), catalysed by reductive dehalogenases (RDases), plays an important role in halogen cycling. Natural organohalides and putative RDase-encoding genes have been reported in Aarhus Bay sediments, however, OHR has not been experimentally verified. Here we show that sediments of Aarhus Bay can dehalogenate a range of organohalides, and different organohalides differentially affected microbial community compositions. PCE-dechlorinating cultures were further examined by 16S rRNA gene-targeted quantitative PCR and amplicon sequencing. Known organohalide-respiring bacteria (OHRB) including Dehalococcoides, Dehalobacter and Desulfotobacterium decreased in abundance during transfers and serial dilutions, suggesting the importance of yet uncharacterized OHRB in these cultures. Switching from PCE to 2,6-DBP led to its complete debromination to phenol in cultures with and without sulfate. 2,6-DBP debrominating cultures differed in microbial composition from PCE-dechlorinating cultures. Desulfobacterota genera recently verified to include OHRB, including Desulfovibrio and Desulfuromusa, were enriched in all microcosms, whereas Halodesulfovibrio was only enriched in cultures without sulfate. Hydrogen and methane were detected in cultures without sulfate. Hydrogen likely served as electron donor for OHR and methanogenesis. This study shows that OHR can occur in marine environments mediated by yet unknown OHRB, suggesting their role in natural halogen cycling.

Keywords: Aarhus Bay marine sediments, tetrachloroethene (PCE), 2,6-Dibromophenol (2,6-DBP), reductive dehalogenation, organohalide respiring prokaryotes, 16S rRNA gene amplicon sequencing

Introduction

Halogenated organic compounds, also termed organohalides, can be man-made or of natural origin. Over 5000 organohalides with a natural origin have been reported (Gribble 1996, 2012, 2015). Marine environments are a large reservoir of natural organohalides, which are produced via biotic and abiotic mechanisms (Gribble 1996, 2012, 2015). Marine organisms such as algae, sponges, corals and microorganisms recruit either substrate-specific halogenases or haloperoxidases to catalyse the biotic formation of organohalides and putative RDase-encoding genes have been reported in Aarhus Bay sediments, however, OHR has not been experimentally verified. Here we show that sediments of Aarhus Bay can dehalogenate a range of organohalides, and different organohalides differentially affected microbial community compositions. PCE-dechlorinating cultures were further examined by 16S rRNA gene-targeted quantitative PCR and amplicon sequencing. Known organohalide-respiring bacteria (OHRB) including Dehalococcoides, Dehalobacter and Desulfotobacterium decreased in abundance during transfers and serial dilutions, suggesting the importance of yet uncharacterized OHRB in these cultures. Switching from PCE to 2,6-DBP led to its complete debromination to phenol in cultures with and without sulfate. 2,6-DBP debrominating cultures differed in microbial composition from PCE-dechlorinating cultures. Desulfobacterota genera recently verified to include OHRB, including Desulfovibrio and Desulfuromusa, were enriched in all microcosms, whereas Halodesulfovibrio was only enriched in cultures without sulfate. Hydrogen and methane were detected in cultures without sulfate. Hydrogen likely served as electron donor for OHR and methanogenesis. This study shows that OHR can occur in marine environments mediated by yet unknown OHRB, suggesting their role in natural halogen cycling.

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Halides are replaced by hydrogen through reductive dehalogenation that often is linked to a specific type of anaerobic respiratory metabolism termed organohalide respiration (OHR). In this process, organohalides serve as the terminal electron acceptors to conserve energy (Dolfin and Tiedje 1987, Mohn and Kennedy 1992, Smidt and De Vos 2004). This process has been documented in bacteria known as organohalide-respiring bacteria (OHRB) (Atashgahi et al. 2016, Fincker and Spormann 2017, Smidt and De Vos 2004). OHR is mediated by reductive dehalogenase (RDase) enzymes (Adrian and Loeffler 2016, Fincker and Spormann 2017). Since the isolation of the first OHRB, Desulfomonile tiedjei (Dolfin and Tiedje 1987, Mohn and Tiedje 1990), a broad diversity of OHRB have been isolated, belonging to three phyla: Chloroflexi, Firmicutes, and Proteobacteria (Atashgahi et al. 2016, Fincker and Spormann 2017, Türkowsky et al. 2018). Members of the genera Dehalococcoides (Dhc) and Dehalogenimonas (Dhg) within the Chloroflexi, and Dehalobacter (Dhb) in the Firmicutes are obligate OHRB based on their restriction to OHR as the sole energy metabolism (Fincker and Spormann 2017, Holscher et al. 2004, Maillard et al. 2003, Moe et al. 2009, Molenda et al. 2016, Muller et al. 2004). Members of Desulfotobacterium (Dsb) in Firmicutes, Sulfurosprin...
illumin (Sul) in Epsilonproteobacteria and various Desulfo bacterota (previously Deltaproteobacteria) species including members of Geobacter (Geo), Desulfolina and Desulfovibrio are considered as facultative OHRB that have a versatile metabolisml including but not restricted to OHR (Liu and Haggblom 2018, Maphosa et al. 2010, Peng et al. 2020a). Intriguingly, up to 10% of all available Desulfo bacterota genomes were found to contain at least one putative RDase gene (Liu and Haggblom 2018). Accordingly, a recent genome-guided study experimentally verified OHR potential in three Desulfo bacterota isolates (Liu and Haggblom 2018).

Using degenerate primers based on sequences of well-characterized RDase genes, different RDase genes have been reported from subsea sediments of the Pacific Ocean close to Peru, Japan, Oregon (United States) and the eastern equator, that were diverse and phylogenetically distinct from their characterised counterparts from contaminated terrestrial environments (Futagami et al. 2009, Futagami et al. 2013). Application of various techniques such as (meta)genomics and (meta)transcriptomics have revealed an enormous diversity of RDase genes in marine sediments (Jochum et al. 2017, Jochum et al. 2018, Petro et al. 2019a, Petro et al. 2019b, Zinke et al. 2017). For instance, meta-transcriptomic analysis of deep subsea sediments from site M59, close to Aarhus Bay, in the Baltic Sea led to the discovery of a trichloroethene (TCE) reductive dehalogenase (tceA)-like gene that was transcribed, indicating the potential for PCE or TCE dechlorination in deep sea sediments as previously described for other environments (Fung et al. 2007, Magnuson et al. 1998, Zinke et al. 2017). Recently, single-cell genomics and metagenomics analyses revealed the presence of RDase genes in sulfate-rich sediments of Aarhus Bay (Fincker et al. 2020, Jochum et al. 2018). This begged the question whether there is actual OHR potential in marine sediments of Aarhus Bay that may prevent accumulation of organohalides and contribute to halide and CO2 recycling back to the sea. It is interesting to note that sulfate reduction has been reported to be the predominant bioprocess in near-surface Aarhus Bay marine sediments (Leloup et al. 2009, Ozoulmez et al. 2020, Petro et al. 2019b), in which reductive dehalogenation is likely to be inhibited by the produced and accumulated sulfide according to previous reports (Azizian et al. 2010b, DeWeerd et al. 1991, Mao et al. 2017, Nelson et al. 2002). Henceforth, taking the presence and absence of sulfate into consideration would add more insights to explore OHR potential in laboratory microcosms.

In this study, we showed that cultures obtained from marine sediments from Aarhus Bay were capable of dechlorinating, debrinating and deiodinating organohalides, further corroborating above-mentioned (metagenome based studies with respect to the predicted occurrence of OHR in marine sediments. Interestingly, the microbial communities in reductively dechlorinating and debrinating enrichment cultures were divergent, suggesting the coexistence of diverse OHRB. Furthermore, enriched microorganisms did not belong to the well-identified OHRB, suggesting the presence of novel dehalogenators in these pristine marine environments.

Materials and Methods

Chemicals

PCE, TCE, cDCE, trans-dichloroethene (tDCE) vinyl chloride (VC), ethene (ETH), 2,4,6-trichlorophenol (2,4,6-TCP), 2,6-dichlorophenol (2,6-DCP), 2,4- dichlorophenol (2,4-DCP), chlorophenol (CP), 2,6-DBP, 3-bromophenol (3-BP), 2-bromophenol (2-BP), 2,4,6-triiodophenol (2,4,6-TIP), 2,6-diiodophenol (2,6-DIP), 2,4-diiodophenol (2,4-DIP), 2-iodophenol (2-IP) and 4-iodophenol (4-IP), phenol, 1,4-dibromobenzene (1,4-DBB), bromobenzene (BB), and benzene were purchased from Sigma-Aldrich. Sulfate (0.5 M) and lactate (0.5 M) stock solutions were prepared by filter sterilization (syringe filter, 0.2 μm, mdimembrane, Ambala Cantt, India). All other (in)organic chemicals were of analytical grade.

Sediment collection and enrichment set-up

Samples were kindly provided by Kasper U. Kjeldsen (Aarhus University). Specifically, samples were collected from a marine sediment core at station M5 in Aarhus Bay (56.103333 N, longitude 10.457933 E), Denmark, and were depth-fractioned into two parts: 3–35 cm and 36–68 cm. Ten grams of sediment from each fraction was transferred into 120 mL serum bottles containing 50 mL anoxic marine medium as previously described (Monserrate and Haggblom 1997, Peng et al. 2020a). Na2S·9H2O (0.48 g/L, 2 mM) was added as reducing reagent and Resazurin (0.005 g/L) as redox indicator. The bottle headspace was exchanged with N2 and CO2 (80 : 20%, 140 KPa), bottles were sealed with Teflon-coated butyl rubber septa and aluminum crimp caps (GRACE, MD, USA) and incubated statically in the dark at 20°C. Some organohalides were added to the marine medium separately and tested, including PCE, 2,6-DBP, 1,4-DBB, 3-BP, 2,4,6-TCP, and 2,4,6-TIP with/without additional SmM sulfate and SmM lactate as the electron donor. Subsequently, experiments focused first on more detailed characterization of PCE dechlorination. The dechlorinating cultures were spiked with PCE (100 μM) as electron acceptor and lactate (5 mM) serving as the electron donor and carbon source. One set of bottles received sulfate (5 mM) as an additional electron acceptor (designated “S”; bottles with only PCE were designated “NS”) (Fig. 1). Two transfers were set as the initial step to enrich for PCE dechlorinating microorganisms. Cultures were transferred when the PCE was reductively dechlorinated to cDCE. For each transfer, 10% (v/v) of the mother cultures was transferred to fresh medium (Fig. 1), with cultures being designated PCE.NS.T1 and PCE.S.T1 for first transfers, and PCE.NS.T2 and PCE.S.T2 indicating the second transfer. Second transfers were subsequently 10-fold serially diluted (SD) according to the dilution-to-extinction principle (NS.SD1, S.SD1, Fig. 1). Highest dilutions for which dehalogenation was observed (NS.SD13, S.SD12, indicated by blue boxes in Fig. 1) were used as inocula for a second series of 10-fold serial dilutions (NS.SD2, S.SD2, Fig. 1). Highest dilutions with active dehalogenation (NS.SD24, S.SD24, Fig. 1) after four spikes of 250 μM PCE and corresponding chemicals (5 mM lactate in NS, 5 mM lactate and 5 mM sulfate in S cultures) in the second serial dilution were used to inoculate a new set of transfers (NS.T1 and S.T1, Fig. 1). These cultures were transferred once more to obtain duplicate sediment-free enrichment cultures (PCE.NS.T2.A/B and PCE.S.T2.A/B, Fig. 1). In addition to dechlorination of the aliphatic organohalide PCE, the potential to dehalogenate aromatic organohalides was also investigated. To this end, 2,6-DBP and 2,6-DCP were selected as representative electron acceptors and injected into fresh cultures separately, to which microbes were transferred from sediment-free PCE dechlorinating enrichments (PCE.NS.T2.A and PCE.S.T2.A). In order to obtain a comprehensive overview of PCE dechlorination and 2,6-DBP dechlorination potential under both sulfate-free and sulfate-amended conditions, the test cultures (PCE and DBP) were further transferred to duplicate cultures (NS.PCE.A/B, NS.DBP.A/B, S.PCE.A/B and S.DBP/C/D). Due to the lack of dechlorination from 2,6-DBP under sulfate-amended conditions (S.DBP/C/D), a new set of duplicate cultures (S.DBP.A/B) was inoculated from the PCE cul-
Figure 1. Experimental outline of reductive dehalogenation by marine sediments from Aarhus Bay M5 station. This experiment was carried out in 5 steps as labelled by (1), (2), (3), (4) and (5). “Sediments” stands for samples from near-surface marine sediments (3-35cm) of Aarhus Bay. Transfer volumes are 10% (v/v) in steps (1), (2), and (3), and 5% (v/v) in steps (4) and (5). Curved arrows in step (3) indicate the transfer pattern in 10 fold dilutions. NS: sulfate-free; S in red: sulfate-amended; Blue boxes in serial dilutions indicate the highest dilution at which dechlorination of PCE to cDCE was observed. Consequently, these are also the cultures that were used for the subsequent transfer; Bold cross marks indicate cultures that were unable to dehalogenate DCP or DBP.

ture (Fig. 1). During the 5th part of the experiment “Dechlorination and Debromination”, duplicates of dechlorinating cultures were spiked five times with PCE (250 μM) and in the same way, debrominating cultures were also spiked five times with 2,6-DBP (100 μM). For the duplicate 2,6-DBP debrominating cultures, additional lactate (5 mM) and lactate/sulfate (5 mM) were also injected into the cultures from the second spike, based on the assumption that debromination was halted due to depletion of lactate. Before each spike, hydrogen and methane were measured in headspace samples, and 2 ml culture was sampled and centrifuged for 5 min at 8000 g. Supernatants were used for metabolite measurements, whereas pellets were used for DNA extraction.

Chemical analyses
Gas chromatography combined with mass spectrometry (GC-MS) was used to measure PCE, TCE and cDCE using an Rt®-Q-BOND column (Retek, PA, USA) and a DSQ MS (Thermo Fisher Scientific). Helium served as carrier gas with a flow rate of 2 ml/min. The split ratio was 30 and the inlet temperature was 100°C. The temperature program included 40°C holding for 1 min, followed by an increase by 40°C/min to 260°C and a final hold at this temperature for 1.5 min. Hydrogen and methane were detected by a Compact GC (Global Analyzer Solutions, Breda, The Netherlands) with a pulsed discharge ionization detector (GC-PDD). Halogenated phenols, benzenes, benzene and phenol were measured using a Thermo Scientific Accela High-performance liquid chromatography (HPLC) system equipped with an Agilent Poroshell 120 EC-C18 column and a UV/Vis detector (set at 210 nM for aromatic halogenated compounds). Short chain fatty acids were measured using a SHIMADZU LC2030 PLUS coupled with a Shodex SUGAR Series® SH1821 column. Sulfate was analysed by using a Thermo Scientific Dionex™ ICS-2100 Ion Chromatography System (Dionex ICS-2100). Sulfide was measured by a photometric method using methylene blue as described previously (Cline 1969).

Genomic DNA isolation and quantitative PCR (qPCR)
Genomic DNA was isolated from pelleted biomass using the DNeasy PowerSoil Kit (QIAGEN, Hilden, Germany) following manufacturer’s instructions. qPCR was used to quantify the copy number of 16S rRNA genes with primers targeting total bacteria (Muyzer et al. 1993), and well-characterized OHRB, including Dehalococcoides (Smits et al. 2004), Dehalogenimonas (Chen et al. 2013), Dehalobacter (Smits et al. 2004), Desulfitobacterium (Smits et al. 2004), Geobacter (Amos et al. 2007) and Sulfurospirillum (Sutton et al. 2015). Furthermore, primers targeting well-characterized functional RDase genes tceA, vcrA, and bvcA were used as listed in Table S1. All reactions were performed in triplicate using a C1000 Thermal Cycler (CFX384 Real-Time system, Bio-Rad Laboratories, Hercules, CA, USA) with iQ™ SYBR Green Supermix (Bio-Rad Laboratories, Hercules, CA, USA) as outlined previously (Peng et al. 2019).

Analysis of microbial composition based on 16S rRNA gene amplicon sequences
Barcoded amplicons of 16S rRNA genes were amplified targeting the V4 region of prokaryotic 16S rRNA genes. The forward primer 515F (5’-GTCGCCAGCMGCCGCGGTAA-3’) and reverse primer 806R (5’-GGACTACACCTGAGGTTATCTAAT-3’) (Caporaso et al. 2011, Walters et al. 2016) were amended at the 5’-end with sample-specific barcodes. The PCR mixture (50 μL) was prepared containing 20 μL 5 x HF Green buffer (Thermo Fisher Scientific, the Netherlands), 1 μL (2 U) of Phusion hot start II High-Fidelity DNA polymerase (Thermo Fisher Scientific), primer mix (500 nM...
for each forward and reverse primer), and 500 nM dNTP (Promega, USA), 10 ng DNA template and nuclease-free water (Promega, USA). The PCR conditions were 98°C, 30 s for pre-denaturation, followed by 25 cycles of 98°C, 10 s for denaturation, annealing at 50°C for 10 s, elongation at 72°C for 10 s, and a final extension at 72°C for 7 min. Three µl of PCR product was analysed by electrophoresis on a 1% (w/v) agarose gel. All samples were amplified in duplicate reactions, and duplicate PCR products were pooled and purified using CleanPCR (cleanNA, the Netherlands) according to the manufacturer’s instructions. The DNA concentration of the purified amplicons was measured by Qubit (Thermo Fisher Scientific). The purified amplicons were pooled in equimolar amounts, including PCR products prepared from synthetic Mock communities of known composition as positive control and nuclease-free water as negative control, and sent for sequencing by HiSeq2000 (GATC-Biotech GmbH, now part of Eurofins Genomics Germany GmbH, Konstanz, Germany).

Analysis of microbial community diversity

The raw sequence data was analysed by NG-Tax 2.0 (Poncheewin et al. 2019, Ramiro-García et al. 2016), which consists of three core processes: barcode-primer filtering, amplicon sequencing variants (ASV) picking and taxonomic assignment. Only the reads completely pairing with primers and barcodes were retained. A threshold of 0.1% relative abundance was used on a per-sample basis to prevent the inclusion of spurious ASVs produced by sequencing and PCR errors. Taxonomic assignment was done based on SILVA 132 SSU Ref (Edgar 2010, Yilmaz et al. 2004). The generated BIOM (Biological Observation Matrix) and tree files were further organised to form phyloseq objects (McMurdie and Holmes 2013). Downstream analyses, including alpha and beta diversity, and microbial composition were performed by Microbiome and Phyloseq R packages (Lahti and Shetty 2017, McMurdie and Holmes 2013).

Statistical Analysis

Statistical analyses and graphics were performed in R (Team 2013) and the built-in libraries ggplot2, tidyrverse, ggpubr and vegan. For microbial diversity analysis, alpha diversity was analysed using Phylogenetic diversity. Observed, Chao1, Shannon and Inverse Simpson indices. Student’s t-test was used to assess significance of observed differences in alpha diversity indices. The function adonis as implemented in vegan (Oksanen et al. 2007) was used for permutational multivariate analysis of variance (PERMANOVA) to assess significance of observed differences in beta diversity based on unifrac distances (Lozupone and Knight 2005, Lozupone et al. 2011), in which the number of permutations was set to 999 by default. In addition, permutation tests for significance in CAPSCALE were also applied following the default settings.

Results

Reductive dechlorination of PCE in Aarhus Bay sediments

Samples from a sediment core taken in Aarhus Bay and depth-fractionated were incubated in mineral marine medium with a range of organohalides separately in the presence (S) and absence (NS) of sulfate (Table 1). Our results revealed the dehalogenating potential of Aarhus Bay sediments for various chlorinated, brominated and iodonated compounds. The microbial composition of PCE dechlorinating cultures exhibited evident differences to cultures to which other organohalides had been added (Fig. S1). Considering the discovery of tceA-like transcripts in sediments of Aarhus Bay (Zinke et al. 2017), PCE dechlorination was initially selected for subsequent experiments to demonstrate OHR in Aarhus Bay sediments, followed up by experiments with 2,6-DBP.

Dechlorination of PCE to cDCE was observed with TCE as intermediate, within 15 days after inoculation with sediment obtained from Aarhus Bay (Fig. 2A). After two transfers and two consecutive serial dilution series, the obtained enrichments still actively dechlorinated PCE to cDCE (Fig. 1B). Thereinto, the most diluted dechlorinating cultures (the fourth cultures in the second serial dilution (NS/S:SD24)) were selected for two additional transfers aiming to enrich the responsible dechlorinating consortium. In the second transfer, cultures incubated in the presence (S) or absence (NS) of sulfate stably dechlorinated PCE to CDCE after three spikes of PCE (Fig. 2C). The overall experimental workflow is shown in Fig. 1, and results of transfers and serial dilutions are provided in Fig. S2 and Fig. S3, respectively.

Detection of known and putative OHRB by quantitative PCR (qPCR) and 16S rRNA gene amplicon sequencing

qPCR analyses revealed the existence of well-known OHRB in the original sample, including Dhc, Dhb, Dwb and Sul ranging from 1.2 (0.8 SD) × 10^8 to 1.5 (0.1 SD) × 10^9 of Geo per gram (Fig. 2A), which accounted for less than 1% of the total 16S rRNA gene copy numbers (9.2 (1.6 SD) × 10^9). Based on 16S rRNA gene amplicon sequence data, relative abundances of these OHRB together were less than 2%, in line with the qPCR results. The 16S rRNA gene copy numbers and relative abundances of these well-characterized OHRB decreased during the enrichment procedure. In particular, Dwb was undetectable after the first serial dilution (Fig. 3A). For all samples, including original sediment fractions and derived cultures, the 16S rRNA gene of Dekalogenimonas (Dhy) and well-characterized RDase genes vcrA, hucA and tceA were below the detection limit. Apparently, the known OHRB were outcompeted by other indigenous dehalogenators during PCE dechlorination. In contrast, the microbial community analysis indicated that certain bacteria, including Halodesulfovibrio in sulfate-free cultures, and Desulfovibrio in sulfate-amended cultures were enriched up to relative abundances of 16.0 (2.3 SD) % and 30.3 (1.7 SD) %, respectively, in Tr1.2.A/B cultures (Fig. 3B). Representative strains of both genera were recently shown to debrominate 2,6-DBP to phenol (Liu and Haggblom 2018). We therefore hypothesized that our enrichments might also possess the ability to debrominate 2,6-DBP to phenol, in line with results of the initial screening (Table 1).

Switching the electron acceptor from PCE to 2,6-DBP

As mentioned above, debromination of 2,6-DBP was observed in the initial screening of the dehalogenation potential of Aarhus Bay sediment. Moreover, members of enriched genera were previously reported to have debromination potential as outlined above. Thus, we tested whether the PCE dechlorinating enrichments retained the capacity of the original sediment for reductive debromination. Indeed, PCE-dechlorinating enrichments debrominated 2,6-DBP under both sulfate-free and sulfate-amended conditions. In contrast, the cultures failed to dechlorinate 2,6-DCP (Fig. 1), which was in agreement with the initial screening (Table 1). More detailed analyses of duplicate cultures revealed that 2,6-DBP was debrominated to phenol with bromophenol as intermediate (Fig. 4A). Five spikes of 100 µM 2,6-DBP each were introduced into the cul-
Table 1. List of halogenated compounds for testing reductive dehalogenation of Aarhus Bay marine sediments

| Aarhus Bay Sediments | Station | Halogenated Compounds | Sulfate Addition | Products |
|----------------------|---------|-----------------------|-----------------|----------|
| 3–35 cm / 36–68 cm   | M5      | Tetrachloroethane (PCE) | + / −           | Trichloroethene (TCE); Cis-dichloroethene (cDCE); Phenol; |
| 3–35 cm / 36–68 cm   | M5      | 2,6-Dibromophenol (2,6-DBP) | + / −           | Bromophenol (BP); Phenol; |
| 3–35 cm              | M5      | 1,4-Dibromobenzene (1,4-DBB) | + / −           | Bromobenzene (BB); Phenol; |
| 3–35 cm              | M5      | 3-Bromophenol (3-BP) | + / −           | Phenol; |
| 3–35 cm              | M5      | 2,4,6-Trichlorophenol (2,4,6-TCP) | + / −           | ND; |
| 3–35 cm              | M5      | 2,4,6-Triodophenol (2,4,6-TIP) | + / −           | 4-Iodophenol (4-IP); Phenol; |

a Different depths of Aarhus Bay marine sediments from one core.
b Cultures incubated with (+) or without (−) sulfate.
c Transformation products detected under both sulfate (+ / −) conditions.
d ND means no dehalogenation.
e Intermediate metabolites.
f Final product after 100% disappearance of the original compound.
g Indicates coexistence of metabolites 4-IP and phenol in a ratio around 1:2.

Figure 2. PCE dechlorination to cDCE in initial microcosms (A), serial dilutions (B) and further transfers (C) in the presence (S) and absence (NS) of sulfate. SD.24:the fourth (i.e. 10–4 dilution) cultures in the second serial dilution in step 3 (Figure 1); Tr2: the second transfers in Step 4 (Figure 1). See also Figure 1 for a detailed scheme of the experimental set-up and history and relatedness of the different cultures. Vertical arrows indicate spikes of PCE. Data shown are average values, and error bars represent the standard deviation of the duplicates in Tr2. Error bars are not always visible due to small standard deviations.

Hydrogen was produced and accumulated up to 29.53 μM after 10 days in sulfate-free cultures after the first spike of 2,6-DBP (Fig. 4B). Then hydrogen was consumed alongside debromination, while methane accumulated after five spikes of 2,6-DBP up to 1.3 mM. In contrast, hydrogen and methane were not detected under sulfate-amended conditions (Fig. 4B). Lactate was utilized with the formation of propionate and acetate at an approximate ratio of 3:2.5 in sulfate-free cultures (Fig. S4A), whereas only acetate was produced and further utilized in sulfate-amended cultures (Fig. S4B). Similarly, hydrogen and methane were obviously produced in sulfate-free PCE-dechlorinating environments after the first 250 μM PCE spike, with particularly rapid hydrogen formation up to 245.13 μM (Fig. 4D). Notably, hydrogen and methane were produced in sulfate-amended cultures up to a detectable level after the third PCE spike, but only at concentrations below 30 μM. Lactate degradation followed a similar trend in PCE dechlorinating cultures as in the 2,6-DBP incubations (Fig. S4C&D). In all cultures where sulfate was added, this was reduced to sulfide.

Microbial diversity and phylum-level composition of dehalogenating enrichments

To assess the microbial community structure in the different cultures, and particularly to compare PCE- and 2,6-DBP dehalogenating cultures, 16S rRNA gene amplicon sequencing was employed. ASV-based alpha diversity (Shannon index and phylogenetic distances) decreased significantly (P < 0.01) in 2,6-DBP dechlorinating cultures compared to the PCE dechlorinating cultures under the corresponding sulfate-free or sulfate-amended conditions (Fig. 5A & B). Beta diversity analysis using weighted Unifrac (W-Unifrac) distances revealed that the microbial community structure was clearly reshaped by changing the electron acceptor from
Figure 3. Microbial community analysis of PCE-dechlorinating cultures by qPCR (A) and 16S rRNA gene amplicon sequence data at genus level (B). 16S rRNA gene copy numbers per millilitre of the cultures except for the marine sediments (Sediments) that are defined per gram of wet sediment. Total: total bacterial 16S rRNA gene copy numbers; Dhc: Dehalococcoides; Dhg: Dehalogenimonas; Dhb: Dehalobacter; Dsb: Desulfotobacterium; Geo: Geobacter; Sul: Sulfurospirillum. Values and error bars represent the averages and standard deviations of the triplicate qPCRs, respectively. Values below the detection limit are shown as one copy per ml. The added horizontal dotted lines represent 10^3, 10^4, and 10^5 copy numbers, respectively. Only taxa that have a relative abundance > 5% in at least one of the samples are shown. Duplicate enrichments are indicated by suffixes 'A' and 'B'. T1 and T2: the first and second transfers in step 2 (Figure 1); SD12 and SD13: the second (10^{-2}) and third (10^{-3}) dilution cultures in the presence and absence of sulfate, respectively, in the first serial dilution in step 3 (Figure 1). See also Figure S2.

Figure 4. Reductive debromination of 2,6-DBP in addition to PCE dechlorination. In parallel cultures, 2,6-DBP and PCE are dehalogenated into phenol (A) and cDCE (C), respectively. Methane and hydrogen (B, D) are measured throughout the experiment in the presence or absence of sulfate. Values and error bars shown in the figure are averages and standard deviation of duplicate cultures. Arrows indicate the spikes of PCE (black) and 2,6-DBP (grey-dotted arrows for sulfate-free cultures, black for sulfate-amended cultures).
PCE to 2,6-DBP, especially in the absence of sulfate (Fig. 5C). Further NMDS analysis was in agreement with W-Unifrac based ordination and displayed differences of microbial composition during dechlorination and dechlorination under sulfate-free or sulfate-amended conditions (Fig. 5D). Among all, sulfate-free debrominating cultures (NS.DBP) showed the biggest distance from the other three types, and the sulfate-amended cultures showed higher similarity regardless of the added organohalide (Fig. 5C and D). Further microbial composition analysis (Fig. 5E) showed that Proteobacteria was the predominant phylum accounting for 83 (6.6 SD) % and 93 (2.6 SD) % in sulfate-amended dechlorinating (S.PCE) and dechlorinating (S.DBP) cultures, respectively. Firmicutes and Proteobacteria were the main phyla in sulfate-free dechlorinating cultures (NS.PCE) accounting for 52 (16 SD) % and 23 (14 SD) %, respectively, whereas Bacteroidetes was predominant in sulfate-free dechlorinating cultures with 83 (5.1 SD) %.

Microbial community dynamics at genus and ASVs levels

To elaborate in more detail on the microbial community changes associated with the enrichment of dechlorinating and dechlorinating cultures, comparisons were done at genus- and ASV level (Fig. 6 & 7). Most notably, we observed the predominance of an unknown genus from Bacteroidetes_BD2_2 in sulfate-free dechlorinating cultures (NS.DBP) accounting for 83 (5.2 SD) % in relative abundance (Fig. 6), which included 11 ASVs (Fig. 7A). Among these, ASV_1808309 accounted for > 50% of all reads in this genus (Fig. 7B). This was followed by Halodesulfovibrio enriched as the second-most predominant genus up to 6.9 (1.4 SD) %. In contrast, Bacillus was the predominant genus with a relative abundance of 31 (18 SD) % in sulfate-free dechlorinating cultures (NS.PCE), with ASV_18083010 and ASV_1808307 accounting for higher proportions than the other three ASVs (Fig. 7B), and Halodesulfovibrio accounted for a relative abundance of 3.7 (1.4 SD) % (Fig. 6).

Notably, Clostridium_sensu_stricto_7 decreased in relative abundance from 70.9 to 1.3% after the 5th addition of PCE in dechlorinating cultures in the absence of sulfate (Fig. 6), which was mainly caused by the decrease of ASV_1808300 (Fig. 7B). Desulfurhopalus increased in relative abundance after the 4th spike and up to 3.9% after the 5th spike in debrominating cultures (NS.DBP), and was stably maintained in dechlorinating cultures (NS.PCE) at 4.1 (1.8 SD) % without sulfate addition. Similarly, Desulfuromusa showed an increasing trend in sulfate-free PCE dechlorinating cultures (NS.PCE) reaching up to 7.8 (2.1 SD) % after 5th spike. Unlike the trend under sulfate-free conditions, Desulfofoplanes became the major genus and was enriched up to 51 (5.8 SD) % after the 5th PCE spike (S.PCE) in the presence of sulfate. Similarly, in dechlorinating cultures, Desulfofoplanes was observed at a stable and high relative abundance of 38 (5.4 SD) % (S.DBP). In addition, Desulfobacter and Bacillus increased in sulfate-amended dechlorinating cultures (S.PCE) reaching up to 17.8% and 10.7% respectively. Desulfuromusa accounted for 40 (4.1 SD) % in sulfate-amended dechlorinating cultures (S.DBP), whereas it represented only a small proportion in sulfate-amended dechlorinating cultures (S.PCE) at 1.1 (0.28 SD) % (Fig. 6), which was the result of a lower relative abundance of ASV_180830167 (Fig. 7B). Overall, several Desulfobacterota taxa, including Desulfobacter, Desulfobacterium, Desulfofoplanes and Desulfuromusa were only present in sulfate-amended cultures, whereas Desulfomicrobium, Halodesulfuvibrio and Methanogenium were only presented under sulfate-free conditions.
Figure 6. Dynamics of microbial community composition during reductive dehalogenation under sulfate-free (NS) and sulfate-amended (S) conditions. Relative abundance of microbial taxa is shown at genus level with a cut-off at 1% in at least one single sample. Samples are numbered from A1 to A5 and B1 to B5, indicating samples collected after each complete dehalogenation, prior to the spike of PCE or 2,6-DBP, respectively. B1 sampled from the sulfate-free PCE dechlorinating cultures (NS.PCE) was absent due to the low quality of the PCR product that did not meet the sequencing requirements.

Figure 7. Phylogenetic analysis (A) and dynamics of microbial composition (B) at ASVs level. The cut-off relative abundance of ASVs is set at 0.1% in at least one single sample. ASVs belonging to the same genus are indicated by the same colour (top 10 genera) or letters. Alphabetic letters in Fig. 6A: A, Desulfothermobacter; B, Desulfothermobacter; C, Desulfothermobacter; D, Halodesulfovibrio; E, Synergistaceae_uncultured; F, Acholeplasma; G, Mollicutes_EUB33-2_uncultured; H, Desulfovibrionales; I, Desulfomicrobiaceae; J, Marinilabiliaceae; K, Bacteroidetes_vadinHA17; L, Lentimicrobiaceae; M, VC2_1_Bac22_uncultured; N, VC2_1_Bac22; O, Bacteria_LCP-89; P, Methanogenium; Q, Methanomicrobiaceae;
Discussion

Putative RDase genes are being increasingly reported from metagenomic analyses of marine sediments. Considering the global distribution of organohalides in marine environments and their excellent potential as electron acceptors for anaerobic respiration, it is tempting to assume that OHR is occurring in Aarhus Bay sediments contributing to recycling halides, carbon and other nutrients. However, experimental verification of OHR potential in these sediments usually has not been achieved. Recent metagenomic and single-cell genomic analysis revealed presence of putative RDase genes in Aarhus Bay sediments, indicating the possibility for alternative energy conservation through reductive dehalogenation in addition to sulfate reduction at the near-surface sulfate-rich zone (Jochum et al. 2017, Jochum et al. 2018). On this basis, we were inspired to investigate OHR potential and the eco-physiology of associated microbial communities.

Reductive dehalogenation of various organohalides by Aarhus Bay marine sediments

Similar to former studies of reductive dehalogenation in marine environments (Ahn et al. 2003, Futagami et al. 2009, Futagami et al. 2013, Kaster et al. 2014, Matturro et al. 2016), we could show that pristine marine sediments from Aarhus Bay could reductively dehalogenate a range of organohalides, including PCE, 2,6-DBP, 1,4-DDB, 3-BP and 2,4,6-TIP (Table 1), suggesting the presence of multiple reductive dehalogenating microorganisms and corresponding RDase genes. Firmicutes were enriched in all dehalogenating cultures (Fig. S1), with members of the Clostridiales being most predominant. These populations may serve as syntrophic partners to dehalogenating populations, for example as hydrogen producer as has been shown previously (Lin et al. 2020, Merlino et al. 2015, Yang et al. 2019). Intriguingly, Lokiarchaeia belonging to Asgard archaea (Spang et al. 2017, Zaremba-Niedzwiedzka et al. 2017), a recently described group of archaea linked to the origin of euakaryotes (Eme et al. 2017), were observed in the original sediment and enriched only in 2,4,6-TIP deiodinating cultures regardless of sulfate addition. To this end, it seems promising to employ reductive deiodination to enrich or even isolate strains from Lokiarchaeota, previously shown to bear putative RDase genes (Spang et al. 2019). In addition, Anaerolineae, belonging to Chloroflexi, were sharply decreased in PCE dehalogenating cultures compared to incubations with other organohalides. Recently, metagenome assembled genomes (MAGs) revealed that Anaerolineae have the potential of reductive dehalogenation (Fincker et al. 2020), however, this has not been experimentally confirmed to date. Finally, members of the Bacteroidetes were enriched only in PCE dechlorinating cultures. Considering that to date there are no representative OHRB identified from this phylum, results presented here provide new leads for the isolation and characterization of yet unknown organohalide respiring prokaryotes.

Microbial composition of PCE dechlorination enrichments

Populations of well-characterized OHRB including Dhc, Dhb, Dsb, Geo, and Sul (Adrian and Loeffler 2016, Atashgahi et al. 2016, Atashgahi et al. 2013, Azizian et al. 2010a, Fincker and Spormann 2017) were found in the marine sediment studied here, but only at a low abundance below 10^6 16S rRNA gene copies/gram sediment. These microbes further decreased in abundance during transfers and serial dilutions indicating they are unlikely responsible for the PCE dechlorination under the conditions used in this study. We also did not detect known genes encoding the enzymes responsible for reductive dehalogenation of PCE and its metabolites, i.e. vca-, buca-, and tceA genes (Chen et al. 2014, Smits et al. 2004), suggesting the presence of other, variant sequences of RDase genes as previously indicated in subsediments (Futagami et al. 2013). The lack of dechlorination beyond cDCE suggests that enzymes required for cDCE and VC dehalogenation were not enriched under the conditions used here as previously observed for other environments (Krajmalnik-Brown et al. 2004, Scheutz et al. 2008). This is a common finding indicating likely evolution of VC-respiring Dehalococcoidia members due to anthropogenic contaminations in terrestrial environments (McMurdie et al. 2009). Microbial community analysis revealed that the original Aarhus Bay sediment sample contained two predominant genera with relative abundances over 5% (Fig. 3B), including an uncultured genus in the Anaerolineae belonging to the Chloroflexi, and the Desulfobacterota genus Desulfatiglans. Recent metagenomic data analysis revealed that members of the class Anaerolineae encoded putative RDase genes in their genomes, suggesting they might have the potential of reductive dehalogenation (Fincker et al. 2020). Furthermore, single-cell genomics has shown that Desulfatiglans-associated microorganisms contain putative RDase genes in their genomes (Jochum et al. 2018). However, in contrast to canonical RDases, the encoded putative RDase proteins contain transmembrane domains at the N-terminus but lacked a TAT signal peptide. Also, no accompanying RDase B gene was observed that normally encodes the membrane anchor for the catalytic subunit of RDases (Jochum et al. 2018). These putative RDases termed as hybrid RDases have not been shown to be functional (Atashgahi 2019). Intriguingly, the relative abundance of Anaerolineae and Desulfatiglans decreased to less than 1% in our highly enriched cultures regardless of the presence of sulfate suggesting they might not be responsible for the observed dehalogenation. In contrast, Clostridium sensu stricto 7 belonging to the Firmicutes, and the Desulfobacterota genus Halodesulfuvibrio were enriched in sulfate-free cultures, whereas other members of the Desulfobacterota including Desulfuvibrio, Desulfoharpos, Desulfoplanes and an unknown genus in the Desulfobulbiae became the predominant genera in sulfate-amended cultures (Fig. 3B & Fig. S3B). To our knowledge, Clostridium members have not been shown to mediate OHR and have been proposed to function as the hydrogen producers facilitating reductive dehalogenation (Lin et al. 2020, Lo et al. 2020, Yang et al. 2019). In contrast, Desulfobacterota representatives, including Halodesulfuvibrio marinesediminis and Desulfuvibrio bizertensis, have been shown to debrominate 2,4,6-tribromophenol and 2,6-DBP into 4-bromophenol (4-BP) and phenol, respectively (Liu and Haggblom 2018). Furthermore, the genome of Desulfkopolus singaporenis was annotated to contain putative RDase genes (GenBank Accession: GCA_900104445.1), but OHR potential in this bacterium has not been experimentally verified (Lie et al. 1999). Henceforth, we speculated that the well-identified OHRB might be outweighed by other potential dehalogenating microbes in our laboratory microcosms.

Switching organohalides from PCE to 2,6-DBP

The sediments of Aarhus Bay were previously reported to have a high Br/Cl ion ratio, and a variety of brominated organic compounds have been identified, implying the potential for debromination may exist in the seafloor sediments (Christensen and Platz 2001, Jorgensen et al. 2020, Zinke et al. 2017). In addition, our initial assessment of dehalogenation capacity corroborated debrominating potential of Aarhus Bay sediments. Further, the genera enriched in PCE-dechlorinating cultures (Fig 2), like Desulfuvibrio,
have previously been reported to include strains that were characterized to debrominate 2,4,6-TBP and 2,6-DBP (Liu and Haggblom 2018). Interestingly, a recent study revealed the possibility of dechlorinating cultures to catalyze debromination (Xu et al. 2022). Indeed, our results are in agreement, as PCE-dechlorinating cultures showed the potential for 2,6-DBP debromination to phenol, whereas 2,6-DCP was not dechlorinated (Fig. 1). Similarly, Peng et al. found marine Desulfobula strains were capable of reductive dechlorination but not reductive debromination (Peng et al. 2020a). This may indicate niche specialization of marine OHRB for reductive debromination that could gain more energy to support bacterial growth than that of reductive dechlorination (Xu et al. 2022).

The formation and consumption of hydrogen was observed in sulfate-free cultures (Fig. 4), indicating that hydrogen likely served as the intermediate electron donor for OHR and methanogenesis (Azizian et al. 2010a, Dolfin and Tiedje 1987) that occurred simultaneously as formerly reported (Aulenta et al. 2002). On the contrary, methane was not generated in sulfate-amended cultures at first that might be due to the fact that sulfate-reducing bacteria outcompeted methanogens due to higher substrate affinity of sulfate-reducing bacteria to hydrogen than methanogens (Kristjansson and Schonheit 1983, Piché-Chouquette and Constant 2019). With the sulfate reduced, methane was detected but at a low concentration below 30 μM (Fig. 4B&D). Interestingly, reductive dechlorination and debromination were not influenced by the presence of sulfate, which was in line with recent reports of marine OHRB belonging to sulfate-reducing bacteria (SRB) (Liu et al. 2020, Liu and Haggblom 2018, Peng et al. 2020a). It is likely that the marine OHRB have developed strategies for concurrent sulfate and organohalide respiration.

Potential OHRB inferred from microbial community analysis

Our results revealed that microorganisms that were previously discovered by metagenome and single-genome assembly as candidate OHRB, such as Desulfatiglans and its relatives (Fincker et al. 2020, Jochum et al. 2018), were not enriched in PCE dechlorination cultures, which might due to their inability to dechlorinate PCE under the conditions used for the experiments here (Fig. 3 and Fig. S3). Furthermore, some of the functionally characterized OHRB, for instance Dehalococcoides, that have been observed in Aarhus Bay sediments based on metagenomic analysis (Fincker et al. 2020), and which strictly depend on hydrogen as electron donor and halogenated compounds as electron acceptor for energy conservation (Maymo-Gatell et al. 1997), were not enriched in our cultures. Their fastidious and restricted metabolism might have rendered them less competitive, being outcompeted by other, more versatile, OHRB, like RDase-containing sulfate reducers (Peng et al. 2020a), in the defined mineral marine medium used in this study (Monserrate and Haggblom 1997). Changing the organohalide electron acceptors significantly reshaped the microbial community structure (Figs 5 and 6), suggesting that different microorganisms might be involved in the dehalogenation of the different chlorinated and brominated compounds tested in our study. Furthermore, the observed decrease in alpha diversity in debrominating cultures suggests that 2,6-DBP or the debrominated phenol might inhibit growth of certain bacteria via the leakage of cellular components, such as K+ and ATP, or even cell membrane destruction (Cooper et al. 2015, Escher et al. 1996, Heipieper et al. 1991, Stasiuk and Kozubek 2008). Intriguingly, transfers of cultures from the initial screening able to dehalogenate 2,6-DBP were no longer able to debrominate 2,6-DBP. As the microbial community analysis revealed the apparent loss of Desulfobulbus in comparison to the transfers from sulfate-amended dechlorinating cultures (Fig. S1 and S5), a likely role of Desulfovibrio for reductive debromination can be hypothesized. Interestingly, members of the genus Bacillus were strongly enriched in sulfate-free dechlorinating cultures. This genus has to date not been characterized to perform reductive dehalogenation. Interestingly, Lim et al. reported that members of the phylum Bacteroidetes were enriched in the presence of natural organohalides (Lim et al. 2018). Similarly, we observed that members of this phylum were also enriched in sulfate-free debrominating cultures, but OHRB belonging to Bacteroidetes remain uncharacterized, providing leads for future attempts to isolate these organisms.

Furthermore, members of several genera recently identified as OHRB were observed, including Desulfuroscia, Halodesulfovibrio and Desulfovibrio (Liu and Haggblom 2018). For each of these genera, several species-level amplicon sequencing variants (ASVs) were observed suggesting that the enriched populations were composed of more than one strain type, such as Desulfuroscia with two ASVs (Fig. 7). To this end, future studies should aim to provide strain-resolved information by meta-omics, such as metagenomics and meta-transcriptomics. Genetic information of new OHRB can be disclosed from genome-resolved binning of metagenome data, providing leads regarding metabolic differences that can guide efforts towards isolation and further characterization of yet unknown dehalogenators.

Conclusions

In conclusion, this work verified the potential for OHR in Aarhus Bay sediments that have previously been shown to be a source of organohalides and putative RDase genes. This is of importance considering the increasing number of studies reporting occurrence of organohalides and putative RDase genes in marine sediments (Fincker et al. 2020, Jorgensen et al. 2020, Peng et al. 2020a, Peng et al. 2020b). Considering the diversity of organohalides naturally produced in marine environments, OHR should play a key role in recycling halides and organic carbon back to the seawater.

Data availability

The nucleotide sequence data has been deposited in the European Bioinformatics Institute under accession number PRJEB50583.

Supplementary data

Supplementary data are available at FEMSEC online.

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Conflict of interest statement. None declared.

References

Adrian L, Loefller F. Organohalide-respiring bacteria. Berlin, Heidelberg: Springer, 2016.
Ahn YB, Rhee SK, Fennell DE et al. Reductive dehalogenation of brominated phenolic compounds by microorganisms associated
with the marine sponge Aplysina aerophoba. Appl Environ Microbiol 2003;69:4159–66.

Amos BK, Sung Y, Fletcher KE et al. Detection and quantification of Geobacter lovleyi strain SZ: implications for bioremediation at tetrachloroethene- and uranium-impacted sites. Appl Environ Microbiol 2007;73:6898–904.

Atashgahi S, Haggbloom MM, Smidt H. Organohalide respiration in pristine environments: implications for the natural halogen cycle. Environ Microbiol 2018;20:934–48.

Atashgahi S, Lu Y, Smidt H. Overview of known organohalide-respiring bacteria—phylogenetic diversity and environmental distribution. In: Adrian L, Löffler FE (eds). Organohalide-Respiring Bacteria. Berlin, Heidelberg: Springer Berlin Heidelberg, 2016, 63–105.

Atashgahi S, Maphosa F, Dogan E et al. Small-scale oxygen distribution determines the vinyl chloride biodegradation pathway in surficial sediments of riverbed hyporheic zones. FEMS Microbiol Ecol 2013;84:133–42.

Atashgahi S. Discovered by genomics: putative reductive dehalogenase with N-terminus transmembrane helixes. FEMS Microbiol Ecol 2019;95:Fiz048.

Augenta F, Majone M, Verbo P et al. Complete dechlorination of tetrachloroethene to ethene in presence of methanogenesis and acetogenesis by an anaerobic sediment microcosm. Biodegradation 2002;13:411–24.

Azizian MF, Marshall IP, Behrens S et al. Comparison of lactate, formate, and propionate as hydrogen donors for the reductive dehalogenation of trichloroethene in a continuous-flow column. J Contam Hydrol 2010a;113:77–92.

Azizian MF, Marshall IP, Behrens S et al. Comparison of lactate, formate, and propionate as hydrogen donors for the reductive dehalogenation of trichloroethene in a continuous-flow column. J Contam Hydrol 2010b;113:77–92.

Bayer K, Scheuermayer M, Fieseler L et al. Genomic mining for novel FADH2-dependent halogenases in marine sponge-associated microbial consortia. Mar Biotechnol 2013;15:63–72.

Cavanaugh JG, Laufer CL, Walters WA et al. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc Natl Acad Sci USA 2011;108:4516–22.

Chen J, Bowman KS, Rainey FA et al. Reassessment of PCR primers targeting 16S rRNA genes of the organohalide-respiring genus Dehalogenimonas. Biodegradation 2014;25:747–56.

Chen K, Huang L, Xu C et al. Molecular characterization of the enzymes involved in the degradation of a brominated aromatic herbicide. Mol Microbiol 2013;89:1121–39.

Christensen JH, Platz J. Screening of polychlorinated diphenyl ethers in blue mussels, marine and freshwater sediments in Denmark. J Environ Monit 2001;3:543–7.

Cline JD. Spectrophotometric determination of hydrogen sulfide in natural waters. Limnol Oceanogr 1969;14:454–8.

Comba F, Kerscher M, Krause T et al. Iron-catalysed oxidation and halogenation of organic matter in nature. Environ Chem 2015;12:381–95.

Cooper M, Wagner A, Wondrousch D et al. Anaerobic microbial transformation of halogenated aromatics and fate prediction using electron density modeling. Environ Sci Technol 2015;49:6018–28.

DeWeerd K, Concannon F, Sufita JM. Relationship between hydrogen consumption, dehalogenation, and the reduction of sulfur oxanions by Desulfitomine tediae. Appl Environ Microbiol 1991;57:1929–34.

Dolfing J, Tiedje JM. Growth yield increase linked to reductive dechlorination in a defined 3-chlorobenzoate degrading methanogenic coculture. Arch Microbiol 1987;149:102–5.

Edgar RC. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 2010;26:2460–1.

Eme I, Spang A, Lombard J et al. Archaea and the origin of eukaryotes. Nat Rev Microbiol 2017;15:711–23.

Escher BJ, Snozzi M, Schwarzenbach RP. Uptake, speciation, and uncoupling activity of substituted phenols in energy transducing membranes. Environ Sci Technol 1996;30:3071–9.

Fincker M, Huber JA, Orphan VJ et al. Metabolic strategies of marine subsurface Chloroflexi inferred from genome reconstructions. Environ Microbiol 2020;22:3188–204.

Fincker M, Spormann AM. Biochemistry of catabolic reductive dehalogenation. Annu Rev Biochem 2017;86:357–86.

Fung JM, Morris RM, Adrian L et al. Expression of reductive dehalogenase genes in Dehalococcoidetes ethylenegens strain 195 growing on tetrachloroethene, trichloroethene, or 2, 3-dichlorophenol. Appl Environ Microbiol 2007;73:4439–45.

Futagami T, Morono Y, Terada T et al. Dehalogenation activities and distribution of reductive dehalogenase homologous genes in marine subsurface sediments. Appl Environ Microbiol 2009;75:6805–9.

Futagami T, Morono Y, Terada T et al. Distribution of dehalogenation activity in subsurface sediments of the Nankai Trough subduction zone. Philos Trans Royal Soc B: Biol Sci 2019;368:20120249.

Gribble GW. A recent survey of naturally occurring organohalogen compounds. Environ Chem 2015;12:396–405.

Gribble GW. Naturally occurring organohalogen compounds—a comprehensive survey. Proc Chem Org Nat Prod 1996.

Gribble GW. Recently discovered naturally occurring heterocyclic organohalogen compounds. Heterocycles 2012;84:157–207.

Gutleben J, Koehler JS, McPherson K et al. Diversity of tryptophan halogenases in sponges of the genus Aplysina. FEMS Microbiol Ecol 2019;195:Fiz108.

Håggblom MM, Ahn YB, Fennell DE et al. Anaerobic dehalogenation of organohalide contaminants in the marine environment. Adv Appl Microbiol 2003;53:61–84.

Hashimoto S, Wakimoto T, Tatsukawa R. Possible natural formation of polychlorinated dibenzo-p-dioxins as evidenced by sediment analysis from the Yellow Sea, the East China Sea and the Pacific Ocean. Mar Pollut Bull 1995;30:341–6.

Heipieper H-J, Kewloeh H, Rehm H-J. Influence of phenols on growth and membrane permeability of free and immobilized Escherichia coli. Appl Environ Microbiol 1991;57:1213–7.

Holscher T, Krajmalnik-Brown R, Ritalahti KM et al. Multiple non-identical reductive-dehalogenase-homologous genes are common in Dehalococcoides. Appl Environ Microbiol 2004;70:5290–7.

Jochum LM, Chen X, Lever MA et al. Depth distribution and assembly of sulfate-reducing microbial communities in marine sediments of Aarhus Bay. Appl Environ Microbiol 2017;83:e01547–17.

Jochum LM, Schreiber L, Marshall IP et al. Single-cell genomics reveals a diverse metabolic potential of uncultivated Desulfatiglans-related Deltaproteobacteria widely distributed in marine sediment. Front Microbiol 2018;9:2038.

Jorgensen BB, Andren T, Marshall IPG. Sub-seafloor biogeochemical processes and microbial life in the Baltic Sea. Environ Microbiol 2020;22:1688–706.

Kaster AK, Mayer-Blackwell K, Pasarelli B et al. Single cell genomic study of Dehalococcoidetes species from deep-sea sediments of the Peruvian Margin. ISME J 2014;8:1831–42.

Krajmalnik-Brown R, HoIscher T, Thomson IN et al. Genetic identification of a putative vinyl chloride reductase in Dehalococcoides sp. strain BAV1. Appl Environ Microbiol 2004;70:6347–51.

Kristjansson J, Schönheit P. Why do sulfate-reducing bacteria outcompete methanogenic bacteria for substrates? Oecologia 1983;60:264–6.
Lahti L, Shetty S. microbiome R package. 2017.
Leloup J, Fossing H, Kohls K et al. Sulfate-reducing bacteria in marine sediment (Aarhus Bay, Denmark): abundance and diversity related to geochemical zonation. Environ Microbiol 2009;11:1278–91.
Leri AC, Mayer LM, Thornton KR et al. Bromination of marine particulate organic matter through oxidative mechanisms. Geochim Cosmochim Acta 2014;142:53–63.
Lie TJ, Clawson ML, Godchaux W et al. Sulfidogenesis from 2-aminoethanesulfonate (taurine) fermentation by a morphologically unusual sulfate-reducing bacterium, Desulforhopalus singularis. FEMS Microbiol Ecol 2018;94:fsy050.
Lin QX, Li ZL, Zhu YY et al. Novel bacterial diversity is enriched with chloroperoxidase-reacted organic matter under anaerobic conditions. FEMS Microbiol Ecol 2018,94:fsy050.
Liu X, Lu CW, Lin WH et al. Microbial communities by denaturing gradient gel electrophoresis analysis or polymerase chain reaction-amplified genes coding for 16S rRNA. ISME J 2002;6:225–36.
Mao X, Polasko A, Alvarez-Cohen L. Effects of sulfate reduction on trichloroethene dechlorination by Dehalococcoides-containing microbial communities. Appl Environ Microbiol 2017,83:169–72.
Magnuson J, Stern R, Gossett J et al. Reductive dechlorination of tetrachloroethene to ethene by a two-component enzyme pathway. Appl Environ Microbiol 1998;64:1270–5.
Maillard J, Schumacher W, Vazquez F et al. Characterization of the corrinoid iron-sulfur protein tetrachloroethene reductive dehalogenase of Dehalobacter restrictus. Appl Environ Microbiol 2003;69:4628–38.
Mao X, Polasko A, Alvarez-Cohen L. Effects of sulfate reduction on trichloroethene dechlorination by Dehalococcoides-containing microbial communities. Appl Environ Microbiol 2017,83:169–72.
Maphosa F, De Vos WM, Smidt H. Exploiting the ecogenomics toolbox for environmental diagnostics of organohalide-respiring bacteria. Trends Biotechnol 2010;28:308–16.
Matturro B, Ubaldi C, Rossetti S. Microbiome Dynamics of a Poly-chlorobiphenyl (PCB) Historically Contaminated Marine Sediment under Conditions Promoting Reductive Dechlorination. Front Microbiol 2016;7:1502.
Maymo-Gatell X, Chien YT, Gossett JM et al. Isolation of a bacterium that reductively dechlorinates tetrachloroethene to ethene. Science 1997;276:1568–71.
McMurdie PJ, Behrens SF, Müller JA et al. Localized plasticity in the streamlined genomes of vinyl chloride respiring Dehalococcoides. PLoS Genet 2009;5:e1000714.
McMurdie PJ, Holmes S. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PloS One 2013;8:e61217.
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Petro C, Zancker B, Starnawski P et al. Marine deep biosphere microbial communities assemble in near-surface sediments in Aarhus Bay. *Front Microbiol* 2019b;10:758.

Piché-Choquette S, Constant P. Molecular hydrogen, a neglected key driver of soil biogeochemical processes. *Appl Environ Microbiol* 2019;85:e02418–18.

Poncheewin W, Hermes GDA, van Dam JCJ et al. NG-Tax 2.0: A semantic framework for high-throughput amplicon analysis. *Front Genetics* 2019;10:1366.

Ramiro-Garcia J, Hermes GDA, Giatsis C et al. NG-Tax, a highly accurate and validated pipeline for analysis of 16S rRNA amplicons from complex biomes. *F1000Research* 2016;5:1791.

Scheutz C, Durant ND, Dennis P et al. Concurrent ethene generation and growth of Dehalococcoides containing vinyl chloride reductive dehalogenase genes during an enhanced reductive dechlorination field demonstration. *Environ Sci Technol* 2008;42:9902–9.

Smidt H, de Vos WM. Anaerobic microbial dehalogenation. *Annu Rev Microbiol* 2004;58:43–73.

Smits TH, Devenoges C, Szymalski K et al. Development of a real-time PCR method for quantification of the three genera *Dehalobacter*, *Dehalococcoides*, and *Desulfotobacterium* in microbial communities. *J Microbiol Methods* 2004;57:369–78.

Spang A, Caceres EF, Ettema TJ. Genomic exploration of the diversity, ecology, and evolution of the archaeal domain of life. *Science* 2017;357:eaaf3883.

Spang A, Stairs CW, Dombrowski N et al. Proposal of the reverse flow model for the origin of the eukaryotic cell based on comparative analyses of Asgard archaeal metabolism. *Nature Microbiol* 2019;4:1138–48.

Stasiuk M, Kozubek A. Membrane perturbing properties of natural phenolic and resorcinolic lipids. *FEBS Lett* 2008;582:3607–13.

Sutton NB, Atashghai S, Saccenti E et al. Microbial community response of an organohalide respiring enrichment culture to permanganate oxidation. *PLoS One* 2015;10:e0134615.

Team RDC. *R: A language and environment for statistical computing*. R Found Stat Comput, 2013.

Turkovsky D, Jehmlisch N, Diekert G et al. An integrative overview of genomic, transcriptomic and proteomic analyses in organohalide respiration research. *FEMS Microbiol Ecol* 2018;94:e0013.

Wagner C, El Omari M, König G. Biohalogenation: nature’s way to synthesize halogenated metabolites. *J Nat Prod* 2009;72:540–53.

Walters W, Hyde ER, Berg-Lyons D et al. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. *mSystems* 2016;1:e00009–15.

Wever R, van der Horst MA. The role of vanadium haloperoxidases in the formation of volatile brominated compounds and their impact on the environment. *Dalton Trans* 2013;42:11778–86.

Wiseman SB, Wan Y, Chang H et al. Polybrominated diphenyl ethers and their hydroxylated/methoxylated analogs: environmental sources, metabolic relationships, and relative toxicities. *Mar Pollut Bull* 2011;63:179–88.

Xu G, Zhao S, Chen C et al. Dehalogenation of polybrominated diphenyl ethers and polychlorinated biphenyls catalyzed by a reductive dehalogenase in *Dehalococcoides mccartyi* Strain MB. *Environ Sci Technol* 2022.

Yang G, Yin Y, Wang J. Microbial community diversity during fermentative hydrogen production inoculating various pretreated cultures. *Int J Hydrogen Energy* 2019;44:13147–56.

Yilmaz P, Parfrey LW, Yarza P et al. The SILVA and “all-species living tree project (LTP)” taxonomic frameworks. *Nucleic Acids Res* 2014;42:D643–8.

Zaremba-Niedzwiedzka K, Caceres EF, Saw JH et al. Asgard archaea illuminate the origin of eukaryotic cellular complexity. *Nature* 2017;541:353–8.

Zinke LA, Mullis MM, Bird JT et al. Thriving or surviving? Evaluating active microbial guilds in Baltic Sea sediment. *Environ Microbiol Rep* 2017;9:528–36.