AEM Accepted Manuscript Posted Online 3 February 2017
Appl. Environ. Microbiol. doi:10.1128/AEM.03384-16
Copyright © 2017 American Society for Microbiology. All Rights Reserved.

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

1 Title: The Effects of sulfate reduction on TCE dechlorination by *Dehalococcoides*-
2 containing microbial communities

3 Running head: Sulfate effects on TCE dechlorination

4 Authors: Xinwei Mao[1], Alexandra Polasko[1] and Lisa Alvarez-Cohen[1,2,*]
5 [1]Department of Civil and Environmental Engineering, College of Engineering, University of
6 California, Berkeley, CA, 94720-1710;
7 [2] Earth and Environmental Sciences Division, Lawrence Berkeley National Laboratory, Berkeley,
8 CA 94720
9
10 *Corresponding author: phone (510) 643-5969; fax (510)643-5264
11 Email: alvarez@ce.berkeley.edu
12
13
14
15
16
17
18
19
20
21
22

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

23 **Abstract**

24 In order to elucidate interactions between sulfate reduction and dechlorination, we
25 systematically evaluated the effects of different concentrations of sulfate and sulfide on
26 reductive dechlorination by isolates, constructed consortia and enrichments containing
27 *Dehalococcoides* sp. Sulfate (up to 5 mM) did not inhibit growth or metabolism of pure
28 cultures of dechlorinator *Dehalococcoides mccartyi* 195, sulfate reducer *Desulfovibrio*
29 *vulgaris* Hildenborough (DvH) or syntroph *Syntrophomonas wolfei* (*S. wolfei*). In
30 contrast, sulfide (5mM) exhibited inhibitory effects on growth of the sulfate reducer and
31 the syntroph, as well as on both dechlorination and growth rates of *D. mccartyi*.
32 Transcriptomic analysis of *D. mccartyi* 195 revealed that genes encoding for ATP
33 synthase, biosynthesis and Hym hydrogenase were down-regulated during sulfide
34 inhibition, while genes encoding for metal-containing enzymes involved in energy
35 metabolism were up-regulated even though the activity of those enzymes (hydrogenases)
36 was inhibited. When electron acceptor (trichloroethene) was limiting and electron donor
37 (lactate) was provided in excess to co-cultures and enrichments, high sulfate
38 concentrations (5 mM) inhibited reductive dechlorination due to the toxicity of generated
39 sulfide. The initial cell ratio of sulfate reducers to *D. mccartyi* (1:3, 1:1 and 3:1) did not
40 affect the dechlorination performance in the presence of sulfate (2mM and 5 mM). In
41 contrast, under electron donor limitation, dechlorination was not affected by sulfate
42 amendments due to low sulfide production, demonstrating that *D. mccartyi* can function
43 effectively in anaerobic microbial communities containing moderate sulfate
44 concentrations (5 mM), likely due to its ability to out-compete other hydrogen-consuming
45 bacteria and archaea.

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

46

47 Key words: reductive dechlorination, sulfate reduction, sulfide generation, inhibition,

48 competition

49 **Importance**

50 Sulfate is common in subsurface environments and has been reported as a co-
51 contaminant with chlorinated solvents at various concentrations. Inconsistent results for
52 the effects of sulfate inhibition on the performance of dechlorination enrichment cultures
53 have been reported in the literature. These inconsistent findings make it difficult to
54 understand potential mechanisms of sulfate inhibition and complicate the interpretation of
55 bioremediation field data. In order to elucidate interactions between sulfate reduction and
56 reductive dechlorination, this study systematically evaluated the effects of different
57 concentrations of sulfate and sulfide on reductive dechlorination by isolates, constructed
58 consortia and enrichments containing *Dehalococcoides sp*. This study provides us with a
59 more fundamental understanding of the competition mechanisms between reductive
60 dechlorination by *D. mccartyi* and sulfate reduction during bioremediation process. It
61 also provides insights on the significance of sulfate concentrations on reductive
62 dechlorination under electron donor/acceptor limiting conditions during *in situ*
63 bioremediation applications. For example, at a TCE contaminated site with high sulfate
64 concentration, proper slow-releasing electron donors can be selected to generate an
65 electron donor limiting environment that favor reductive dechlorination and minimize
66 sulfide inhibition effect.

67

68

Accepted Manuscript Posted Online

AEM
Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

69 **Introduction**

70 Perchloroethene (PCE), trichloroethene (TCE) and their daughter products
71 dichloroethene (DCE) and vinyl chloride (VC) are common soil and groundwater
72 contaminants with established toxicity and mutagenicity towards many organisms.[1-3] *In*
73 *situ* bioremediation processes that stimulate the growth of anaerobic microbial
74 communities capable of reductively dechlorinating these contaminants to harmless ethene
75 (ETH) are of great interest.[4] Among reported dechlorinating species, *Dehalococcoides*
76 *mccartyi* is the only known bacterium that can reductively dechlorinate PCE and TCE all
77 the way to ethene.[1] *D. mccartyi* requires H_2 as its exclusive electron donor, acetate and
78 CO_2 as carbon sources and vitamin B_{12} as a co-factor. [5-7] Although reductive
79 dechlorination can occur under a variety of redox conditions,[8] dechlorination commonly
80 only accounts for a small fraction of electron flow in microbial communities during
81 bioremediation.[9-11] Other terminal electron accepting processes (TEAPs), such as sulfate
82 reduction, iron reduction, nitrate reduction, methanogenesis, homoactogenesis, and
83 volatile fatty acids formation typically account for a large fraction of the electron flow in
84 these systems (Table S1).

85 Sulfate is common in subsurface environments and is often reported as a co-
86 contaminant with chlorinated solvents at various concentrations (0.2- 30 mM).[12-17] The
87 effects of sulfate and its reduction product sulfide on other terminal electron accepting
88 processes have been explored in anaerobic digestion, sulfate reduction, nitrification, as
89 well as reductive dechlorination processes.[17-21] Hydrogen sulfide (H_2S) has been shown
90 to inhibit the growth of sulfate reducing bacteria at 16 mM due to both its intrinsic
91 toxicity and indirect toxicity by precipitation with iron as ferric sulfide.[19]

Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

92 The dechlorination of solvents under sulfate reducing conditions is complicated
93 and less well studied. Although successful organic-stimulated bioremediation of solvents
94 has been observed in aquifers containing sulfate, the typical approaches involve injecting
95 an excess of electron donor in order to deplete sulfate and to avoid competition for
96 hydrogen between dechlorination and sulfate reduction.[22-23] This approach was shown to
97 be successful at some field sites; however, it has proven to be unsuccessful at sites with
98 high sulfate concentrations or complex geochemical conditions. [15, 24-26]

99 There are a limited number of laboratory studies with detailed information on the
100 effects of sulfate on dechlorination.[21,26-28] In addition, some conflicting results due to
101 sulfate addition, ranging from enhanced dechlorination[27,29,30] to inhibited or incomplete
102 dechlorination[15, 27, 29,31,32] as well as no observed effect on dechlorination [16,25] have been
103 reported over the past decade. A review of published field data from TCE-contaminated
104 sites with sulfate concentrations ranging from 39 mg L^{-1} to 4,800 mg L^{-1} reported the
105 overall trend that as sulfate concentrations increased, dechlorination reactions became
106 incomplete or delayed. [26] In addition, among these previous studies, there have only been
107 a few that used microbial communities with the confirmed presence of *D. mccartyi*
108 [15,28,30,32,33] and cellular quantification has been lacking. Further work is needed to clarify
109 the significance of sulfate concentrations on reductive dechlorination under electron
110 donor/acceptor limiting conditions. In addition, the effects of sulfide, the sulfate
111 reduction product, on dechlorination needs to be systematically evaluated.

112 In this study, we hypothesize two main mechanisms for the observed failure of
113 complete dechlorination during bioremediation in sulfate-containing environments: 1)
114 inhibition of enzymes involved in dechlorination by the sulfate reduction product sulfide;

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

115 2) the predominance and faster growth kinetics of sulfate reducing bacteria, compared
116 with *D. mccartyi* at high H_2 concentrations (electron acceptor limitation). In order to test
117 these hypotheses, we investigated the inhibitory effect of sulfate and sulfide on i) pure *D.*
118 *mccartyi* strain and supporting microorganisms, ii) constructed syntrophic consortia at
119 different cell ratios and electron donor/ acceptor limited conditions, and iii) a
120 methanogenic dechlorinating enrichment culture with high and low sulfate amendments.
121 Transcriptomic analysis of *D. mccartyi* was used to investigate gene expression patterns
122 during sulfide inhibition in order to better understand the mechanism of inhibition. This
123 study provides a fundamental understanding of the effects of sulfate reduction on
124 reductive dechlorination by *D. mccartyi*.

125 **Material and Methods**

126 *Bacterial cultures and growth condition*

127 *Dehalococcoides mccartyi* strain 195 (strain 195) was grown in defined medium
128 with H_2/CO_2 (90:10) headspace, 0.6 mM TCE as electron acceptor and 2 mM acetate as
129 carbon source [6] (Figure 1 A). *Desulfovibrio vulgaris* Hildenborough (DvH) was grown in
130 the same defined medium with N_2/CO_2 headspace, 10 mM lactate as electron donor and 5
131 mM sulfate as electron acceptor. *Syntrophomonas wolfei* (*S. wolfei*) was grown on
132 crotonate in 160 mL serum bottles as described previously.[34] Bacterial co-cultures of *S.*
133 *wolfei* and strain 195 (*S. wolfei* and strain 195 were inoculated at 5% of the total liquid
134 volume, respectively) were sustainably maintained on 5 mM butyric acid (5% vol/vol
135 inoculation) with 0.6 mM TCE as described previously.[35] Bacterial co-cultures of DvH
136 and strain 195 (DvH/195, 5% vol/vol inoculation) were sustainably maintained on 5 mM

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

137 lactic acid (5% vol/vol inoculation) with 0.6 mM TCE as described previously.[36] The
138 methanogenic dechlorinating community ANAS was previously enriched from
139 contaminated soil obtained from Alameda Naval Air Station (CA). The culture has been
140 maintained in the laboratory for over 15 years in a continuously stirred semi-batch fed
141 reactor, and its community structure and dechlorination performance have been
142 previously described. [37-39]

143 In order to study the competition between reductive dechlorination and sulfate
144 reduction under electron acceptor limitation (electron donor in excess), strain 195 and
145 DvH were grown in defined medium with H_2/CO_2 headspace (Figure 1 C) with 0.7 mM
146 TCE and 2 mM or 5 mM sulfate (Table S2). For electron donor limitation experiments,
147 tri-cultures containing *S. wolfei*/DvH/195 were constructed in defined medium (Table S2)
148 with 7.0 mM butyric acid, 0.7 mM TCE and 2 mM (or 5 mM) sulfate and N_2/CO_2 (80:20,
149 vol/vol) headspace (Figure 1 D). For both electron donor and acceptor limiting
150 conditions, TCE (0.7mM per dose) was amended to the cultures when the previous dose
151 was depleted. All experiments were performed in triplicate. After three sub-culturing
152 events (5% vol/vol inoculation), tri-culture *S. wolfei*/DvH/195 cells were harvested
153 during late exponential phase (day 6) and analyzed by scanning electron microscopy
154 (SEM) as described previously.[35]

155 *Chemical analysis*

156 Chloroethenes and ethene were measured by FID-gas chromatograph using 100
157 µL headspace samples, and hydrogen and carbon monoxide were measured by RGD-gas
158 chromatography using 300 µL headspace sample as described previously.[37, 40] The mass
159 of each compound was calculated based on gas-liquid equilibrium by using Henry's law

Accepted Manuscript Posted Online

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

160 constants at 34°C. Organic acids, including butyrate and acetate, were analyzed with a
161 high-performance liquid chromatograph as described previously.[37] Sulfate concentration
162 was measured by suppressed Ion Chromatography (Dionex ICS 1100) on a Dionex
163 IonPac AERS500 column (4mm) with 4.5 mM Na_2CO_3/0.8 mM $NaHCO_3$ as eluent.
164 Sulfide concentration was measured at the end of experiments by methylene blue
165 method.[41] Trace metals concentrations were analyzed on an Agilent Technologies 7700
166 series ICP-MS.[42]

167 *DNA extraction and cell number quantification*

168 1.5 mL liquid samples were collected during the incubation for cell density
169 measurements and cells were harvested by centrifugation (21,000 × *g*, 10 min at 4°C).
170 Genomic DNA was extracted from cell pellets using Qiagen DNeasy Blood and Tissue
171 Kit according to the manufacturer's instructions for Gram-positive bacteria. qPCR using
172 SYBR Green-based detection reagents was applied to quantify gene copy numbers of
173 each bacterium with *S.wolfei* 16S rRNA gene primers (forward primer 5'-
174 GTATCGACCCCTTCTGTGCC-3', and reverse primer 5'-
175 CCCCAGGCGGGATACTTATT-3'), [43] DvH 16S rRNA gene primers (forward primer
176 5'-AATCGGAATCACTGGGCGTA-3' and reverse primer 5'-
177 CCCTGACTTACCAAGCAGCC-3'), [36] and *D. mccartyi tceA* gene primers (forward
178 primer 5'-ATCCAGATTATGACCCTGGTGAA-3' and reverse primer 5'-
179 GCGGCATATATTAGGGCATCTT-3'), as previously described.[44] Cell number
180 calculation was normalized based on target gene copy numbers in each genome of the
181 bacterium.

AEM
Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

182 *RNA preparation and transcriptome analysis*

183 100 mM stock solution of sulfide-S (the sum of all speciation of H_2S-S, HS^--S
184 and S^{2-}-S) was prepared from $Na_2S \cdot 9H_2O$ in the defined culture medium. 10 mM sulfide-
185 S was amended to strain 195 cultures on day 4 during mid-log growth phase when 50%
186 of TCE was degraded. Cultures were sampled on day 6 when control bottles exhibited
187 late exponential growth (around 75% of 78 μmol TCE was dechlorinated). In order to
188 collect sufficient material for transcriptomic microarray analysis, 60 bottles of sulfide-S
189 amended strain 195 cultures and 18 bottles of control bottles (strain 195, no sulfide-S
190 addition) were inoculated and grown from triplicate bottles of the isolate. For each
191 biological triplicate, cells from 20 bottles were collected by vacuum filtration on day 6
192 for the experimental group and the control (300 mL culture per filter, 0.2-μm autoclaved
193 GVWP filter (Durapore membrane, Millipore, Billerica, MA)). Each filter was placed in
194 a 2 mL orange-cap micro-centrifuge tube, frozen with liquid nitrogen and stored at -80 °C
195 until further processing. RNA extraction and preparation were described previously.[35]

196 *Transcriptomic microarray analysis*

197 The Affymetrix GeneChip microarray used in this study has been described
198 previously.[45] Briefly, the chip contains 4,744 probe sets that represent more than 98% of
199 the ORFs from four published *Dehalococcoides* genomes (strain 195, VS, BAV1, and
200 CBDB1). cDNA was synthesized from 9 μg RNA**,** then each cDNA sample was
201 fragmented, labeled and hybridized to each array. All procedures were performed with
202 minimal modifications to the protocols in section 3 of the Affymetrix GeneChip
203 Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology
Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

204 http://www.affymetrix.com). Microarray data analysis methods were described
205 previously.[36,39]

206 **Results**

207 *Sulfate and sulfide effects on axenic cultures*

208 An environmentally high sulfate concentration (5 mM) did not affect cell growth
209 or dechlorination rates of strain 195 (data not shown), a bacterium unable to reduce
210 sulfate to sulfide. We also tested the effect of sulfide (the reduction product of sulfate) on
211 cell growth of strain 195, and found that with 5% inoculation, it took 6, 10 and 14 days to
212 dechlorinate 75 µmol TCE in the presence of 0 mM, 2 mM and 5 mM sulfide,
213 respectively. The cell yield of strain 195 decreased about 65% as sulfide concentrations
214 increased from 0 to 5 mM (Supporting material Figure S1 A). For *S. wolfei* (another
215 bacterium incapable of sulfate reduction) grown with crotonate as electron donor, cell
216 growth was not inhibited by 5 mM sulfate addition while 5 mM sulfide decreased cell
217 yields by 40% compared to the control group (Supporting material Figure S1 C). For the
218 DvH isolate that is capable of sulfate reduction, when sulfide concentrations were above
219 10 mM, cell growth was inhibited (Supporting material Figure S1 B).

220 *Effect of sulfate reduction on dechlorination under electron acceptor limitation*

221 Syntrophic co-culture DvH /195 grows sustainably on lactate and TCE with DvH
222 fermenting lactate to acetate and H_2 that are used by strain 195 as carbon source and
223 electron donor for reductive dechlorination of TCE, respectively.[36] DvH is capable of
224 reducing sulfate and hence produce sulfide. In this study, 12 mM lactate was amended to
225 the co-culture initially as electron donor, while 5 mM sulfate and 0.55 mM TCE were

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

226 added as electron acceptors (Figure 1 B). Based on stoichiometry, 11.1 mM lactate would
227 be required to reduce both electron acceptors: 10 mM lactate for sulfate reduction to
228 sulfide and 1.1 mM lactate for TCE reduction to ethene, creating electron acceptor
229 limitation. Aqueous H_2 concentrations increased to 1.4 ± 0.6 μM on day 2 in the sulfate-
230 fed co-culture (Figure 2 A), compared to 43.1 ± 3.7 μM in the control group without
231 sulfate amendment (lactate fermentation only, data not shown). When H_2 in the co-
232 culture dropped below 0.1 μM on day 4, another 5mM lactate was amended to the culture
233 and H_2 slightly increased to above 1.0 μM on day 5, indicating that lactate fermentation
234 was proceeding. However, TCE dechlorination rates decreased by 62% from day 4 to day
235 9, and no cell growth was observed. On day 9, another 2 mM lactate was added to the co-
236 culture and H_2 slightly increased to 2.0 μM, but both dechlorination and cell growth
237 stalled from day 9 to day 16 (Figure 2 B). The 5mM sulfate was depleted within 4 days.
238 On day 5 no sulfate was detected while sulfide concentration was measured to be 4.8 ±
239 0.7 mM (Figure 2 C). At the end of the experiment (day 16), the cell number ratio of
240 strain 195 to DvH was about 1:6 in contrast to the no sulfate control (co-culture grown on
241 lactate and TCE), where the ratio was 4.3:1, similar to previously reported ratios.[36]
242 Sulfide can precipitate metals that are necessary nutrients and hence make them
243 inaccessible to the cells.[46] In order to demonstrate that the lack of dechlorination
244 observed in this study was due to sulfide inhibition instead of trace metal insufficiency
245 caused by sulfide precipitation, at the end of the experiment (day 16) the headspace of the
246 experimental bottles were flushed for 40 minutes with sterilized nitrogen gas to remove
247 sulfide and bottles were re-amended 0.5 mM TCE and 1 mM lactate. Complete TCE
248 dechlorination was observed after five days (data not shown). In addition, at the end of

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

249 the experiment (day 16), trace metals concentrations in the liquid medium were observed
250 to be at the same micro molar levels as in the positive controls (no sulfate amendment).
251 To further investigate the effect of sulfate reduction on reductive dechlorination
252 under electron acceptor limitation, the activity of co-culture DvH/195 was quantified at
253 different inoculum cell ratios (Table S2) with excess H_2 as electron donor (H_2/CO_2
254 headspace, 90:10 vol:vol, Figure 1 C). When 2 mM sulfate was amended to the co-
255 culture, no negative effects on dechlorination or cell growth were observed compared to
256 the positive control (no sulfate amendment) among different cell ratios, while sulfate was
257 fully reduced to sulfide (Supporting material Figure S2). However, when 5 mM sulfate
258 was amended to the co-culture, all sulfate was reduced by the end of the experiment at all
259 cell ratios with reduced product sulfide (supporting material Figure S3) and TCE
260 degradation stalled after day 4 (Figure 3 A and supporting material Figure S3). Both
261 sulfate reduction rates and growth rates of DvH were higher when inhibition of
262 dechlorination occurred, and > 99.5% of consumed electron equivalents (i.e. H_2) went to
263 sulfate reduction (Table 1) rather than to dechlorination.

264 *Effect of sulfate reduction on dechlorination under electron donor limitation*

265 In order to study the competition for H_2 by dechlorination and sulfate reduction
266 under electron donor limitation, we maintained a tri-culture of *S. wolfei*/DvH/195 on
267 5mM butyrate, 0.7 mM TCE and 2 mM sulfate (Figure 1 D) with different initial cell
268 ratios of DvH to strain 195 (Table S2). In these cultures, *S. wolfei* ferments butyrate to
269 acetate (used for biosynthesis) and H_2, which is competed by 195 for dechlorination and
270 DvH for sulfate reduction, thus maintaining the requisite low H_2 concentrations to sustain
271 energetically unfavorable butyrate degradation by *S. wolfei*. DvH does not use butyrate

Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

272 as an electron donor for sulfate reduction. Based on stoichiometry (equations in Table
273 S1), 5.4 mM butyrate would be required to fully reduce each of the electron acceptors: 2
274 mM sulfate to hydrogen sulfide and 0.7 mM TCE to ethene. 5 mM butyrate was first fed
275 to the tri-culture to generate electron donor limiting conditions. Another 0.7 mM TCE
276 and 1 mM butyrate were subsequently amended to the culture when the previous dose of
277 TCE was depleted. During the experimental period, H_2 remained between 0.03 to 0.13
278 µM for all cell ratios (Supporting material Figure S4), which is above the threshold for
279 either dechlorination or sulfate reduction [47,48] and was comparable to that maintained in
280 the control group (without sulfate addition). TCE dechlorination rates were not
281 considerably affected by the sulfate additions (2 mM or 5 mM) for all initial cell ratios
282 (Figure 3 B, supporting material Figure S4, S5). With equal starting cells of DvH and
283 strain 195, the sulfate reduction rates (14.3 ± 0.3 µmol d^{-1}) decreased to about half of
284 those in the electron acceptor limited condition (28.4 ± 1.7 µmol d^{-1}) at 2 mM sulfate, and
285 to about one quarter with 5 mM sulfate at electron acceptor limited condition, while only
286 26.8% - 28.0% of sulfate was reduced in all three cell ratios (Supporting material Figure
287 S5).

288 In order to study the continuous competition of sulfate reduction and
289 dechlorination under electron donor limiting condition, we constructed tri-culture *S.*
290 *wolfei*/DvH/195 with initial cell ratios of 0.08:1:1 (Table S2). 5 mM butyrate, 2 mM
291 sulfate and 0.7 mM TCE were amended and the tri-culture was routinely sub-cultured
292 into fresh medium (10% vol/vol) every 14 days after TCE was fully reduced to ethene
293 (data not shown). After three subculture events, we monitored TCE dechlorination
294 performance and cell growth in the tri-culture (Supporting material Figure S6). Strain 195

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

295 increased to 1.9 ± 0.2 $\times 10^8$ mL^{-1}, which was similar to the control group with no sulfate
296 amendment (1.8 ± 0.2 $\times 10^8$ mL^{-1}), while *S. wolfei* (1.2 ± 0.3 $\times 10^7$ mL^{-1}) increased to 50%
297 higher than the control (0.8 ± 0.1 $\times 10^7$ mL^{-1}). DvH cell numbers increased to 1.4 ± 0.2
298 $\times 10^7$ mL^{-1} on day 10, then decreased to 0.8± 0.1 $\times 10^7$ mL^{-1} by the end of the experiment
299 (62% lower than that in the initially constructed tri-culture 2.1 ± 0.1 $\times 10^7$ mL^{-1}). The cell
300 ratio (*S. wolfei*/DvH/195) was stably maintained at 1:1:16 and the dechlorination rate was
301 not affected by sulfate addition (2 mM) after the three sub-cultures. Interestingly, cell
302 aggregates were observed in the late exponential phase of each subculture event
303 (Supporting material Figure S7).

304 *Effects of Sulfate on dechlorination in a groundwater enrichment*

305 A methanogenic reductive-dechlorinating enrichment culture (ANAS) was used to
306 test sulfate effects on dechlorination under different electron limiting conditions (Figure
307 4). Two sulfate concentrations (2 mM and 5 mM) were amended to ANAS with 20 mM
308 lactate and 0.2 mM TCE for electron acceptor limitation. When electron acceptor was
309 limiting, H_2 was produced within two days and achieved mM levels. TCE dechlorination
310 stalled on day 6 at both sulfate concentrations, and H_2 levels dropped to < 2 nM (data not
311 shown). In order to avoid electron donor limitation, another 20 mM lactate was re-
312 amended to the bottles on day 6, and TCE reduction resumed within two days in the 2
313 mM sulfate cultures while H_2 levels remained above 20 nM. However, no further TCE
314 reduction was observed in the 5 mM sulfate cultures although H_2 levels (6.0 nM) were
315 above the threshold of dechlorination by day 8.

316 Under electron donor limitation, micro molar levels of H_2 (~0.15 μM) was
317 intermittently added to maintain the low H_2 concentrations expected. Dechlorination rates

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

318 were the same with sulfate amendments as in the control (Figure 4 B) with *cis*-DCE (81.7
319 ± 3.3 % with 5mM sulfate, 90.8 ± 1.5% with 2mM sulfate) as the main reduction product
320 on day 8 (data not shown). Sulfate reduction rates decreased significantly to 3.7 ± 0.2
321 μmol d^{-1} (at 2mM sulfate) and 7.0 ± 1.3 μmol d^{-1} (at 5 mM sulfate) compared to the
322 electron acceptor limitation at 15.3 ± 1.1 μmol d^{-1} (with 2mM sulfate) and 43.3 ± 0.8
323 μmol d^{-1} (with 5 mM sulfate). Methane production occurred in the control (data not
324 shown) but was not observed in the enrichment with sulfate amendments within the
325 experimental period (8 days) due to the low aqueous H_2 concentrations (< 100 nM),
326 consistent with previous research.[47]

327 *Transcriptomic study of* strain 195 *with sulfide inhibition*

328 The effects of sulfide addition on *D. mccartyi* 195 gene expression were studied
329 in order to better understand the inhibition mechanism. 10 mM Sulfide was amended to
330 strain 195 at mid-log phase of growth on day 4. Cell samples were collected after 48
331 hours additional incubation (day 6), when control bottles (without sulfide amendment)
332 reached late exponential growth phase. TCE dechlorination rates were slower in the
333 sulfide amended bottles than in the controls (0.3 mmol/L/d versus 1.3 mmol /L/d).
334 Transcriptomic analysis showed that 115 genes were significantly down regulated, while
335 207 genes were significantly up-regulated (≥ two-fold change) in the presence of sulfide.

336 The short-term exposure to 10 mM sulfide did not change the expression pattern
337 of genes encoding for dehalogenases (Table S3). However, down-regulated genes include
338 Hym [Fe]-hydrogenase (DET0146-0148), ATP synthase (DET0558-0565), and genes
339 related to biosynthesis (Table S3). Up-regulated gene expressions were observed in a
340 subset of the genes encoded for ferrous iron transport protein (DET0095-0097),

Accepted Manuscript Posted Online
AEM
Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

341 phosphate ABC transporters (DET 0138-0142), and genes related to nitrogen regulation
342 and transport (DET1124-1125).

343 **Discussion**

344 Inconsistent results for the effects of sulfate inhibition on the performance of
345 dechlorination enrichment cultures have been reported in the literature. El Mamouni et al.
346 [31] reported that 10 mM sulfate addition to soil had no significant effect on TCE
347 dechlorination by indigenous microorganisms while higher sulfate concentrations (15 and
348 20 mM) yielded slower dechlorination. Heimann et al.[30] reported that 2.5 mM sulfate
349 inhibited dechlorination by a mixed anaerobic culture by reducing the H_2 supply to low
350 nanomolar H_2. Conversely, sulfate did not affect dechlorination when rapid fermentation
351 of lactate resulted in accumulation of hydrogen to levels >100 nM. Aulenta et al. [27]
352 reported that 3.7 mM sulfate adversely affected the rate of reductive dechlorination of an
353 enriched dechlorinating community. These inconsistent findings make it difficult to
354 understand potential mechanisms of sulfate inhibition and complicate the interpretation of
355 bioremediation field data.

356 *Inhibition mechanism*

357 This study demonstrates that sulfide rather than sulfate exhibits inhibitory effects
358 on dechlorination and growth of *D. mccartyi*, fermenting bacterium *S. wolfei* and sulfate
359 reducing bacterium DvH. The cell yield of strain 195 decreased significantly at high
360 sulfide concentrations (5 mM) while TCE dechlorination slowed, indicating that *D.*
361 *mccartyi* decoupled growth from dechlorination when sulfide was introduced to the
362 system at moderate to high concentrations. This result agrees with a previous study
363 showing that sulfate did not inhibit *D.mccartyi* FL2 at high concentrations (10 mM).[21]

Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

364 Sulfide exerts inhibitory effects on a variety of cultures with different thresholds.[21, 49-53] A
365 previous study reported that insoluble metal sulfide formation from mg/L concentrations
366 of heavy metals deactivated sulfate reducing bacteria by acting as a physical barrier to the
367 cells.[46] At remediation sites, sulfate reduction can overlap with iron reduction which can
368 lead to precipitated iron sulfide. The biogenic iron sulfide may reduce part of the sulfide
369 toxicity and also perform abiotic chloroethene degradation when electron donor is in
370 excess.[54, 55] The overall TCE remediation may benefit from the resulting iron sulfide
371 formation. However, this abiotic chloroethene degradation process was not investigated
372 in this study. In our experimental set-up, *D. mccartyi* was grown as planktonic cells and
373 trace metals were supplied at micro molar levels, below those needed to generate
374 significant insoluble precipitation. In addition, our results demonstrated that sulfide
375 inhibition is reversible, similar to the study conducted by Samhan-Arias[56], indicating that
376 inhibition was not due to trace metal deficiency. In the pH range (7.0~7.3) used in these
377 experiments, H_2S and HS^- each counts for half of sulfide present in the culture.[49] In some
378 organisms, the toxicity of H_2S has been attributed to its ability to inhibit cytochrome c-
379 oxidase in a similar manner to hydrogen cyanide (HCN) that prevents cellular respiration
380 and inhibits the activity of a number of metal-containing enzymes by forming a complex
381 bonds with metals.[57] Although *D. mccartyi* genomes do not encode for cytochromes, they
382 do encode for many metal-containing enzymes, including the critical reductive
383 dehalogenases. The down-regulated genes for ATP synthase, biosynthesis and Hym
384 hydrogenase during sulfide inhibition agree with the physiological observation of lower
385 cell yields and reduced dechlorination rates in strain 195. A similar gene expression
386 pattern of membrane-bound electron transferring complexes was observed in a previous

Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

387 transcriptomic study of DvH during inhibition by nitrate reducing bacteria.[58] In contrast,
388 sulfide inhibition of metal-containing enzymes resulted in up-regulated expression of
389 genes encoding for metal-containing enzymes involved in energy metabolism. The added
390 counter ion sodium (in the form of sodium sulfide) was unlikely be inhibitory to the
391 growth of strain 195, because in the sulfate inhibition experiment, we tested up to 10 mM
392 sodium sulfate, and did not observe any inhibitory effect on growth or dechlorination
393 performance of strain 195.

394 *Sulfate effects on dechlorination during electron acceptor limitation*

395 Faster growth kinetics of sulfate reducing bacteria under electron acceptor (TCE)
396 limitation caused sulfide accumulation that inhibits the growth of *D. mccartyi* at high
397 initial sulfate concentrations (5 mM). The results from the DvH/195 co-culture with
398 lactate and sulfate addition showed that 5 mM initial sulfate inhibited both dechlorination
399 and growth of strain 195. The same inhibition effect on dechlorination was also observed
400 when DvH/195 was supplied with excess electron donor and in the ANAS enrichment
401 culture supplied with lactate. This inhibition is consistent with previous observations that
402 5 mM sulfide inhibited dehalogenation in soil free microcosms,[29] and a recent field-scale
403 enhanced reductive dechlorination (ERD) study that also showed reductive
404 dechchlorination was negatively impacted with sulfate concentration above 5 mM when
405 ethanol was supplied in excess as electron donor.[15] While another recent field-scale study
406 showed that sulfate below 2 mM was not inhibitory to reductive dechlorination in
407 hyporheic zones[16], which agrees with our observation that low sulfate concentration (2
408 mM) did not inhibit DvH/195 or ANAS enrichment. No methane production was
409 observed in sulfate-supplied ANAS under electron acceptor limitation, due to the low H_2

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

410 production (~10 nM) compared to the control (~100 nM). Sulfate reducing bacteria
411 (SRB) outcompete methanogens for H_2, even at 2 mM initial sulfate.

412 *Sulfate effect on dechlorination under electron donor limitation*

413 The dechlorination rate and cell yield of strain 195 was little affected at tested
414 sulfate concentrations (2 ~ 5 mM) in the tri-culture under electron donor limitation
415 (Figure 1 D). The sulfate reduction rate by DvH was slower compared to the electron
416 acceptor limitation (Figure 1 C, Table 1) due to the competition with strain 195 for H_2,
417 which agrees with a previous report using an enrichment culture growing at electron
418 donor limiting condition.[25] Reductive dechlorination accounted for 36~38% of consumed
419 electrons while sulfate reduction accounted for 62~64% of consumed electrons. Similar
420 observations were made using sediment slurries, in which the presence of PCE accounted
421 for approximately 50% of the reducing equivalents with the remainder directed to sulfate
422 reduction.[59] On the contrary, in the cases where dehalogenation was inhibited (Table 1),
423 hydrogen was provided in excess to the system to avoid hydrogen competition between
424 sulfate reduction and dehalogenation. Therefore, accumulated sulfide was the reason for
425 inhibited dehalogenation.

426 The long term maintenance of tri-culture *S.wolfei*/DvH/195 consortia at electron
427 donor limiting condition showed with the same initial cell inoculation of DvH and 195,
428 195 became dominant after several sub-culturing event, demonstrating *D. mccartyi* out-
429 competes DvH for available H_2 even in sulfate-rich (5 mM) environments. This finding is
430 consistent with a recent study with a butyrate-fed dechlorinating enrichment culture in
431 which *D. mccartyi* became the predominant species regardless of the sulfate
432 concentrations (0.6 to 11.2 mM).[32] Further, the H_2 utilization half velocity coefficient for

Accepted Manuscript Posted Online

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

433 sulfate reduction ($K_{S\text{-}H2,\ sul}$) is reported to be 0.2~2.4 μM, [60-61] while for reductive
434 dechlorination the reported value ($K_{S\text{-}H2,\ dechlorition}$) is in the range of 2~7 nM,[61,62]
435 indicating reductive dechlorination has a much higher H_2 affinity and can outcompete
436 sulfate reduction at lower hydrogen concentrations. SEM photos of the tri-culture
437 (Supporting material Figure S7) growing on butyrate (electron donor limitation) show
438 cell aggregate formation between the fermenting syntrophs and the H_2-consuming
439 bacteria, demonstrating the cells are tend to form physical proximity during the
440 syntrophic condition under electron donor limitation. This result is similar to a previous
441 study that cell aggregates formed between *S. wolfei* and strain 195 during syntrophic
442 growth.[35]

443 The observed dechlorination rate of the sulfate-supplied ANAS enrichment
444 culture under electron donor limitation was similar to the control group (no sulfate
445 added). This agrees with the observations using constructed consortia, which demonstrate
446 that reductive dechlorination rates are not affected by sulfate amendment (2mM and 5
447 mM) under electron donor limitation. Also, the higher abundance of *D. mccartyi* (>30%)
448 in the enrichment culture compared to the co- and tri-cultures may be another reason that
449 dechlorination out-competes sulfate reduction.[38]

450 *Cell ratio effect*

451 Few studies have examined the effect of cell ratios of sulfate reducers to
452 dechlorinators on dechlorination performance.[63] In sulfate-rich environments, sulfate
453 reducers may be the dominant species compared to *D. mccartyi*, resulting in competition
454 for H_2. This study showed that different initial cell ratios of sulfate reducing bacteria to
455 *D. mccartyi* (from 0.3 to 3.0) resulted in no significant differences in dechlorination

AEM
Applied and Environmental Microbiology
Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

456 profiles or cell growth (Table 1), demonstrating that initial cell ratios are not the most
457 critical factor for controlling inhibition of reductive dechlorination in sulfate reducing
458 environments.

459 In conclusion, sulfide instead of sulfate is responsible for the inhibitory effects on
460 dechlorination and growth by *D. mccartyi*. Under electron acceptor limited conditions,
461 sulfate concentrations are the key factor that determines the extent of dechlorination, with
462 high sulfate concentrations exhibiting inhibition due to the toxicity of the sulfate
463 reduction product sulfide. Under electron donor limited conditions, *D. mccartyi* can
464 successfully dechlorinate in anaerobic microbial communities regardless of sulfate
465 concentrations, demonstrating the ability of *D. mccartyi* to effectively compete against
466 other hydrogen-consuming bacteria. The inhibitory concentrations of sulfide on
467 *Dehalococcoides* strains could be incorporated to current kinetic modeling in order to
468 better predict reductive dechclorination process at sulfate reducing environment during
469 bioremediation practice.

470

471 **Acknowledgement**

472 This work was funded through research grants from NIEHS (P42-ES04705-14) and NSF
473 (CBET-1336709)

474 **Tables and Figures**
475

476 Figure 1. Ecological interactions between strain195, DvH and *S. wolfei* in constructed
477 consortia and potential inhibitory effects of sulfate/sulfide. A) Potential inhibitory effects
478 of sulfate/sulfide on strain 195; B) DvH and 195 in a syntrophic co-culture with lactate as

Accepted Manuscript Posted Online

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

479 electron donor and sulfate addition; C) DvH and 195 with H_2 fed in excess as electron
480 donor and sulfate addition; D) DvH, 195 and *S. wolfei* tri-culture (*S. wolfei*/ DvH/195)
481 with butyrate as electron donor, and TCE and sulfate as electron acceptors. Acetate is the
482 carbon source for growth of 195. Dashed lines indicate potential inhibitory effect.
483
484
485 Figure 2. Co-culture (DvH/195) electron acceptor limitation experiment. A) TCE
486 dechlorination activity and H_2 production with arrows showing lactate amendments of
487 5mM and 2 mM; B) cell numbers; C) sulfate and sulfide concentrations. Symbols
488 represent the mean of biological triplicates and error bars indicate standard deviation.
489 Absence of error bars indicates error was smaller than the symbol.
490
491 Figure 3. Constructed consortia amended with 5 mM sulfate along with TCE and H_2. A
492 and C: co-culture DvH/195 (inoculum ratio 1:1) with H_2/CO_2 headspace; B and D: Tri-
493 culture *S.wolfei*/DvH/195 (inoculum ratio 0.08:1:1) with 6 mM butyrate. Symbols
494 represent the mean of biological triplicates and error bars indicate standard deviation.
495 Absence of error bars indicates error was smaller than the symbol.
496
497 Figure 4. TCE dechlorination profile of enrichment culture ANAS with different initial
498 sulfate concentrations: A) with 20 mM lactate amendment and B) H_2 amendment as
499 electron donor. Sulfate consumption during the experiment: C) with 20 mM lactate
500 amendment and D) H_2 amendment as electron donor. Error bars indicate standard
501 deviation of biological triplicate. Arrow indicates 20 mM lactate amendment to the
502 culture on day 6.
503

AEM
Applied and Environmental Microbiology
Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology
Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

504 **Reference**

505 1. Bhatt P, Kumar MS, Mudliar S, Chakrabarti T. 2007. Biodegradation of chlorinated
506 compounds - A review. Critical Reviews in Environ. Sci. Technol 37 (2): 165-198.
507 2. U.S.EPA. 2011. IRIS Toxicolgical Review of Trichloroethylene.
508 https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=237625. (accessed on
509 Jan 23, 2017)
510 3. Moran MJ, Zogorski JS, Squillace PJ. 2007. Chlorinated solvents in groundwater of
511 the United States. Environ. Sci. Technol 41(1): 74-81.
512 4. Pandey J, Chauhan A, Jain RK. 2009. Integrative approaches for assessing the
513 ecological sustainability of in situ bioremediation. FEMS Microbiol Rev 33: 324-375.
514 5. Maymó-Gatell X, Anguish T, Zinder SH. 1999. Reductive dechlorination of
515 chlorinated ethenes and 1,2-dichloroethane by "*Dehalococcoides ethenogenes*" 195.
516 Appl. Environ. Microbiol 65 (7): 3108-3113.
517 6. He J, Holmes VF, Lee PK, Alvarez-Cohen L. 2007. Influence of vitamin B-12 and
518 cocultures on the growth of *Dehalococcoides* isolates in defined medium. Appl.
519 Environ. Microbiol 73 (9): 2847-2853.
520 7. Yi S, Seth EC, Men YJ, Stabler SP, Allen RH, Alvarez-Cohen L, Taga ME. 2012.
521 Versatility in Corrinoid Salvaging and Remodeling pathways supports corrinoid-
522 dependent metabolism in *Dehalococcoides mccartyi*. Appl. Environ. Microbiol 78
523 (21): 7745-7752.
524 8. Bradley PM, Chapelle FH. 2010. Biodegradtion of chlorinated ethenes. *In Situ*
525 *Remediation Of Chlorinated Solvent Plumes. SERDP/ESTCP Environmental*
526 *Remediation Technology*, Stroo, H. F.; Ward, C. H., Eds. Springer: Heidelberg,
527 Germany. pp 39-67.
528 9. Yu SH, Semprini L. 2002. Comparison of trichloroethylene reductive dehalogenation
529 by microbial communities stimulated on silicon-based organic compounds as slow-
530 release anaerobic substrates. Water Research 36 (20): 4985-4996.
531 10. Ma X, Novak PJ, Clapp LW, Semmens MJ, Hozalski RM. 2003. Evaluation of
532 polyethylene hollow-fiber membranes for hydrogen delivery to support reductive
533 dechlorination in a soil column. Water Research 37 (12): 2905-2918.

Accepted Manuscript Posted Online

534 11. Lee IS, Bae JH, Yang Y, McCarty PL. 2004. Simulated and experimental evaluation
535 of factors affecting the rate and extent of reductive dehalogenation of chloroethenes
536 with glucose. J. Contam. Hydrol 74 (1–4): 313-331.
537 12. Bagley DM, Gossett JM. 1990. Tetrachloroethene transformation to trichloroethene
538 and *cis*-1,2-dichloroethene by sulfate-reducing enrichment cultures. Appl Environ
539 Microbiol 56 (8): 2511-2516.
540 13. Lee M. D. 1998. New Perspectives on Microbial Dehalogenation of chlorinated
541 solvents: Insights from the Field. Annu Rev Microbiol 52: 423-452.
542 14. Miao Z, Brusseau M L, Carroll K C, Carreón-Diazconti C, Johnson B. 2012. Sulfate
543 reduction in groundwater: characterization and applications for remediation.
544 Environ. Geochem. Health 34(4):539-550.
545 15. Pérez-de-Mora A, Zila A, McMaster M L, Edwards E A. 2014. Bioremediation of
546 Chlorinated Ethenes in Fractured Bedrock and Associated Changes in Dechlorinating
547 and Nondechlorinating Microbial Populations. Environ. Sci. Technol 48: 5770−5779.
548 16. Freitas J G, Rivett M O, Roche R S, Durrant M, Walker C, Tellam J H. 2015.
549 Heterogeneous hyporheic zone dechlorination of a TCE groundwater plume
550 discharging to an urban river reach. Sci Total Environ 505: 236–252.
551 17. Zanaroli G, Negroni A, Häggblom MM, Fava F. 2015. Microbial dehalogenation of
552 organohalides in marine and estuarine environments. Curr Opin Biotechnol 33: 287-
553 295.
554 18. Chen Y, Cheng J J, Creamer K S. 2008. Inhibition of anaerobic digestion process: A
555 review. Bioresour Technol 99 (10): 4044-4064.
556 19. Reis MAM, Almeida JS, Lemos PC, Carrondo MJT. 1992. Effect of Hydrogen
557 Sulfide on Growth of Sulfate Reducing Bacteria. Biotech and Bioeng 40 (5): 593-600.
558 20. Joye S B, Hollibaugh J T. 1995. Influence of Sulfide Inhibition of Nitrification on
559 Nitrogen Regeneration in Sediments. Science 270 (5236): 623-625.
560 21. He J, Sung Y, Krajmalnik-Brown R, Ritalahti K M, Löffler F E. 2005. Isolation and
561 characterization of *Dehalococcoides* sp. Strain FL2, a trichloroethene (TCE)- and 1,2-
562 dichloroethene-respiring anaerobe. Environ Microbiol 7 (9): 1442-1450.
563 22. Morse JJ, Alleman BC, Gossett JM, Zinder SH, Fennell DE, Sewell GW. 1997. A
564 Treatability Test for Evaluating the Potential Applicability of the Reductive

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

AEM
Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

565 Anaerobic Biological In Situ Treatment Technology (RABITT) to Remediate
566 Chloroethenes. A Draft Technical Protocol Developed for the Environmental Security
567 Technology Certification Program, Department of Defense. US Government Printing
568 Office, Washington, DC.
569 23. Aulenta F, Majone M, Tandoi V. 2006. Enhanced anaerobic bioremediation of
570 chlorinated solvents: environmental factors influencing microbial activity and their
571 relevance under field conditions. J. Chem. Technol. Biotechnol 81 (9): 1463-1474.
572 24. Fennell DE and Gossett JM. 2003. Microcosms for site-specific evaluation of
573 enhanced biological reductive dehalogenation, in Dehalogenation: Microbial
574 Processes and Environmental Applications, ed. By Häggblom MM and Bossert ID.
575 Kluwer Academic, Boston, MA, pp. 385-420.
576 25. Aulenta F, Pera A, Rossetti S, Papini MP, Majone M. 2007. Relevance of side
577 reactions in anaerobic reductive dechlorination microcosms amended with different
578 electron donors. Water Research 41: 27-38.
579 26. Pantazidou M, Panagiotakis I, Mamais D, Zikidi V. 2012. Chloroethene
580 biotransformation in the presence of different sulfate concentrations. Ground Water
581 Monit Remediation 32: 106-119.
582 27. Aulenta F, Beccari M, Majone M, Papini MP, Tandoi V. 2008. Competition for H_2
583 between sulfate reduction and dechlorination in butyrate-fed anaerobic cultures.
584 Process Biochemistry 43: 161-168.
585 28. Berggren DRV, Marshall IPG, Azizian MF, Spormann AM, Semprini L. 2013.
586 Effects of sulfate reduction on the bacterial community and kinetic parameters of a
587 dechlorinating culture under chemostat growth conditions. Environ. Sci. Technol 47
588 (4): 1879-1886.
589 29. Hoelen T P, Reinhard M. 2004. Complete biological dehalogenation of chlorinated
590 ethylenes in sulfate containing groundwater. Biodegradation 15: 395-403.
591 30. Heimann AC, Friis AK, Jakobsen R. 2005. Effects of sulfate on anaerobic
592 chloroethene degradation by an enriched culture under transient and steady-state
593 hydrogen supply. Water Research 39: 3579-3586.
594 31. El Mamouni R, Jacquet R, Gerin P, Agathos SN. 2002. Influence of electron donors
595 and acceptors on the bioremediation of soil contaminated with trichloroethene and

Accepted Manuscript Posted Online

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

596 nickel: laboratory- and pilot-scale study. Water Sci and Technol 45 (10): 49-54.
597 32. Panagiotakis I, Mamais D, Pantazidou M, Rossetti S, Aulenta F, Tandor V. 2014.
598 Predominance of *Dehalococcoide*s in the presence of different sulfate concentrations.
599 Water Air Soil Pollut 225: 1785-1799.
600 33. Atashgahi S, Lu Y, Zheng Y, Saccenti E, Suarez-Diez M, Ramiro-Garcia
601 J, Eisenmann H, Elsner M, J M Stams A, Springael D, Dejonghe W, Smidt H. 2016.
602 Geochemical and microbial community determinants of reductive dechlorination at a
603 site biostimulated with glycerol. Environ Microbiol doi: 10.1111/1462-2920.13531.
604 34. Beaty PS, McInerney MJ. 1987. Growth of *Syntrophomonas wolfe*i in pure culture on
605 crotonate. Archives of Microbiology 147 (4): 389-393.
606 35. Mao X, Stenuit B, Polasko A, Alvarez-Cohen L. 2015. Efficient metabolic exchange
607 and electron transfer within a syntrophic trichloroethene-degrading coculture of
608 Dehalococcoides mccartyi 195 and Syntrophomonas wolfei. Appl Environ Microbiol
609 81(6): 2015-2024.
610 36. Men YJ, Feil H, Verberkmoes NC, Shah MB, Johnson DR, Lee PK, West KA, Zinder
611 SH, Andersen GL, Alvarez-Cohen L. 2011. Sustainable syntrophic growth of
612 *Dehalococcoides ethenogenes* strain 195 with *Desulfovibrio vulgaris* Hidenborough
613 and *Methanobacterium congoense*: global transcriptomic and proteomic analyses.
614 ISME J 6 (2): 410-421.
615 37. Lee PKH, Johnson DR, Holmes VF, He J, Alvarez-Cohen L. 2006. Reductive
616 dehalogenase gene expression as a biomarker for physiological activity
617 of *Dehalococcoides* spp. Appl Environ Microbiol 72 (9): 6161-6168.
618 38. Brisson VL, West KA, Lee PK, Tringe SG, Brodie EL, Alvarez-Cohen L. 2012.
619 Metagenomic analysis of a stable trichloroethene-degrading microbial community.
620 ISME J 6(9): 1702-14.
621 39. West KA, Lee PKH, Johnson DR, Zinder SH, Alvarez-Cohen L. 2013. Global gene
622 expression of *Dehalococcoides* within a robust dynamic TCE-dechlorinating
623 community under conditions of periodic substrate supply. Biotechnol and Bioeng 110
624 (5): 1333-1341.
625 40. Freeborn RA, West KA, Bhupathiraju VK, Chauhan S, Rahm BG, Richardson RE,
626 Alvarez-Cohen L. 2005. Phylogenetic analysis of TCE-dechlorinating consortia

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Accepted Manuscript Posted Online

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

627 enriched on a variety of electron donors. Environ Sci Technol 39 (21): 8358-8368.
628 41. Cline JD. 1969. Spectrophotometric determination of hydrogen sulfide in natural
629 waters. Limnology and Oceanography 14: 454–458.
630 42. Brisson VL, Zhuang WQ, Alvarez-Cohen L. 2016. Bioleaching of rare earth elements
631 from monazite sand. Biotechnol Bioeng 113(2):339-48.
632 43. Sieber JR, Sims DR, Han C, Kim E, Lykidis A, Lapidus AL, McDonnald E, Rohlin L,
633 Culley DE, Gunsalus R, McInerney MJ. 2010. The genome of *Syntrophomonas*
634 *wolfei*: new insights into syntrophic metabolism and biohydrogen production. Environ.
635 Microbiol 12 (8): 2289-2301.
636 44. Johnson DR, Lee PKH, Holmes VF, Alvarez-Cohen L. 2005. An internal reference
637 technique for accurately quantifying specific mRNAs by real-time PCR with
638 application to the *tceA* reductive Dehalogenase gene. Appl. Environ. Microbiol 71 (7):
639 3866-3871.
640 45. Lee PKH, Cheng D, Hu P, West KA, Dick GJ, Brodie EL, Andersen GL, Zinder SH,
641 He J, Alvarez-Cohen L. 2011. Comparative genomics of two newly
642 isolated *Dehalococcoides* strains and an enrichment using a genus microarray. ISME
643 J 5:1014-1024.
644 46. Utgikar VP, Harmon SM, Chaudhary N, Tabak HH, Govind R, Haines JR. 2002.
645 Inhibition of sulfate-reducing bacteria by metal sulfide formation in bioremediation of
646 acid mine drainage. Envion. Toxicol 17(1):40-48.
647 47. Yang YR, McCarty PL. 1998. Competition for hydrogen within a chlorinated solvent
648 dehalogenating anaerobic mixed culture. Environ. Sci. Technol 32 (22): 3591-3597.
649 48. Lovley DR, Goodwin S. 1988. Hydrogen concentrations as an indicator of the
650 predominant terminal electron-accepting reactions in aquatic sediments. Geochimica
651 et Cosmochica Acta 52 (12): 2993-3003.
652 49. Karhadkar PP, Audic J-M, Faup G.M., Khanna P. 1987. Sulfide and sulfate inhibition
653 of methanogenesis. Water Res 21 (9): 1061-1066.
654 50. Okabe S, Nielsen P H, Jones W L, Characklis W G. 1995. Sulfide product inhibition
655 of *Desulfovibrio desulfuricans* in batch and continuous cultures. Water Research 29
656 (2): 571-578.
657 51. Magnuson, J.K., Stern, R.V., Gossett, J.M., Zinder, S.H. and Burris, D.R. 1998.

Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

658 Reductive dechlorination of tetrachloroethene to ethene by a two-component enzyme
659 pathway. Appl. Environ. Microbiol 64: 1270-1275.
660 52. Jin RC, Yang GF, Zhang QQ, Ma C, Yu JJ, Xing BS. 2013. The effect of sulfide
661 inhibition on the anammox process. Water Res 47 (3): 1459-1469.
662 53. Zhou Z, Xing C, An Y, Hu D, Qiao W, Wang, L. 2014. Inhibitory effects of sulfide
663 on nitrifying biomass in the anaerobic-anoxic-aerobic wastewater treatment process. J
664 Chem Technol Biotechnol 89:214-219.
665 54. He Y T, Wilson J T, Su C, Wilkin R T. 2015. Review of Abiotic Degradation of
666 Chlorinated Solvents by Reactive Iron Minerals in Aquifers. Ground Water Monit
667 Remediat 35: 57-75.
668 55. Hyun S P, Hayes K F. 2015. Abiotic reductive dechlorination of *cis*-DCE by ferrous
669 monosulfide mackinawite. Environ Sci Pollut Res 22:16463–16474.
670 56. Samhan-Arias AK, Garcia-Bereguain MA, Gutierrez-Merino C. 2009. Hydrogen
671 sulfide is a reversible inhibitor of the NADH oxidase activity of synaptic plasma
672 membranes. Biochem Biophys Res Commun 388 (4):718-722.
673 57. Truong DH, Eghbal MA, Hindmarsh W, Roth SH, O'Brien PJ. 2006. Molecular
674 Mechanisms of Hydrogen Sulfide Toxicity. Drug Metabolism Review 38 (4):733-744.
675 58. Haveman S A, Greene E A, Voordouw G. 2005. Gene Expression analysis of the
676 mechanism of inhibition of *Desulfovibrio vulgaris* Hildenborough by nitrate-reducing,
677 sulfide-oxidizing bacteria. Environ Microbiol 7(9): 1461-1465.
678 59. Mazure CS, Jones W J. 2001. Hydrogen Concentrations in Sulfate-Reducing
679 Estuarine Sediments during PCE Dehalogenation. Environ. Sci. Technol 35: 4783-
680 4788.
681 60. Sonne-Hansen J, Westermann P, Ahring B K. 1999. Kinetics of Sulfate and Hydrogen
682 Uptake by the Thermophilic Sulfate-Reducing Bacteria *Thermodesulfobacterium* sp.
683 Strain JSP and *Thermodesulfovibrio* sp. Strain R1Ha3. Appl Environ Microbiol 65(3):
684 1304-1307.
685 61. Kouznetsova I, Mao X, Robinson C, Barry DA, Gerhard JI, McCarty PL. 2010.
686 Biological reduction of chlorinated solvents: Batch-scale geochemical modeling. *Adv.*
687 Water Res 33 (9): 969-986.
688 62. Cupples AM, Spormann AM, McCarty PL. 2003. Growth of a *Dehalococcoides*-like

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online

689 microorganism on vinyl chloride and cis-dichloroethene as electron acceptors as
690 determined by competitive PCR. Appl Environ Microbiol 69 (2): 953-959.
691 63. Drzyzga O, Gerritse J, Dijk JA, Elissen H, Gottschal JC. 2001. Coexistence of a
692 sulphate-reducing *Desulfovibrio* species and the dehalorespiring *Desulfitobacterium*
693 *frappieri* TCE1 in defined chemostat cultures grown with various combinations of
694 sulphate and tetrachloroethene. Environ Microbiol 3(2): 92-99.
695

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

AEM Applied and Environmental Microbiology

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology

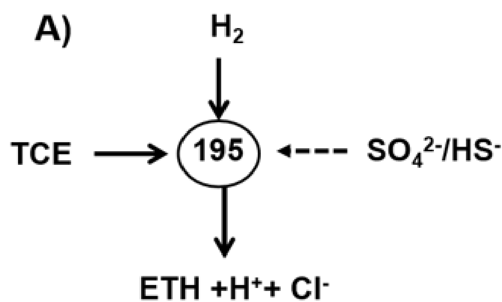
Table 1. Substrate utilization rates and electron equivalent consumption by constructed consortia.

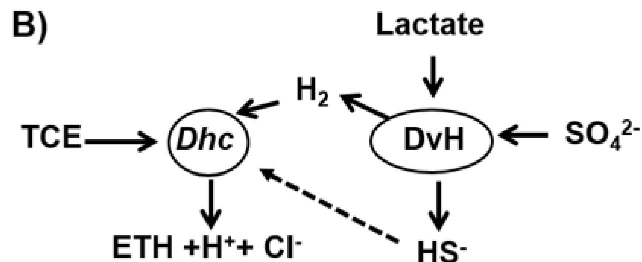
electron donor	Consortiua members	DvH: 195:*S. wolfei* ratio[a]	Limiting substrate	SO_4^{2-} mM	Cl^- release rate[b] μmol Cl^- d^{-1}	Sulfate reduction rate μmol sulfate d^{-1}	specific dechlorination rate $\times10^{-10}$ μmol $cell^{-1}$ d^{-1}	specific sulfate reduction rate $\times10^{-9}$ μmol $cell^{-1}$ d^{-1}	Consumed electron equiv ratio of sulfate reduction to dechlorination[c]	Inhibition effect
H_2	DvH/195	3:1	acceptor	2	20.2 ± 0.3	30.0 ± 1.5	6.2 ± 1.2	4.2 ± 0.6	1.8:1	NO
		1:1			22.1 ± 0.4	28.4 ± 1.7	6.5 ± 1.1	4.4 ± 0.3	1.7:1	
		1:3			22.6 ± 0.3	27.9 ± 1.1	5.5± 0.7	3.8 ± 0.2	1.6:1	
H_2	DvH/195	3:1	acceptor	5	1.1 ± 0.1	40.4 ± 0.5	5.5 ±0.5	2.1 ± 0.3	346:1	YES
		1:1			1.4 ± 0.3	41.3 ± 0.8	5.7 ±0.1	3.2 ± 0.7	282:1	
		1:3			1.6 ± 0.4	37.6 ± 2.1	5.1 ±0.3	2.2 ± 0.5	199:1	
Butyrate	*S.wolfei*/DvH /195	3:1:0.08	donor	2	29.8 ± 0.9	15.0 ±1.1	4.6 ± 0.7	5.0 ± 1.3	1.6:1	NO
		1:1:0.08			25.7 ± 0.6	14.3 ± 0.3	4.6 ± 0.3	7.1 ± 1.8	1.8:1	
		1:3:0.24			25.1 ±0.1	13.8 ±0.7	4.2 ± 0.3	6.3 ± 1.1	1.6:1	
Butyrate	*S.wolfei*/DvH /195	3:1:0.08	donor	5	23.2 ±0.7	13.1±1.1	8.0 ± 2.1	6.6 ± 1.2	1.8:1	NO
		1:1:0.08			23.1 ±0.3	10.7 ± 2.1	5.3 ± 1.1	5.3 ± 0.7	1.8:1	
		1:3:0.24			23.7 ±0.1	11.9 ± 0.8	5.4 ± 0.7	4.0 ± 0.3	1.6:1	

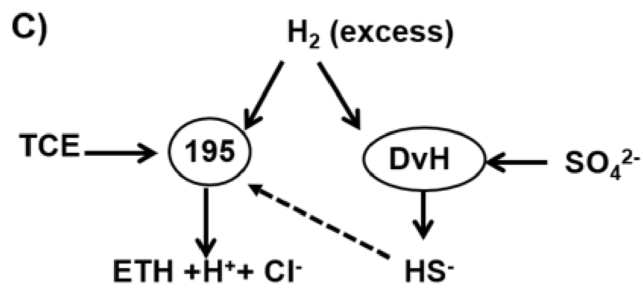
[a.] Initial cell ratios from table S2.
[b.] Cl^- release rates were calculated for 14-day experimental period in each experiment (Figure S2 to S5). Sulfate reduction rate was calculated for the same experimental period.
[c.] Consumed electron equivalents calculated based on the half reactions listed in table S1 with 2:1 electrons per Cl^- released and 8:1 electrons per sulfate reduced.

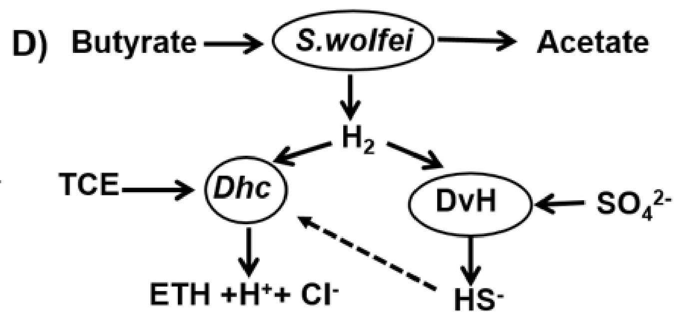
Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online
Applied and Environmental Microbiology
AEM
Applied and Environmental Microbiology









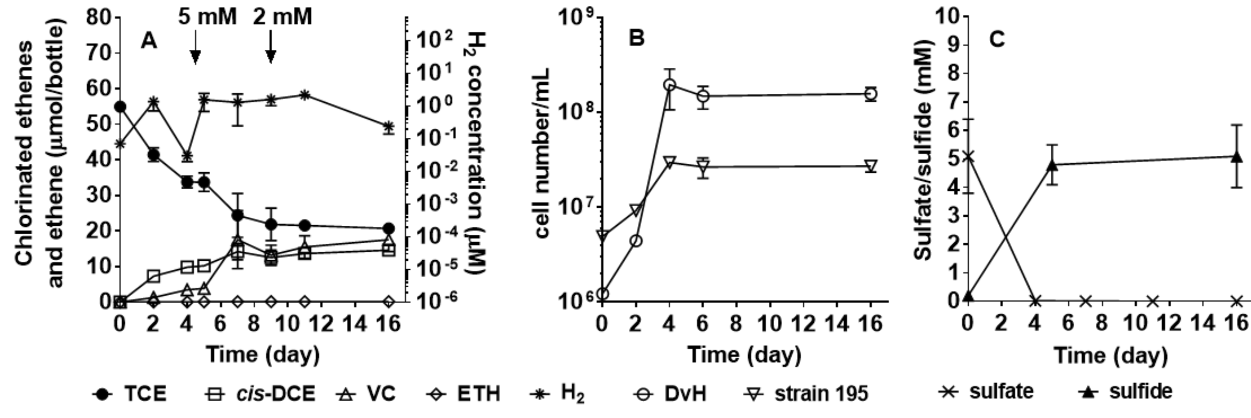
Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online

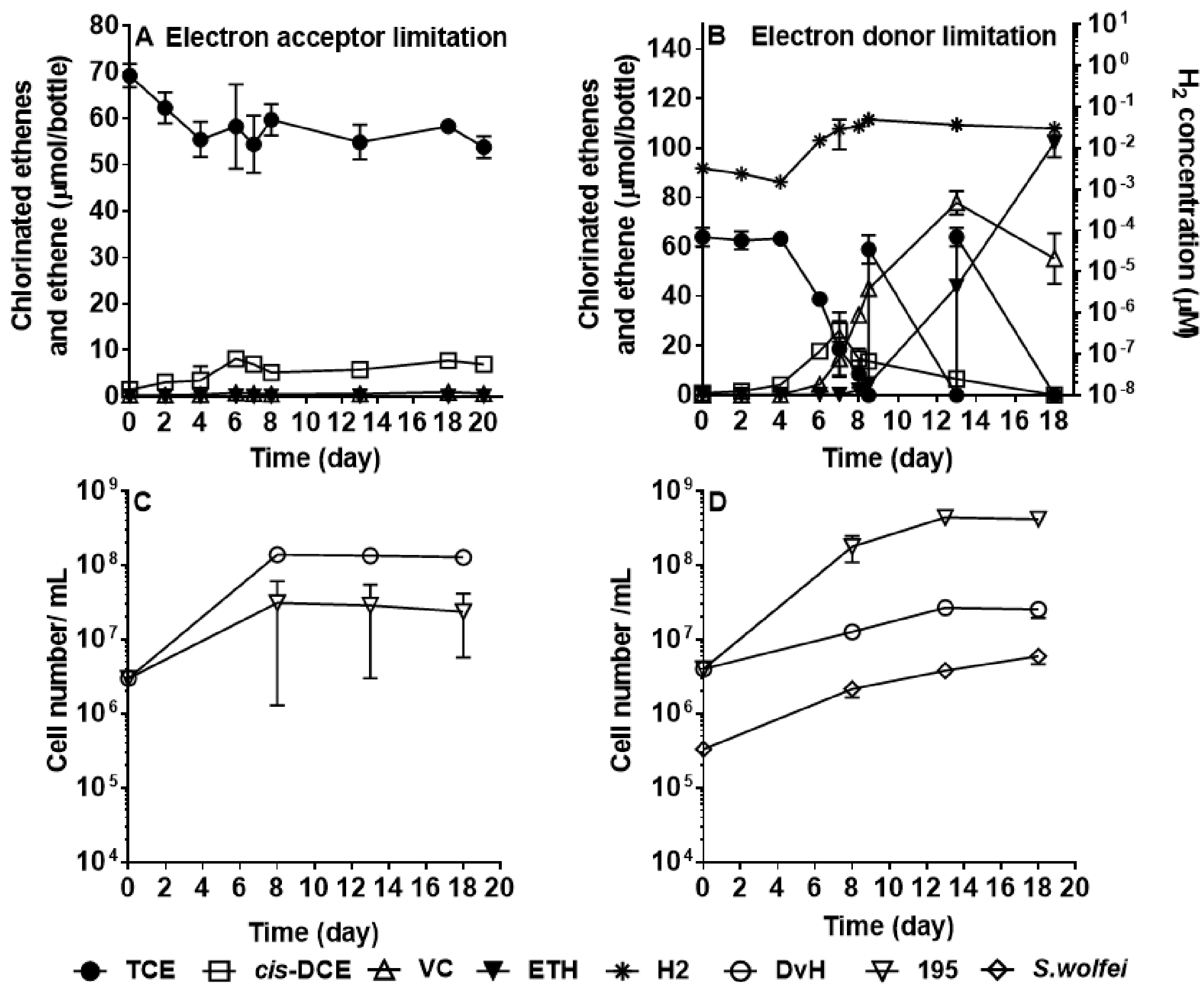
Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology



Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley



Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley

Accepted Manuscript Posted Online

Applied and Environmental Microbiology

AEM

Applied and Environmental Microbiology

Downloaded from http://aem.asm.org/ on February 28, 2017 by University of California, Berkeley