Metabolic Interactions Supporting Effective TCE Bioremediation under Biogeochemical Conditions

Grant 1R01ES024255-01

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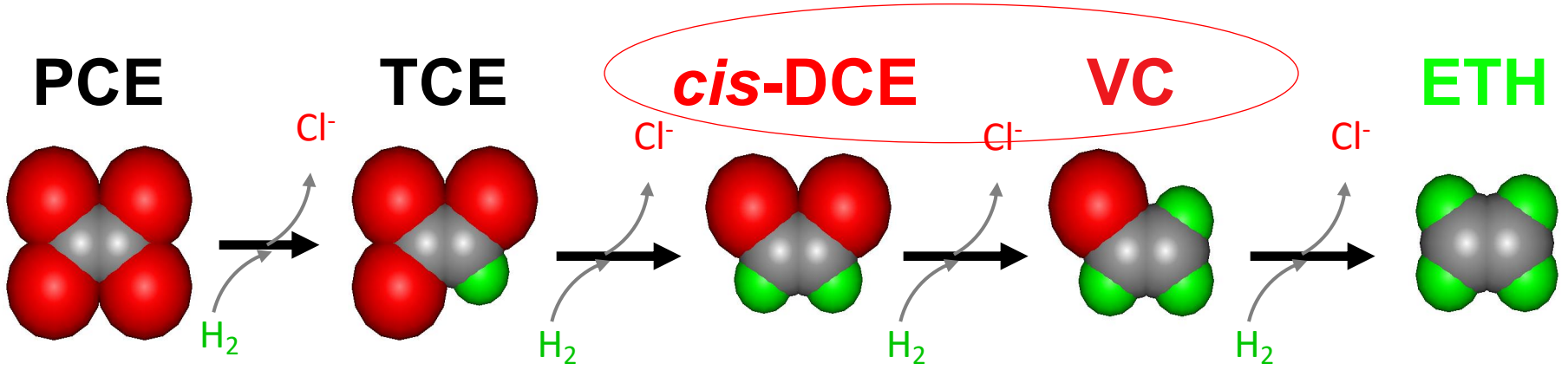
Berkeley
UNIVERSITY OF CALIFORNIA

Anaerobic Microbial Reductive Dechlorination

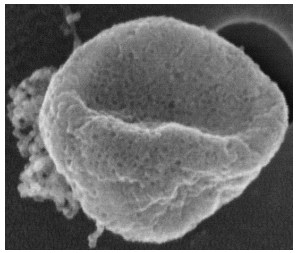


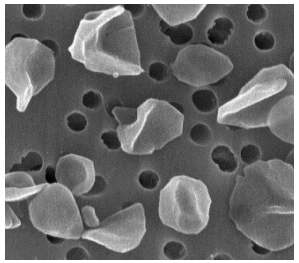
Clostridium, Dehalobacter, Dehalospirillum, Desulfitobacterium, Desulfomonile, Desulfuromonas, Sulfurospirillum, Geobacter, etc

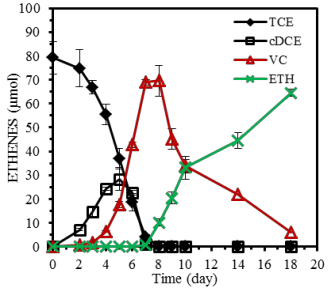
Partial dechlorination

Dehalococcoides mccartyi (Dhc)

Complete dechlorination

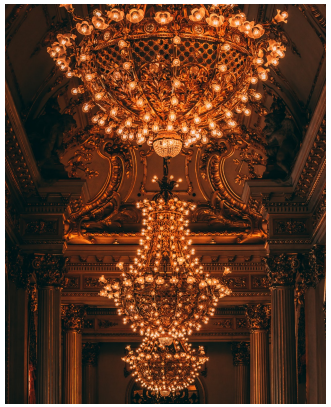




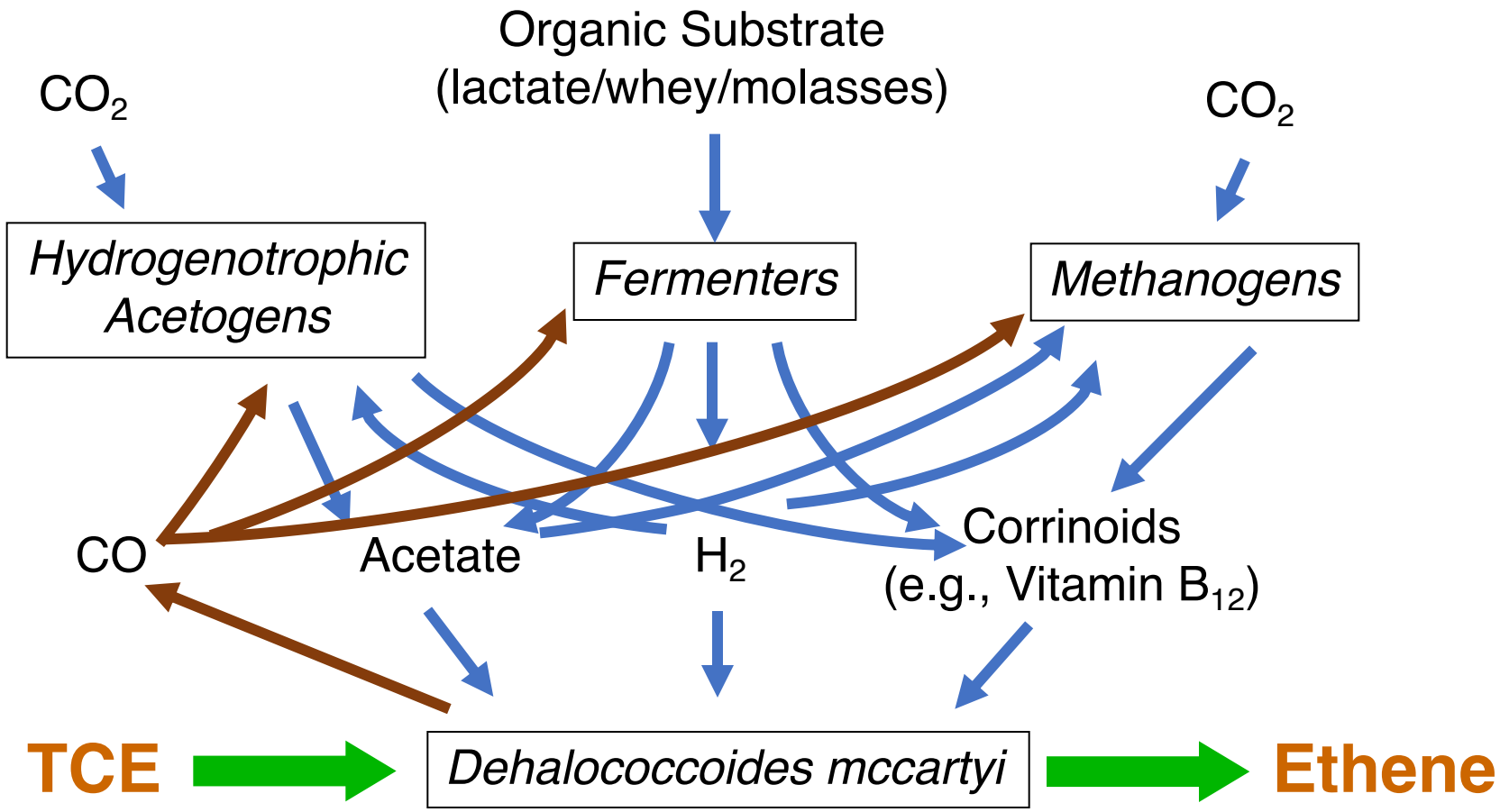


- Electron acceptors: *chlorinated ethenes*
- Electron donor: H_2
- Carbon source: *acetate*, CO_2
- Coenzymes: *corrinoids (vitaminB_{12})*
- Toxic waste: *CO*

Interactions in Dechlorinating Communities

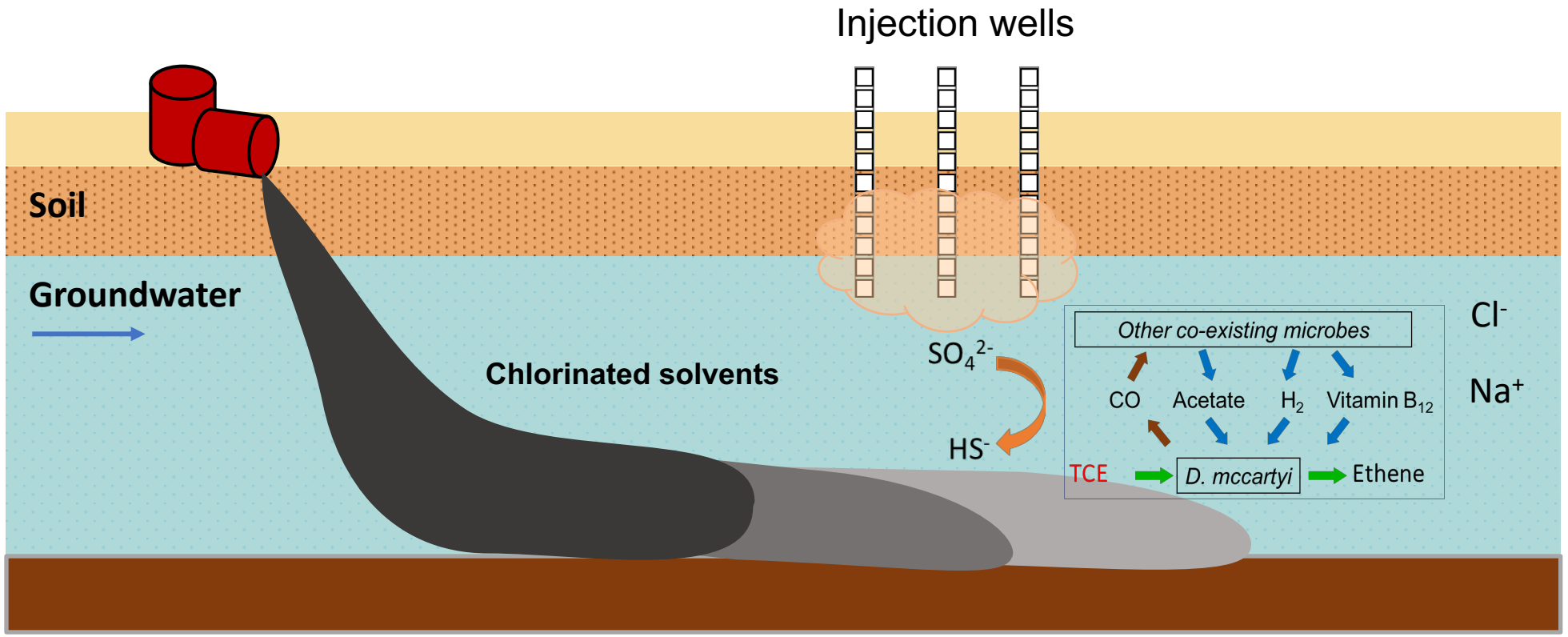


Dhc does not live alone in nature.



Geochemical Perturbations on TCE Bioremediation

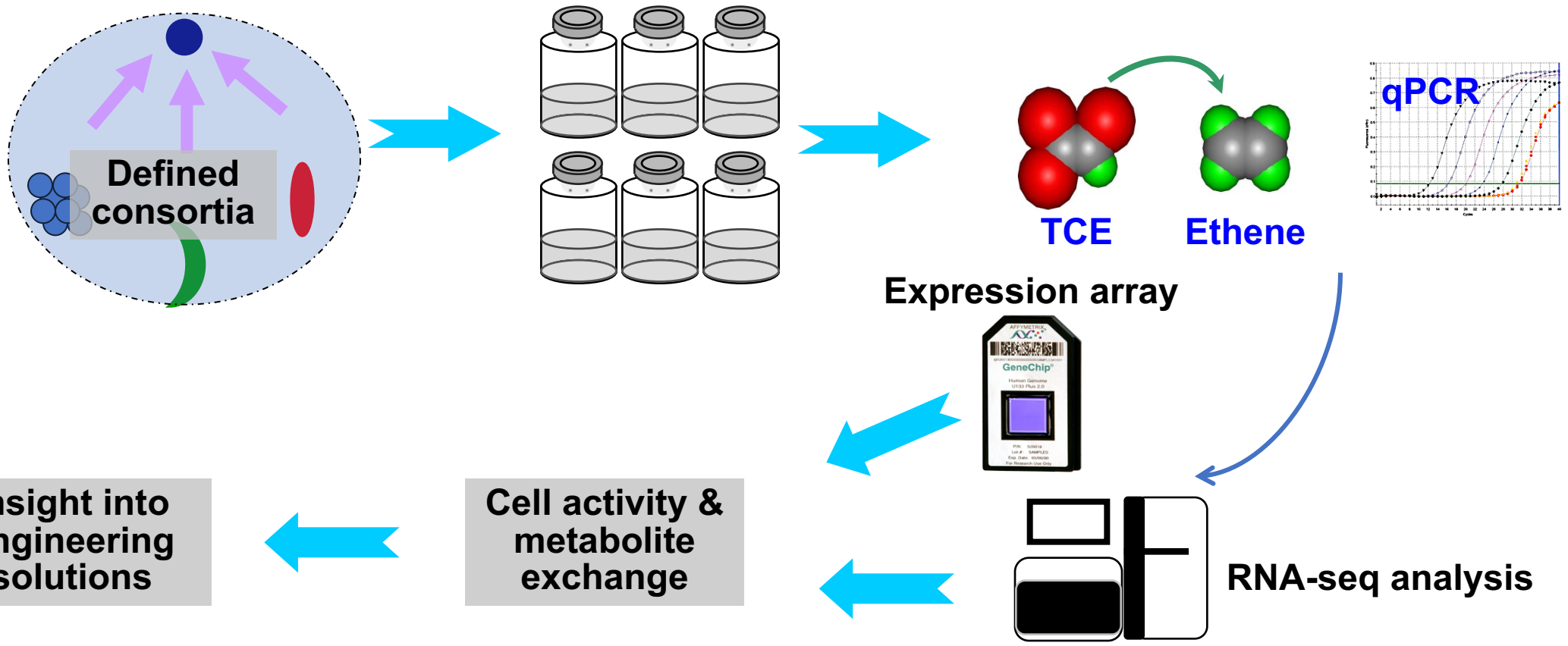
Important to determine how environmental conditions affect material exchanges in TCE-dechlorinating communities.



Technical Objectives and Approach

1) Construct defined consortia representing major interactions crucial to TCE-bioremediation

2) Investigate consortia performance in the presence of sulfate reduction or high salinity



4) Possible solutions to overcome the perturbation.

3) Apply either microarray or RNA-seq to elucidate the effects of perturbation on metabolism and functions of Dhc.

Effects of Sulfate Reduction on TCE-dechlorination

Sulfate Effects

- Sulfate is prevalent in groundwater.
- Sulfate-reducing bacteria often occur in the same niche with dechlorinating bacteria.

$$SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O$$

- Lack of consistent understanding of sulfate's effects on TCE dechlorination.
- Two testing hypotheses:
  - Inhibitory effects of sulfate or sulfide
  - Competition of electron donor (H_2)

Toxicity effects

Pure culture

Electron donor competition

Two scenarios: 1) electron acceptor limiting, 2) electron donor limiting

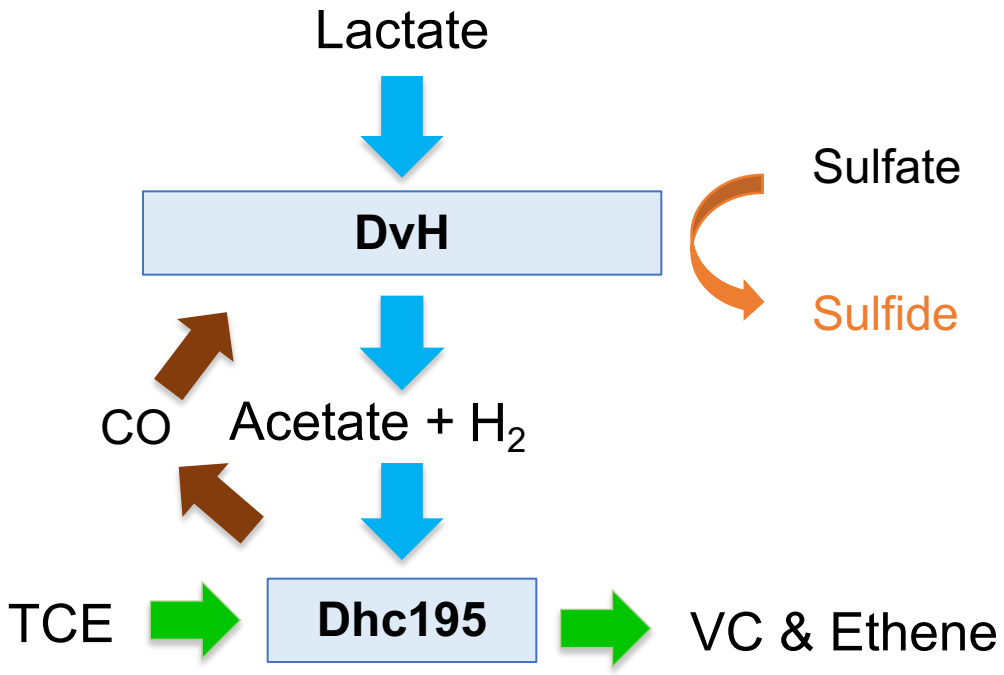
Consortia

Complex enrichment

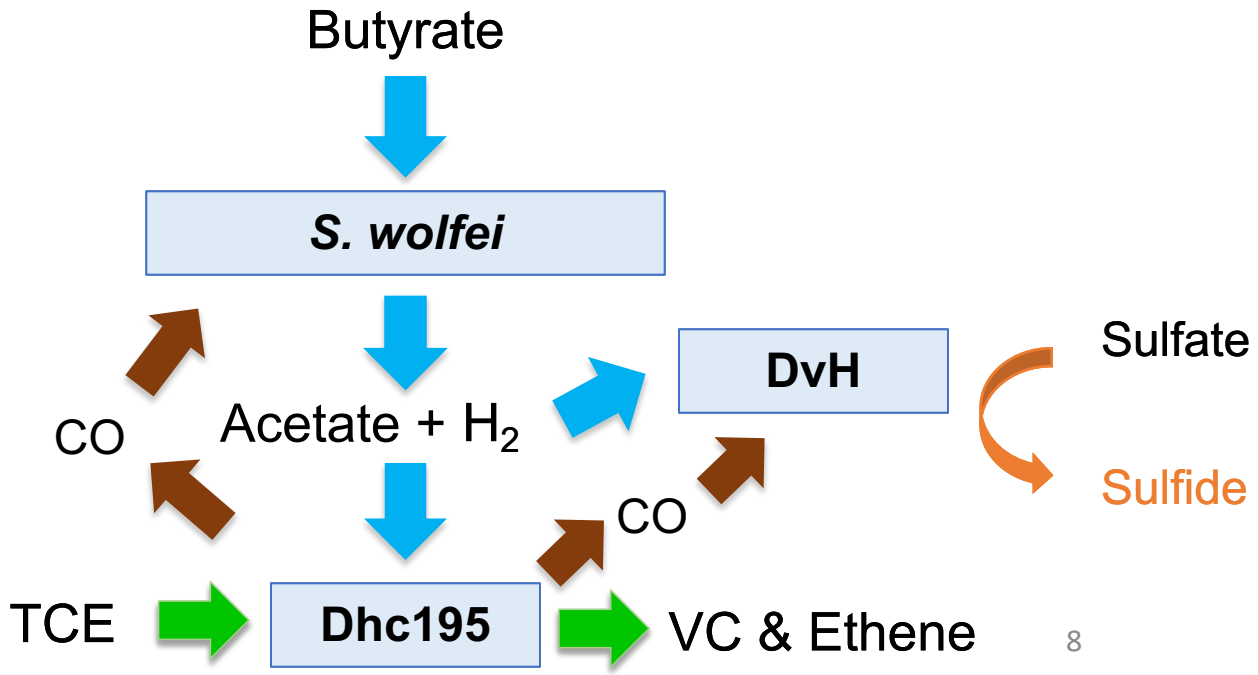
Two Types of Syntrophic Consortia

Bacterium	Function
Desulfovibrio vulgaris Hildenborough (DvH)	Fermentation, sulfate reduction
Syntrophomonas wolfei (S. wolfei)	Fermentation
Dehalococcoides mccartyi strain 195 (Dhc 195)	TCE dechlorination

Scenario 1: electron acceptor limiting



Scenario 2: electron donor limiting



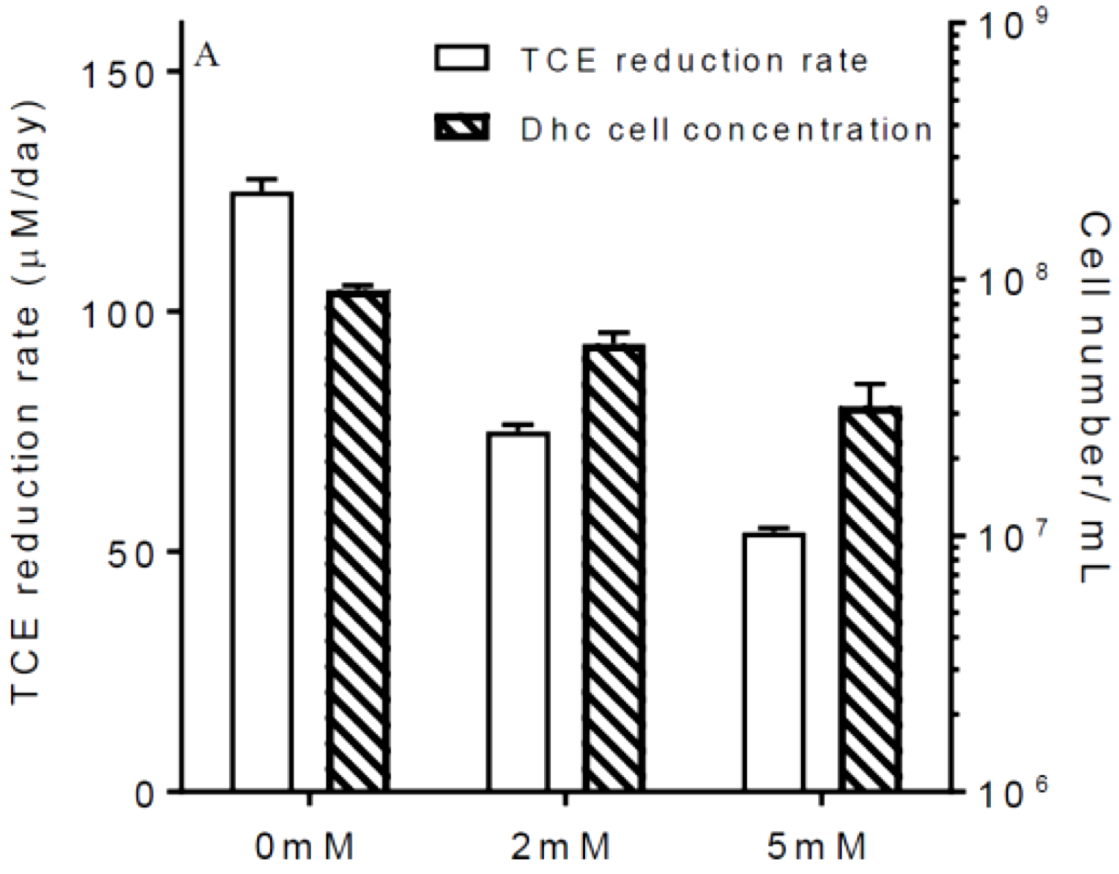
Inhibitory Effects on Syntrophic Consortia Members

$$SO_4^{2-} \rightarrow HS^-$$

Axenic cultures	Function	Sulfate		Sulfide	
Dhc 195	TCE dechlorination	5 mM	No effects	5 mM	Decreased yield by 65%
S. wolfei	Fermentation	5 mM	No effects	5 mM	Decreased yield by 40%
DvH	Fermentation, sulfate reduction	N/A		>10 mM	Cell growth inhibited

Mao et al. Appl. Environ. Microbiol. 2017

Sulfide Inhibition on Dhc195

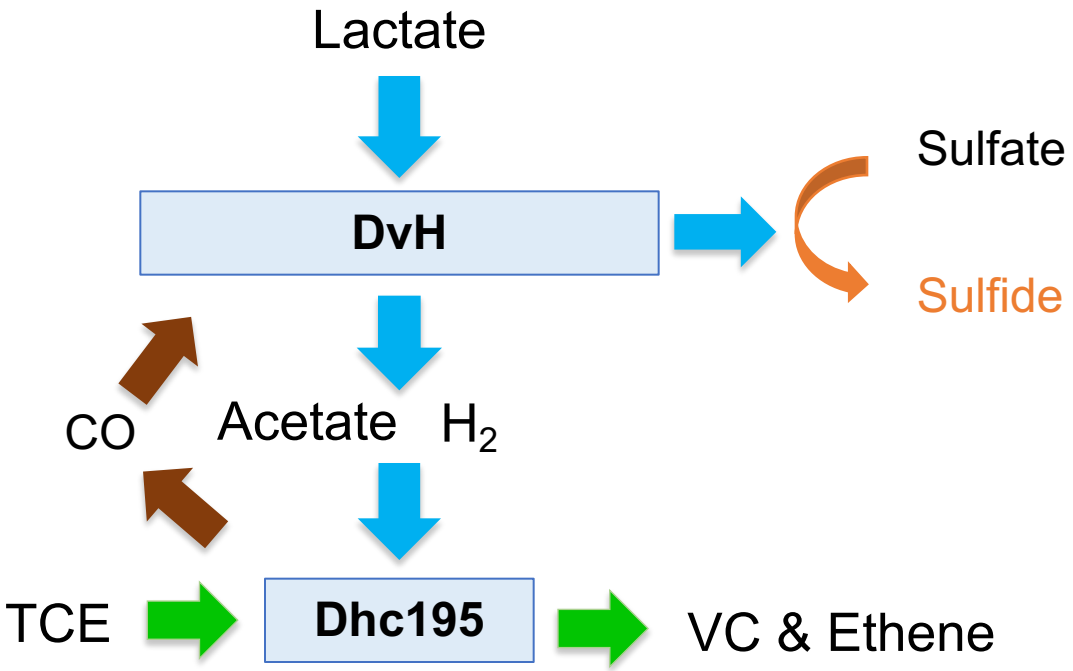


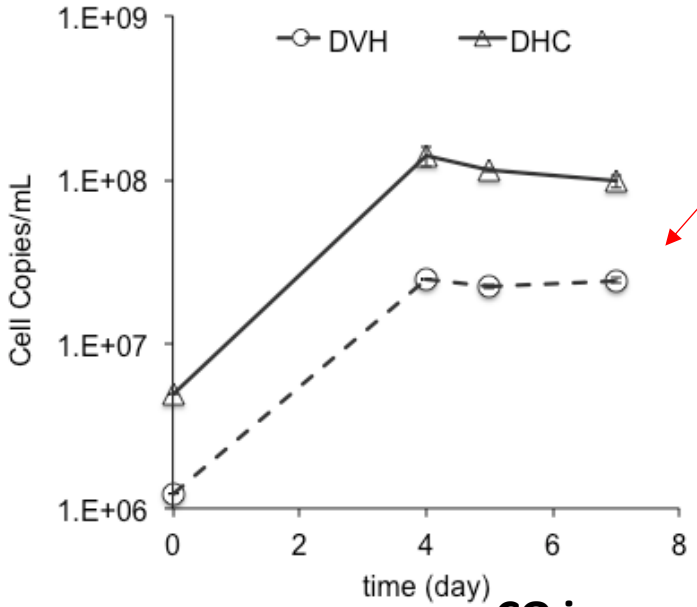
- Decreased TCE dechlorination rates.
- Decoupled growth from dechlorination when sulfide was introduced.
- Transcriptomic analysis using microarray indicates the gene expression changes in ATP synthase, biosynthesis, and metal-containing enzymes.

Mao et al. Appl. Environ. Microbiol. 2017

Effects of Sulfate Reduction on TCE-dechlorination

Scenario 1: electron acceptor limiting

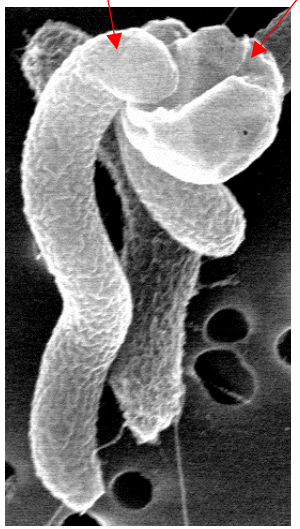


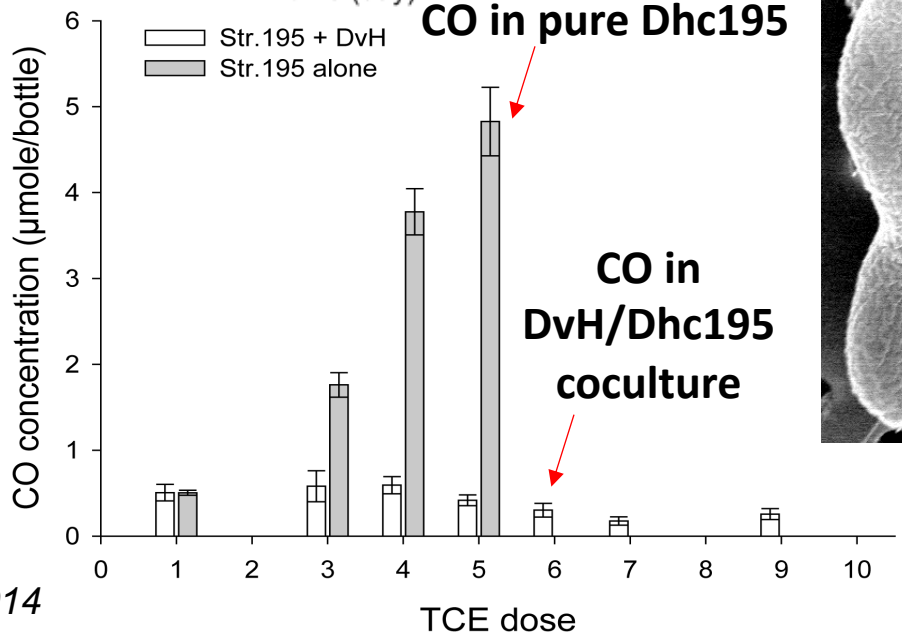


Dhc195:DvH: ~5:1

DvH

Dhc195



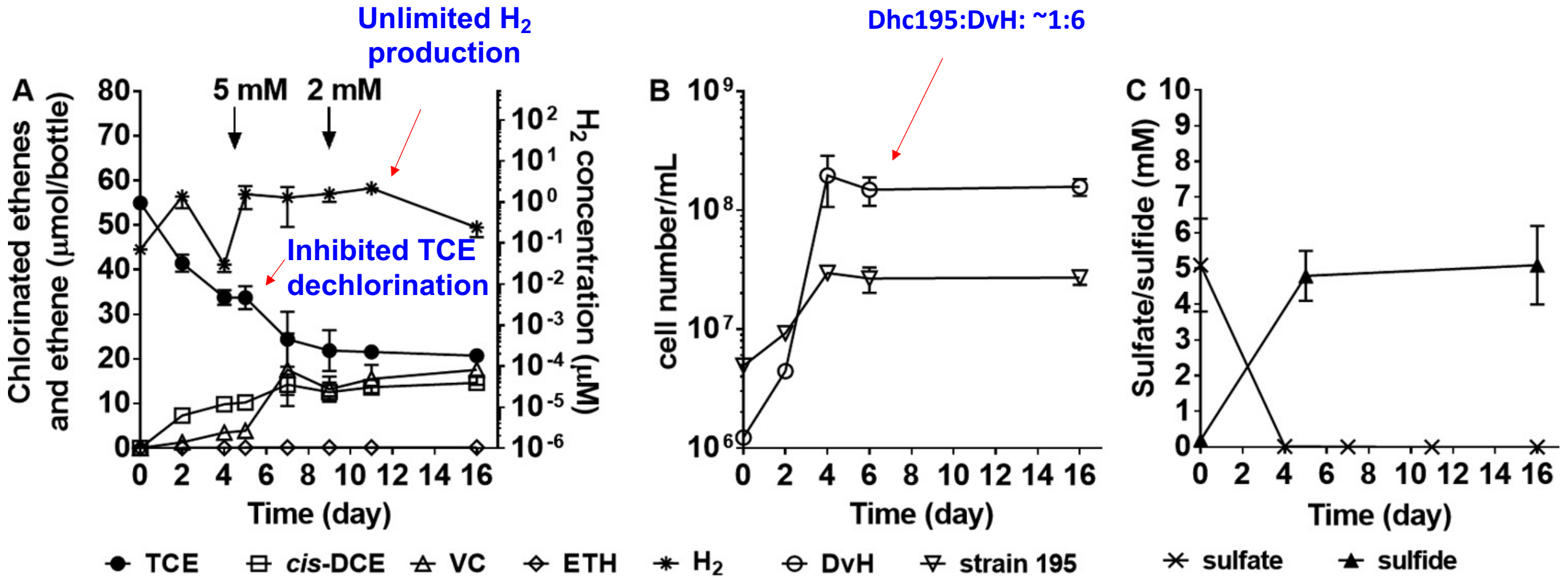


CO in pure Dhc195

CO in DvH/Dhc195 coculture

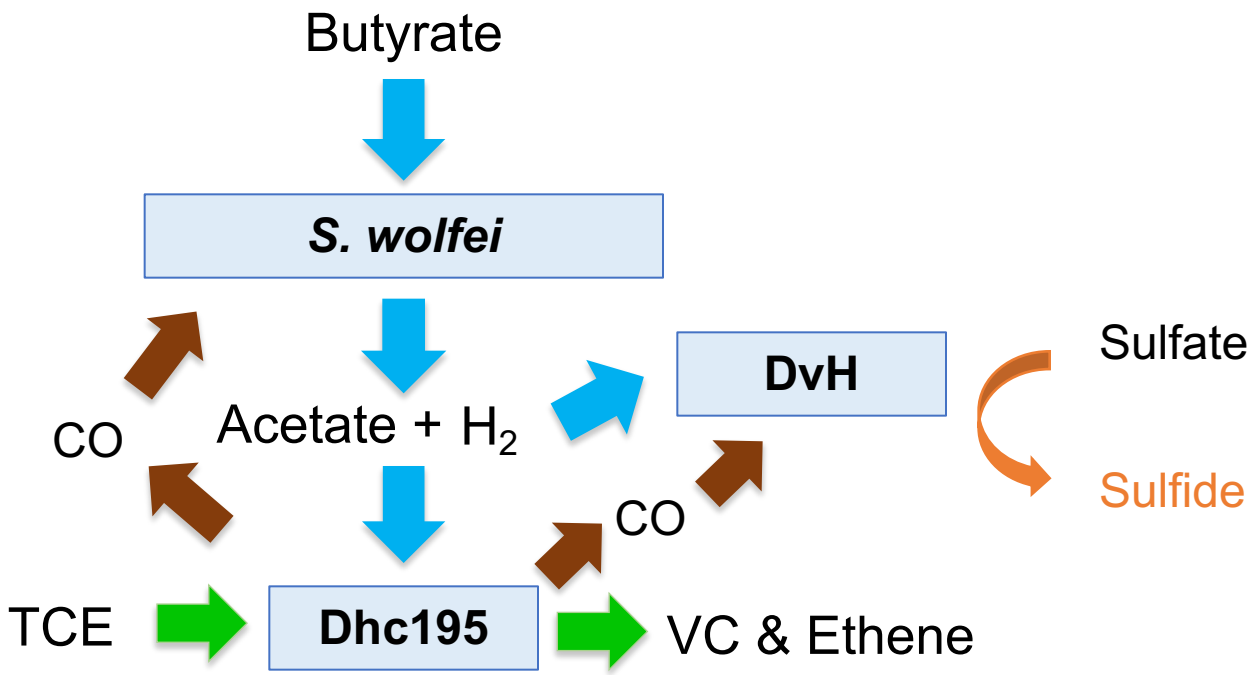
Men et al., ISME J, 2012 , Polasko et al., AGU,2014, Zhuang et al., PNAS 2014

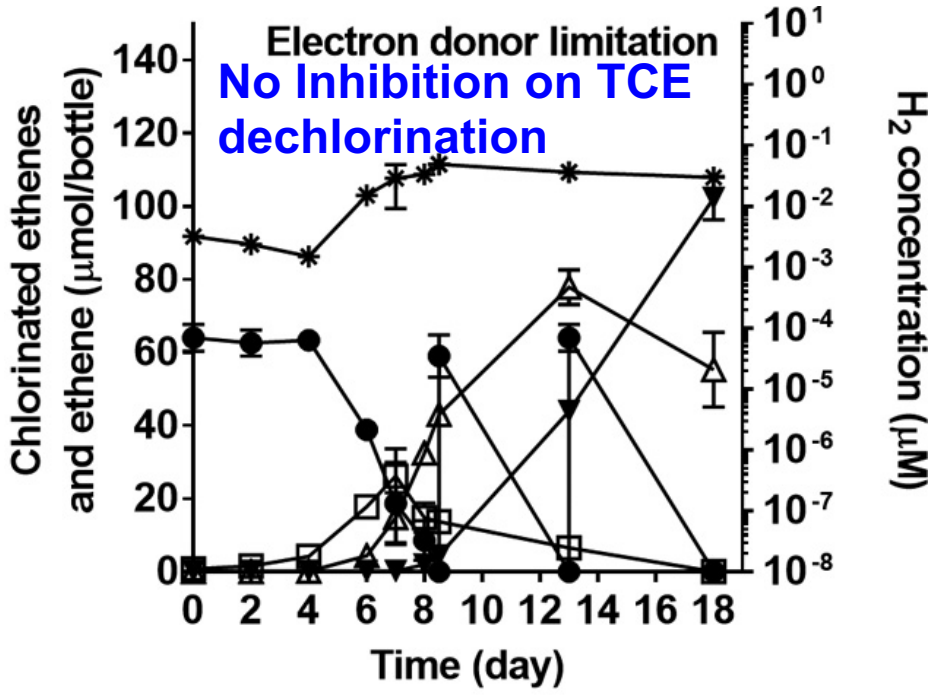
Co-Culture DvH/Dhc195 under Electron Acceptor Limitation

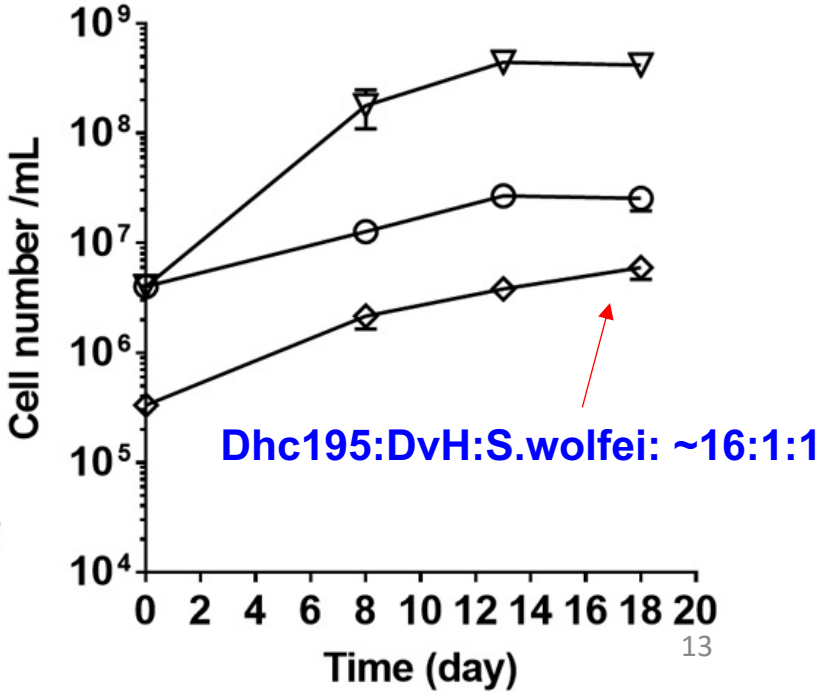


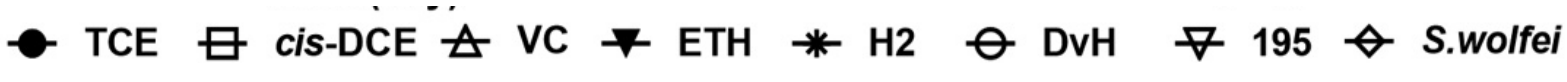
Mao et al. Appl. Environ. Microbiol. 2017

Tri-Culture *S. wolfei*/DvH/Dhc195 under Electron Donor Limitation



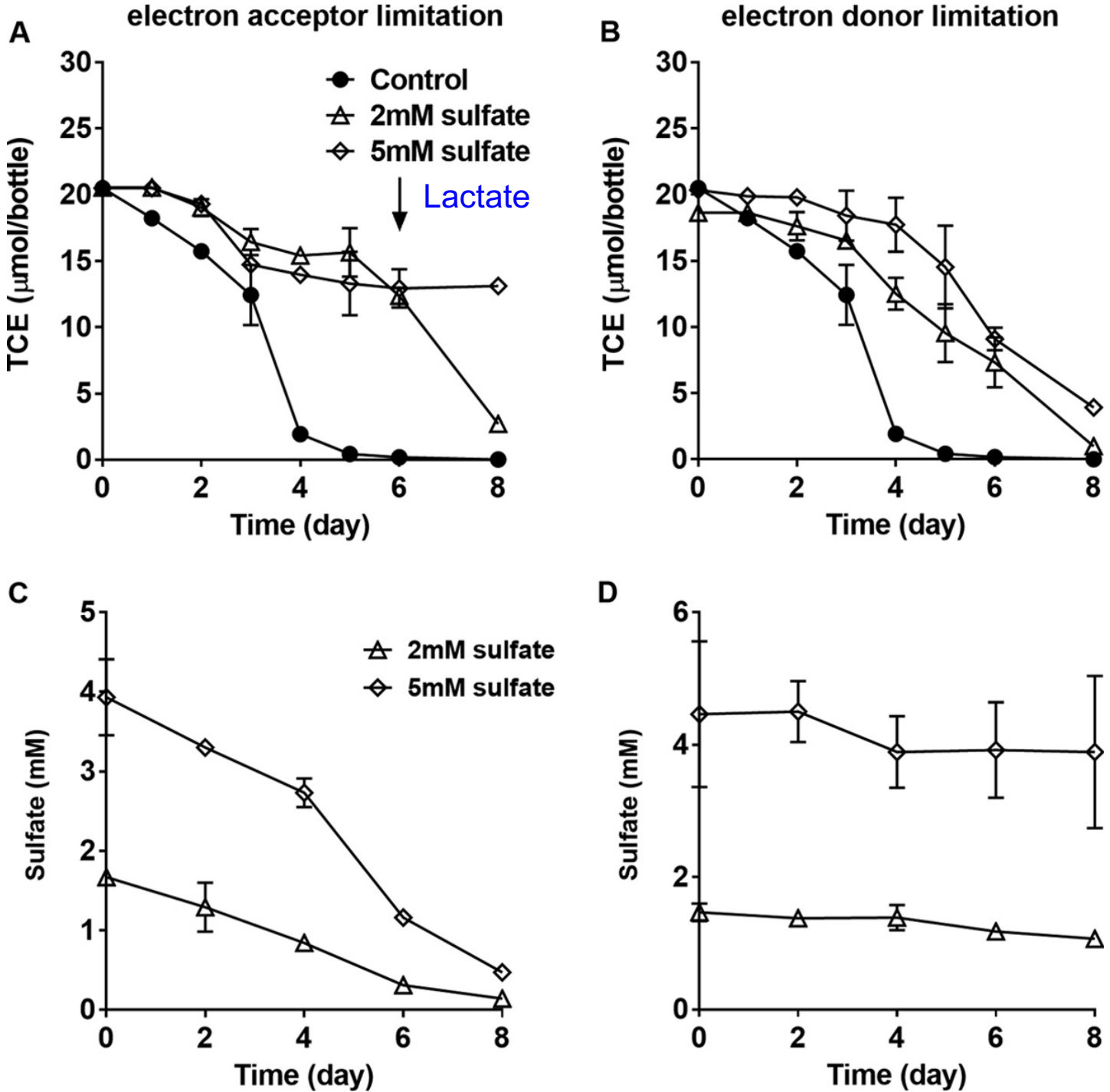






Mao et al. Appl. Environ. Microbiol. 2017

Effects of Sulfate Reduction on TCE-Dechlorinating Enrichment Culture



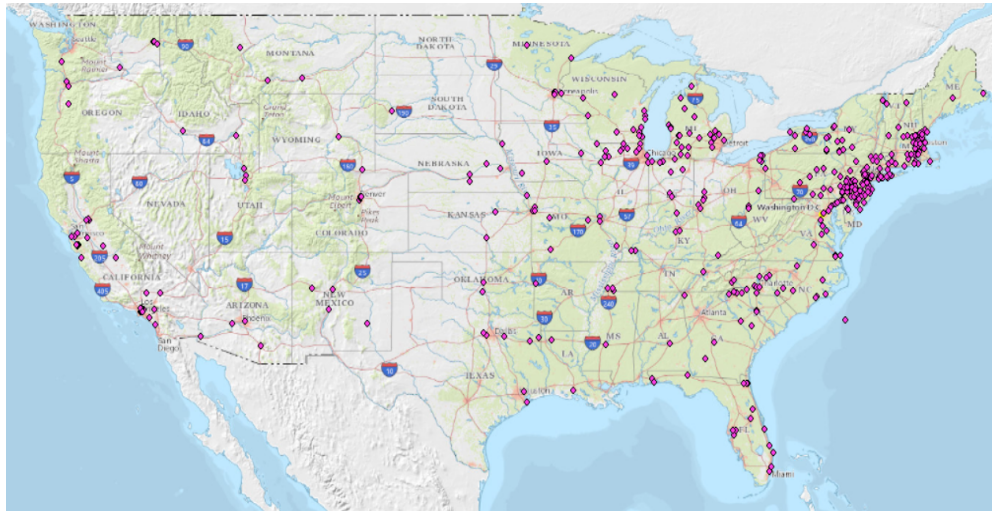
- Enrichment culture showed similar inhibitory patterns as the defined consortia under the two limitation conditions.
- Methane production occurred in the control culture but not in sulfate amended groups due to low H_2 concentration.

Mao et al. Appl. Environ. Microbiol. 2017

Effects of Salinity on TCE-dechlorination

Salinity Effects on TCE bioremediation

- TCE is present at 389 National Priorities List (NPL) sites, many of which are along the coast.
- Effects of salinity on TCE bioremediation are unknown.
- Two testing hypotheses:
  - ➢ Salt stress at the cellular level of Dhc
  - ➢ Salt stress on the metabolic interactions



Bacterium	Function
DvH	Fermentation
Pelosinus fermentans R7 (**PfR7**)	Fermentation, corrinoid production
Dhc 195	TCE dechlorination

Inhibitory effects

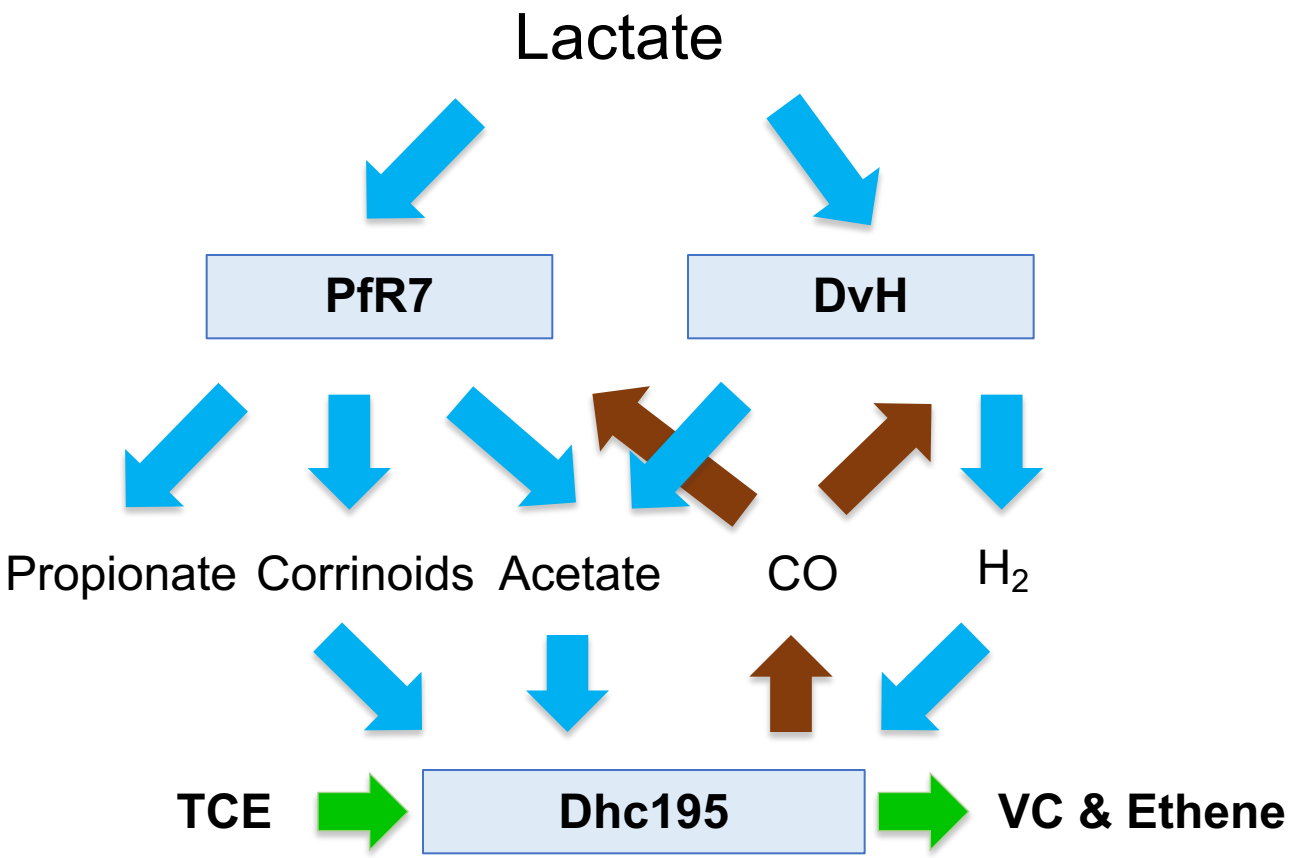
Pure culture → Consortia

Salinity stress

Two scenarios:

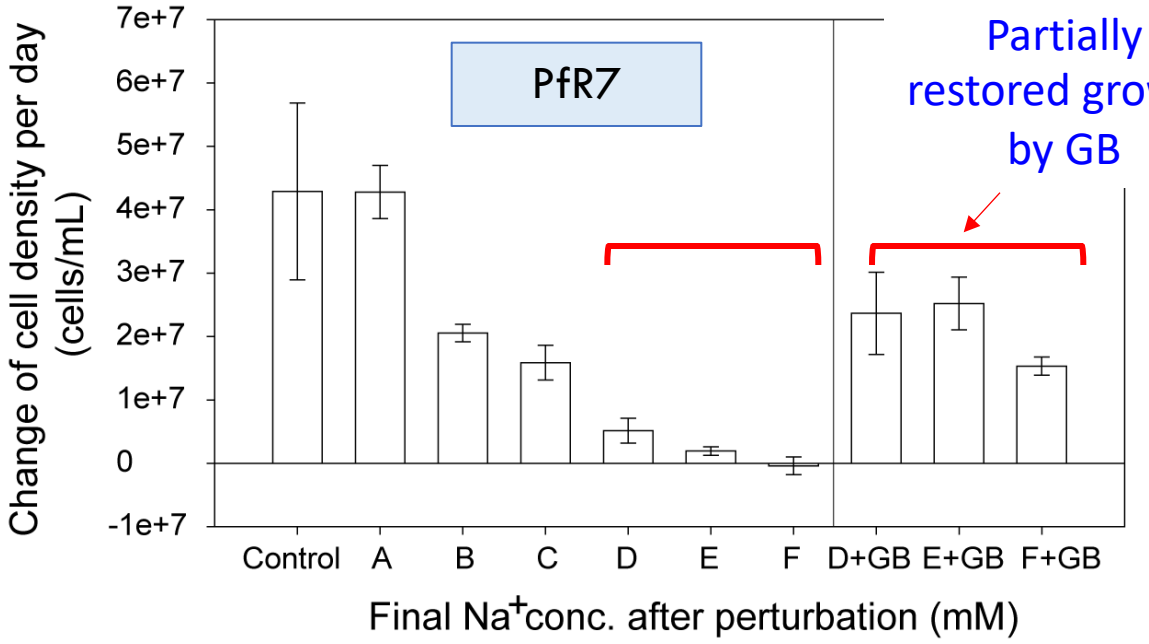
1) Existing salinity in groundwater
2) Salinity perturbation

Tri-Culture of PfR7/DvH/Dhc195 under Salt Stress

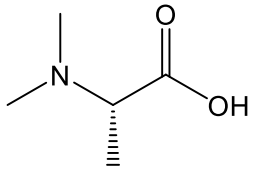


Salt Stress on Consortium Members

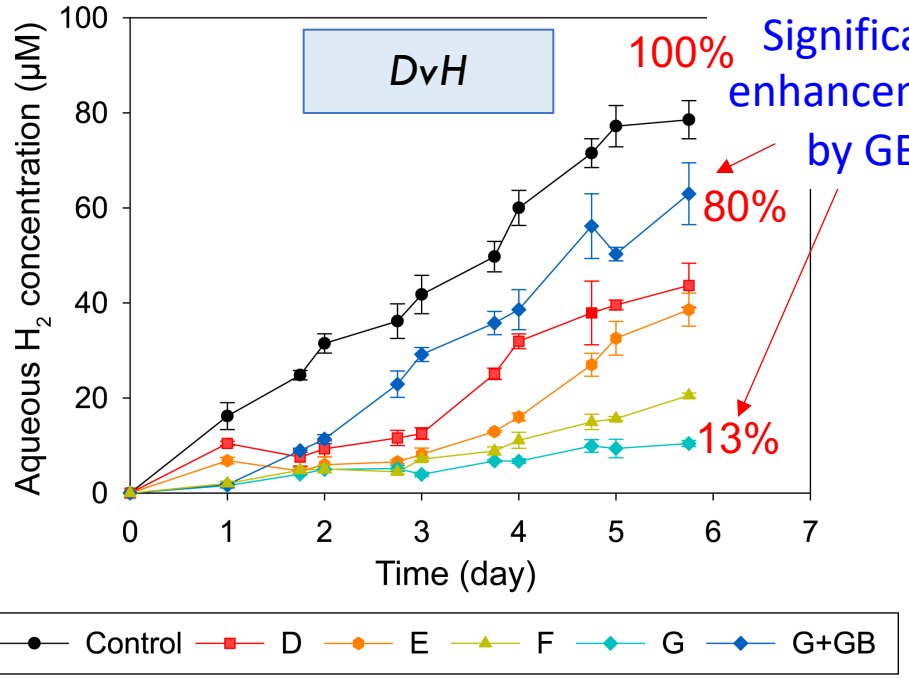
Group	Control	A	B	C	D	E	F	G	H	I	J
Na^+ conc. after perturbation (mM)	50	183	227	271	315	359	404	448	492	536	580
Limiting factor for TCE dechlorination	N/A		PfR7 Limiting		PfR7 & DvH Limiting			Dhc195 Limiting			



Partially restored growth by GB



Glycine betaine (GB) 2 mM



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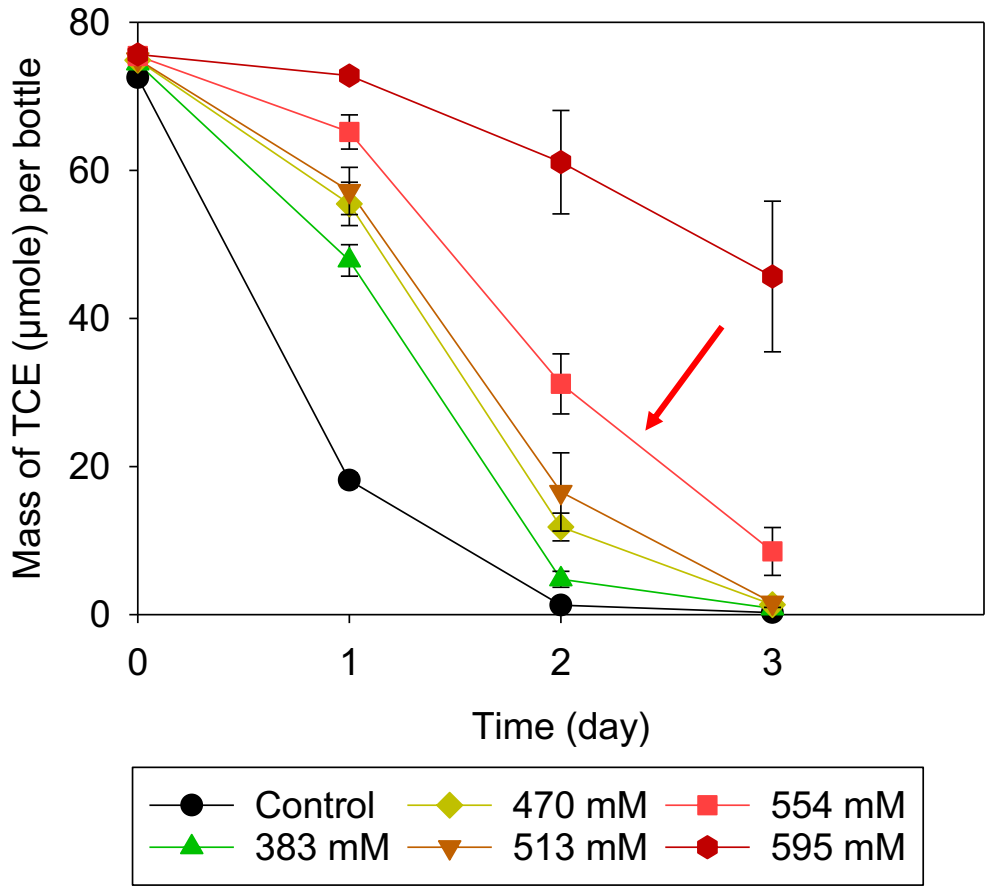
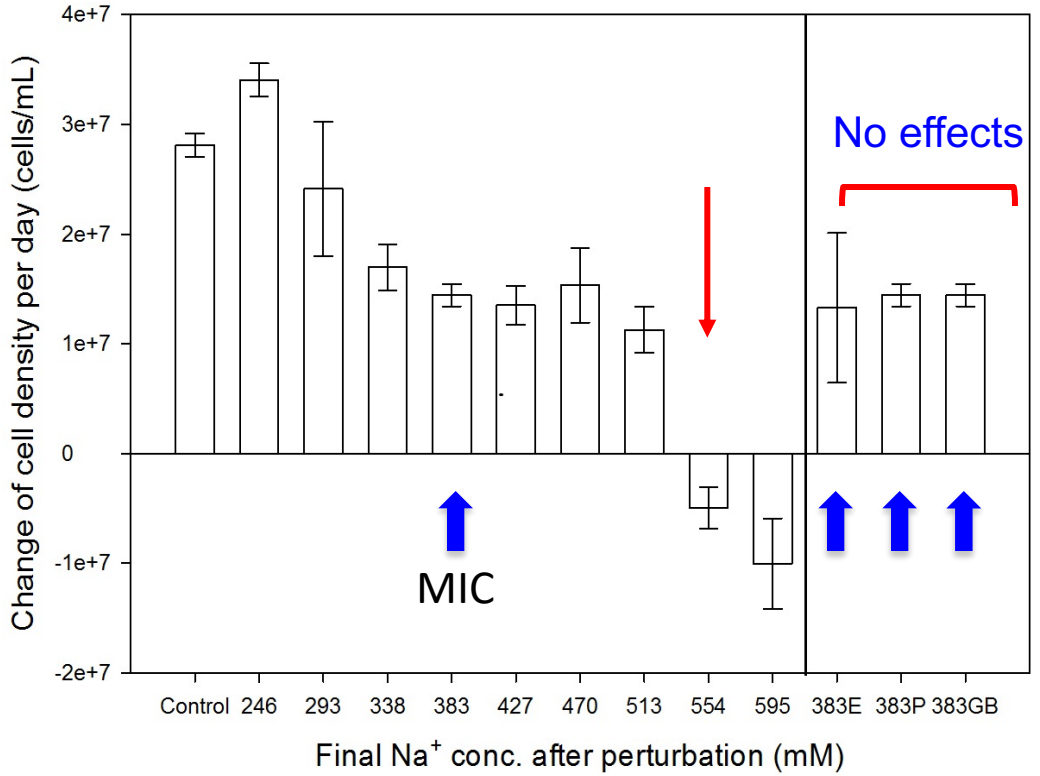
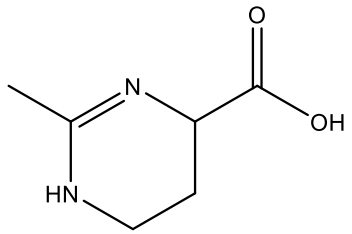
Overall Salt Stress Response of Dhc195 Pure Culture



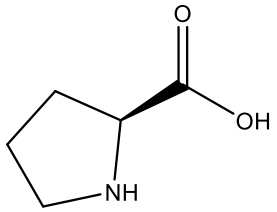


MIC = Minimum inhibitory concentration

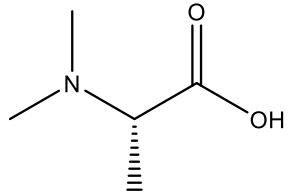
stressor concentration that decreases the overall yield by 50%



Ectoine



Proline



Glycine betaine

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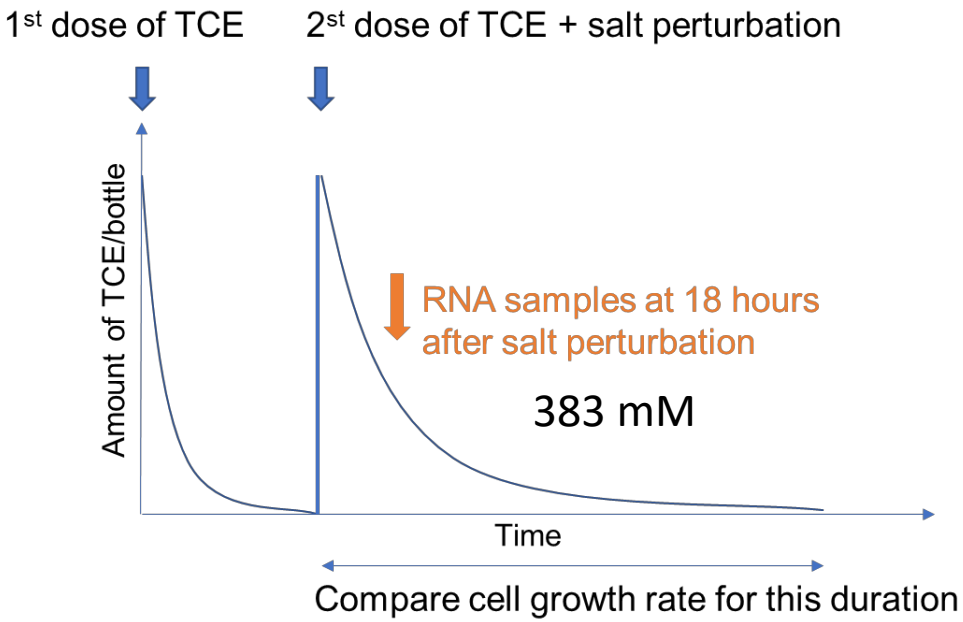
Transcriptional Responses of Dhc195 Pure Culture under Salinity Perturbation



Schematic diagram for Dhc195 pure culture salt perturbation experiment

- Biosynthesis:
  - Acetyl-CoA synthesis
  - Pyruvate synthesis
  - Glutamate/glutamine biosynthesis
  - DNA/RNA synthesis
  - Riboflavin metabolism
  - tRNA synthetase
- Energy metabolism:
  - NADH dehydrogenases
  - ATP synthases
  - ABC transporters

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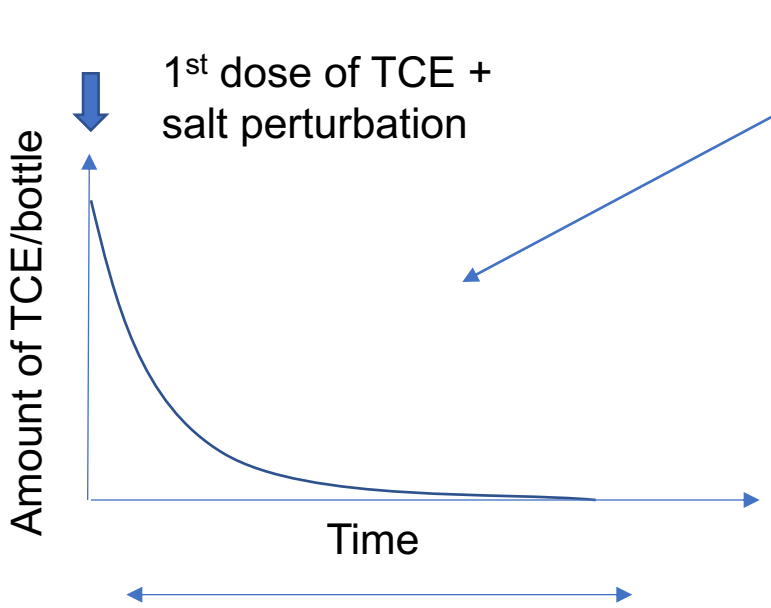
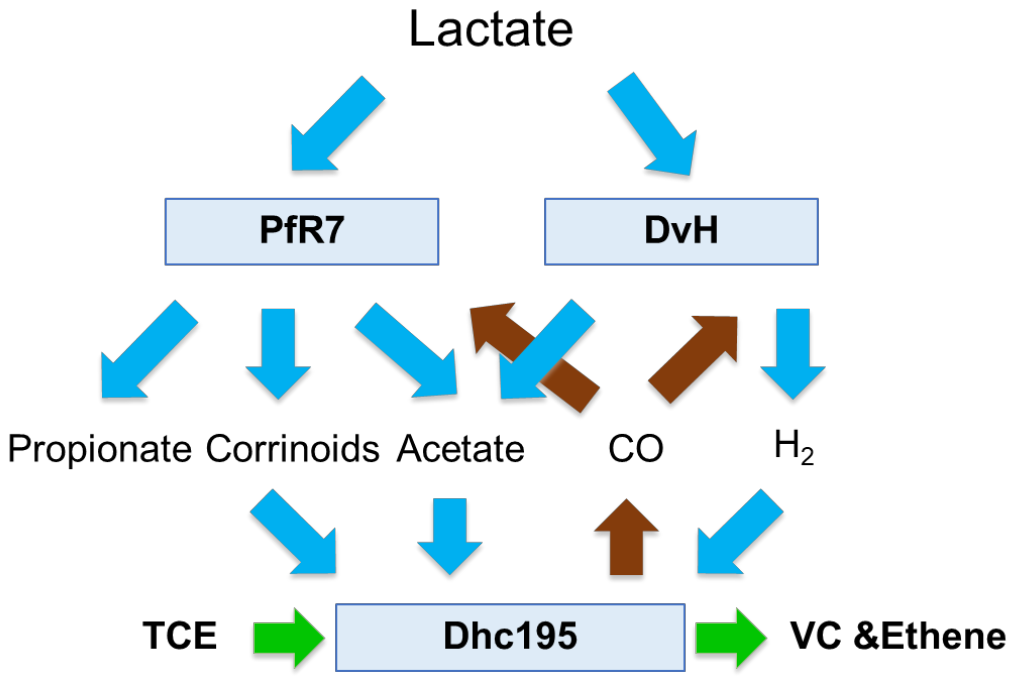
Effects on Metabolic Interactions under Salt Stress (I)

Group	Control	A	B	C	D	E	F	G	H	I	J
Na^+ conc. after perturbation (mM)	50	**183**	**227**	**271**	**315**	359	404	448	492	536	580
Limiting factor for TCE dechlorination	N/A		PfR7 Limiting		PfR7 & DvH Limiting			Dhc195 Limiting			



Compare dechlorination kinetics & cell growth during this period



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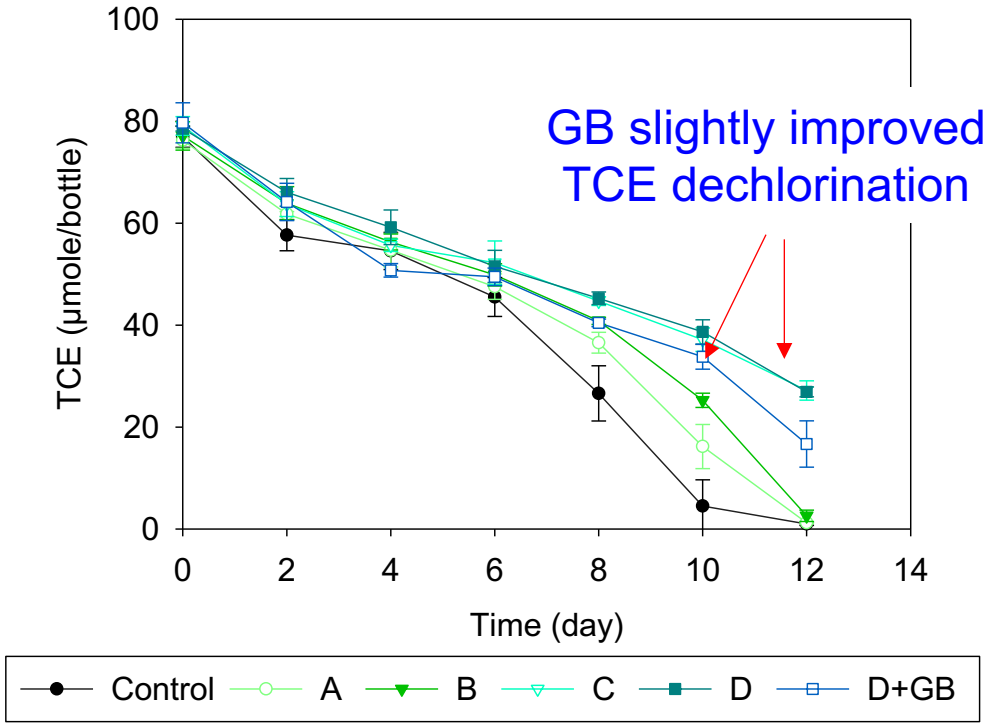

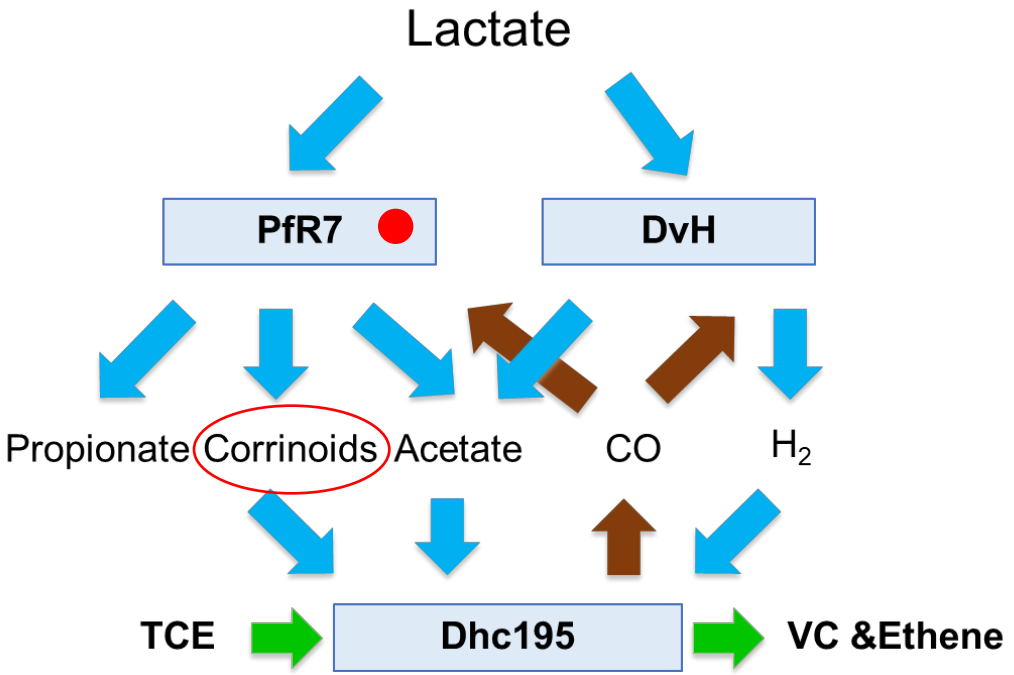

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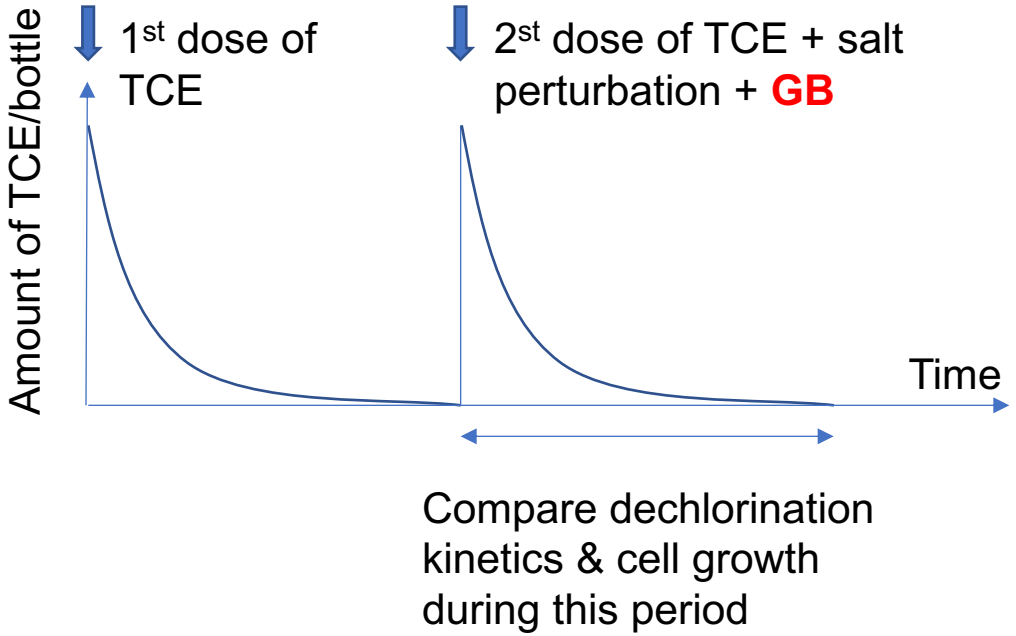
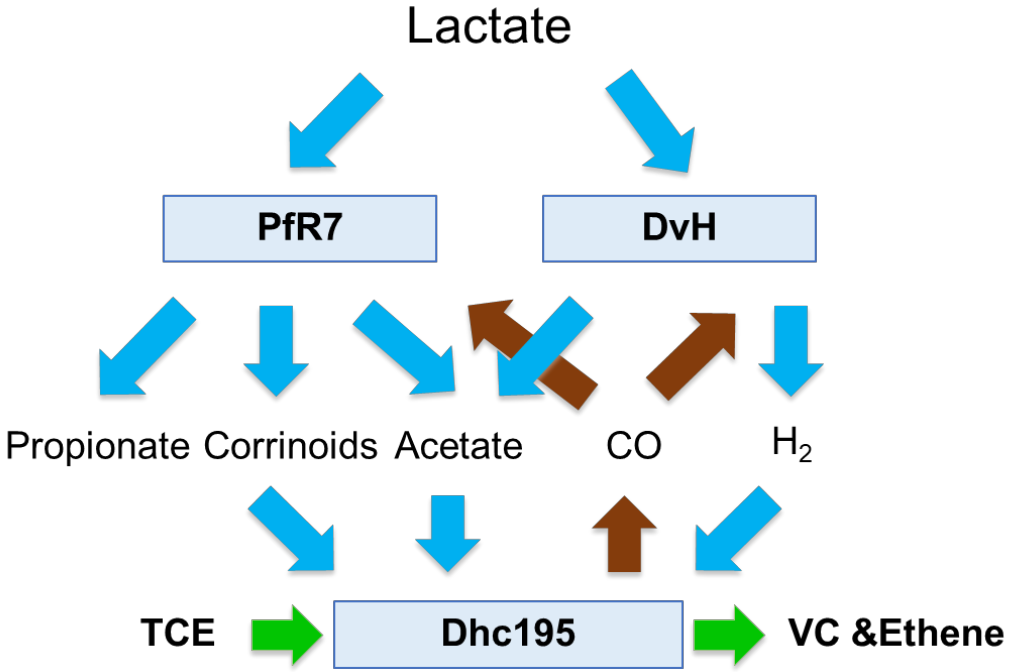
Effects on Metabolic Interactions under Salt Stress (II)

Group	Control	A	B	C	D	E	F	G	H	I	J
Na^+ conc. after perturbation (mM)	50	183	227	271	**315**	**359**	**404**	**448**	492	536	580
Limiting factor for TCE dechlorination	N/A		PfR7 Limiting		PfR7 & DvH Limiting			Dhc195 Limiting			





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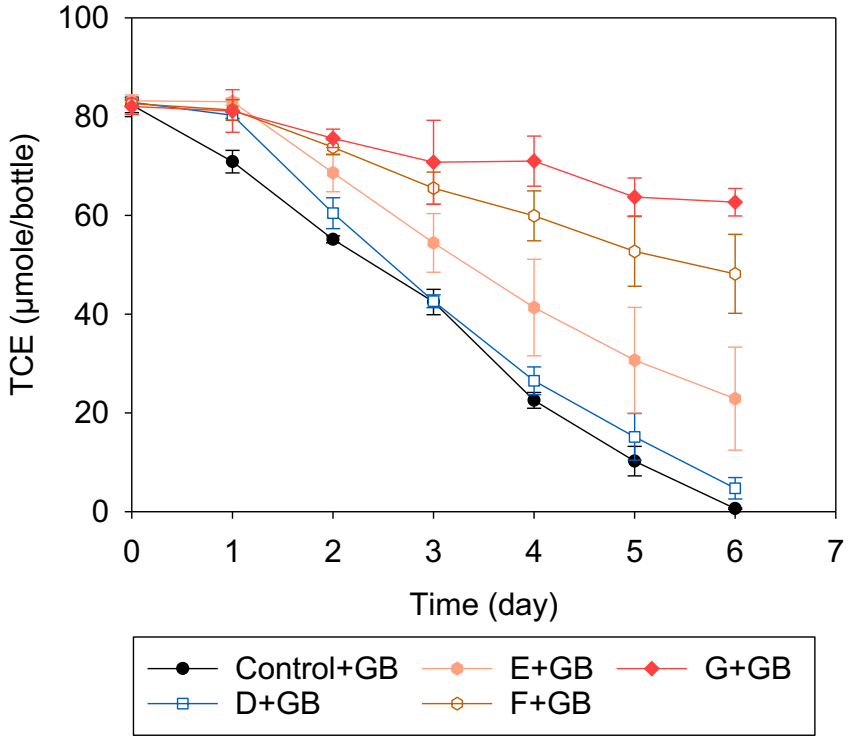
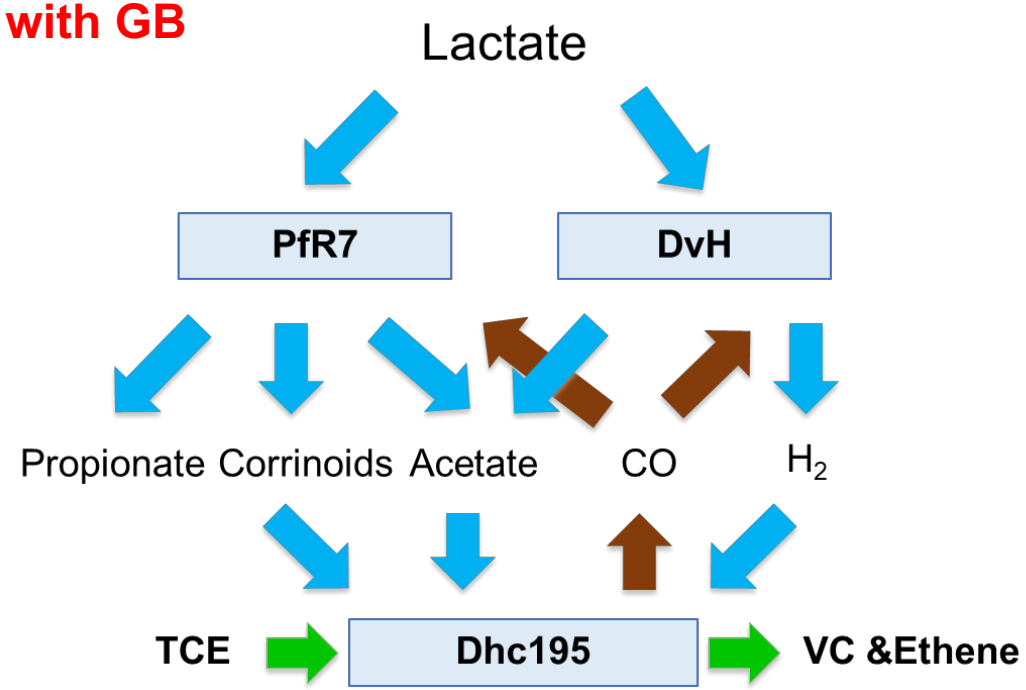


Amendment with GB



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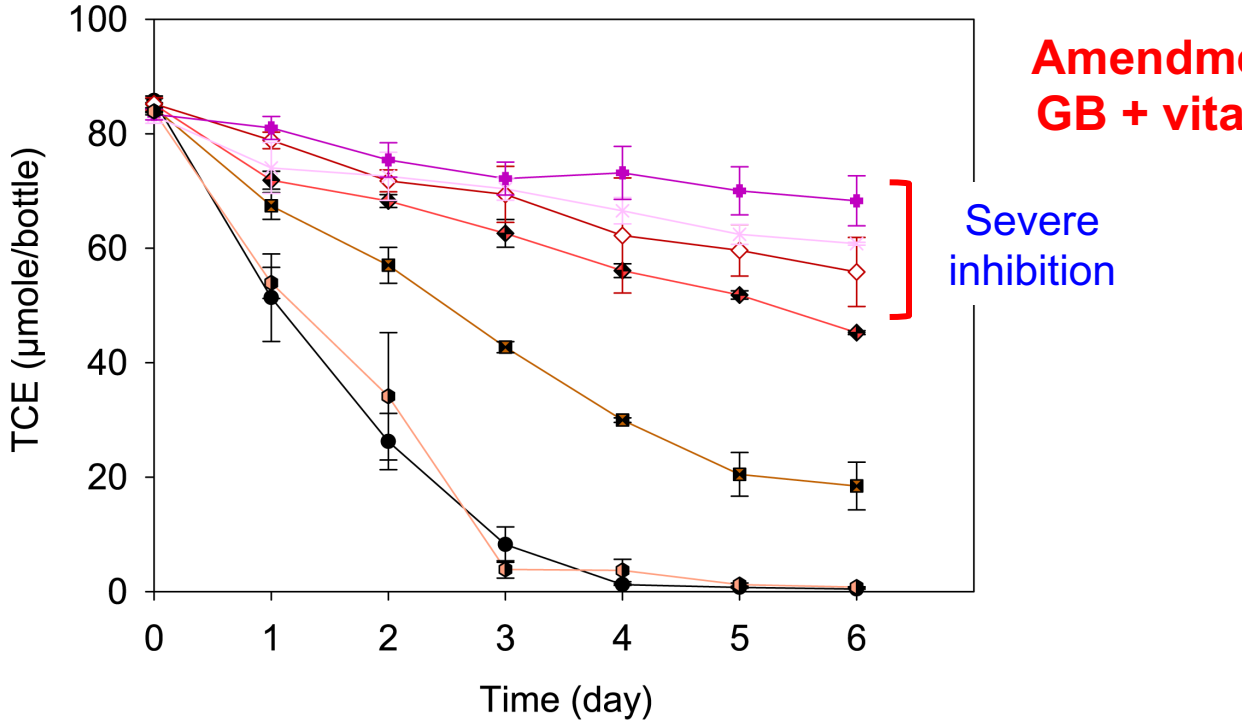
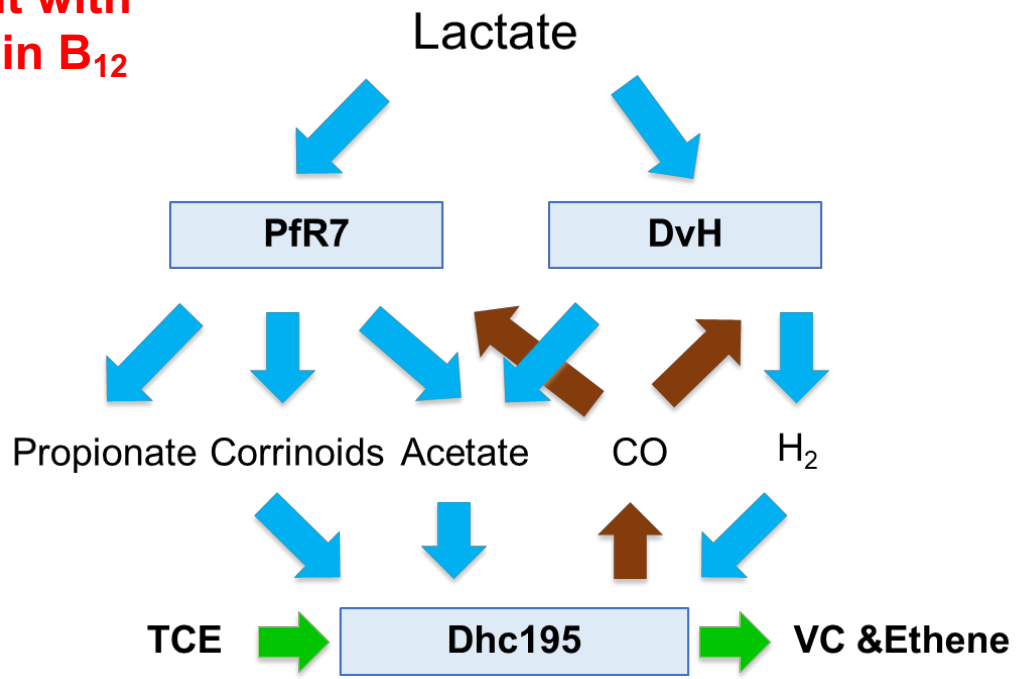


Amendment with GB + vitamin B_{12}



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Summary

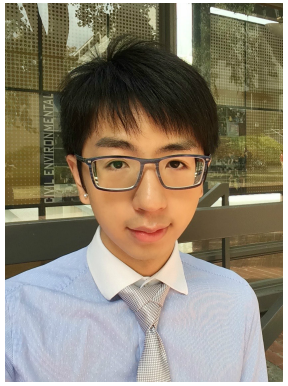
- **Sulfate effects**
  - Sulfide (5mM) inhibited TCE dechlorination and growth of Dhc195.
  - When hydrogen was abundant, sulfate-reducing bacterial activity generated sulfide that inhibited TCE dechlorination.
  - The sulfate-reduction activity can be limited by using slow fermentable substrates to prioritize TCE dechlorination.

- **Salt stress**
  - Dhc195 has a relatively higher tolerance to salt stress compared to supporting bacteria that formed syntrophic interactions with Dhc.
  - The salt stress mostly caused the transcriptional changes in genes encoding catabolism, tRNA, amino acid, and nucleic acid biosynthesis in Dhc.
  - Osmoprotectant, i.e., GB can be used to ameliorate the inhibition on the supporting bacteria.
  - Biostimulation with medium containing cobalamin and GB is necessary to sustain the bioremediation performance under salt perturbation at concentrations up to 400 mM.

Acknowledgements

Alvarez-Cohen's lab at UC Berkeley

Prof. Lisa Alvarez-Cohen



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Thank you!
Questions?

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