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Acetylene Fuels TCE Reductive Dechlorination by Defined *Dehalococcoides*/*Pelobacter* Consortia

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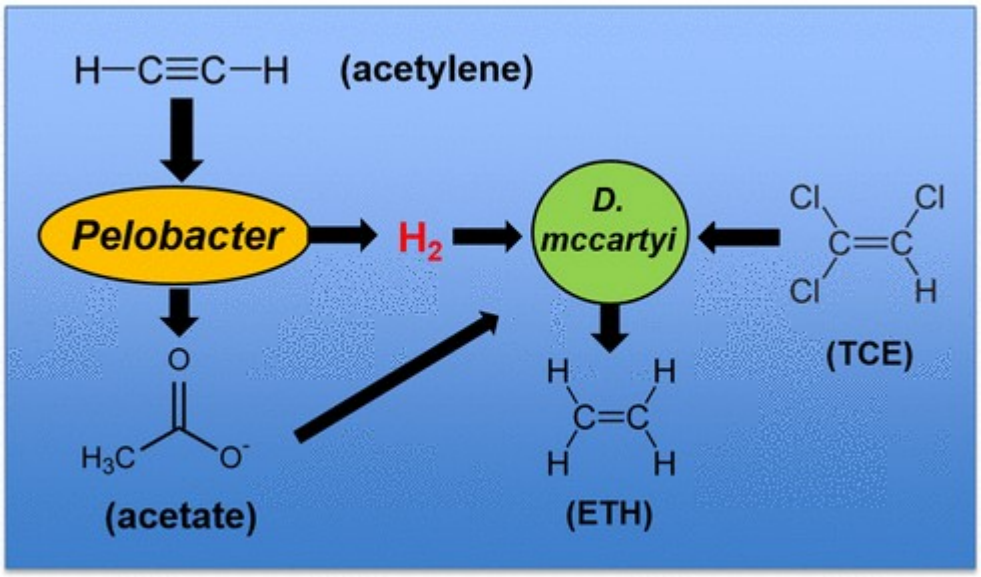
Abstract



Acetylene (C_2H_2) can be generated in contaminated groundwater sites as a consequence of chemical degradation of trichloroethene (TCE) by in situ minerals, and C_2H_2 is known to inhibit bacterial dechlorination. In this study, we show that while high C_2H_2 (1.3 mM) concentrations reversibly inhibit reductive dechlorination of TCE by *Dehalococcoides mccartyi* isolates as well as enrichment cultures containing *D. mccartyi* sp., low C_2H_2 (0.4 mM) concentrations do not inhibit growth or metabolism of *D. mccartyi*. Cocultures of *Pelobacter* SFB93, a C_2H_2-fermenting bacterium, with *D. mccartyi* strain 195 or with *D. mccartyi* strain BAV1 were actively sustained by providing acetylene as the electron donor and carbon source while TCE or *cis*-DCE served as the electron acceptor. Inhibition by acetylene of reductive dechlorination and methanogenesis in the enrichment culture ANAS was observed, and the inhibition was removed by adding *Pelobacter* SFB93 into the consortium. Transcriptomic analysis of *D. mccartyi* strain 195 showed genes encoding for reductive dehalogenases (e.g., *tceA*) were not affected during the C_2H_2-inhibition, while genes encoding for ATP synthase, biosynthesis, and Hym hydrogenase were down-regulated during C_2H_2

inhibition, consistent with the physiological observation of lower cell yields and reduced dechlorination rates in strain 195. These results will help facilitate the optimization of TCE-bioremediation at contaminated sites containing both TCE and C_2H_2.

Introduction

Groundwater contamination by trichloroethene (TCE) poses a serious threat to human health and can lead to the generation of vinyl chloride (VC), a known human carcinogen(1) via abiotic and biotic reduction processes.(2, 3)*Dehalococcoides mccartyi* is the only known species of bacteria that can completely dechlorinate TCE to the benign product ethene (C_2H_4).(4)*D. mccartyi* is a strict hydrogenotroph characterized by specific requirements for an exogenous supply of key compounds, including hydrogen (H_2), acetate, corrinoids, biotin, and thiamine, of all which are usually provided by other microbial members of anaerobic food webs within dechlorinating communities.(5-8)*D. mccartyi* has also been studied with respect to its genetic regulation, physiological characteristics and metabolic interactions with other microbial species.(5, 9-12)*D. mccartyi* strain 195 (strain 195) can use TCE and *cis*-dichloroethene (*cis*-DCE) as terminal electron acceptors for the oxidation of H_2 to form VC, but VC is further reduced to ethene cometabolically by strain 195, and is not linked to energy generation.(5) In contrast, *D. mccartyi* strain BAV1 can conserve energy by metabolically dechlorinating both *cis*-DCE and *trans*-DCE to VC, and VC to ethene.(10)

Acetylene (C_2H_2) can be generated at TCE-contaminated sites as a consequence of the abiotic degradation of TCE by zerovalent iron or by in situ reaction with other minerals such as iron sulfide (FeS)- or Co (II)-containing sulfides.(13-15) Acetylene inhibits a variety of microbial processes including fermentation,(16) methanogenesis,(17) methane oxidation,(18) nitrogen fixation (as a competitive inhibitor of nitrogenase(19)), and both nitrification and denitrification(20) by interaction with the metal components of enzymes such as hydrogenases, oxidoreductases, and monooxygenases.(21, 22) Therefore, acetylene has commonly been used in experiments measuring nitrogen fixation (acetylene reduction assay) and denitrification (acetylene blockage of N_2O reductase assay).(23) Acetylene has also been shown to inhibit biotic reductive dechlorination of TCE to DCE and VC in laboratory experiments, and thus can cause TCE accumulation at groundwater remediation sites.(24) The levels of acetylene inhibition on microbial processes are strongly dependent on its concentration.(24)

However, acetylene can be used as a substrate by anaerobic bacteria in estuarine sediments and enrichment cultures.(25, 26) For example, *Pelobacter acetylenicus* can metabolize acetylene via acetylene hydratase and acetaldehyde dismutatse enzymes generating H_2, ethanol, and acetate as end products.(27-30) H_2 and acetate formed from acetylene fermentation could therefore potentially serve as the electron donor and carbon source to fuel reductive dechlorination by *D. mccartyi*. *Desulfovibrio vulgaris*

Hildenborough and *Syntrophomonas wolfei* have been shown to serve as successful syntrophic partners with *D. mccartyi* to support its reductive dechlorination as well as its growth via interspecies H_2 transfer.(8, 31)

In this study, we hypothesized that the inhibitory effect of acetylene on reductive dechlorination could be removed by adding a *Pelobacter* strain to *D. mccartyi*-containing cultures performing TCE-respiration. Moreover, we expected that the fermentation products of acetylene metabolism would provide *D. mccartyi* with electron donors needed to carry out reductive dechlorination. The proposed ecological interactions between *D. mccartyi* strains and *Pelobacter* strain SFB93 are illustrated in Figure 1. In order to test this hypothesis, we systematically evaluated (i) the inhibition effect of TCE on *Pelobacter* strain SFB93; (ii) acetylene inhibition on two *D. mccartyi* strains and a dechlorinating enrichment; and (iii) cell growth in cocultures of *Pelobacter* strain SFB93 with *D. mccartyi* strain 195 or BAV1 using acetylene as electron donor with TCE or VC as electron acceptor.

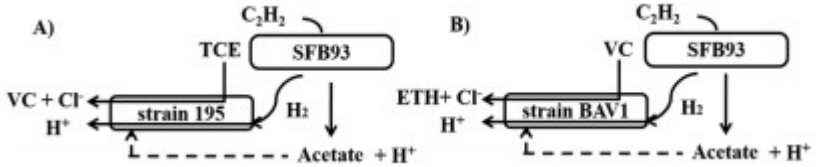


Figure 1. Ecological interactions between A) *D. mccartyi* strain 195 and *Pelobacter* SFB93; B) *D. mccartyi* strain BAV1 and *Pelobacter* SFB93. Solid lines indicate the substrate is used for energy production. Dotted lines indicate the substrate is used for biomass production.

Materials and Methods

Bacterial Cultures and Growth Conditions

D. mccartyi strain 195 (strain 195) was grown in defined medium with H_2/CO_2 headspace (80:20), 0.6 mM TCE as electron acceptor, and 2 mM acetate as carbon source.(6)*D. mccartyi* strain BAV1 (BAV1) was grown in the same defined medium with H_2/CO_2 headspace, 1.2 mM *cis*-DCE as electron acceptor, and 2 mM acetate as carbon source. An isolate from San Francisco Bay, *Pelobacter* strain SFB93 (SFB93), was grown in modified artificial bay water medium (ABW) with N_2 headspace and acetylene (0.1–1.5%) as electron donor and the carbon source.(30) Bacterial cocultures of strains 195 and SFB93 (10% vol/vol inoculation of each bacterium) were initially established in 60 mL serum bottles containing 25 mL ABW medium with TCE supplied at a liquid concentration of 0.1 mM (corresponding to 3 μmol TCE per bottle), 0.1 mM acetylene (corresponding to 0.15 mL gas), and N_2 headspace incubated at 34 °C without agitation. The cocultures were subsequently transferred (5% vol/vol inoculation) to generate subcultures after 20 μmol TCE was reduced to VC and 36 μmol acetylene was depleted. After three subcultures, we measured reductive dechlorination performance and cell growth of the coculture with multiple feedings of TCE and acetylene. In order to establish the coculture of BAV1 and SFB93, two consecutive additions of acetylene (88.1 μmol in total) were first amended to SFB93 in 20

mL carbonate media(32) with N_2 headspace. After acetylene was consumed, BAV1 (2.5 mL 10% inoculation) and 13.5 ± 1.7 μmol VC (0.4 mM) were inoculated to develop the coculture with subsequent amendment of 22.8 μmol acetylene (0.8 mM) and incubation at 28 °C without agitation.

The dechlorinating enrichment culture used in this study (ANAS) has been maintained in our laboratory for over 15 years in a continuously stirred semibatch fed reactor amended with 25 mM lactate and 0.1 mM TCE as previously described.(33, 34) Two distinct *D. mccartyi* strains have been isolated from ANAS: (1) strain ANAS1 which contains the TCE reductive dehalogenase gene *tceA*, and (2) strain ANAS2 which contains the VC reductive dehalogenase gene *vcrA*.(35) The community structure and dechlorination performance of ANAS has been previously described.(33, 34, 36)

Chemical Analysis

Chloroethenes, ethene, and acetylene were measured by FID-gas chromatography using 100 μL headspace samples, and hydrogen was measured by RGD-gas chromatography using 300 μL headspace samples. (33, 37) The mass of each compound was calculated based on gas–liquid equilibrium by using Henry's law constants at 34 and 28 °C according to mass (μmol/bottle) = $C_l \times V_l + C_g \times V_g$.

DNA Extraction and Cell Number Quantification

Liquid samples (1.5 mL) were collected for cell density measurements and cells were harvested by centrifugation (21 000*g*, 10 min at 4 °C). Genomic DNA was extracted from cell pellets using Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions for Gram-positive bacteria. qPCR using SYBR Green-based detection reagents was applied to quantify gene copy numbers of strain 195 with *D. mccartyi tceA* gene primers (forward primer 5′-ATCCAGATTATGACCCTGGTGAA-3′ and reverse primer 5′-GCGGCATATATTAGGGCATCTT-3′), as previously described.(38)

RNA Preparation and Transcriptome Analysis

For the transcriptomic data, 5 mL acetylene (corresponding to 1.3 mM in liquid phase) were added to strain 195 cultures on day 5 during mid log growth phase when 50% of TCE was degraded. Cultures were sampled on day 7 when control bottles without acetylene exhibited late exponential growth (around 75% of 78 μmol TCE was dechlorinated, i.e., ~20 μmol TCE remained) and no further TCE degradation was evident in experimental bottles. In order to collect sufficient material for transcriptomic microarray analysis, 60 bottles of acetylene-inhibited strain 195 cultures and 18 control bottles (strain 195, without acetylene) were inoculated and grown from triplicate bottles (160 mL serum bottles, with H_2/CO_2 headspace) of the isolate. For each sampling, triplicate subsamples of cells from 20 bottles were collected by vacuum filtration on day 6 from the experimental and control groups [300 mL culture per filter, 0.2-μm autoclaved GVWP filter

(Durapore membrane, Millipore, Billerica, MA)]. Each filter was placed in a 2 mL orange-cap microcentrifuge tube, frozen with liquid nitrogen and stored at −80 °C until further processing. RNA extraction and preparation were described previously.(8)

Transcriptomic Microarray Analysis

Our use of the Affymetrix GeneChip microarray has been reported previously.(35) Briefly, the chip contains 4,744 probe sets that represent more than 98% of the ORFs from four published *Dehalococcoides* genomes (strain 195, VS, BAV1, and CBDB1). cDNA was synthesized from 9 μg RNA, then each cDNA sample was fragmented, labeled, and hybridized to each array. All procedures were performed with minimal modifications to the protocols in section 3 of the Affymetrix GeneChip Expression Analysis Technical Manual (Affymetrix, Santa Clara, CA http://www.affymetrix.com). Microarray data analysis methods were previously described.(31, 34)

Results

Inhibitory Effects of Acetylene and TCE on Isolates

We evaluated the effects of TCE on growth of *Pelobacter* strain SFB93 and observed no inhibition of growth at any of the tested concentrations (0.1–0.5 mM). Unsurprisingly, there was no evidence of TCE consumption by strain SFB93 during these incubations (data not shown). No inhibition of strain 195 dechlorination was observed by C_2H_2 concentrations <0.4 mM while partial inhibition (58%) occurred at 0.8 mM acetylene, and complete inhibition at 1.3 mM (Figure S1 of the Supporting Information, SI). In order to determine whether the inhibition of TCE dechlorination was reversible, actively dechlorinating strain 195 was amended with 1.3 mM acetylene. After 4 days, a subset of these cultures were amended with *Pelobacter* strain SFB93 cells. Acetylene was consumed within 3 days in the SFB93-amended samples (data not shown), and TCE reduction resumed at a rate of 63% of the initial no-acetylene control with all TCE reduced by the end of the experiment (Figure 2). In contrast, in the culture without *Pelobacter* amendment no additional TCE was removed, demonstrating that acetylene inhibition of TCE dechlorination by strain 195 is reversible.

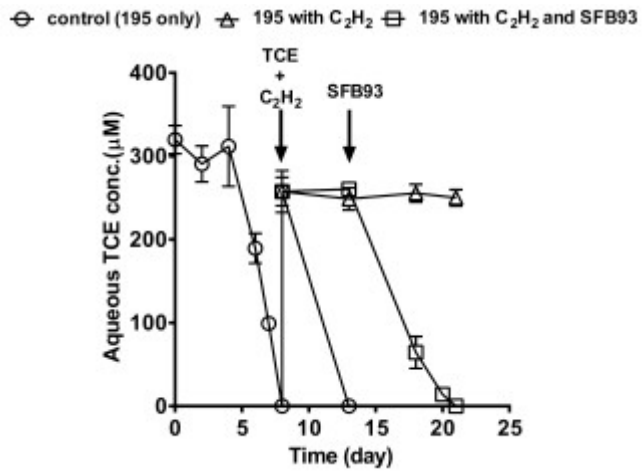


Figure 2. C_2H_2 inhibition on TCE reduction by *D. mccartyi* strain 195. Symbols represent the mean of biological triplicates, and error bars are standard deviations. Absence of bars indicates error was smaller than the symbol.

Acetylene Fuels Dechlorination by *D. mccartyi* Strains

An anaerobic coculture consisting of strain 195 and strain SFB93 was established with acetylene serving as the electron donor and carbon source (Figure 3). While strain 195 metabolically reduces TCE to VC using H_2 as electron donor, strain SFB93 ferments acetylene to ethanol, acetate, and H_2.(26, 30) In dechlorinating communities, ethanol is rapidly oxidized to acetate and H_2, thereby providing additional electron donors and carbon sources to dechlorinators, sulfate-reducers, and methanogens. Complete fermentation of 1 mol acetylene can generate 1 mol hydrogen, which then can serve as electron donor to reduce 0.5 mol TCE to VC. In the SFB93/195 coculture, 26.2 μmol of TCE was reduced and 25.0 ± 2.4 μmol VC accumulated as the major reduction product, with trivial amounts of *cis*-DCE and ethene (Figure 3). Acetylene was readily consumed within 2 days of each amendment. Aqueous H_2 concentrations ranged from 114 to 217 nM during TCE-dechlorination, well above the H_2 threshold for reductive dechlorination.(39) By the end of the experiment, strain 195 cell concentrations reached $3.3 \pm 0.1 \times 10^7$ cell mL^{-1}, with a cell yield of $3.7 \pm 0.3 \times 10^7$ cells μmol^{-1} Cl^- released, demonstrating that acetylene fermenting strain SFB93 can provide the H_2 and acetate required to support the growth of dechlorinating strain 195.

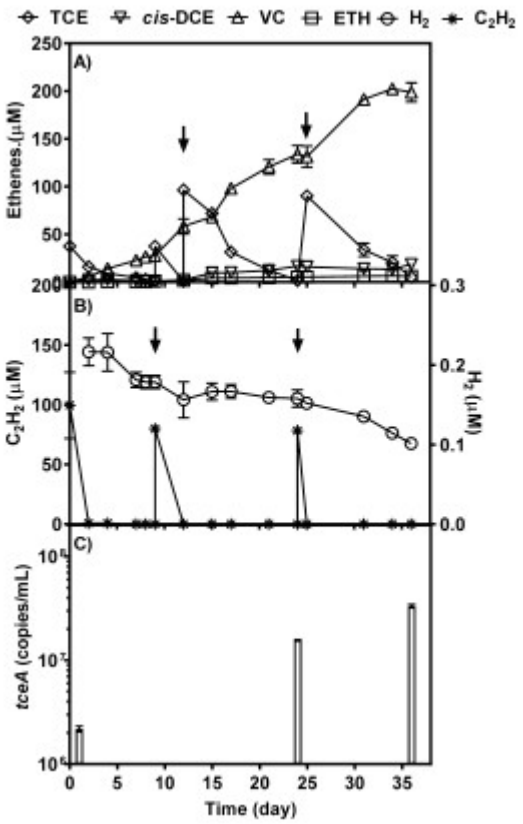


Figure 3. Coculture *D. mccartyi* strain 195 grown with *Pelobacter* SFB93. A) TCE reduction and VC production; B) C_2H_2 and H_2 concentrations; and C) cell numbers of strain 195. Arrows indicate TCE A) and C_2H_2 B) amendments to the coculture. Symbols represent the mean of biological triplicates, and error bars are standard deviations. Absence of bars indicate error was smaller than the symbol.

We also explored the coculture growth of *D. mccartyi* strain BAV1 with strain SFB93 on VC and acetylene. Unlike strain 195, BAV1 gains energy from dechlorination of VC to ethene.(10) However, similar to strain 195, BAV1 does not transform acetylene and cannot use it as an electron donor for reductive dechlorination (Figure 4 A). When BAV1 was cocultured with SFB93, acetylene was consumed within 1 day (data not shown), and VC was completely reduced to ethene with a reduction rate of 0.9 ± 0.1 μmol day^{-1} (Figure 4B).

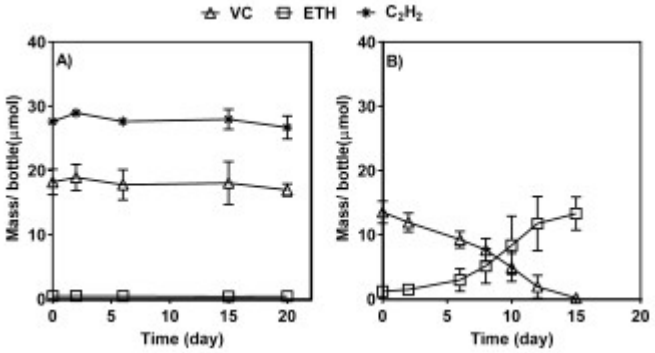


Figure 4. VC reduction by A) *D. mccartyi* strain BAV1 with C_2H_2 as the sole electron donor and N_2 headspace; B) coculture of *D. mccartyi* strain BAV1 and *Pelobacter* strain SFB93 with C_2H_2 as sole electron donor and N_2 headspace. Symbols represent the mean of biological triplicates, and error bars are standard deviations. Absence of bars indicates that the error was smaller than the symbol.

Effect of Acetylene on *D. mccartyi*-Containing Enrichment Cultures

A methanogenic reductive-dechlorinating enrichment culture (ANAS) was selected to examine the inhibition of acetylene on the culture's capacity for reductive dechlorination. During these experiments, ANAS was reducing TCE primarily to VC rather than to ethene. When ANAS was amended with acetylene in the absence of any other electron donor (e.g., lactate), only a small amount of TCE dechlorination (34%) to DCE was observed (Figure 5A) with no consumption of acetylene (Figure 5B). In contrast, when SFB93 was added to the ANAS culture, acetylene was completely consumed and had to be reamended twice to provide sufficient electron donors for reduction of TCE to VC and ethene (Figure 5C,D). No methane production occurred in the absence of strain SFB93 (Figure 5A), although significant methanogenesis occurred in its presence (Figure 5C). Acetylene is a known inhibitor of methanogenesis,(17) but this inhibition can be reversed by the presence of active populations of acetylene-fermenting bacteria, as was recently demonstrated with anaerobic freshwater lake sediments.(30)

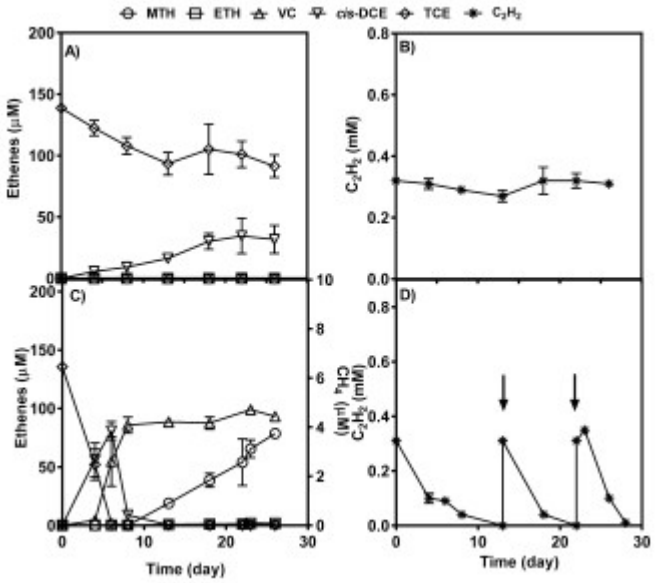


Figure 5. Reductive dechlorinating enrichment culture ANAS with 0.3 mM C_2H_2 as sole electron donor and carbon source. A) and B) ANAS without *Pelobacter* amendment; C) and D) ANAS with *Pelobacter* strain SFB 93 amendment. Arrows indicate C_2H_2 reamendment. Symbols represent the mean of biological triplicates, and error bars are standard deviations. Absence of bars indicates error was smaller than the symbol.

We also tested the acetylene inhibition effects on the ANAS enrichment when lactate was supplied as external electron donor. This was done with both low (0.3 mM) and high (1.0 mM) acetylene amendments. In the 0.3 mM acetylene amendment, no inhibition of TCE reduction or methane production was observed (Figure S2), while with 1.0 mM acetylene, the TCE reduction

rate decreased by 57%. In both cases, acetylene was not consumed throughout the experiment (Figure S3A,B). However, when strain SFB93 was amended to ANAS, complete reductive dechlorination was observed and acetylene was consumed and required replenishment (Figure S3C,D), but no methane production was observed confirming the inhibition of acetylene to methanogenesis. The results demonstrate acetylene was efficient at blocking methanogenesis in lactate-fed cultures of ANAS when cocultured with SFB93, but not in cultures that were solely fed acetylene (Figure 5).

Transcriptomic Analysis of Acetylene Inhibition on *D. mccartyi* Strain 195

The effects of acetylene on *D. mccartyi* strain 195 gene expression were analyzed to better understand the mechanism(s) of its inhibition. Transcriptomic analysis showed that 298 genes were significantly (i.e., ≥2-fold change) down regulated, while 173 genes were significantly up-regulated in the presence of acetylene (Figure 6). The short-term exposure to acetylene did not change the expression pattern of most genes encoding for dehalogenases (Table S1), with the exception of DET1545 whose expression under both conditions was quite low (<700). However, down-regulated genes include ATP synthase (DET0558–0565), proton-translocating NADH-quinone oxidoreductase (DET0928–0933), Hym [Fe]-hydrogenase (DET0145–0148), ribosome proteins (DET0473–0493), and genes related to biosynthesis (Table S1), suggesting an interruption of the electron transport chain. The transcriptomic results agree with the lower cell yields observed in experimental bottles ($0.8 \pm 0.2 \times 10^8$ cells/mL) compared to controls with no acetylene exposure ($1.6 \pm 0.3 \times 10^8$ cells/mL) (two-tailed student's *t* test, $P = 0.03$). Up-regulated gene expressions were observed in a subset of the genes encoding for nitrogen regulation and transport (DET 1124–1125, DET1154–1158), ferrous iron transport protein (DET0095–0097, DET1503–1505), phosphate ABC transporters (DET 0138–0142), and a membrane-bounded molybdopterin oxidoreductase (DET0102–0103).

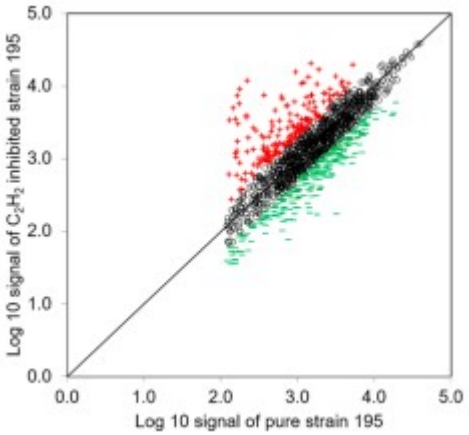


Figure 6. Microarray signal intensities of transcripts from strain 195 (*x*-axis) versus C_2H_2 amended strain 195 (*y*-axis). Colored points represent statistically significant differential transcription: average intensity> 200, $p < 0.05$, more than 2-fold difference, genes significantly up-regulated (red, +) or down-regulated (green, −) in C_2H_2 amended strain 195 versus strain 195. All measurements are averages from three biological replicates.

Discussion

In this study, we found that acetylene can inhibit reductive dechlorination in *D. mccartyi*-containing cultures, but only when applied at relatively high concentrations (>0.4 mM). Moreover, that inhibition was eliminated when acetylene-consuming *Pelobacter* strain SFB93 was present (Table 1). One proposed mechanism of acetylene inhibition on reductive dechlorination is that acetylene directly inhibits reductive dehalogenase required for TCE and VC reduction. However, no differential gene expressions of reductive dehalogenase *tceA* were observed in acetylene inhibited cells compared to the control bottles. This unchanged transcriptomic profile of reductive dehalogenase was also observed during sulfide inhibition experiments with strain 195 (unpublished data), and during comparisons of strain 195 while in exponential and stationary phase growth.(12) This finding suggests the transcriptional control and cell growth become decoupled when cells are exposed to the inhibitors, making it problematic to use reductive dehalogenase (RDase) expression levels as accurate biomarkers to indicate the physiological activity of *D. mccartyi* under field conditions.

Table 1. C_2H_2 Inhibition Effects on *D. mccartyi*-Containing Cultures

cultures	e^-donor	e^- acceptor conc. (mM)	C_2H_2 aqueous conc. (mM)	*Pelobacter* strain SFB 93	C_2H_2 inhibition on RD[a]
strain 195	H_2	0.3 (TCE)	0–1.3	no	yes, >0.8 mM
strain 195	H_2	0.3 (TCE)	1.3	yes	no
strain 195/SFB 93		0.25 (TCE)	0.1 (×3 doses)	yes	no
strain BAV1/SFB 93		0.3 (VC)	0.4	yes	no
ANAS		0.14 (TCE)	0.3	no	yes
ANAS		0.14 (TCE)	0.3	yes	no
ANAS	lactate	0.14 (TCE)	1.0	no	yes
ANAS	lactate	0.14 (TCE)	1.0	yes	no

[a]RD: reductive dechlorination.

Acetylene inhibition of hydrogenase activity has been suggested to be responsible for the inhibited growth of many hydrogenase containing microorganisms, such as cyanobacteria and nitrogen fixing bacteria. There are other biological functions of hydrogenases. For example, bidirectional hydrogenases can control excess reducing equivalents, especially in photosynthetic microorganisms (e.g., cyanobacteria). Hydrogenases may also be involved in membrane-linked energy conservation through the generation of a transmembrane proton motive force in nitrogen-fixing bacteria.(40) For example, acetylene is known to inhibit Fe/Ni-containing hydrogenases(21) and the same down-regulation pattern of [Fe]-hydrogenase (Hym) was observed in this study. In fact, genes involved in energy conversion by the electron transport chain (e.g., ATP synthase, proton-translocating NADH-quinone oxidoreductase) were also significantly down-regulated in acetylene inhibited strain 195, which agrees with the physiological observations of lower cell yields and reduced dechlorination rates of strain 195 during acetylene inhibition. Acetylene inhibited TCE dechlorination by *D. mccartyi* strain 195 and the ANAS enrichment culture at

levels above 0.3 mM (Table 1), at concentrations similar to that previously reported (0.2 mM) for inhibiting a VC-dechlorinating consortium.(24)

Phylogenetic analysis of 16S rRNA genes showed that *Pelobacter* strain SFB93 has 96% similarity to *Pelobacter acetylenicus*, and 97% sequence similarity to strain *P. carbolinicus*, which are able to grow by fermentation with acetate and hydrogen as end products.(30, 41, 42) Strain SFB93 also contains acetylene hydratase (AH), the enzyme capable of metabolizing acetylene.(30, 43) In the coculture studies, we demonstrated that strain SFB93 can ferment acetylene to hydrogen to support TCE reduction to VC by strain 195 and VC reduction to nontoxic end-product ethene by strain BAV1 as well as acetate to support cell growth. Specifically, the dechlorination rate (0.6 μmol TCE·d^{-1}) of the cocultured 195/SFB93 was similar to cocultured strain 195 with *Desulfovibrio desulfuricans* (0.4 μmol d^{-1}; He et al. 2007), and comparable to other sustainable growing *D. mccartyi*- containing syntrophic cocultures (2.1–11 μmol d^{-1}).(8, 31) The cell yield of strain 195 (3.7 ± 0.3 × 10^7 cells μmol^{-1} Cl^- released) was comparable to previous *D. mccartyi*-containing coculture studies: 3.3 × 10^7 to 4.2 × 10^8 cells μmol^{-1} Cl^- released.(8) During our coculture experiments, H_2 concentrations remained at levels between 100 to 210 nM (Figure 3B) indicating that the H_2 generation rate was at about the same level as its consumption rate, and that the growth of the two species was strictly coupled via interspecies hydrogen transfer.

No reductive dechlorination, methanogenesis, or hydrogen production was observed in ANAS when acetylene was present as the only potential electron donor. This was likely due to the absence of *Pelobacter* in the microbial enrichment.(36, 37) By introducing strain SFB93, acetylene promoted the complete reduction of TCE to VC and methane was produced, demonstrating that the fermentation of acetylene by *Pelobacter* strain SFB93 to hydrogen and acetate supports the reductive dechlorination and other processes in the enrichment (e.g., methanogenesis).

Previous studies showed that acetylene can inhibit methanogens at relatively low concentrations of 42–48 μM.(24, 44) In our study, we found methanogenesis was inhibited at 300 μM acetylene, but that inhibition was also entirely eliminated in the presence of strain SFB93 (Figure 5C, Figure S3), demonstrating that acetylene inhibition on methanogenesis can also be removed by *Pelobacter* fermentation. However, at higher acetylene concentrations (~1 mM), methanogenesis was inhibited even with *Pelobacter* present (Figure S3C), indicating that the methanogens in ANAS are more sensitive to acetylene inhibition than *D. mccartyi* strains.

While acetylene is known to inhibit biological reductive dehalogenation of TCE, we have now demonstrated that through the intersession of acetylene-fermenting bacteria this inhibition can be eliminated. Moreover, under these conditions acetylene can also serve as a carbon source and hydrogen donor to fuel microbial dehalogenation of TCE and its daughter products to yield a desired, innocuous end-product (ethene). We suggest that a future focus

upon acetylene and the bacteria that ferment it may help further our understanding of the present constraints encountered in bioremediation efforts of subsurface environments contaminated with compounds like TCE that lead to a buildup of more toxic VC. Such investigations could lead to practical solution to this widespread and vexing environmental contamination problem.

Acknowledgments

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