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Published in:
Journal of Environmental Monitoring

Link to article, DOI:
[10.1039/c9em00544g](https://doi.org/10.1039/c9em00544g)

Publication date:
2020

Document Version
Peer reviewed version

[Link back to DTU Orbit](#)

Citation (APA):
Murray, A. M., Maillard, J., Rolle, M., Broholm, M. M., & Holliger, C. (2020). Impact of iron- and/or sulfate-reduction on a cis-1,2-dichloroethene and vinyl chloride respiring bacterial consortium: experiments and model-based interpretation. *Journal of Environmental Monitoring*, 22, 740-750. <https://doi.org/10.1039/c9em00544g>

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**Impact of iron- and/or sulfate-reduction on a *cis*-1,2-dichloroethene and vinyl chloride
respiring bacterial consortium: experiments and model-based interpretation**

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Environmental Science: Processes & Impacts Accepted Manuscript

Keywords

Degradation kinetics, microbial communities, biogeochemical modeling, organohalide respiration, chlorinated ethenes

Environmental Significance

Organohalide respiration is a natural environmental process that can be utilized for bioremediation of subsurface contaminants, e.g. chlorinated ethenes. This is a well-studied process, however the impacts of other subsurface microbial processes on organohalide respiration are much less well defined. This study was conducted in order to enhance understanding of the role of the microbial community on the rate of organohalide respiration, with specific emphasis on competing terminal electron accepting processes, namely sulfate and Fe(III) reduction. The different impacts from each sulfate-reducing and Fe(III)-reducing bacterial guild member on *cis*-1,2-dichloroethene and vinyl chloride dechlorination rates show that the dynamics within a microbial community containing organohalide-respiring bacteria cannot always be accurately characterized by broad, categorical, inter-guild trends, but are rather the result of intricate interactions between individual community members. This finding furthers understanding of how to best approach application of biodegradation of organic contaminants.

Abstract

Process understanding of microbial communities containing organohalide-respiring bacteria (OHRB) is important for effective bioremediation of chlorinated ethenes. The impact of iron and sulfate reduction on *cis*-1,2-dichloroethene (cDCE) and vinyl chloride (VC) dechlorination by a consortium containing the OHRB *Dehalococcoides* spp. was investigated using multiphase batch experiments. The OHRB consortium was found to contain endogenous iron- and sulfate-reducing bacteria (FeRB and SRB). A biogeochemical model was developed and used to quantify the mass transfer, aquatic geochemical, and microbial processes that occurred in the multiphase batch

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4 42 system. It was determined that the added SRB had the most significant impact on contaminant
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6 43 degradation. Addition of the SRB increased maximum specific substrate utilization rates, k_{max} , of
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9 44 cDCE and VC by 129% and 294%, respectively. The added FeRB had a slight stimulating effect on
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11 45 VC dechlorination when exogenous SRB were absent, but when cultured with the added SRB,
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13 46 FeRB moderated the SRB's stimulating effect. This study demonstrates that subsurface microbial
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1. Introduction

Chlorinated ethenes have been used at dry-cleaning facilities and in industry as metal degreasers for decades and are common groundwater contaminants. Naturally occurring organohalide-respiring bacteria (OHRB) can anaerobically degrade chlorinated ethenes by using them as electron acceptors paired often with hydrogen as an electron donor¹. Sequential dechlorination of perchloroethene (PCE) and trichloroethene (TCE) yields the daughter products *cis*-1,2-dichloroethene (cDCE), vinyl chloride (VC), and ultimately the non-toxic ethene. PCE and all of its daughter products except ethene are toxic to humans, and thus it is advantageous to harness the capabilities of OHRB, both for natural attenuation and engineered bioremediation systems². However, at many contaminated sites, sequential chlorinated ethene degradation inexplicably stops during one of the last two steps of the process: cDCE or VC accumulates, and the non-toxic endpoint is not achieved³.

The degradation stall can be due to a number of factors, including lack of specific functional genes in the OHRB or unfavorable environmental conditions, like low pH or lack of nutrients/vitamins⁴.

Evidence from the laboratory, the field, and kinetic models suggests that, all other conditions being ideal, the degradation stall may be due to competition for electron donor in the groundwater microbial community (Fennell and Gossett, 1998). Hydrogen is produced by fermenting and syntrophic bacteria and can also be used in terminal electron accepting processes such as iron and sulfate reduction (Fennell and Gossett, 1998; Yang and McCarty, 1998). Previous studies support that iron- and sulfate-respiration directly impact organohalide-respiration (OHR)^{8,9}, and competition for electron donor is often posited as the mechanism of impact. However, the extent of enhancement and/or inhibition by other reduction processes appears to be more complex than competition for electron donor, and can depend on the rates of hydrogen production and consumption¹⁰, sulfide concentration¹¹, iron mineralogy¹², and aqueous Fe(III) speciation¹³.

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4 73 Sulfate reduction has even been statistically correlated to increased chlorinated ethene degradation
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10 75 Most kinetic OHRB laboratory studies are composed of carefully curated consortia, where only
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12 76 those community members that support the OHRB growth are maintained, and do not include
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14 77 quantification of these microbial community and geochemistry processes ^{15–17}. Geochemical
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16 78 processes, such as the formation of iron complexes, have only been considered in a handful of
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18 79 modeling efforts to describe community interactions, and these models have not been developed
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20 80 based on dedicated experiment and modeling work ^{9,18,19}. Microbial activity in the subsurface is also
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22 81 limited by mass transfer processes, such as kinetic dissolution of chlorinated ethenes from non-
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24 82 aqueous phases ²⁰ and diffusive/dispersive processes, yet this is rarely included in lab-scale models
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26 83 ^{21,22}. Therefore, exploration of the impact of sulfate- and iron-reducing bacteria (SRB and FeRB) on
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28 84 chlorinated ethene degradation by OHRB, while accounting for geochemical and mass transfer
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38 87 In this study, we conducted electron acceptor limited batch experiments that included a chlorinated
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44 90 organic liquid phase (hexadecane spiked with cDCE) acting as contaminant source, and an aqueous
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46 91 phase in which biodegradation and chemical reactions occurred. The relatively simple OHRB
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48 92 consortium was perturbed by the addition of other electron acceptors (Fe(III) and sulfate) to the
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50 93 medium and FeRB and SRB to the inoculum, to form batch communities with different microbial
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52 94 guilds. We then interpreted the chemical and biomolecular experimental data using a kinetic
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54 95 biogeochemical model, whose foundational approach was developed in Murray et al., 2019. In
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56 96 contrast to Murray et al., 2019, the current study is conducted with the chlorinated ethenes cDCE
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and VC, rather than PCE, which are less reliably biodegradable *in situ*, is conducted over a span of multiple months rather than a few days, and contains a more complex microbial community. Furthermore, the model was significantly expanded to account for multiple dechlorination steps, utilize more types of data, and include sampling of multiple physical phases. This model which allowed us to accurately account for the fundamental physics of the batch systems in the current study, including mass transfer limitation between the different phases; quantify the rates of bacterial reduction processes; and distinguish the different physical, biological, and chemical processes that impact dechlorination of cDCE and VC by OHRB.

2. Materials and methods

2.1 Microbial cultures

The OHRB consortium, SL2, used in this study originated from a chlorinated ethene-contaminated aquifer and was enriched and maintained over several years²³. The consortium contains the genus *Dehalococcoides* that is capable of sequentially respiring cDCE to VC and ethene. *Shewanella oneidensis* strain MR-1²⁴ was used as a model FeRB and *Desulfovibrio vulgaris* Hildenborough²⁵ was used as a model SRB. *S. oneidensis* and *D. vulgaris* were selected because both can use hydrogen and neither can use acetate as an electron donor^{26–28}. *D. vulgaris* is also capable of Fe(III) reduction, but does not grow when using this electron acceptor^{29,30}.

2.2 Experimental setup

The base medium was prepared and sterilized in the same manner described in Murray et al.³¹. Acetate, ferric citrate, and sulfate stocks were each added such that in the finished medium approximately 2 mM of carbon source, 5 mM of iron source, and 5 mM of sulfate source were present (Table S1). 60 mL of medium was added to each 125 mL batch bottle, and the headspace was replaced with a 4:1 mixture of hydrogen to carbon dioxide through 0.22 μ m sterile filters to a final pressure of 1.5 bar. Bottles were sealed with butyl rubber stoppers. An electron balance

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4 121 showed that 134% more donor was added to the system than was needed for all added electron
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6 122 acceptors. This excess of hydrogen was added directly to the system, rather than produced by
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8 123 fermenting populations in the microbial community, to ensure that all bacterial processes were
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10 124 electron acceptor limited. In the case that fermenting populations are present in the microbial
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12 125 community to produce hydrogen, this extra step in the process can serve to keep the hydrogen
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14 126 concentration low and thus electron donor limited^{6,32}.
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19 127 To avoid that the cells were exposed to toxic concentrations of cDCE, cDCE dissolved in
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21 128 hexadecane was used as a source in the system. Hexadecane is immiscible with water and remains
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23 129 as a distinct second liquid phase in the experimental batch system. The cDCE/hexadecane formed
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25 130 an organic layer between the aqueous and gaseous compartments in the bottle. As chlorinated
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27 131 ethenes are degraded in the aqueous phase, they diffuse from the organic layer to the gaseous and
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29 132 aqueous phases to maintain equilibrium at a non-toxic concentration in the aqueous phase. To avoid
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31 133 trace amounts of chloroform often present in commercially available cDCE, cDCE was produced in
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33 134 the laboratory using a *Dehalobacter restrictus* culture that degraded PCE to cDCE. When the PCE
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35 135 was exhausted and only cDCE remained, the organic layer was recovered from these cultures,
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37 136 diluted to approximately 200 mM cDCE with hexadecane, and sterilized via autoclaving. An aliquot
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42 137 of 2 mL of the cDCE stock solution in hexadecane was added to each experimental batch, which
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44 138 created a thin (approximately 5 mm) organic layer between the aqueous and gaseous phases. To
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46 139 ensure that the aqueous and gaseous phases did not intersect and that the only phase boundaries
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48 140 were the organic-aqueous and organic-gaseous boundaries, the batches were not agitated.
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52 141 Aqueous samples of 1 mL were taken at regular intervals for chemical and molecular analyses. The
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54 142 sampling interval was three days for the first month of the experiment and one week for the
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56 143 remainder. Sampling continued for each experimental batch until dechlorination of all chlorinated
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ethenes was complete. Dechlorination was ascertained to be complete when chloride measurements produced the same value in consecutive sampling events.

SL2, *S. oneidensis*, and *D. vulgaris* were cultivated as separate inocula in a dark 30 °C room. Cell counts were measured in each inoculum using universal qPCR. The same number of OHRB consortium cells were added to each experimental batch. The numbers of FeRB and SRB cells added were scaled to be 0.01 of the OHRB consortium cells. Four batch configurations were setup to investigate the OHRB activity and to explore the community interactions with the SRB and FeRB guilds. A schematic of the batch inocula configuration is included in Figure S1. Batch 1 consisted of the OHRB consortium alone, with neither FeRB nor SRB added. Batch 2 consisted of only the OHRB consortium and added SRB, Batch 3 consisted of only the OHRB consortium and added FeRB, and Batch 4 consisted of all three guilds together. All experimental batches were conducted in duplicate, statically, in a dark, 30 °C room.

2.3 Molecular methods

Cell counts of each bacterial guild inoculum were quantified by quantitative polymerase chain reaction (qPCR) using universal primers (SI) in order to inoculate each experimental batch with the appropriate inocula ratio. A MIC apparatus (Bio Molecular Systems) was used for analysis of the qPCR data. SRB and FeRB inocula were diluted with base medium prior to inoculation. PCR and terminal restriction fragment length polymorphism (T-RFLP) analyses were completed as described in the SI. Fragment counts of the modeled guilds (OHRB, SRB, indigenous SRB (SRBi), and FeRB) were selected and converted to cell counts based on the number of 16S rRNA gene copies per genome for each species^{24,25,33}. These relative abundances based on cell number were then normalized to this selected portion of the community. Because fragments lengths of the indigenous FeRB (FeRBi) were identical to that of the exogenous FeRB, it was not possible to distinguish between these two populations using T-RFLP. Thus, the FeRBi and exogenous FeRB are modeled

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4 168 as one guild, and are referred to throughout this study as FeRB. Similarly, strains of OHRB could
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6 169 not be distinguished using the T-RFLP method (Berggren et al., 2013). Despite these limitations,
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9 170 the T-RFLP method was selected for use here to demonstrate the utility of a comparatively simple,
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11 171 cost-effective molecular method that can be applied to field and laboratory microbial community
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17 173 **2.4 Analytical methods**

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19 174 Total Fe(II) concentrations were assessed using a colorimetric ferrozine assay ³⁴. Samples were
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23 176 and were analyzed immediately. Chloride concentrations were assessed via silver ion titration with
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26 177 a Chlor-o-counter (Flohr Instrument, Nieuwegein, Netherlands). Sulfate concentrations were
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28 178 measured by ion chromatography – suppressed conductivity detection (ICS-5000, Thermo Fisher).
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30 179 Samples were pretreated with a cleanup-cartridge (1 cc, Dionex OnGurad II Na, ThermoScientific)
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32 180 to remove divalent metal ions, which protects the column and suppressor. Samples were diluted five
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34 181 times in a 5% zinc acetate solution directly upon removal to trap sulfide as zinc sulfide and were
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36 182 frozen at -20°C until analysis. A sample of 1 mL was taken from each batch to measure pH prior to
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39 183 inoculation and at the end of the time series to ensure that the medium remained buffered at pH 7
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42 184 throughout the experimental duration. The gas composition in each batch headspace was measured
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44 185 by gas chromatography (Agilent Technologies 7890B system equipped with Optima 624 LB) ³⁶.
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46 186 Peak areas were corrected using the molar mass of each compound and are representative of the
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49 187 relative headspace composition. The proportions of cDCE, VC, and ethene were normalized as
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51 188 relative proportions of these three compounds.

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54 189 **2.5 Biogeochemical model**

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56 190 The modeling tool used to interpret the batch experiments is founded on an approach that couples
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59 191 MATLAB® and the geochemical code PHREEQC ³⁷ via the IPhreeqc module ^{38,39}. A detailed

description of the coupling between the two codes to describe degradation, microbial community dynamics, and geochemical reactions is provided in Murray et al., 2019³¹. In this study, the model was used to describe the following processes:

- cDCE and VC dechlorination and microbial activity of OHRB;
- reduction of other electron acceptors (Fe(III) and sulfate) as well as microbial growth and decay of SRB and FeRB;
- geochemical reactions including equilibrium aqueous speciation, mineral precipitation, and kinetic abiotic redox reactions;
- kinetic mass transfer of volatile compounds between the aqueous, organic, and gaseous phases;
- sample removal from both aqueous and gaseous phases during the course of the experiments.

A set of differential equations is used to describe the transfer of moles of cDCE, VC, ethene and hydrogen between the gaseous, organic, and aqueous phases, represented by the slim black arrows on the conceptual schematic in Figure 1 (Equations S1-S5). Dual Monod kinetics (Equation S6) are used to describe chlorinated ethene respiration by OHRB (thick black arrows, Figure 1), sulfate reduction by SRB and SRBi (green and dark yellow arrows, respectively, Figure 1) and Fe(III) reduction by FeRB (red arrows, Figure 1). First order kinetics are used to model Fe(III) reduction by SRB and SRBi (green and dark yellow arrows, respectively, Figure 1, Equation S8)²⁹, and speciation reactions (Table S2) were used to determine the bioavailable portion of Fe(III)-citrate⁴⁰. Hydrogen is consumed in all bacterial processes (blue lines, Figure 1). Abiotic Fe(III) reduction by biogenic sulfide (Equation S9-S10) is included in the model to proceed as first order with respect to Fe(III) and 0.5 order with respect to sulfide (dotted black lines, Figure 1)⁴¹. Precipitation of Fe(II)

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6 216 PHREEQC reaction module (dotted black lines, Figure 1) ⁴². Chemical reactions included in the
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9 217 model are listed in Table 1. Expressions for mass transfer, aqueous speciation, biotic Fe(III) and
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11 218 sulfate reduction, bacterial growth and decay, abiotic Fe(III) reduction, and FeS_(s) precipitate
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13 219 formation are included in the SI (Equations S1-S11).

221 *Figure 1. Conceptual model of the processes that occur in the experiment. Slim, black arrows:*
222 *mass transfer processes; $\kappa_{i,o-aq}$ is the mass transfer coefficient between the organic and*
223 *aqueous phases, and $\kappa_{i,o-g}$ between the organic and gas phases, for compound i . Thick arrows*
224 *indicate microbial processes: black: chlorinated ethene dechlorination by OHRB, red: iron*

reduction by FeRB (both added FeRB and FeRB native to the OHRB consortium), and dark green and dark yellow: sulfate and iron reduction by added SRB and SRBi (SRB native to the OHRB consortium), respectively. Blue lines: hydrogen used in these reduction processes. Dotted black lines: abiotic geochemical processes. Note that Fe(II) and Fe(III) speciation is not explicitly included in the conceptual model.

Table 1. Stoichiometry of biogeochemical reactions included in the model. R_{ED} = electron donor half-reaction, R_{EA} = electron acceptor half reaction, R_{Bio} = biomass synthesis half reaction. See SI for kinetic description of $R_{EA}:Fe^{3+}$ for SRB and SRBi, $R_{Abio}:Fe^{3+}$, and $R_{precip}:FeS_{(s)}$.

Reaction	Group	Stoichiometry
R_{ED}	OHRB, FeRB, SRB, SRBi	$\frac{1}{2}H_2 \rightarrow H^+ + e^-$
R_{Bio}	OHRB, FeRB, SRB, SRBi	$\frac{1}{2}CH_3COO^- + \frac{1}{4}CO_2 + \frac{1}{4}NH_4^+ + \frac{5}{4}H^+ + e^- \rightarrow \frac{1}{4}C_5H_7O_2N + H_2O$
$R_{EA}: cDCE$	OHRB	$\frac{1}{2}C_2H_2Cl_2 + H^+ + e^- \rightarrow \frac{1}{2}C_2H_3Cl + \frac{1}{2}H^+ + \frac{1}{2}Cl^-$
$R_{EA}: VC$	OHRB	$\frac{1}{2}C_2H_3Cl + H^+ + e^- \rightarrow \frac{1}{2}C_2H_4 + \frac{1}{2}H^+ + \frac{1}{2}Cl^-$
$R_{EA}: Fe^{3+}$	FeRB, SRB, SRBi	$Fe^{3+} + e^- \rightarrow Fe^{2+}$
$R_{EA}: SO_4^{2-}$	SRB, SRBi	$\frac{1}{8}SO_4^{2-} + \frac{19}{16}H^+ + e^- \rightarrow \frac{1}{16}H_2S_{(g)} + \frac{1}{16}HS^- + \frac{1}{2}H_2O$
$R_{Abio}: Fe^{3+}$	Abiotic	$2Fe^{3+} + HS^- \rightarrow 2Fe^{2+} + S^0 + H^+$
$R_{precip}: FeS_{(s)}$	Abiotic	$Fe^{2+} + HS^- \rightarrow FeS_{(s)} + H^+$

Dechlorination of cDCE to ethene was modelled as a two-step process, with production and consumption of the intermediate VC. Competitive inhibition by cDCE reduction was included in

the kinetic description for the dechlorination of VC to ethene¹⁷. The change in VC concentration in the aqueous phase is described by Equation (1):

$$\frac{dC_{VC,aq}}{dt} = L(t) \left[k_{max,cDCE} X \left(\frac{C_{cDCE,aq}}{C_{cDCE,aq} + K_{S,cDCE}} \right) \left(\frac{C_{H2,aq}}{C_{H2,aq} + K_{S,H2}} \right) - k_{max,VC} X \left(\frac{C_{VC,aq}}{C_{VC,aq} + K_{S,VC} \left(1 + \frac{C_{cDCE,aq}}{K_{CI,cDCE}} \right)} \right) \left(\frac{C_{H2,aq}}{C_{H2,aq} + K_{S,H2}} \right) \right] \quad (1)$$

where $dC_{VC,aq}/dt$ [mol L⁻¹ h⁻¹] is the temporal change in the VC concentration in the aqueous phase; $C_{cDCE,aq}$, $C_{VC,aq}$, and $C_{H2,aq}$ [M] are the aqueous concentrations of cDCE, VC, and hydrogen; $k_{max,cDCE}$ and $k_{max,VC}$ [mol cell⁻¹ s⁻¹] are the maximum specific reduction rates of cDCE and VC; X [cells L⁻¹] is the biomass concentration; t [s] is time; $K_{S,cDCE}$, $K_{S,VC}$, and $K_{S,H2}$ [M] are the half-saturation constants for cDCE, VC, and hydrogen; and $K_{CI,cDCE}$ [M] is the competitive inhibition coefficient. As described in¹⁷, $K_{CI,cDCE}$ is equivalent to $K_{S,cDCE}$. Similarly to Murray et al., 2019³¹, the maximum specific reduction rates of the bacterial guilds were fitted for during model development. Simple manual calibration of the key kinetic parameters to the experimental observations was performed in this study. The normalized root mean square error (NRMSE) was minimized to obtain the best fit. The function $L(t)$ (Equation 2) describes the lag-time for each batch^{40,43,44}:

$$L(t) = \begin{cases} = 0 & t \leq t_{lag} \\ = 1 & t > t_{lag} \end{cases} \quad (2)$$

where the t_{lag} [s] is the time from the start of the batch until the lag time ends and the exponential growth phase begins. The lag function was used to simulate the processes carried out by the OHRB and SRBi guilds, and was not needed for the SRB and FeRB guilds.

The mass transfer of the volatile compounds cDCE, VC, ethene, and hydrogen across the organic-gaseous and organic-aqueous phase boundaries and the evolution of their amounts in the different phases were modeled by:

$$\left(\frac{dn_i}{dt}\right)_{org-j} = V_j(C_{i,j}^{eq} - C_{i,j})\left(\frac{\kappa_{i,org-j}A}{V_{org}}\right) \quad (3)$$

where $(dn_i/dt)_{org-j}$ [mol s⁻¹] is the change in moles of compound i through time, t [s], across the boundary between the organic phase and phase j , V_j [L] is the volume of phase j , $C_{i,j}$ [M] and $C_{i,j}^{eq}$ [M] are the concentration and equilibrium concentration of compound i in phase j , $\kappa_{i,org-j}$ [dm s⁻¹] is the mass transfer coefficient, and A [dm²] is the cross-sectional area of the phase interface²¹.

Gas and aqueous phase samples were collected in this experiment. The modeling approach accounts for the removal of mass from the aqueous phase and the gaseous headspace by:

$$n_i = C_{i,gas}V_{gas} + C_{i,org}V_{org} + C_{i,aq}V_{aq} - C_{i,aq}V_{S,aq} \quad (4)$$

$$n_i = C_{i,gas}V_{gas} + C_{i,aq}V_{aq} + C_{i,org}V_{org} - \left(\frac{C_{i,gas}V_{gas}}{V_{gas} + V_{S,gas}}\right)V_{S,gas} \quad (5)$$

where n_i [mole] is the remaining moles of compound i after the sample is removed, $C_{i,j}$ [M] is the concentration of compound i in phase j , V_j [L] is the volume of phase j , and $V_{S,aq}$ and $V_{S,gas}$ [L] is the volume of the removed sample. The volume of the headspace does not change when a gas sample is collected; $V_{S,gas}$ can most accurately be described as the volume by which the headspace increases before the sampling syringe is removed. The concentrations of the compound in each phase after

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sample removal was then calculated ⁴⁵ using the partition coefficients $K_{gas-org}$ and K_{org-aq} [-] (Table S3) and the pressure calculated based on the change in moles of volatile compounds in the gas phase.

3. Results

The OHRB consortium was naturally capable of iron- and sulfate-reduction. These processes were promoted by the addition of Fe(III) and sulfate to the growth medium. Each batch experiment therefore did not differ in the processes present in the batch system but by the rate of those processes and the changes in the microbial community composition. We collected aqueous and gaseous samples and analyzed for chlorinated ethenes, chloride, Fe(II), sulfate, and community composition. First we present the changes in sulfate concentration and microbial community, then the multiple complementary datasets and the model simulations, and lastly we describe and discuss the impact of the community composition on the cDCE and VC respiration kinetics.

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3.1 Sulfate-reducing *Desulfovibrio* populations

A decrease in the sulfate concentration through time was observed in all batches, including those where no SRB was added (Figure 2). There are clear similarities in Batches 1 and 3, where SRB were not expressly added via the batch inoculum, versus Batches 2 and 4, where SRB were expressly included in the batch inoculum. In Batches 1 and 3, the sulfate concentration decreased slowly in the first 6 days, decreased more rapidly from day 6, and all sulfate was consumed by day 16. In Batches 2 and 4, the sulfate concentration began to decline between days 0 and 3 and all sulfate was consumed within the first 9 days of the experiment. The disappearance of sulfate coincides with visual observation of black, iron-sulfide precipitate formation in each batch.

Figure 2. Sulfate concentration and relative abundance for all experimental batches. Color legend indicates fragment lengths identified through T-RFLP analysis. Fragments of length 126-128: *Dehalococcoides* spp. (OHRB), 205-206: exogenous *Desulfovibrio vulgaris* (SRB), 208-210: endogenous *Desulfovibrio* spp. (SRBi), 234: exogenous and endogenous *Shewanella oneidensis* (FeRB). All other terminal fragments with other lengths indicate other populations

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4 295 *present in the microbial community (see SI for more details). Note that the time scale on the*
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7 296 *x-axis has been selected to show observed trends in the data and is not consistent between*
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9 297 *panels. Original batch data is presented; original and duplicate batch data are presented in*
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15 299 In all experimental batches, T-RFLP shows that fragments attributed to *Dehalococcoides* spp.
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17 300 comprise between 60-80% of the total fragments present in the microbial community at the
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21 302 relative abundance and fragments of length 205-206 (Batches 2 and 4) and length 208-210 (Batches
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23 303 1 and 3) increase in relative abundance. Fragments of length 205-206 were attributed to the
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25 304 endogenous *Desulfovibrio* spp. and fragments of length 208-210 were attributed to the added SRB,
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27 305 *Desulfovibrio vulgaris* Hildenborough (see SI). Based on the correlation between the sulfate
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35 309 *Desulfovibrio* population indigenous to the OHRB consortium (the aforementioned SRBi). Both
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37 310 populations reduced sulfate in a period of approximately 10 days, but the SRBi appear to have
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49 316 Fragments of length 234, attributable to *Shewanella oneidensis*, the added FeRB, were also found in
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consortium (see SI), and exogenous and endogenous populations in Batches 3 and 4 could not be distinguished using T-RFLP. It is also of note that there is excellent agreement between the duplicates for each batch, in both sulfate concentration and microbial community composition (Figure S2).

3.2 Physical and biogeochemical data and process simulations

The multiple, complementary datasets gathered for each of the experimental batches and their simulated development through time are depicted in Figure 3 (example for Batch 3, all batches and duplicates in SI, all NRMSE values in SI). All batches and their duplicates demonstrate a relatively slight increase in the chloride concentration (Figure 3a) within the first 9 days of the experiment, which is speculated to be the result of residual activity in the inoculum. The measured chloride concentration then plateaued for a period of approximately one month before increased concentrations of this OHR product were detected. After the plateau, the chloride concentration increased rapidly for a period of approximately three weeks and then increased less rapidly for the remainder of the experiment. It appears that the change in behavior of the aqueous chloride concentration increase aligns with the disappearance of cDCE and appearance of ethene in the headspace (Figure 3a and Figure 3d). Sulfate and Fe(II) concentrations (Figure 3c) showed exhaustion of the electron acceptors sulfate and Fe(III) within the first two weeks of each experimental duration. The evolution of these aqueous chemical parameters were captured fairly well in the case of Fe(II) and very well in the case of chloride and sulfate. Abiotic reduction and mineral precipitation reactions in the model simulations in this study are discussed in detail in Murray et al., 2019³¹. Lastly, it is important to note that the x-axis in Figure 3c is scaled differently than the other panels; all sulfate and Fe(III) in all batches was reduced before chloride appreciation began.

Figure 3. Data and simulations for aqueous chloride concentration (a), microbial population relative abundance (b), aqueous total Fe(II) and sulfate concentrations (c), and relative headspace composition of chlorinated ethenes (d). Example shown for Batch 3 (OHRB, FeRB, and SRBi), other batches and all batch duplicates in SI (Figures S3-S8). Relative abundance (b) data are normalized to the four selected guilds OHRB, FeRB, SRB, and SRBi. Time axis is scaled for panel (c) to best display the change in concentration that occurs at the beginning of the time series.

Normalized relative microbial community abundance data (Figure 3b) depict the same trend seen in Figure 2; the dominant population shifts from OHRB at the beginning of the experiment to SRB or SRBi, and then shifts back to OHRB after approximately 1.5 months. The model is able to simulate these shifts in dominance, both for SRBi (Figure 3b) and for SRB (SI). Measurement of the relative headspace composition (Figure 3d) shows that nearly all cDCE is transformed to VC, which is further transformed to ethene. The abrupt disappearance of cDCE in the headspace and the gradual transformation of VC to ethene suggest that competitive inhibition is only present in one direction (i.e. cDCE inhibits VC dechlorination)¹⁸. Simulations using this one-way competitive inhibition successfully capture the transitions from cDCE to VC and from VC to ethene.

The example of the multiple datasets and simulations for Batch 3 presented in Figure 3 demonstrates that the processes occurring in the experimental batch system are accurately described by the proposed model. Successful simulation of both the aqueous chemical species and the microbial community relative abundance show quantitative understanding of how the reduction processes, aquatic geochemistry, and bacterial growth are linked. Likewise, simulation of the chloride concentration and the relative headspace evolution demonstrate understanding of how chloride production is attributed to each step in the two-step dechlorination process. Additionally, capturing the dynamic changes in the headspace composition demonstrates that the model is able to accurately capture mass transfer in the multiphase system.

3.3 Microbial process comparison and dechlorination kinetics

Because the concentration of the intermediate VC was established, the contribution of each chlorinated ethene degradation step to the aqueous chloride measured concentration could be distinguished. The evolution of chlorinated ethene degradation in the batches can then be inferred from the chloride concentration evolution. When comparing the chloride concentrations of each batch (Figure 4), there appears to be a difference in the duration of the concentration plateau,

hereafter called the OHR lag phase, between Batches 1 and 3 and Batches 2 and 4. However, differences in the lag duration between duplicates in Batches 3 and 4 (SI) indicate this is unlikely the result of the microbial community composition. Lag parameters used for each batch are included in the SI. When adjusted for the difference in the lag period, duplicate batches show agreement in the shape of the chloride production curve.

Figure 4. Chloride evolution data (points) and simulations (lines) for all batches. Filled data points and solid lines represent batches without exogenous FeRB (Batch 1 & 2); open data points and dashed lines represent batches with exogenous FeRB (Batch 3&4). Black data points and lines represent batches without exogenous SRB (Batch 1&3); green data points and lines represent batches with exogenous SRB (Batch 2&4).

Particularly in the later parts of the curve, the observed increase in the measured chloride concentration was more rapid in Batches 2 and 4, those with exogenous SRB, than in Batches 1 and 3 (Figure 4). In the later points during the time series, cDCE is depleted and all produced chloride

is attributable to VC respiration. The shift from the period where cDCE dechlorination dominates to where VC dechlorination dominates is apparent by a shift in the slope of each simulated chloride curve (Figure 4).

Each batch and its duplicate were simulated using the same electron acceptor maximum specific utilization rates $k_{max,cDCE}$ and $k_{max,VC}$ (Table 2, Figure S3), affirming that when the lag phase is accounted for, there is agreement between the duplicates. By including a lag period of 3 d for SRBi, both SRB guilds were able to be fit using the same $k_{max,SRB}$, $K_{S,SRB,SO4}$, and $K_{S,SRB,H2}$ (Table 2, Table S2). Similarly, the endogenous and exogenous FeRB were able to be simulated by the same set of $k_{max,FeRB}$, $K_{S,FeRB,Fe3}$, and $K_{S,FeRB,H2}$ kinetic parameters (Table 2, Table S2).

Table 2. Maximum specific substrate utilization rates determined for each batch and its duplicate

Batch	Inoculum	$k_{max,cDCE}$ mol _{cDCE} cell ⁻¹ s ⁻¹	$k_{max,VC}$ mol _{VC} cell ⁻¹ s ⁻¹	$k_{max,FeRB}$ mol _{Fe3} cell ⁻¹ s ⁻¹	$k_{max,SRB}$ mol _{SO4} cell ⁻¹ s ⁻¹
1	OHRB	7×10^{-20}	8.5×10^{-21}		
2	OHRB + SRB	9×10^{-20}	2.5×10^{-20}	1×10^{-18}	7×10^{-18}
3	OHRB + FeRB	7×10^{-20}	1.0×10^{-20}		
4	OHRB + SRB + FeRB	8×10^{-20}	1.6×10^{-20}		

Batches 1 and 3 were simulated by the same cDCE dechlorination rate, which was also the slowest of all four batches. The $k_{max,cDCE}$ for Batches 2 and 4, which included the exogenous SRB, were 129% and 114% higher, respectively, than in Batches 1 and 3. The $k_{max,VC}$ varied for each batch, and was slowest in Batch 1. The $k_{max,VC}$ for Batches 2, 3, and 4 were 294%, 118%, and 188% higher, respectively, than for Batch 1. The two highest $k_{max,cDCE}$ and $k_{max,VC}$ were found for Batches 2 and 4, which included the exogenous SRB. Model simulations also show that aqueous hydrogen concentrations never decreased below 9.7×10^{-5} M, confirming that the systems were indeed electron acceptor limited.

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Simulation results for the biotic consumption of electron acceptors in Figure 5 show how the microbial processes occurred at different times during the experimental duration. The same maximum Fe(III) and sulfate utilization rates for the FeRB and SRB/SRBi, respectively, were able to capture the behavior of these guilds, and the extent of these processes then impacted cDCE and VC dechlorination, which occurred later in the time series. The maximum consumption rate for the OHRB processes is much lower than that of the SRB and FeRB processes, and the duration of these OHRB processes is much longer. The abrupt decrease in the cDCE and increase in VC dechlorination rates is due to the choice to model the system as inhibited in one direction. The inclusion of exogenous SRB and subsequent increase in the $k_{max,VC}$ impacts the shape of the VC dechlorination rate curve. In Batches 2 and 4, which included the exogenous SRB, the VC consumption rate is sustained at a higher level for a period of time, whereas in Batches 1 and 3, without the exogenous SRB, the VC rate steadily decreases until the end of the experiment.

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Figure 5. Simulated consumption rates for all microbial processes in each experimental batch. Main panels include all microbial processes for each batch. Insert panels include only chlorinated ethene degradation processes for each batch. Note that Fe(III)-reduction by FeRB is displayed on the secondary y-axis. The OHRB consortium contains the endogenous FeRB and SRBi. Simulation results only; no data shown.

3.4 Discussion

The processes that support or inhibit cDCE and VC dechlorination are of particular importance from an applied environmental perspective, as *in situ* biodegradation of these compounds does not always reliably occur. These daughter compounds often accumulate in natural aquatic environments and may pose an even higher risk than the original contamination⁴⁶. Batch experiments conducted

with a *Dehalococcoides*-containing consortium capable of sequential dechlorination of cDCE to VC and ethene can shed light on the impact of other subsurface respiration guilds on these OHR processes. In order to obtain a fundamental understanding of the biogeochemical and mass transfer processes occurring in the system, multiple complementary datasets were collected and subsequently interpreted with a comprehensive modeling approach that includes degradation kinetics, aquatic chemistry, and interphase mass transfer.

Based on quantification of the maximum specific substrate utilization rates, $k_{max,cDCE}$ and $k_{max,VC}$, and the comparison of the cDCE and VC degradation rates (Figure 5), it appears that the primary effect of the addition of exogenous SRB is the stimulation of VC dechlorination. Addition of exogenous FeRB appears to have a slight stimulation effect on VC dechlorination and has a moderating effect when added in combination with exogenous SRB.

Sulfate reduction and cDCE dechlorination have been documented to proceed concurrently in a variety of systems, including batch experiments, flow-through experiments, and subsurface environments^{14,32,47,48}. Concurrent cDCE dechlorination and sulfate reduction was not observed in the batches in this study; rather, cDCE dechlorination only began after an apparent lag phase of approximately one month, which coincided with the depletion of other electron acceptors in the medium. A lag phase of such length has not been observed with this consortium during regular cultivation and culture transfer, where these electron acceptors are not present in the medium. The stimulating effect on dechlorination caused by the exogenous SRB may be due to the rapid depletion of sulfate, and further stimulation may be seen with less sulfate in the medium. The converse may also be true, that the activity of the exogenous SRB stimulated dechlorination, and increased SRB activity would further stimulate the dechlorination by OHRB after recovery from the apparent lag phase. In a chemostat study conducted by Berggren et al., 2013³², the addition of sulfate to an OHRB-containing community resulted in decreased dechlorination kinetics of

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chlorinated ethenes by OHRB. The rate of VC dechlorination in particular decreased. This decrease correlated with a shift in abundance of strains of *Dehalococcoides mccartyi* present in the community. Because the abundance of strains cannot be distinguished with T-RFLP, it may be that, in the current study, sulfate-reduction spurs a shift in the *Dehalococcoides* sp. populations that can carry out chlorinated ethene reduction at different rates.

Iron reduction has been found to correlate with decreased VC dechlorination in field investigations⁸ and with decreased dechlorination of chlorinated ethenes in laboratory experiments⁴⁹. In this study, the most pronounced effect from the addition of exogenous FeRB corresponds well with these findings; in Batches 2 and 4, the stimulating effect of exogenous SRB in Batch 4 is less than in Batch 2. In Batches 1 and 3, where the exogenous SRB are not present, additional FeRB have a slight stimulating effect on VC dechlorination. SRB and FeRB are functionally and phylogenetically diverse^{29,50,51}, and it may be that the FeRB guild interacts with other populations (OHRB, SRB) differently depending upon the configuration of the microbial community. The FeRB may thus impact the OHRB in different ways.

Abiotic dechlorination of chlorinated ethenes has also been documented to occur, facilitated by iron-sulfide precipitates^{52,53}. However, these reactions occur on a time-scale much greater than that of the experimental duration in the current study, and were not included in the conceptual and numerical models.

By using an electron acceptor limited system, community dynamics other than competition for electron donor may impact OHR. Many incomplete metabolic pathways have been identified in *Dehalococcoides*⁵⁴, and the dependency of OHRB on other members of the microbial community is well-established^{15,55}. The exogenous SRB may fulfill some such types of metabolic interdependency, for instance the added strain of *Desulfovibrio vulgaris* may synthesize a corrinoid

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cofactor that promotes faster dechlorination than the corrinoid provided in the growth medium⁵⁶. It may also be the case that the endogenous SRB were present at a higher initial concentration than the SRBi, and thus could have a greater impact on the OHRB.

4. Conclusion

This study demonstrates that, even in a batch system, the dechlorination activity impacts and is in turn impacted by continuous feedback between microbial kinetics, aquatic chemistry, and mass transfer limitations. When all of these elements were taken into account, the effect of the community composition on the dechlorination rate was ascertained. Microbial communities that contained exogenous SRB performed cDCE and VC dechlorination at 129% and 294% faster rates, respectively, than the case where only endogenous SRB (SRBi) were present. Augmenting the community with additional FeRB stimulated VC dechlorination in presence of only SRBi, but moderated the stimulating effect of exogenous SRB. The different impacts from each SRB and FeRB guild member on the cDCE and VC dechlorination rates show that the dynamics within an OHRB-containing microbial community cannot always be accurately characterized by broad, categorical, inter-guild trends, but are rather the result of intricate interactions between individual community members.

Conflict of interest

There are no conflicts of interest to declare.

Acknowledgements

This study was funded by a DTU alliance PhD scholarship granted to Alexandra Murray. Massimo Rolle acknowledges the support of the Independent Research Fund Denmark (grant DFF-7017-00130).

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