Changing our views on old ideas to promote sustainable bioremediation: *Fe(III) reduction and electron donors in complete TCE dechlorination*

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Overview

- A (very brief) background on me and my research and consulting work
- Scope of the problem: what is needed?

- Current dogma in Cl-solvent remediation
  - Fe(III) reduction is a competitive process
  - More time and more electrons
  - *Dehalococcoides* are the only organisms that generate ethene

- Experimental Data
  - Acetate as the sole electron donor with sustainable usage:
    - Low concentration electron donor addition
    - Less methane generated
  - Overlapping respiratory processes
    - Concurrent Fe(III) reduction and complete dechlorination
  - DHC and Non-*Dehalococcoides* complete dechlorination
    - Unique microbial community associated with this strategy

- Conclusions

I am primarily an academic researcher

- Ph.D. Microbiology, UMASS Amherst
- Assistant Professor University of Illinois CEE
- Associate Professor Clemson EEES
- Kavli Fellow, National Academy of Sciences
- Scientific advisory boards:
  - Battelle Chlorinated Solvents/In Situ Remediation (Monterrey Conference)
  - UMASS/AEHS Contaminated Soils Conference
  - SURF Academic liaison
  - DuPont working group on advanced geochemistry in remediation
- Research:
  - Remediation: especially bioremediation
  - Mixed biological/abiotic reactions for contaminant transformation
  - Explosives biodegradation under Fe(III)-reducing conditions
  - Combined Fe(III) reduction and complete dechlorination
  - Sustainable remediation
  - Biofuels using unbalanced fermentation
  - Mineral recovery from wastewater
  - Advanced methane production in waste systems

I am also a consultant

Finneran Environmental Consulting *specialty remediation services*

- Many academics consult on their area of expertise
- We can provide answers to questions when you (the practitioners) have reached their limit of expertise, or have just hit a brick wall in the data analyses and interpretation
- As an example – my firm:
  - I used to work for GeoSyntec (2001-2004)
  - I have been consulting for 8 years (One client has been with me since 2008, and I have current projects with that firm)
  - I am now on the Tersus Scientific Advisory Board
  - I have been retained once by Carus Corp. as an “on call” bioremediation expert
  - I see my strength as bridging the academic-consulting world because I have worked in both
  - I provide specialty remediation consulting on all aspects of biological and chemical remediation, biogeochemistry, field sampling plans, remediation work plans, and data analyses and interpretation to assist my clients
  - I describe my work like the TV show “House, M.D”… people (on TV…) go to him when they have reached the end of their tether for diagnostic medicine; I do diagnostic remediation analyses and design…

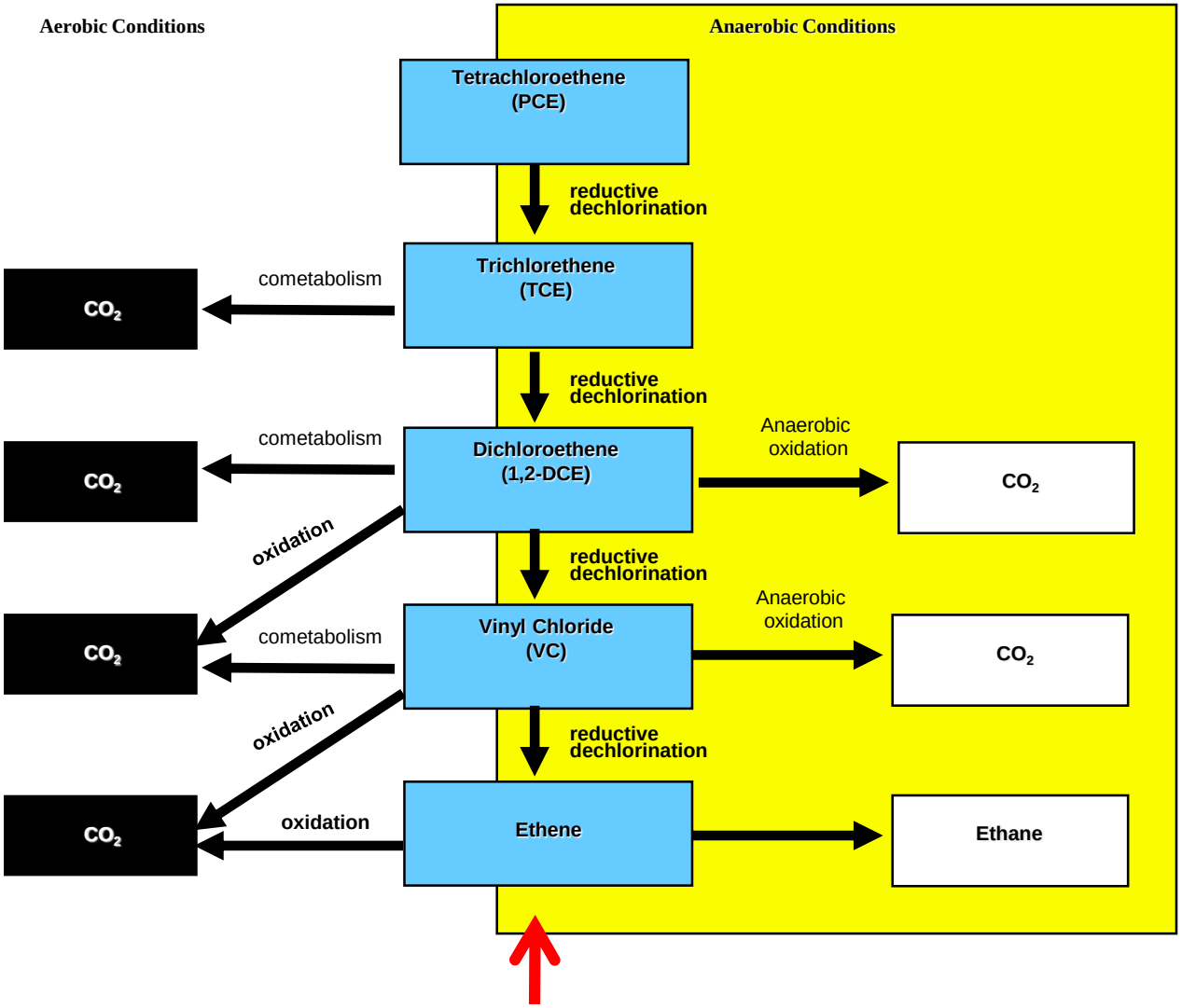
The Problem

Entrenched Ideas in Remediation

There is no such thing as "turn key" remediation. If you ever see or hear that, think long and hard before working with the person or the company stating it

Would you stick with a doctor who claimed "turn key" services for you or your family's health…?

Chlorinated solvent remediation



Issue: the complete reductive pathway has significant geochemical and microbiological limitations → are these real or just an artifact of limited understanding

Issues in remediation

- Only one microbial species has been identified that completely reduces cis-DCE and VC to ethene (*Dehalococcoides mccartyi*; strain specific)
  - This organism works within a limited geochemical range

- Complete dechlorination has been correlated to sulfate reduction and methanogenesis (i.e. low redox potential!) – ***this is incorrect***

- Fe(III) reduction has long been considered a completely competitive process – ***this is also incorrect***

- Fe(III) reduction is typically the most dominant anaerobic metabolic process in subsurface environments → by negating this process we are missing the largest pool of microbial diversity

- Acetate is the key carbon intermediate in all organic matter oxidation
  - Strategies predicated on acetate as an amendment “include” a greater diversity of organisms

Issues in remediation

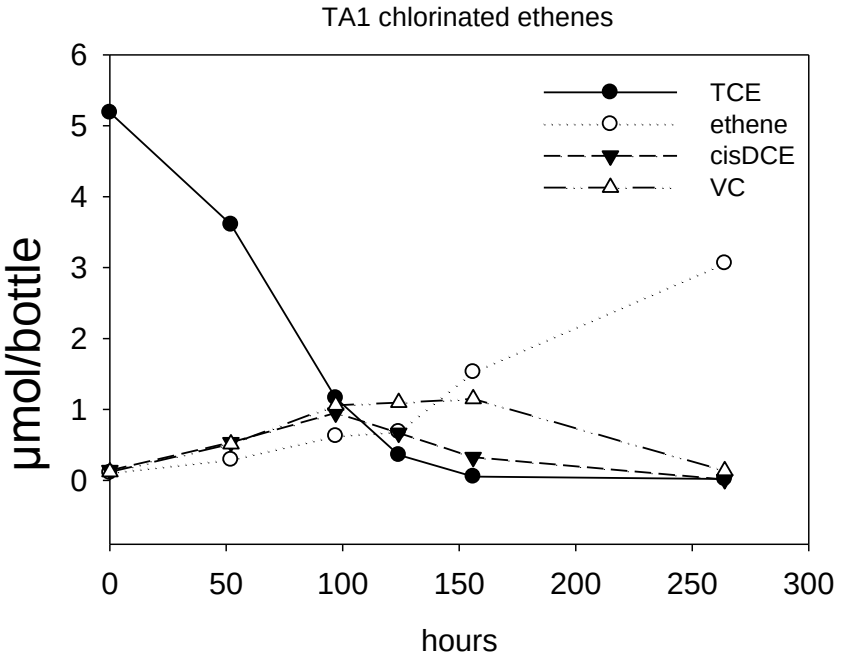
- Electron donors are added at much too high a concentration
- The “more time and more electrons” culture has been pervasive in the industry
- Our data (presented here and published) demonstrate that low electron donor is as good as or better than high electron donor
  - *This has implications for performance and cost; it is possible to reduce TCE to ethene faster, while saving time and money, for the site*
- This generates methane → a potent greenhouse gas and an absolute waste product
- Acetate is generally considered a poor electron donor for complete dechlorination, even though it is simple to use and sustains a very diverse microbial community

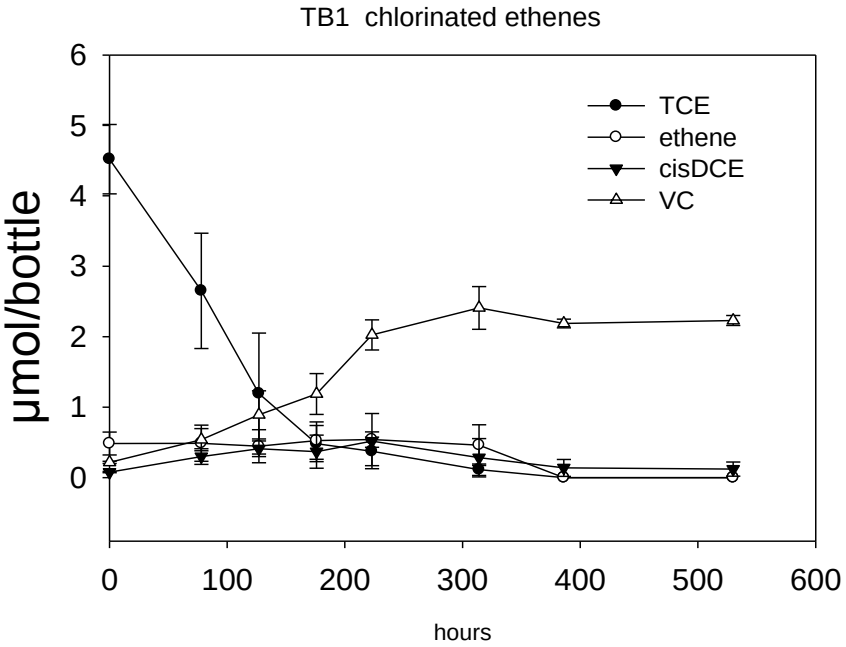
TCE Contaminated Site

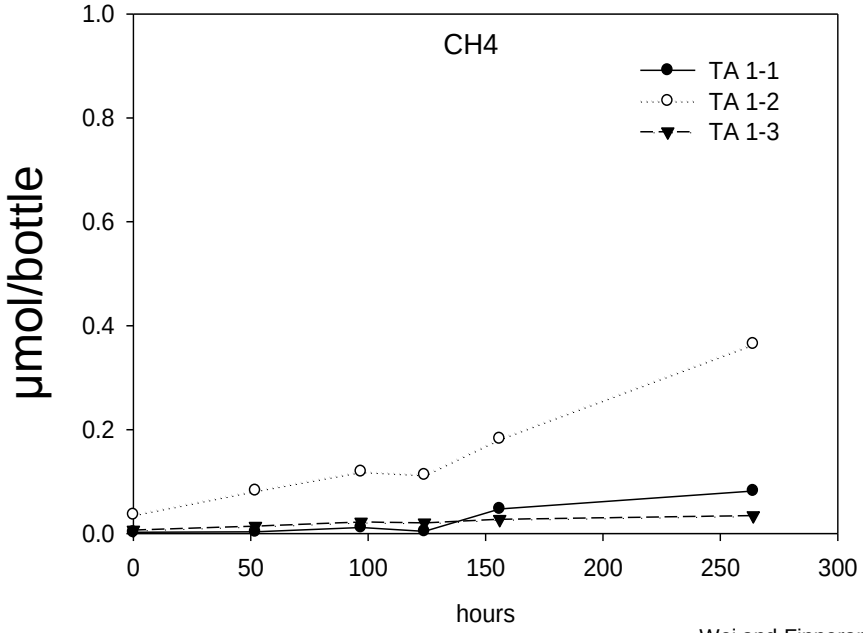
Connecticut

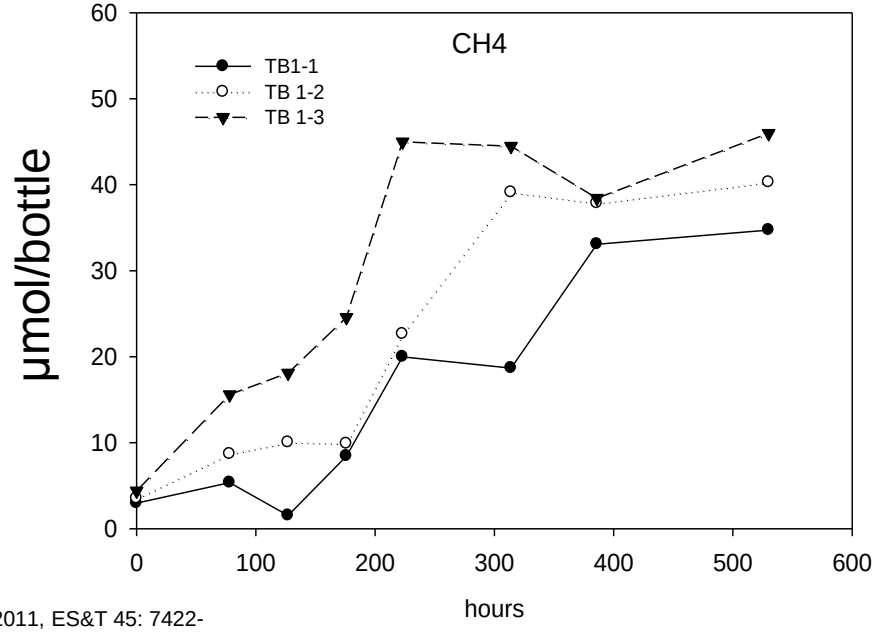
Aquifer Material

TCE reduction (and daughter products) and methane generation in *acetate amended* sediment;
TA = stoichiometric (low) acetate; TB= 10X necessary acetate



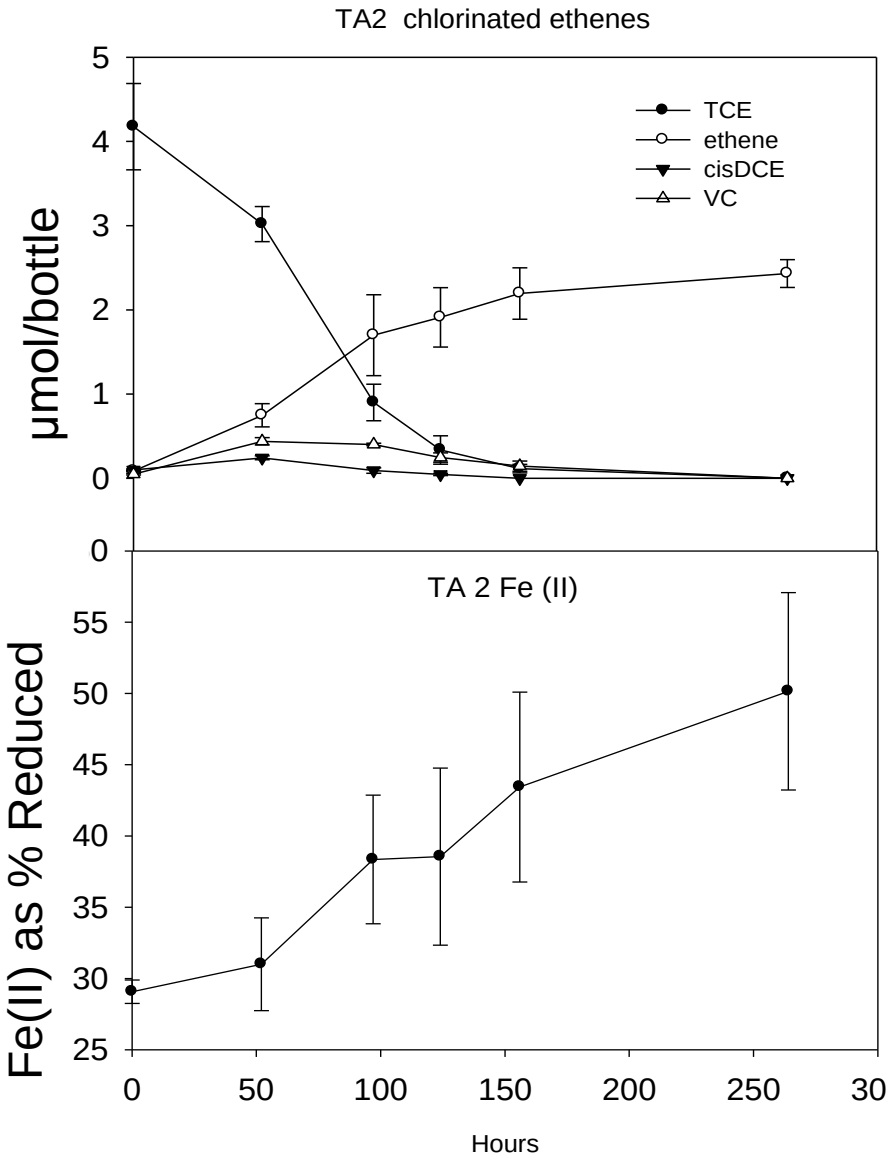


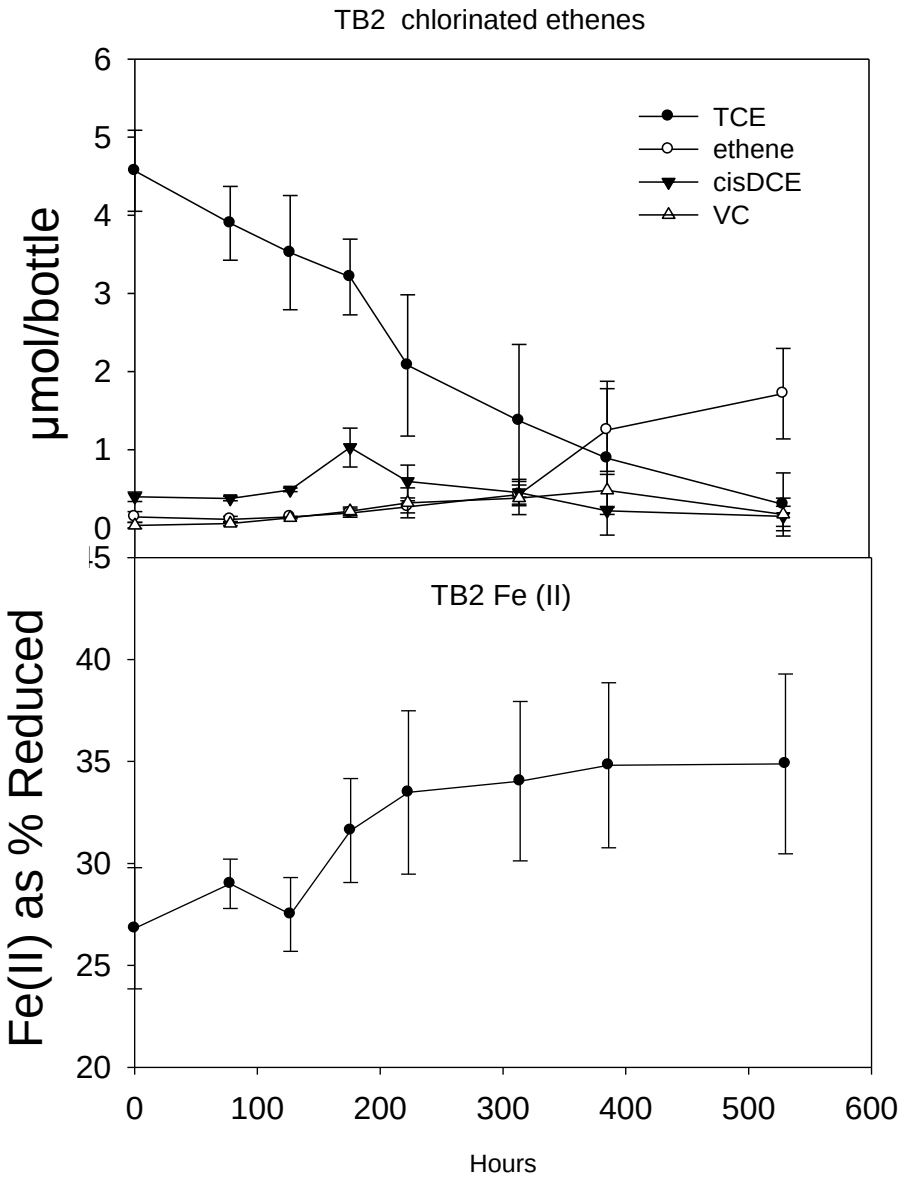




Wei and Finneran, 2011, ES&T 45: 7422-

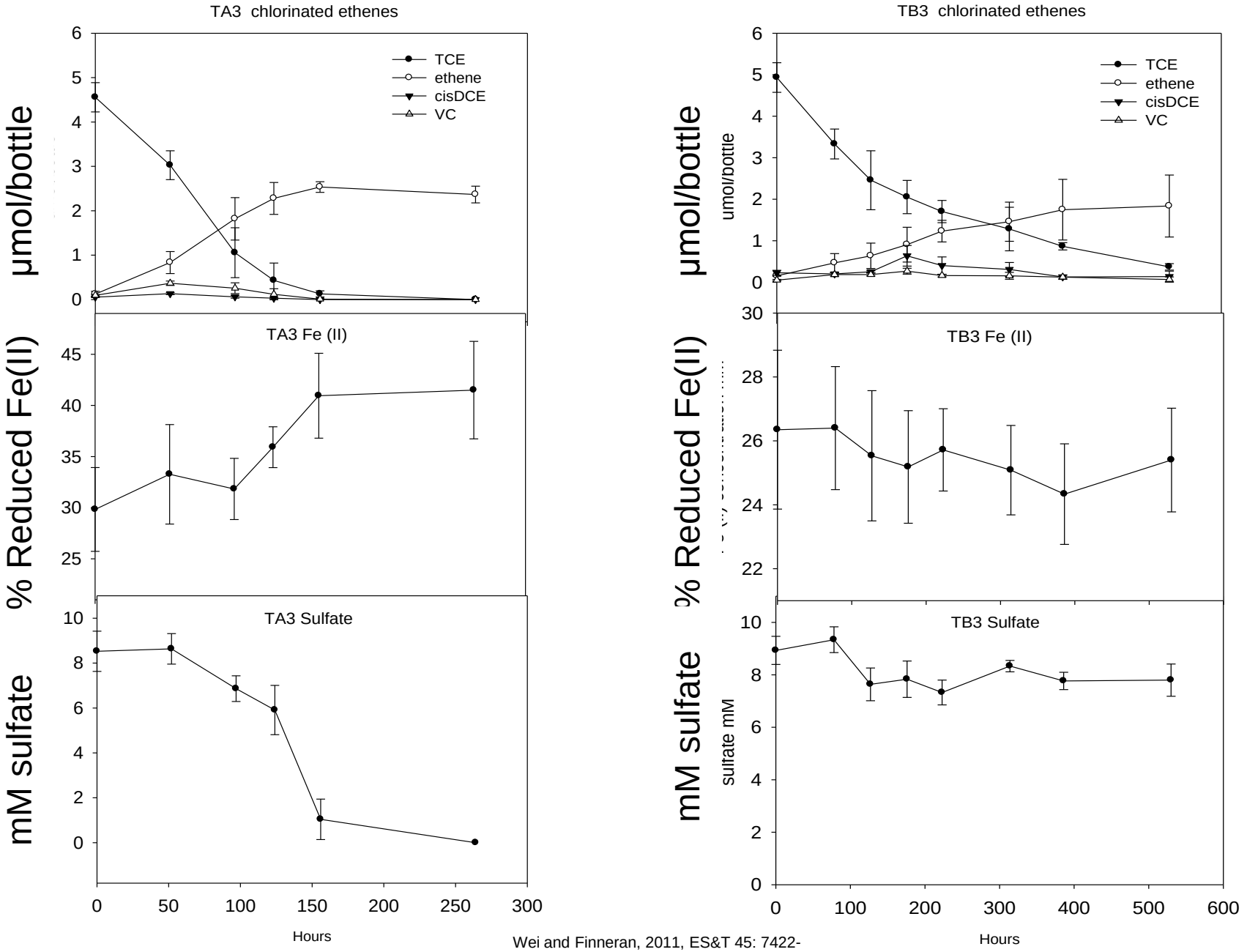
TCE reduction (and daughter products) and Fe(III) reduction in *acetate + Fe(III) amended* sediment





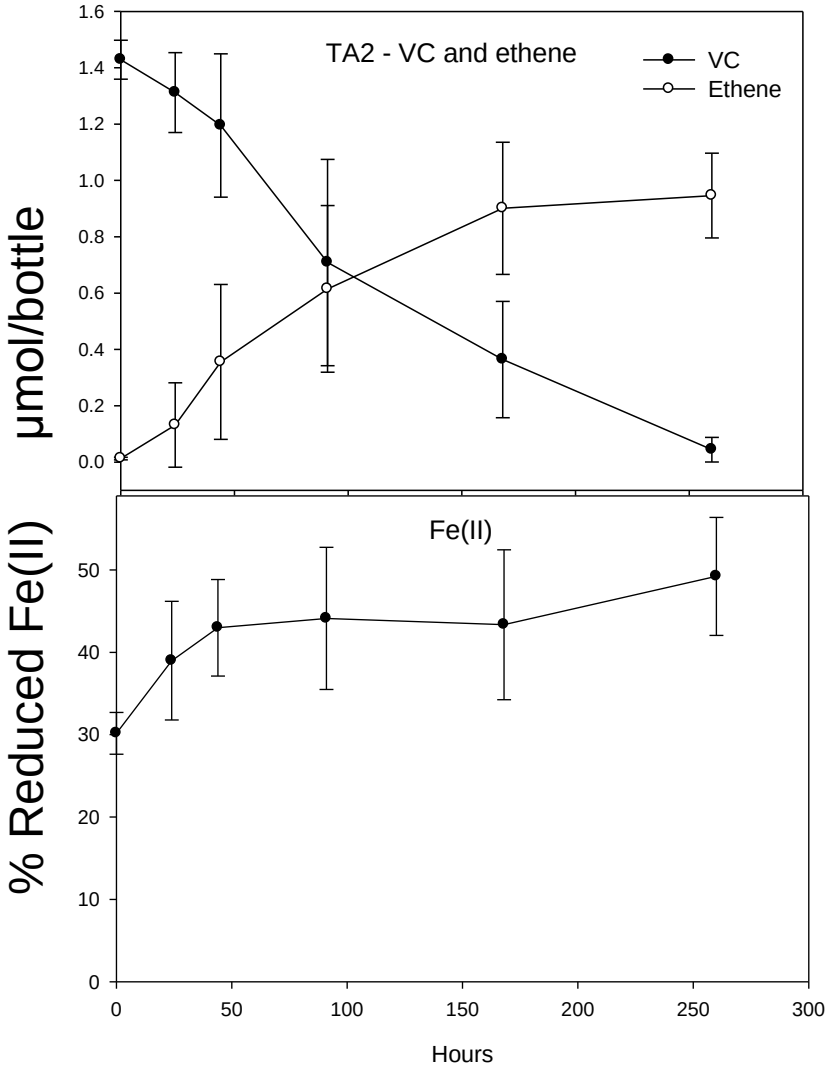
Wei and Finneran, 2011, ES&T 45: 7422-7428

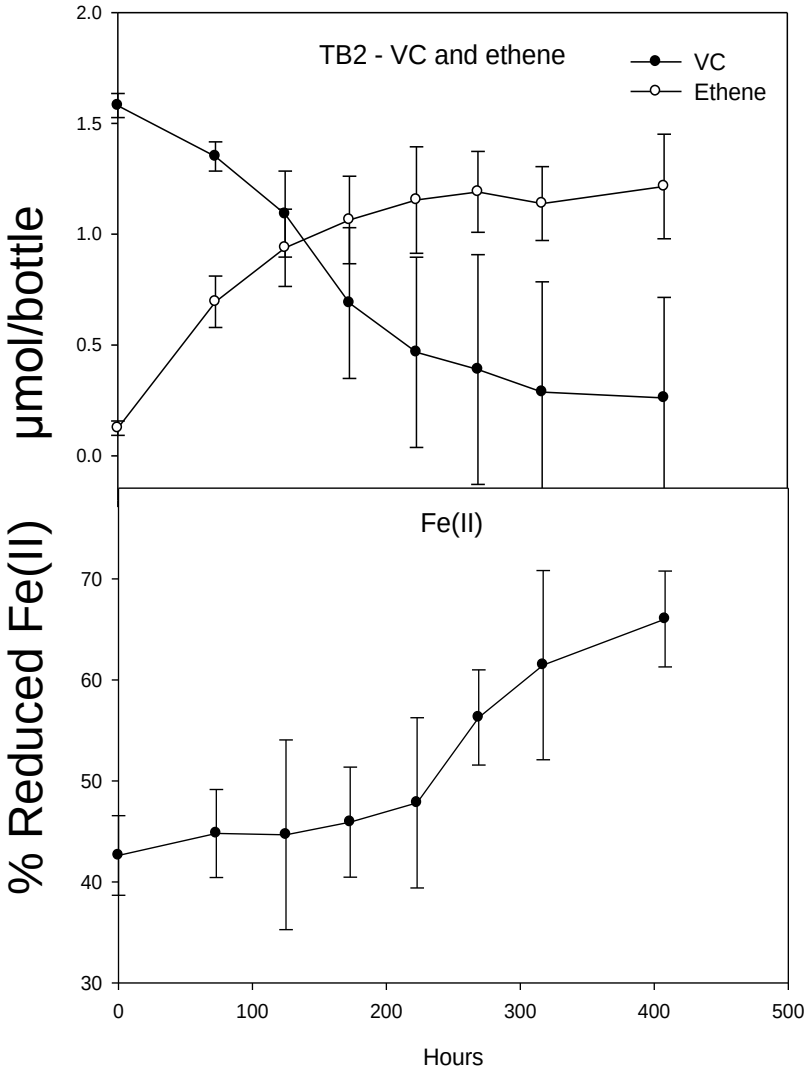
TCE reduction (and daughter products), Fe(III) reduction, and sulfate reduction in *acetate + Fe(III) + sulfate amended* sediment



Wei and Finneran, 2011, ES&T 45: 7422-

VC reduction (and ethene production) and Fe(III) reduction in *acetate + Fe(III) amended* sediment





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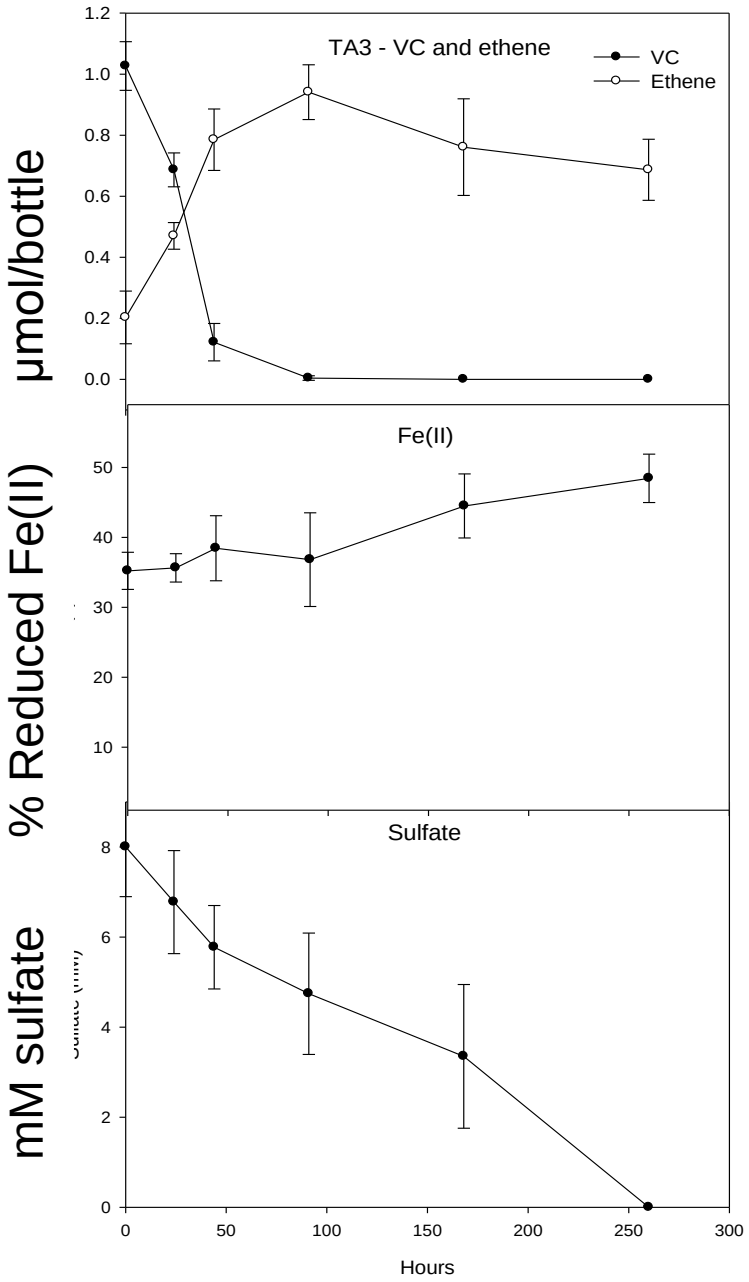

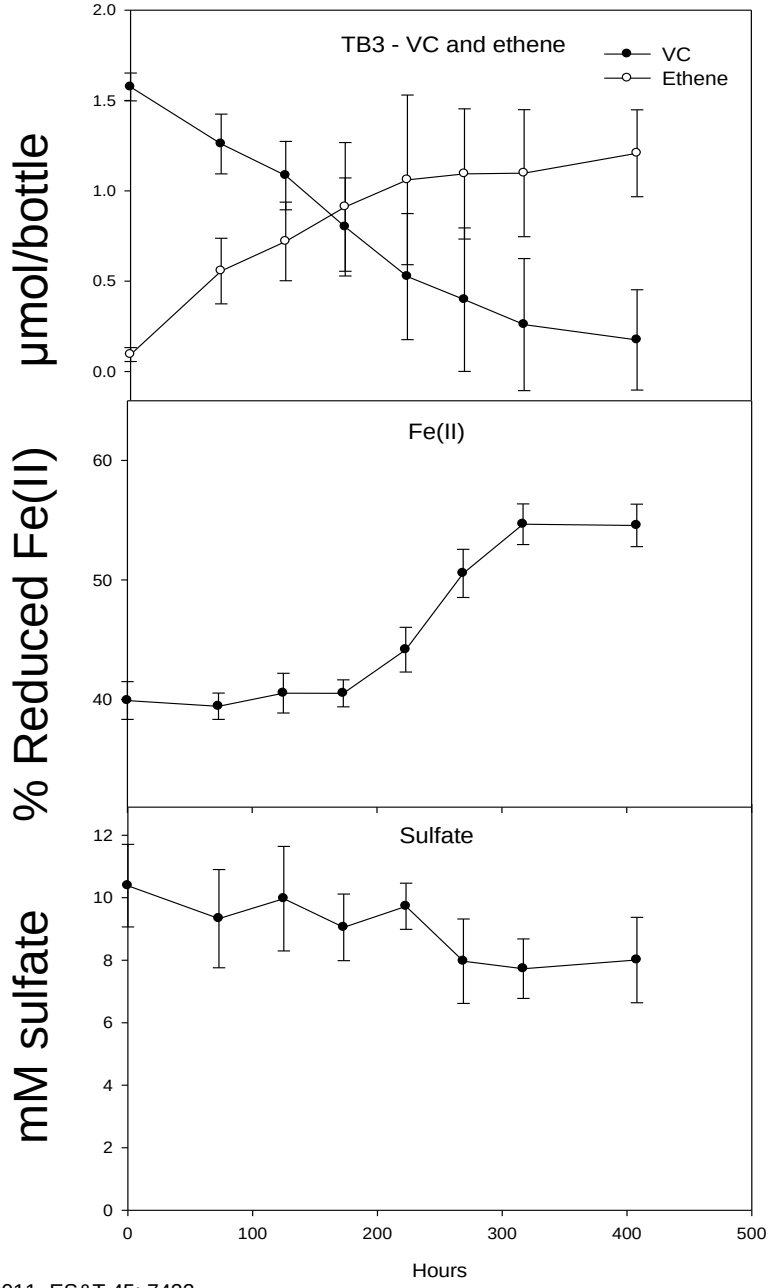

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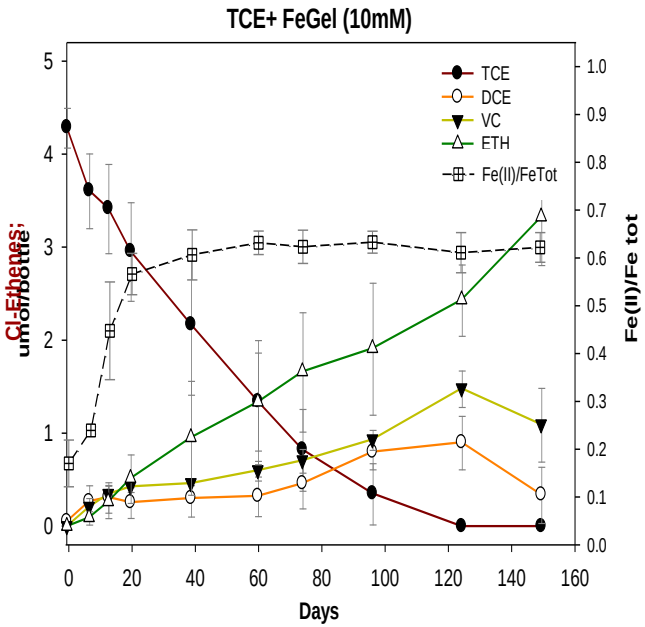
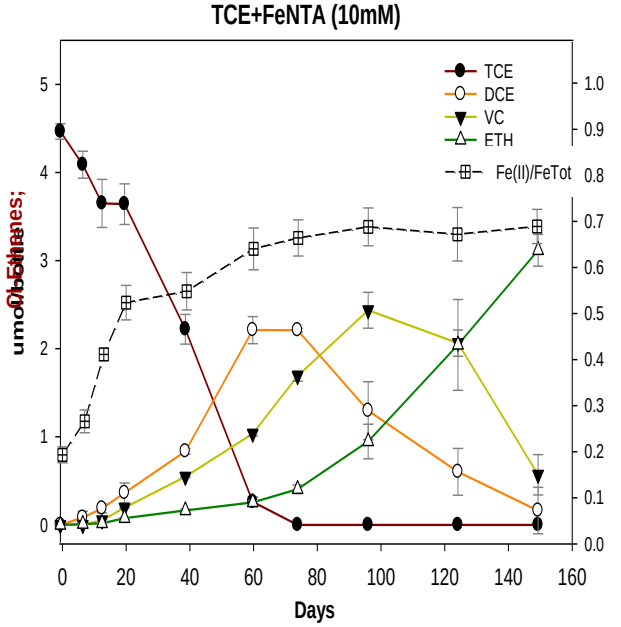
Fe(III) dependent “inhibition” can be due to the form of Fe(III) used in experiments

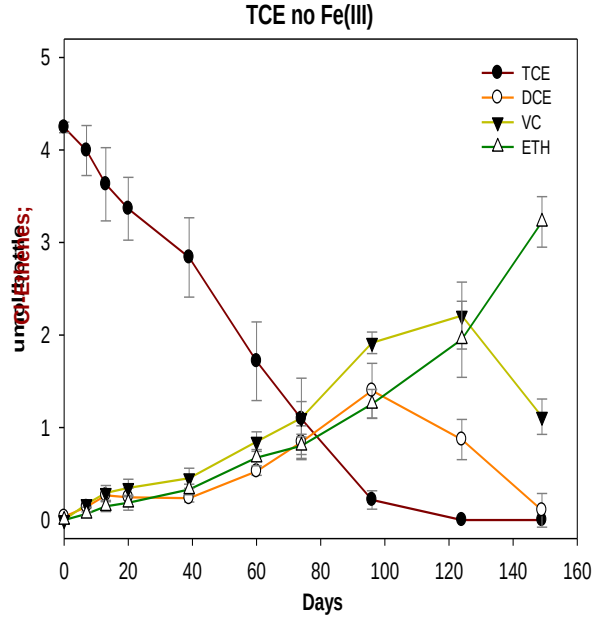
TCE+FeGel



TCE+FeNTA



TCE no Fe(II)



- **The TCE degradation rates and daughter product distributions were different in the incubations amended with different forms of Fe(III).**
- **FeNTA reduction did not inhibit TCE degradation, but stimulated TCE degradation rates.**
- **Different microbial community structures might develop, depending on the Fe(III) speciation and bioavailability.**

Wei and Finneran, 2011, ES&T 45: 7422-7428

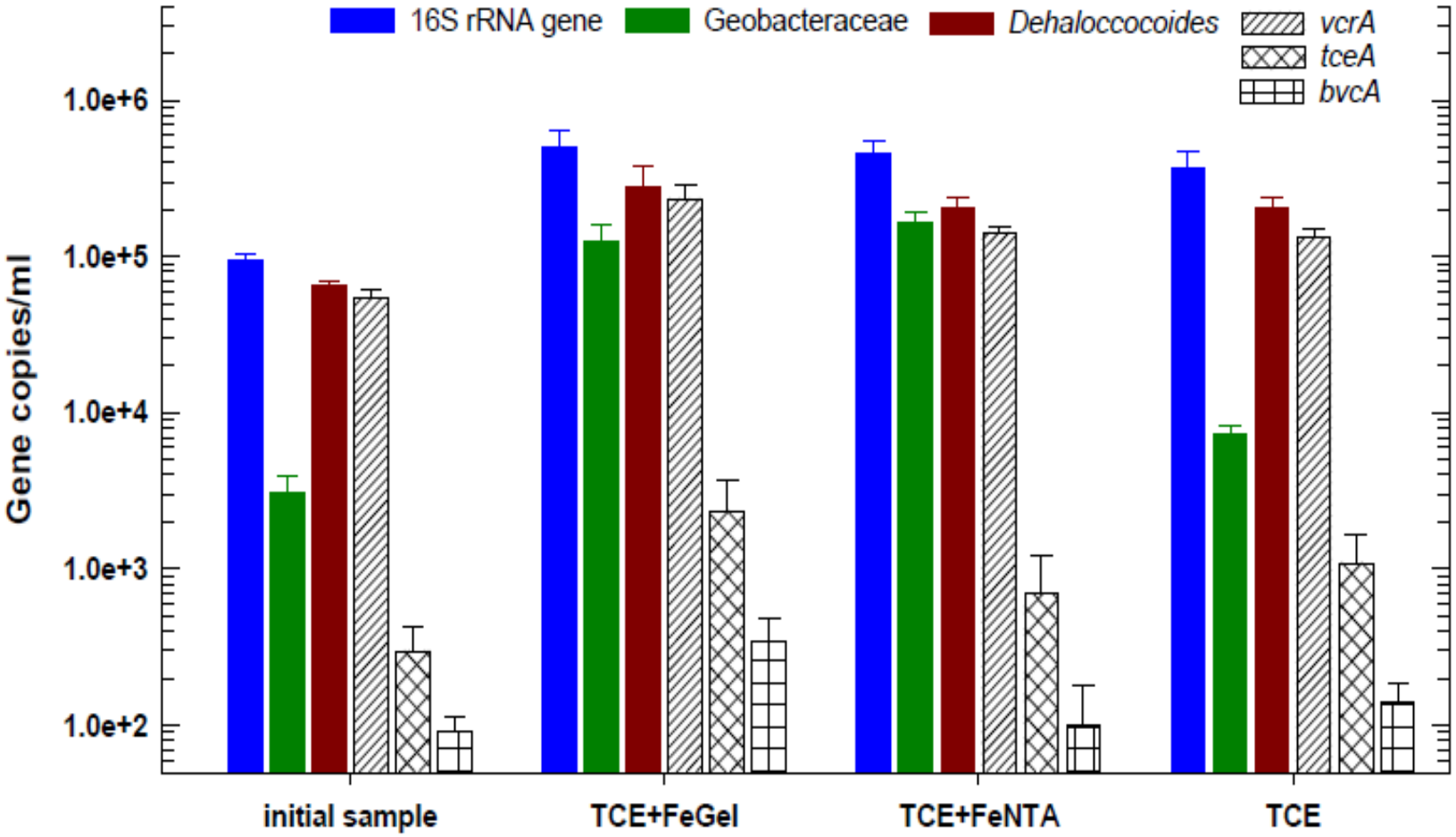
Microbial communities that develop in these Fe(III) reduction/complete dechlorination environments reflect two dominant groups

clone groups		closest relative in NCBI BLAST search	closest known relative	% in TCE only	% in TCE + FeGel	% in TCE + FeNTA
Dehalococcoides	G1	Uncultured *Dehalococcoides sp* . clone DcocHCBPCE_48 (FJ810793.1) (99%)	*Dehalococcoides sp.* GT (CP001924.1) (99%)	24.5	21.3	12.3
	G2	*Dehalococcoides sp.* JN18_V4_B (EF059527.1) (99%)		5.3	N.A	N.A
Geobacter	G3	Uncultured *Geobacter sp.* clone LrhB63 (AM159369.1) (99%)	*Geobacter humireducens* (AY187306.1) (98%)	N.A	11.7	19.2
	G4	*Geobacter lovleyi* (AY914177.1) (98%)		5.3	9.6	11.6
	G5	Uncultured *Geobacter sp.* clone: FH-33 (AB293279.1) (94%)	*Geobacter humireducens* (AY187306.1) (93%)	N.A	11.7	7.5
	G6	*Geobacter thiogenes* strain K1 (NR_028775.1) (98%)		N.A	2.1	1.4
other iron-reducing clones	G7	Iron-reducing enrichment clone CI-A4 clone CI-A4 (DQ676996.2) (100%)	n.a.	N.A	4.3	11.6
	G8	Iron-reducing bacterium enrichment culture clone HN-HFO29 (FJ269093.1) (100%)	n.a.	N.A	4.3	8.2
	G9	Iron-reducing enrichment clone CI-A9 clone CI-A9 (DQ677001.1) (99%)	n.a.	N.A	7.4	4.1
other clones	G10	*Desulfosporosinus sp.* 063 (GQ214051.1) (98%)		2.1	11.7	7.5
	G11	Uncultured bacterium clone 127 (FJ535060.1) (93%)	n.a.	N.A	N.A	6.2
	G12	Uncultured bacterium clone FRC-AI_600 (EF507958.1) (97%)	n.a.	6.4	4.3	4.1
	G13	Uncultured *Dechlorosoma sp.* , isolate ALISEMBF34R34 (FM877971.1) (99%)	n.a.	5.3	5.3	2.1
	G14	Uncultured bacterium clone J3A10 (GU139289.1) (96%)	*Desulfovibrio butyratiphilus* strain BSY-C (AB303306.1) (94%)	N.A	1.1	1.4
	G15	Uncultured bacterium clone AN108 (GQ859927.1) (100%)	*Desulfosporosinus sp.* 063 (GQ214051.1) (99%)	N.A	3.2	1.4
	G16	*Variovorax sp.* P-59, strain P-59 (AM411933.1) (97%)		N.A	N.A	1.4
	G17	Uncultured bacterium clone FW2_121B (GQ263435.1) (99%)	n.a.	11.7	1.1	N.A
	G18	Uncultured bacterium clone SINI1098 (HM126754.1) (99%)	*Azospira sp.* Cu-d-1 (EF016458.1) (99%)	8.5	N.A	N.A
	G19	*Azoarcus sp* . (AF482683.1) (99%)		5.3	N.A	N.A
	G20	Uncultured Firmicutes bacterium clone GASP-MB3S2_C03 (EF665657.1) (94%)	n.a.	9.6	1.1	N.A
	G21	Uncultured bacterium clone JH-WH18 (DQ351911.1) (98%)	n.a.	4.3	N.A	N.A
	G22	Uncultured bacterium clone FFCH16890 (EU132251.1) (97%)	n.a.	5.3	N.A	N.A
	G23	Uncultured bacterium clone AKIW782 (DQ129364.1) (98%)	*Acetonema longum* DSM 6540(T) (AJ010964.1) (89%)	1.1	N.A	N.A
	G24	Uncultured Clostridia bacterium clone X9Ba76 (AY607206.1) (98%)	*Clostridium aldrichii* strain P-1 (NR_026099.2) (96%)	5.3	N.A	N.A

- ***Dehalococcoides* and *Geobacter* species were two major groups in these incubations.**
- **FeNTA stimulated more DIRB, including *Geobacter lovleyi* SZ, which can transform TCE to *cis*-DCE with concurrent Fe(III) reduction.**

Wei and Finneran, 2011, ES&T 45: 7422-

Quantitative PCR data (QPCR) demonstrate that the DHC and Fe(III)-reducers are developing together

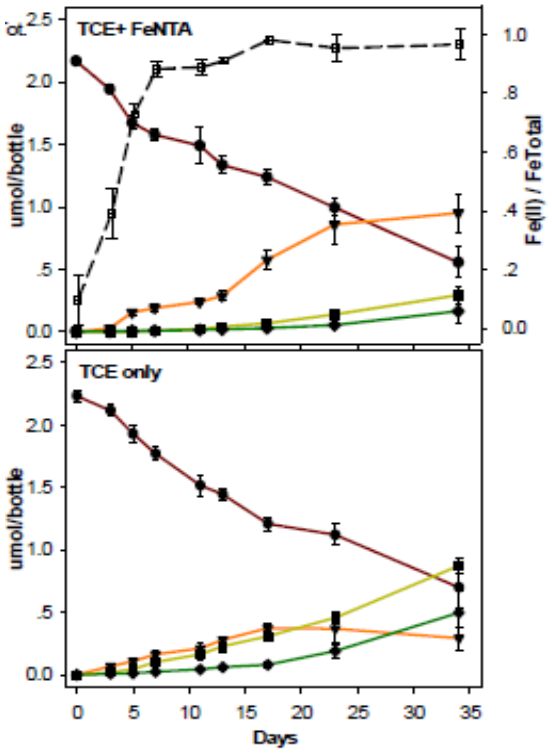


- **Both *Dehalococcoides* and *Geobacteraceae* were enriched in TCE+Fe(III) incubations.**
- **FeI(III) reduction did not inhibit the enrichment of *Dehalococcoides* species.**
- **The *vcrA*-carrying strains was the dominant *Dehalococcoides* in all the samples; the highest number was found in FeGel amended sediment.**

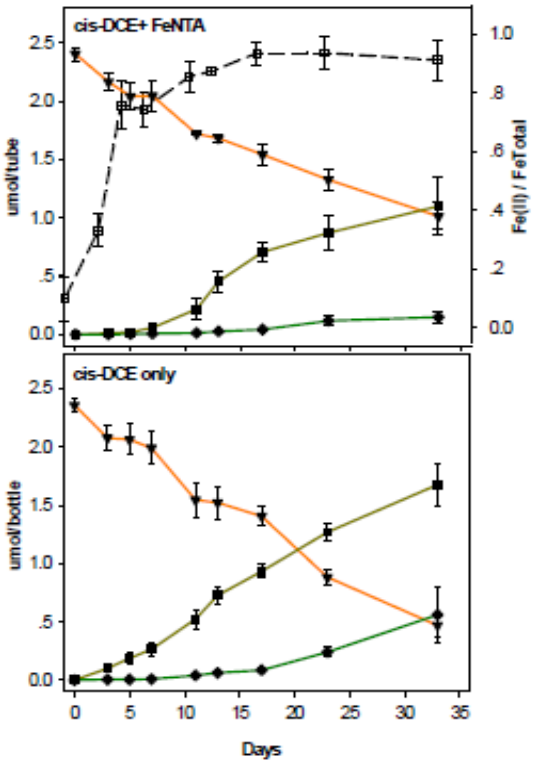
Wei and Finneran, 2011, ES&T 45: 7422-

It is all about the dissolved molecular H_2 steady state!

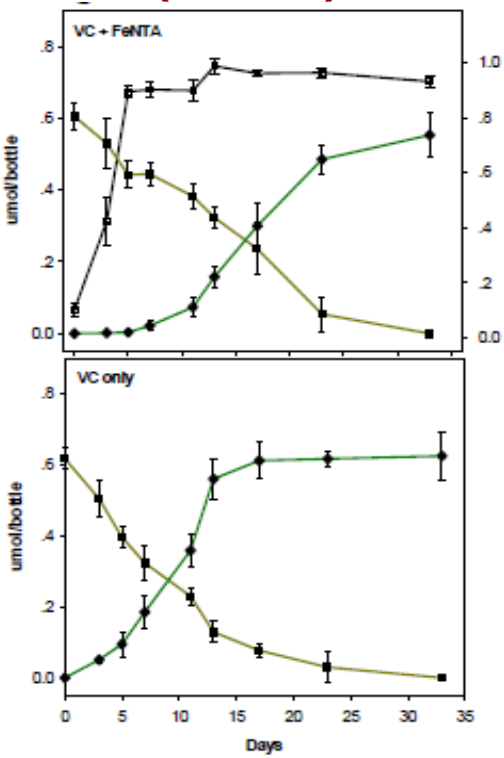
TCE (±FeNTA)

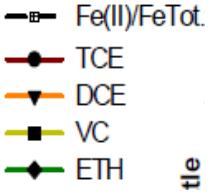


cis-DCE (±FeNTA)

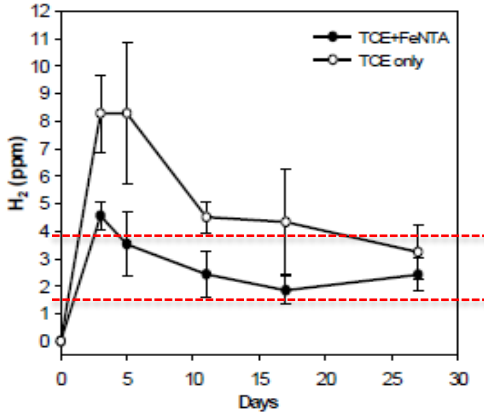


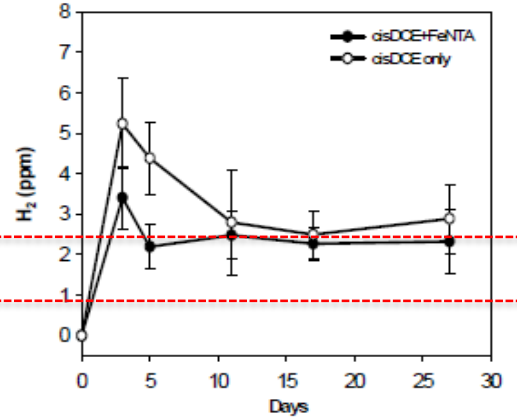
VC (±FeNTA)

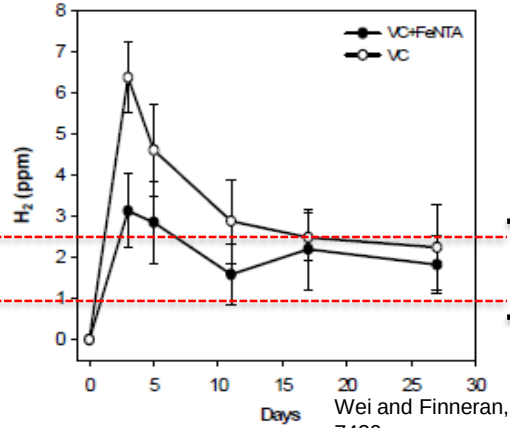




H_2 production and consumption



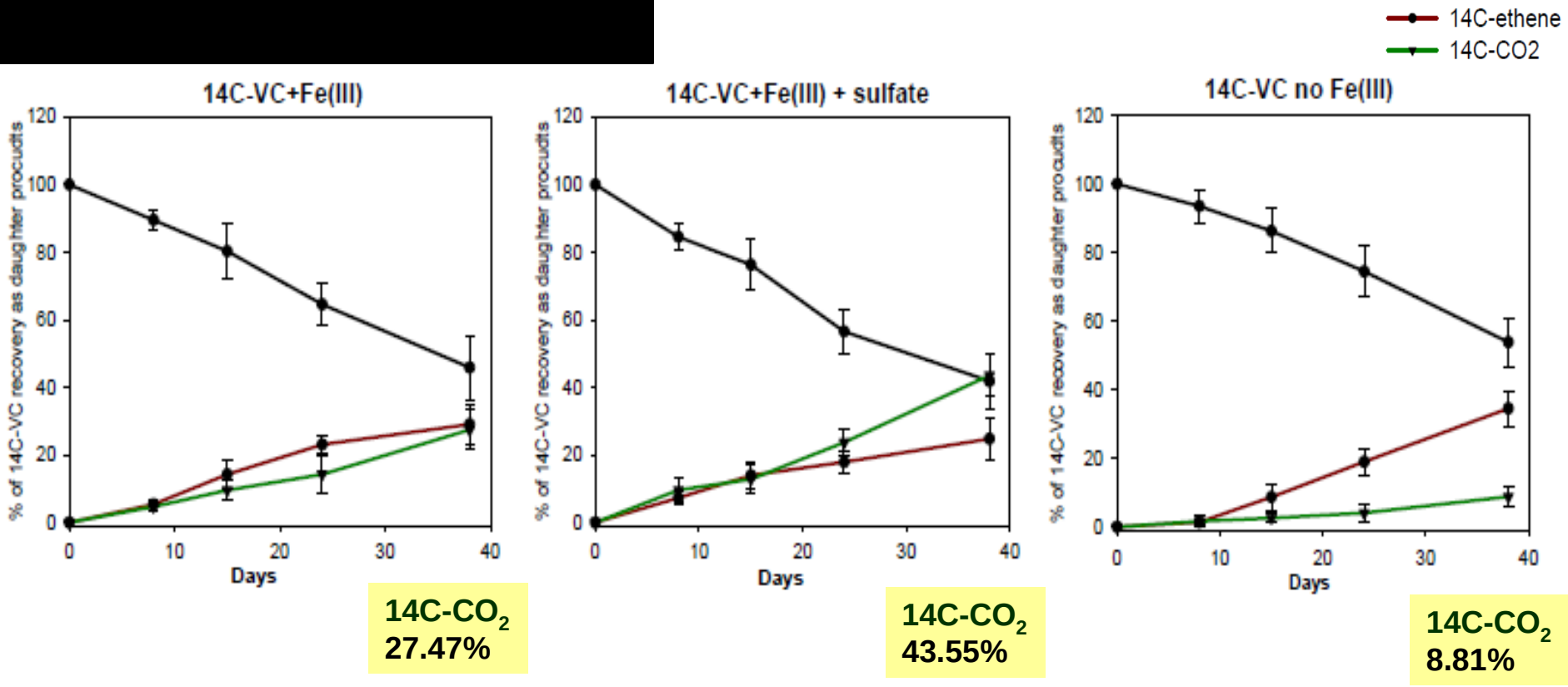




This narrow concentration range is the optimal H_2 threshold for complete dechlorination; Fe(III) reducers help poise the H_2 at the most appropriate concentration

Wei and Finneran, 2011, ES&T 45: 7422-

Perhaps some fraction of the chlorinated solvents is being mineralized to CO_2 by Fe(III) reducers, which accounts for poor mass balances in the presence of Fe(III)



Wei and Finneran, 2013, Biodegradation 24: 413-425

The fraction of electron equivalents going to complete dechlorination is relatively small, which argues for adding less electron donor (rather than more) depending on the site

Table 1 Distribution of electron consumption in reductive dechlorination, Fe(III) reduction and methanogenesis in enrichment culture incubations with different starting acetate concentrations

TEAPs	A. TCE + Fe(III) + stoichiometric acetate		B. TCE + Fe(III) + 10× Acetate		C. VC + Fe(III) + stoichiometric acetate		D.VC + Fe(III) + 10× acetate	
	Distribution of e^- (%)	e^- use/total (%)	Distribution of e^- (%)	e^- use/total (%)	Distribution of e^- (%)	e^- use/total (%)	Distribution of e^- (%)	e^- use/total (%)
Dechlorination	7.01 (0.68)	3.53 (0.18)	4.71 (1.06)	0.38 (0.07)	1.64 (0.16)	0.88 (0.11)	0.86 (0.15)	0.08 (0.01)
Fe(III) reduction	86.77 (3.9)	44.04 (4.96)	58.84 (3.28)	4.85 (0.46)	89.79 (2.52)	48.04 (3.76)	57.42 (3.49)	5.17 (0.24)
Methanogenesis	6.22 (1.07)	3.20 (0.91)	36.45 (2.25)	2.99 (0.13)	8.57 (2.62)	4.54 (1.23)	41.72 (3.42)	3.77 (0.51)
e^- used/total e^- available		50.77		8.22		53.46		9.02

Results are presented as percent (%) of electron equivalents theoretically available from the complete oxidation of acetate. The values are the estimated fraction of electrons recovered in each terminal electron accepting process, with the remainder theoretically remaining as acetate

Wei and Finneran, 2013, Biodegradation 24: 413-425

TCE Contaminated Site South Carolina

Influence of electron donor concentration

The issue: site electron donor concentration was already high, but more electron donors were added

The approach: use clean water to dilute the electron donor concentration (also found massive NAPL plume during my remediation investigation that was missed for almost a decade!)

Upgradient edge:
TCE and cis-DCE (300mg/L)
High electron donor
Acetate 3-4mM
Dissolved Hydrogen 18nM
High molecular mass electron donors added in 2011
Complete dechlorination minimal to absent
Chlorinated solvents mixed with petroleum hydrocarbons

Other critical site data:
Mn(IV) high
Fe(III) high
pH controlled by Fe(III) reduction

~ 4 years GW travel time

Downgradient edge:
TCE, cis-DCE, and VC lower
Ethene present at 80% stoichiometry of VC, so complete dechlorination more active
Low electron donor
Acetate 100-200µM
Dissolved Hydrogen 1.8nM
Not influenced by 2011 electron donor amendment

Essentially, we need to make the upgradient area mimic the downgradient area, and to do so we are going to dilute the plume using de-aerated, recirculated water (if we need to… NAPL removal may lead to MNA)

We got to run some lab studies…

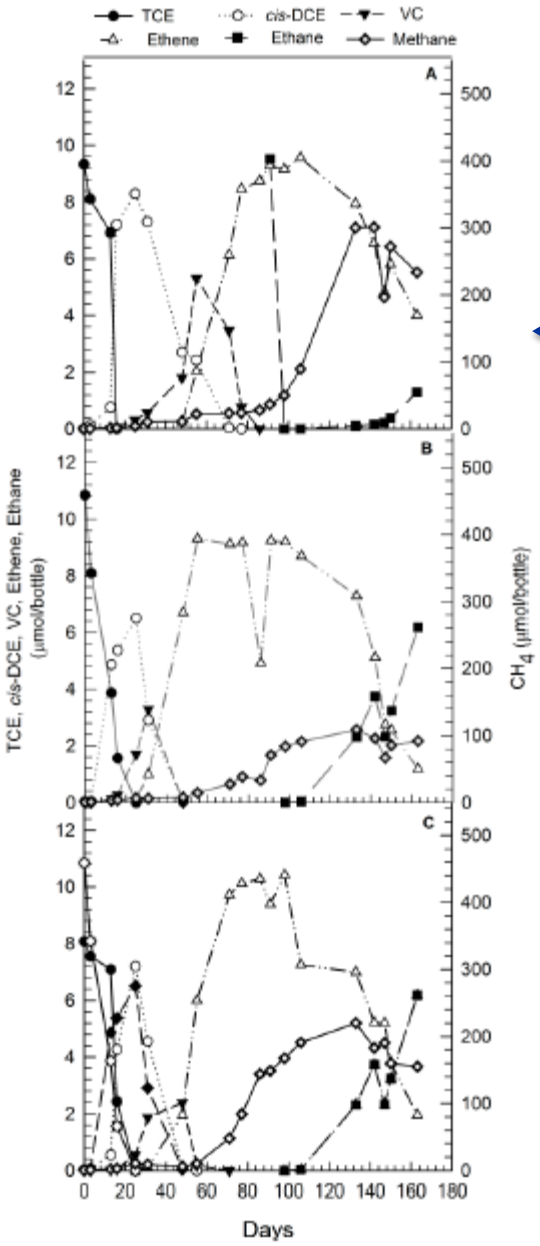
Streambed sediments were collected at the outflow of a TCE contaminated site (TCE, cis-DCE, and VC in the sediment)

Relatively high organic carbon content given that it is shallow sediment

Table 1: Experimental matrix for TCE in batch incubations of stream bed sediments

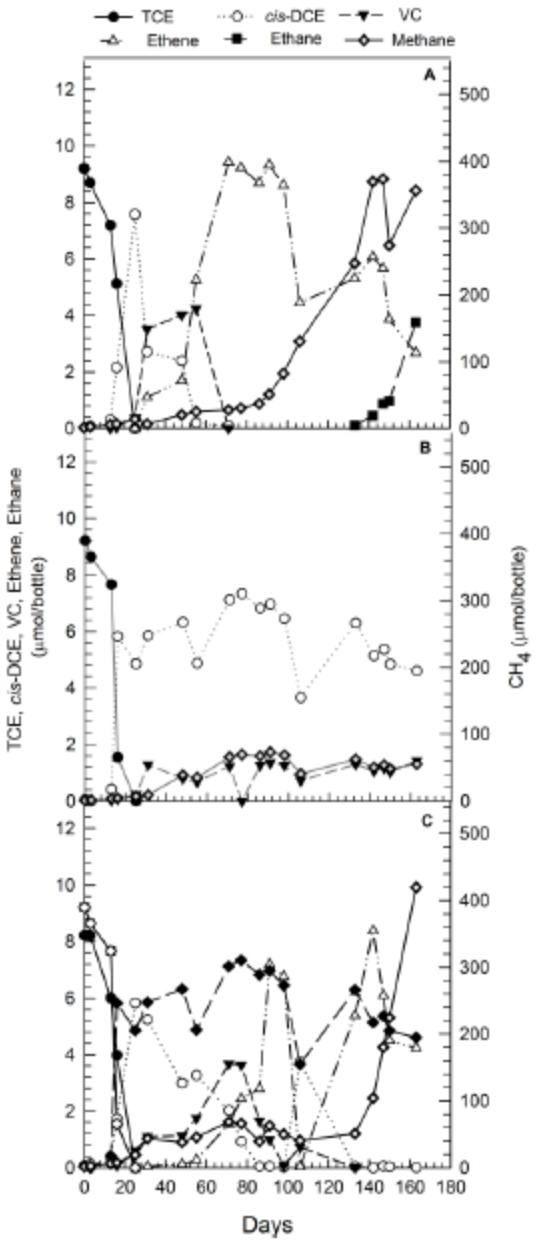
	TEA		Electron Donor		
Treatment	Amount TCE (µmol/bottle)	$V_{TCE,liq}$ (mL)	H_2 produced from Electron Donor (µmols)	Amount Substrate (mg)	Amount Substrate (µL)
Killed	10	1.23	0	0	0
No amend	10	1.23	0	0	0
1 x Acetate	10	1.23	30	0.45	50
5 x Acetate	10	1.23	150	2.25	250
10 x Acetate	10	1.23	300	4.5	500
1 x "RNAS Newman Zone"	10	1.23	30	1.15	23
5 x "RNAS Newman Zone"	10	1.23	150	5.73	115
10 x"RNAS Newman Zone"	10	1.23	300	11.45	229
1 x "EOS Concentrate 598B42"	10	1.23	30	0.24	25
5 x "EOS Concentrate 598B42"	10	1.23	150	1.21	126
10 x "EOS Concentrate 598B42"	10	1.23	300	2.42	252
1 x "CAP 18 ME"	10	1.23	30	0.59	13
5 x "CAP 18 ME"	10	1.23	150	2.94	64
10 x "CAP 18 ME"	10	1.23	300	5.88	128

Unamended versus acetate amended, TCE as the primary contaminant

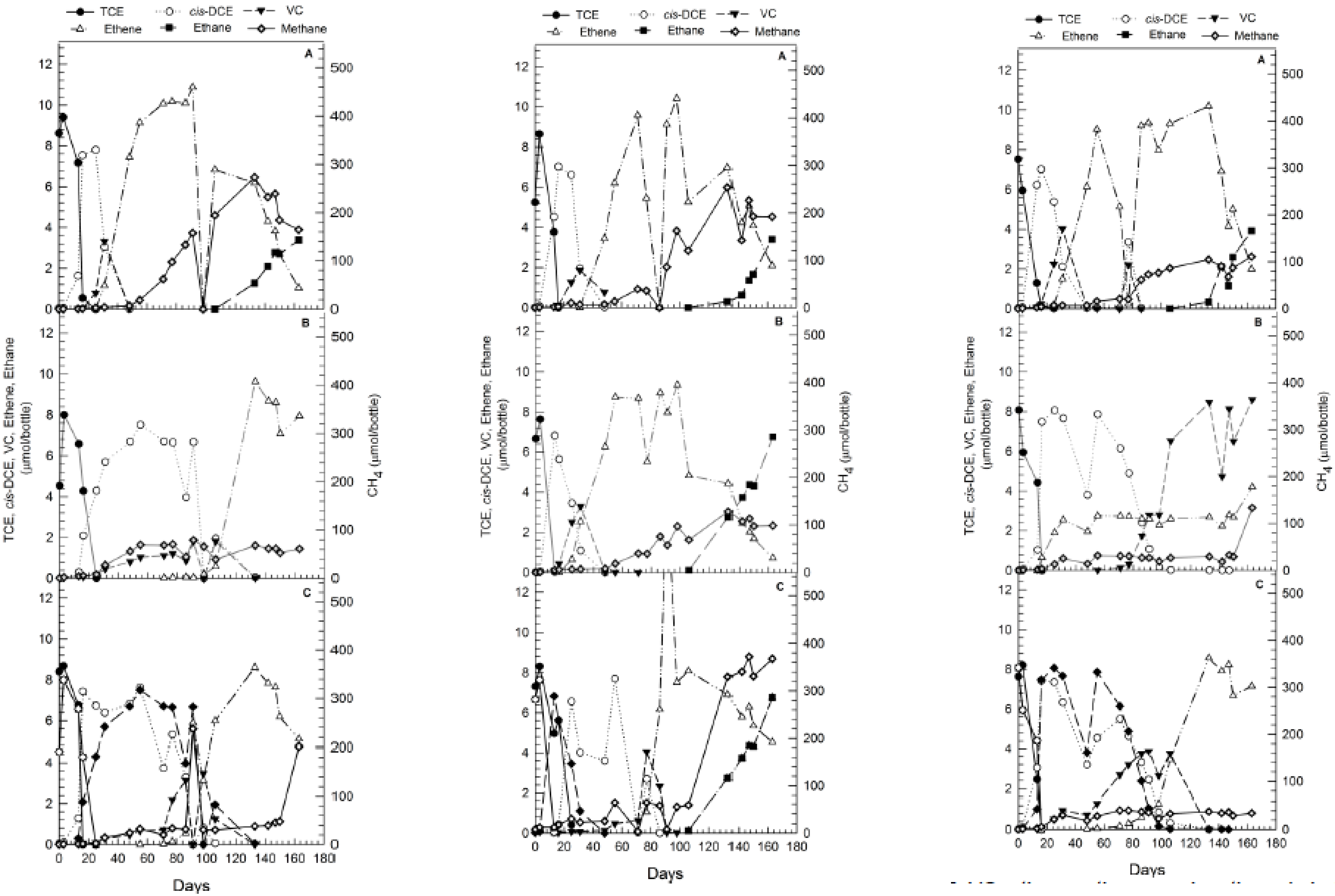


TCE was completely reduced without adding an electron donor; this was not an electron donor limited site

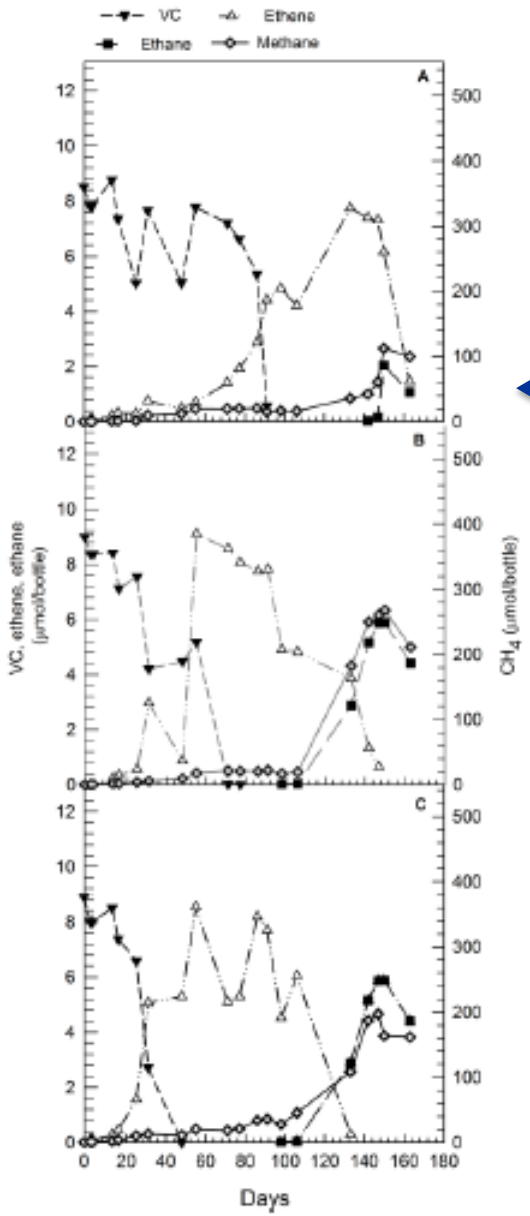
Even the low molecular mass compound acetate actually slowed the rate and extent of complete dechlorination; previous approaches at this site used high molecular mass vegetable oil based electron donors (***which was not even fermented***…!)



Newman Zone (left), EOS (middle), and CAP18 (right) amended (all at 5X-10X stoichiometry)
TCE as the primary contaminant

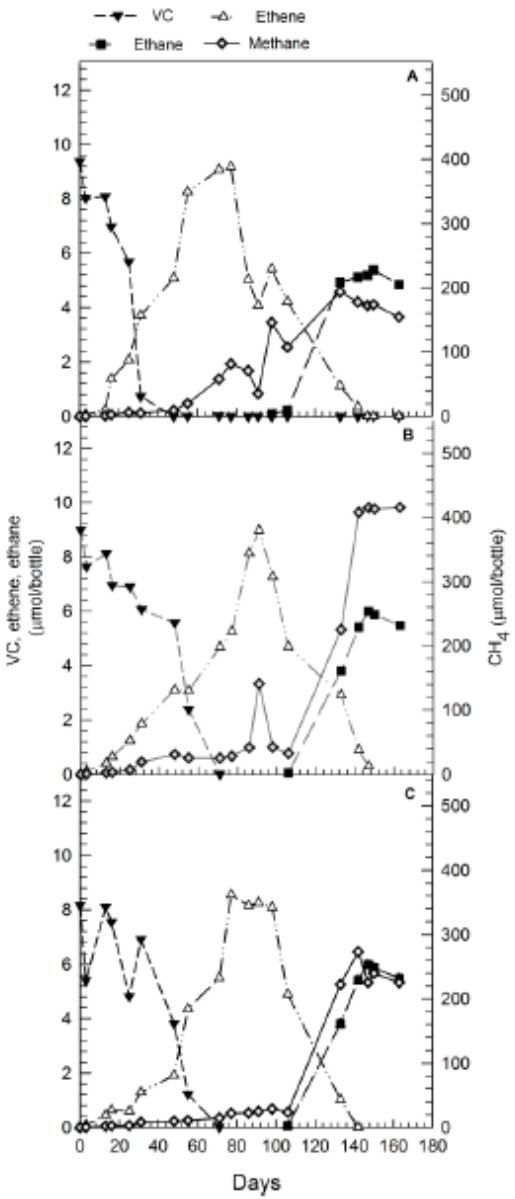


Unamended versus acetate amended, VC as the primary contaminant

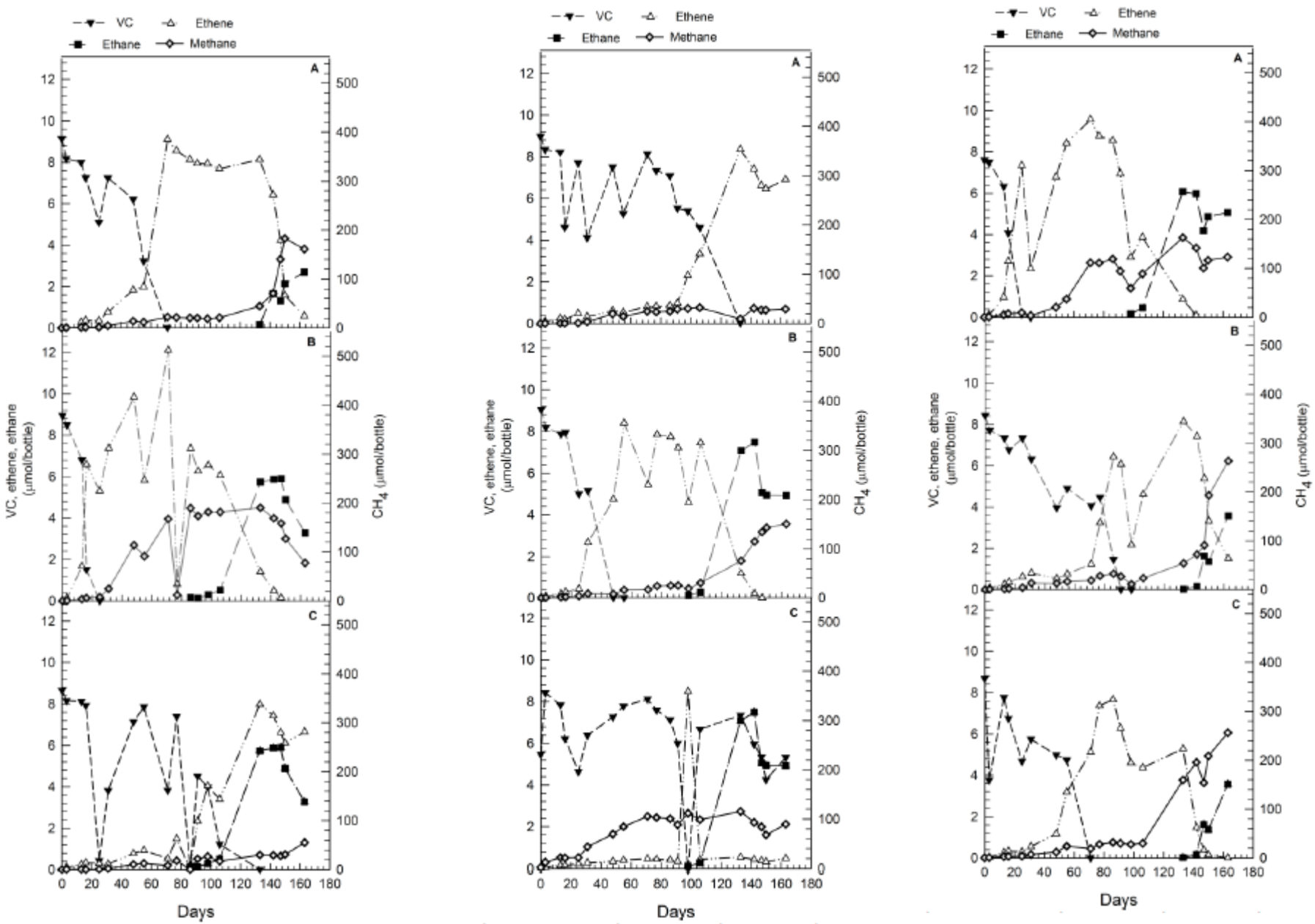


VC was completely reduced without electron donor; this was not an electron donor limited site

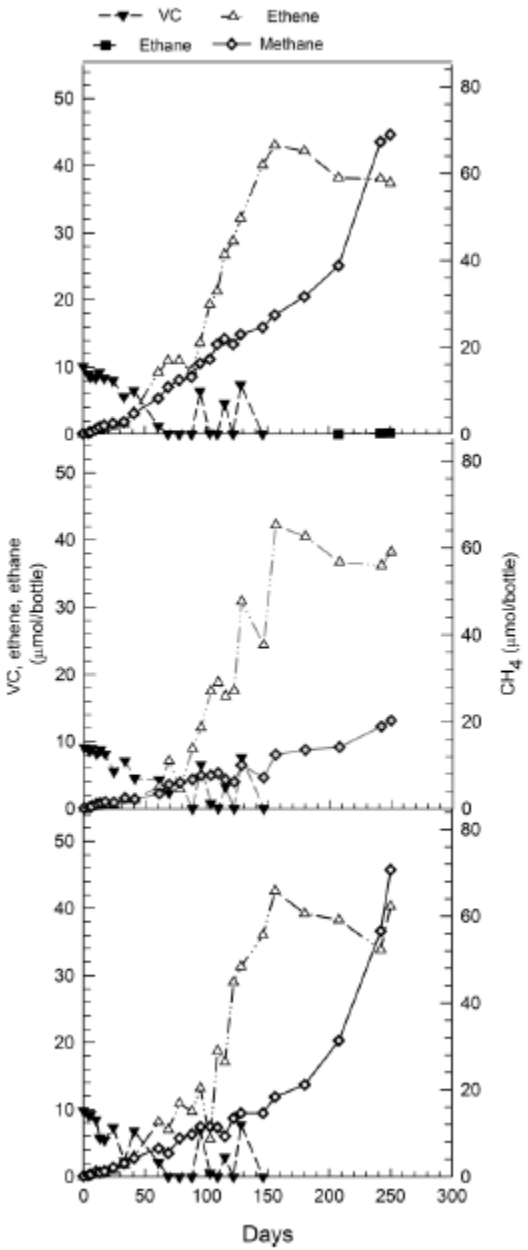
As with TCE, the low molecular mass compound acetate actually slowed the rate and extent of complete dechlorination; previous approaches at this site used high molecular mass vegetable oil based electron donors; methane was generated



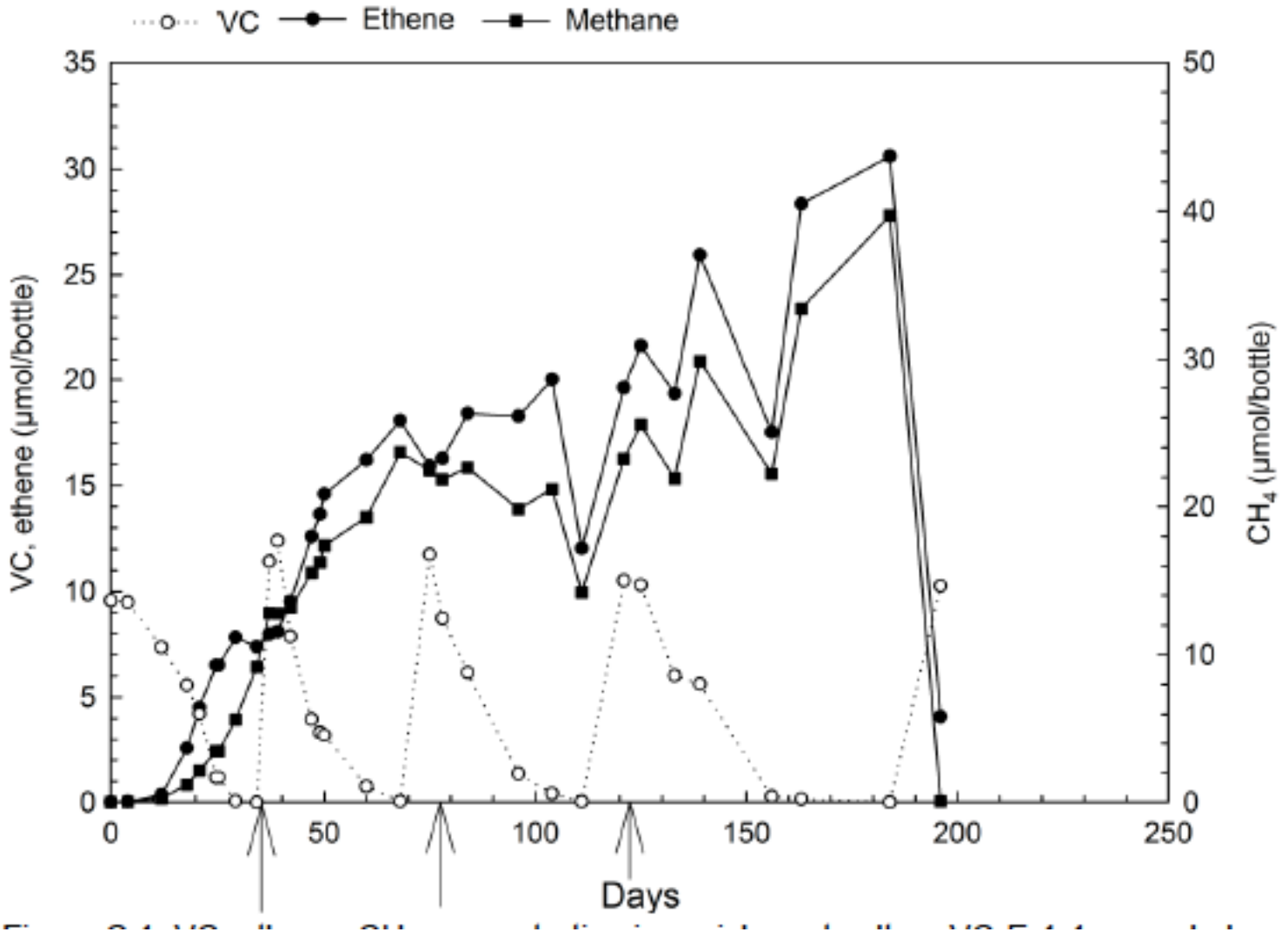
Newman Zone (left), EOS (middle), and CAP18 (right) amended (all at 5X-10X stoichiometry)
VC as the primary contaminant



Unamended with VC as the primary contaminant, stimulated Fe(III) reduction with 10mmol/g ferrihydrite and completely reduced VC to ethene



Enrichment culture developed: VC reducer with stimulated Fe(III) reduction,10mmol/g ferrihydrite



Conclusions

- It is clear that the actual biogeochemical processes tied to complete reductive dechlorination are poorly understood and more work is necessary to characterize these for basic sciences and remediation practices
- Adding low concentration electron donor (stoichiometric) actually increased the extent of complete dechlorination in aquifer material (this is very different than the standard accepted practice)
- Fe(III) was concurrently reduced with ethene production (this overturns years of speculation that it is a strictly competitive process)
- Fe(III), sulfate, and chlorinated ethenes can be simultaneously reduced under this “electron donor competitive” system
- Acetate is a good electron donor for complete dechlorination, but what is the mechanism?
  - H_2 from acetate or novel community?
  - Limited methane production (less carbon footprint to waste)
- <u>This has real implications for bioremediation</u> → lower donor cost and better kinetics/extent, less methane (i.e. more sustainable)

Thank You!

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