



Leading Science · Lasting Solutions

Flying Below the Radar – What Are Those Other Microbes Doing?



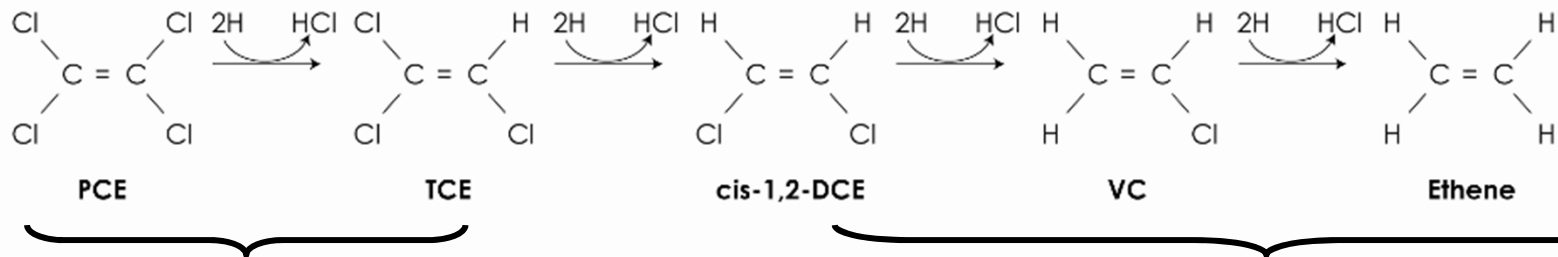
Sandra Dworatzek,
Jeff Roberts,
Phil Dennis, and
Peter Dollar
April 10, 2018

siremlab.com



Dhc is the Rockstar!

Can accumulate if Dhc are
absent wrong strain



Dehalobacter
Dehalospirillum
Desulfitobacterium
Desulfuromonas
Geobacter
+*Dehalococcoides*

Only
Dehalococcoides

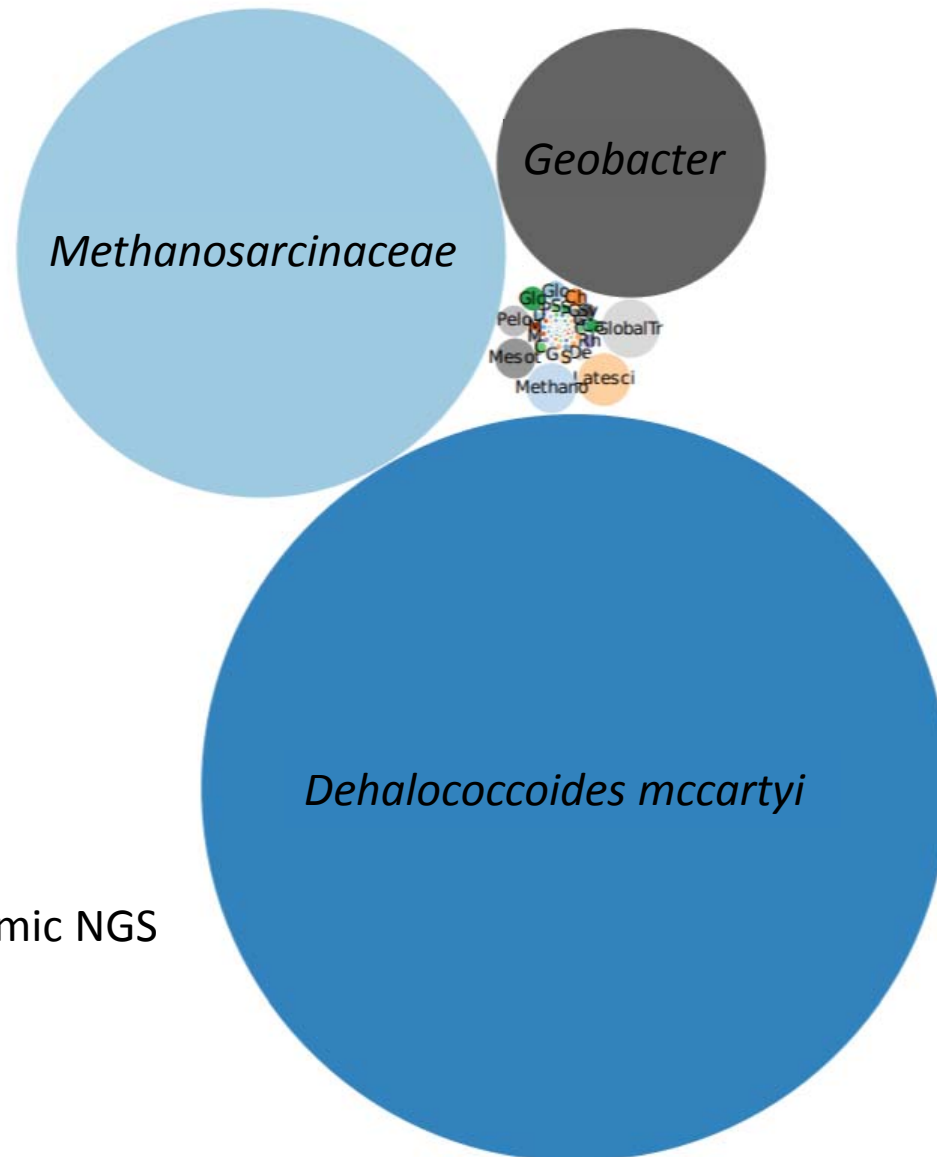


Molecular biological tools

- qPCR - Quantifies the abundance of specific microorganisms and functional genes
- Metagenomics/16S Amplicon Sequencing (Next Generation DNA Sequencing)
 - Direct genetic analysis of all microorganisms in a sample



Composition of KB-1 a dehalorespiring culture

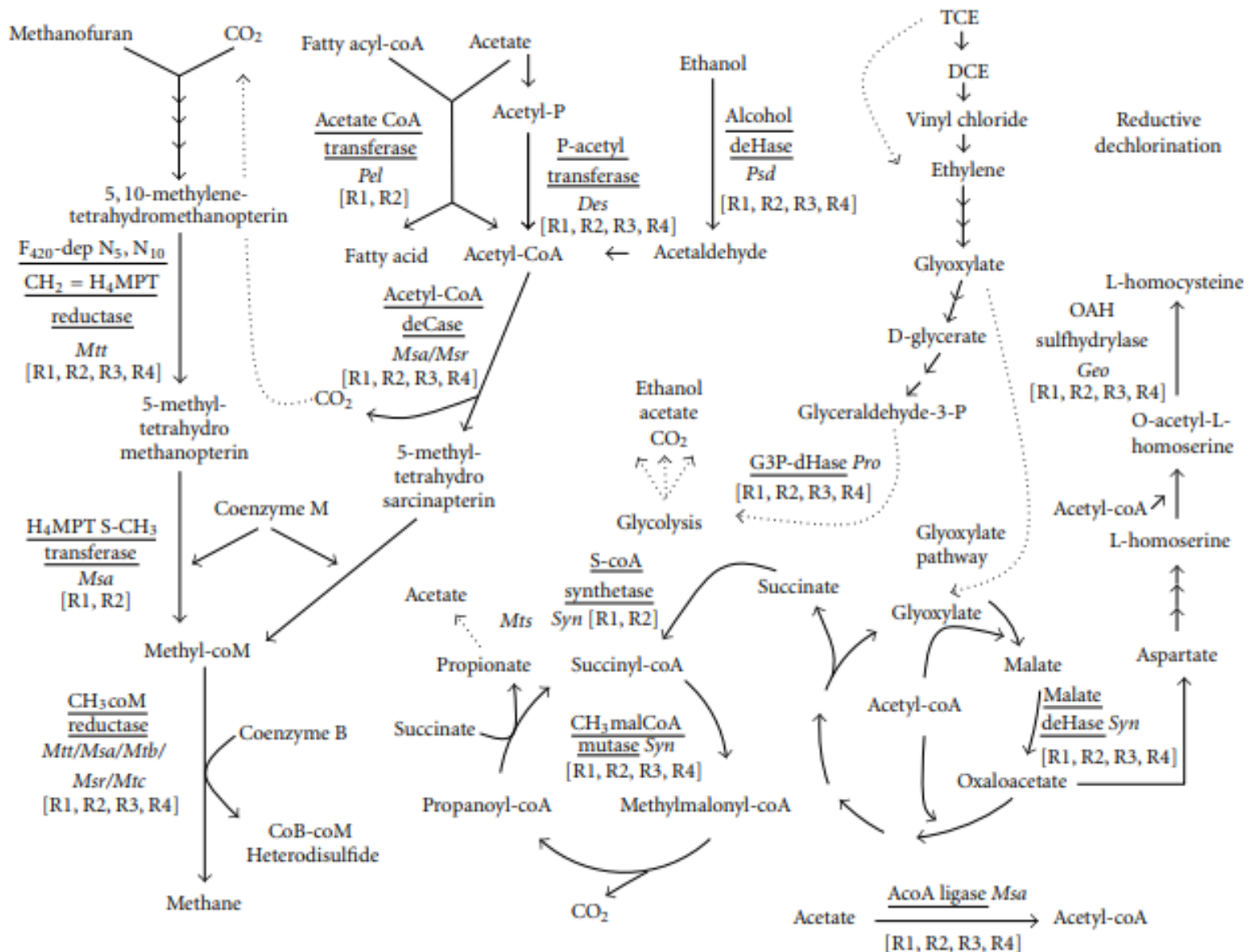


determined by Metagenomic NGS



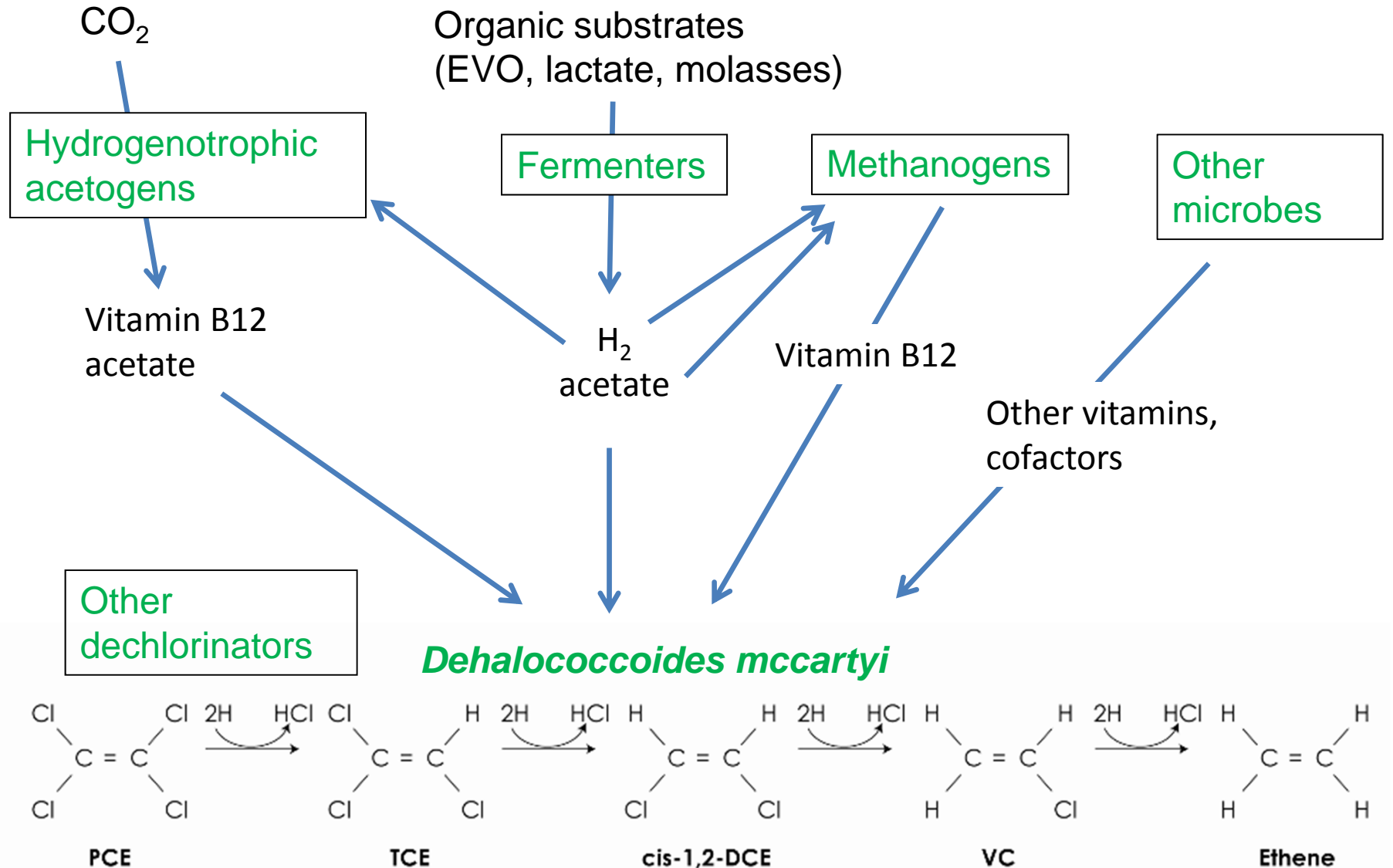


So a better picture might be:





Dhc do not live alone, but thrive in a community



■ ■ Many other organisms are required for successful bioremediation

- Other Dechlorinators
 - *Geobacter*
 - *Dehalobacter*
 - *Dehalogenimonas*
- *Fermenters*
 - *Bacteriodetes*
 - *Sporomusa*
- Methanogens
- Sulfate and nitrate reducers
- Others?



Importance of *Geobacter*

“A potential advantage of bioremediation technology is that microorganisms-which can attack the contaminant at or near the DNAPL water interface, may provide an effective, efficient, and less costly approach to DNAPL source zone remediation”

IRTC Remediation of DNAPLs Team



Geobacter

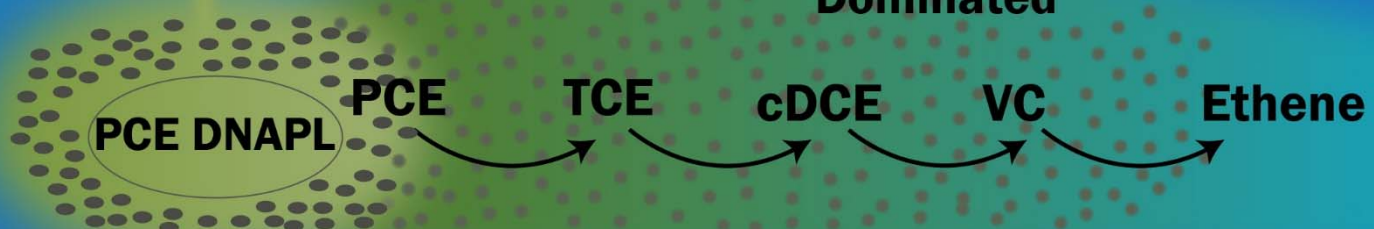


Dehalococcoides



**High Concentration Dissolved
PCE *Geobacter* Dominated**

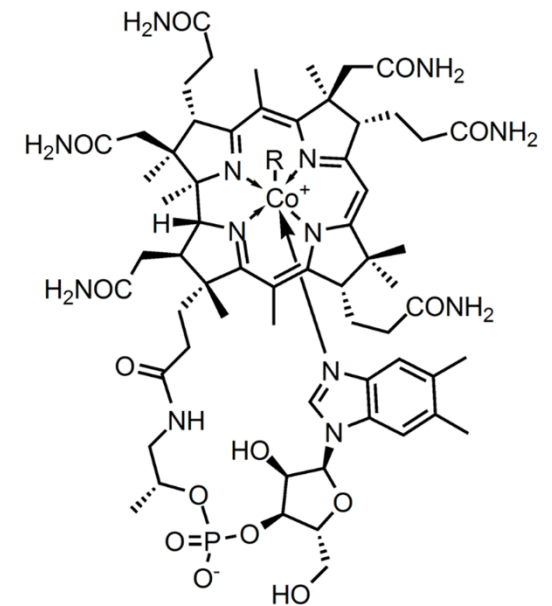
**Lower Concentration CVOC
Plume *Dehalococcoides*
Dominated**





Vitamin B12

- Vitamin B12 (Cyanocobalamin) is an essential enzyme co-factor
- *Dhc* don't have the machinery to make B12 and must rely on the microbial community to supply it
- Many known B12 synthesizing organisms;
 - Methanogens and acetogens
 - *Sporomusa*
 - *Geobacter*



R = 5'-deoxyadenosyl, Me, OH, CN



Geobacter Vitamin B12 production

- *Geobacter lovleyi* has been determined to produce the right type of vit B12 required for Dhc rdases



Unexpected Specificity of Interspecies Cobamide Transfer from *Geobacter* spp. to Organohalide-Respiring *Dehalococcoides mccartyi* Strains

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Dehalococcoides mccartyi strains conserve energy from reductive dechlorination reactions catalyzed by corrinoid-dependent reductive dehalogenase enzyme systems. *Dehalococcoides* lacks the ability for *de novo* corrinoid synthesis, and pure cultures require the addition of cyanocobalamin (vitamin B₁₂) for growth. In contrast, *Geobacter lovleyi*, which dechlorinates tetrachloroethene to *cis*-1,2-dichloroethene (*cis*-DCE), and the nondechlorinating species *Geobacter sulfurreducens* have complete sets of cobamide biosynthesis genes and produced 12.9 ± 2.4 and 24.2 ± 5.8 ng of extracellular cobamide per liter of culture suspension, respectively, during growth with acetate and fumarate in a completely synthetic medium. *G. lovleyi*-*D. mccartyi* strain BAV1 or strain FL2 cocultures provided evidence for interspecies corrinoid transfer, and *cis*-DCE was dechlorinated to vinyl chloride and ethene concomitant with *Dehalococcoides* growth. In contrast, negligible increase in *Dehalococcoides* 16S rRNA gene copies and insignificant dechlorination occurred in *G. sulfurreducens*-*D. mccartyi* strain BAV1 or strain FL2 cocultures. Apparently, *G. lovleyi* produces a cobamide that complements *Dehalococcoides*' nutritional requirements, whereas *G. sulfurreducens* does not. Interestingly, *Dehalococcoides* dechlorination activity and growth could be restored in *G. sulfurreducens*-*Dehalococcoides* cocultures by adding 10 μ M 5',6'-dimethylbenzimidazole. Observations made with the



Flying under the radar: *Dehalogenimonas*

- Originally identified in a tetrachloroethane degrading consortia with unique characteristics
- Found to degrade a wide range of chlorinated compounds:
 - Chlorinated ethanes (1,1,2,2-TECA, 1,2-DCA)
 - Chlorinated propanes (1,2,3-TCP)
 - Chlorinated ethenes (*trans*-DCE and VC)
 - Chlorinated benzenes
- Now being found at many sites





Flying under the radar: *Dehalobacter*

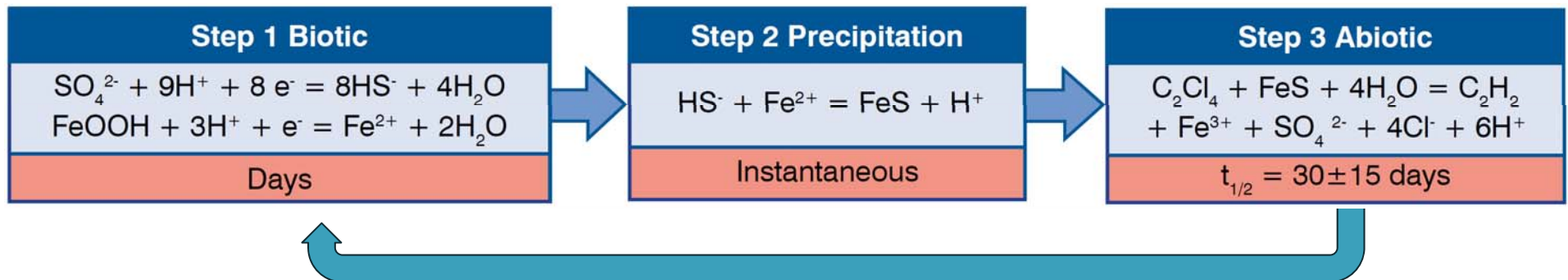
- Found to degrade a wide range of contaminants
 - Chlorinated ethenes
 - Chlorinated ethanes (1,1,1-TCA)
 - Chlorinated methanes
 - CFCs
 - Chlorinated benzenes





Biogeochemical Reduction of Chlorinated Solvents

Although defined as “abiotic” processes, degradation of CVOCs by reactive minerals often involves a combination of biologically mediated and geochemical reactions:



Geobacter and sulfate reducing bacteria (SRB) regenerate Fe^{3+} & SO_4^{2-} in biotic reactions



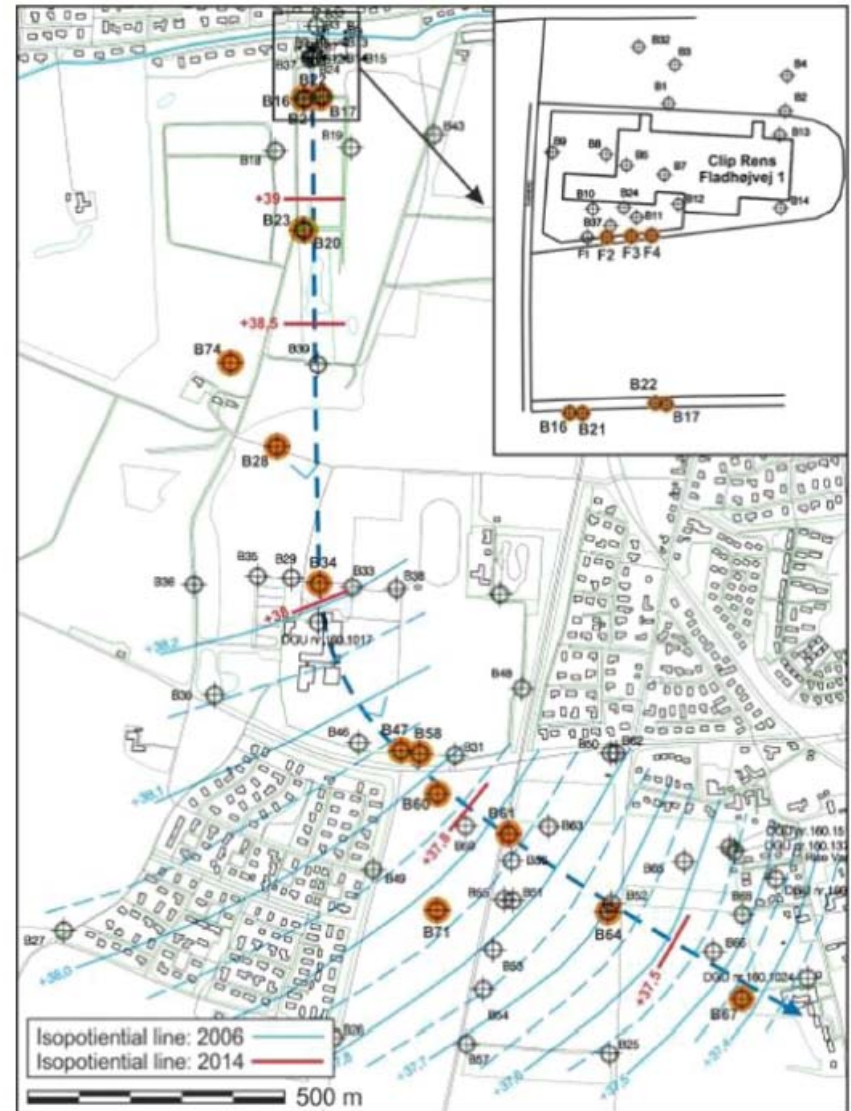


CASE STUDIES :
1) DENMARK POST THERMAL SITE



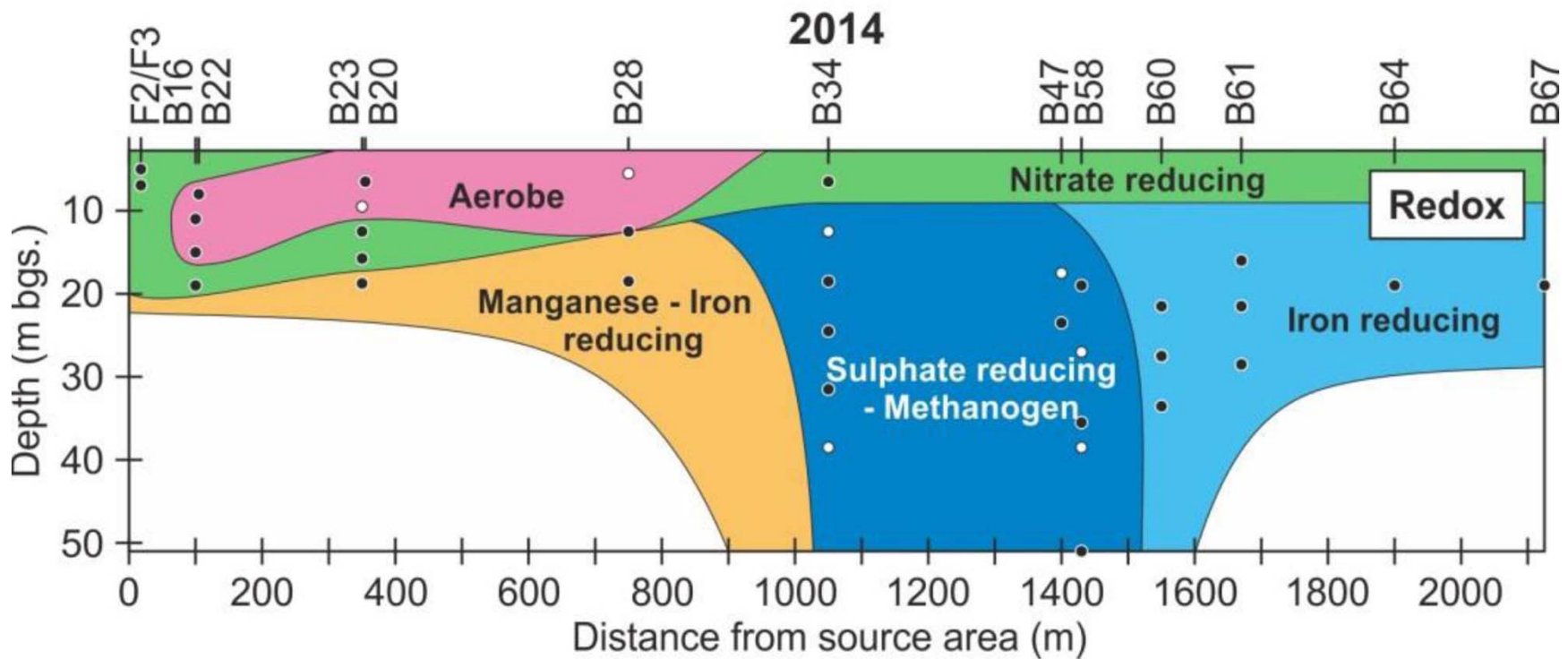
Denmark Post-Steam Injection Site

- PCE (dry cleaner) source zone treated with steam—currently in MNA
- Characterization by molecular and isotopic methods (CSIA)
- Study determined likely degradation mechanisms included:
 - Reductive dechlorination
 - Biogeochemical (pyrite)
 - Aerobic degradation

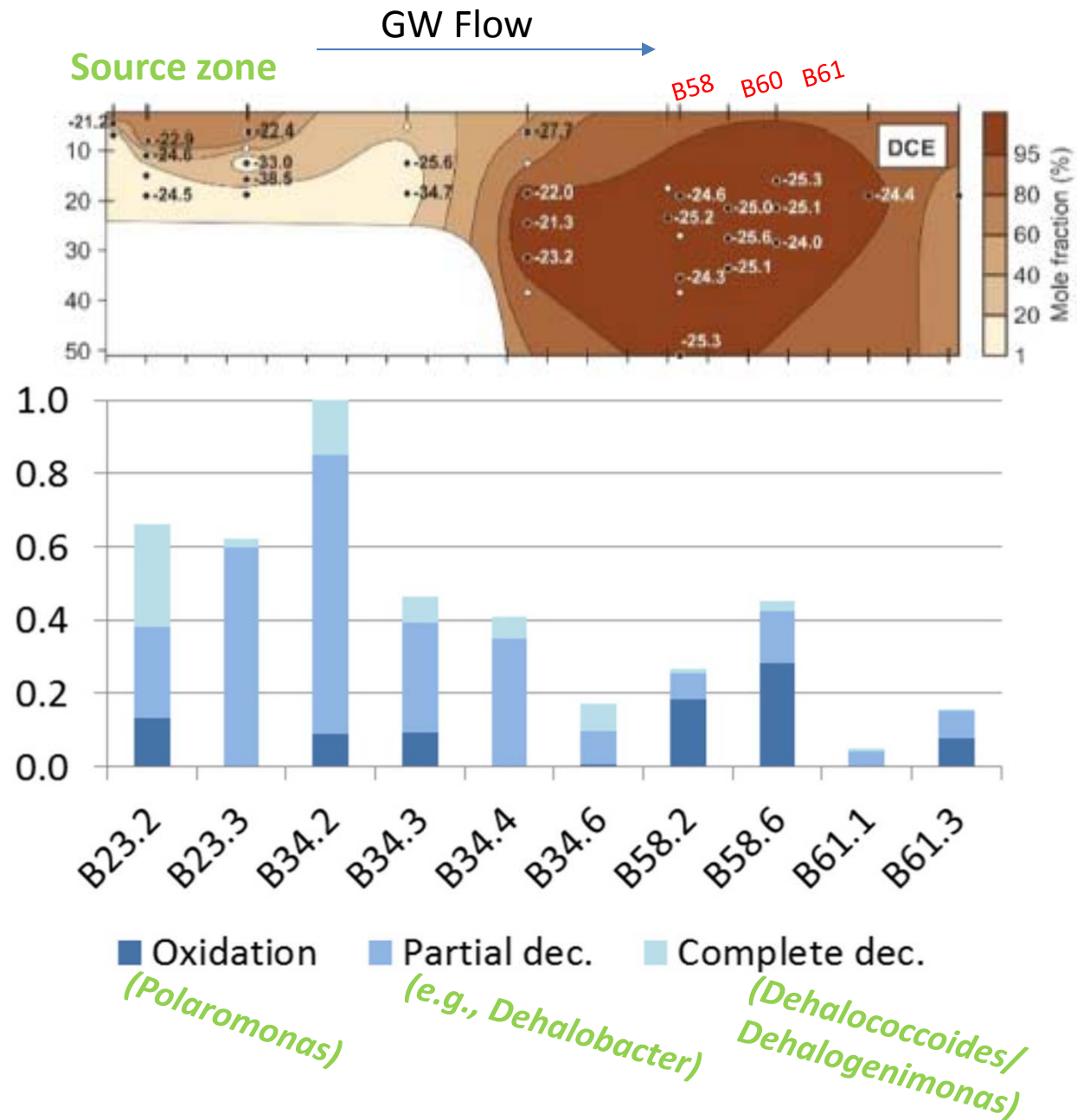




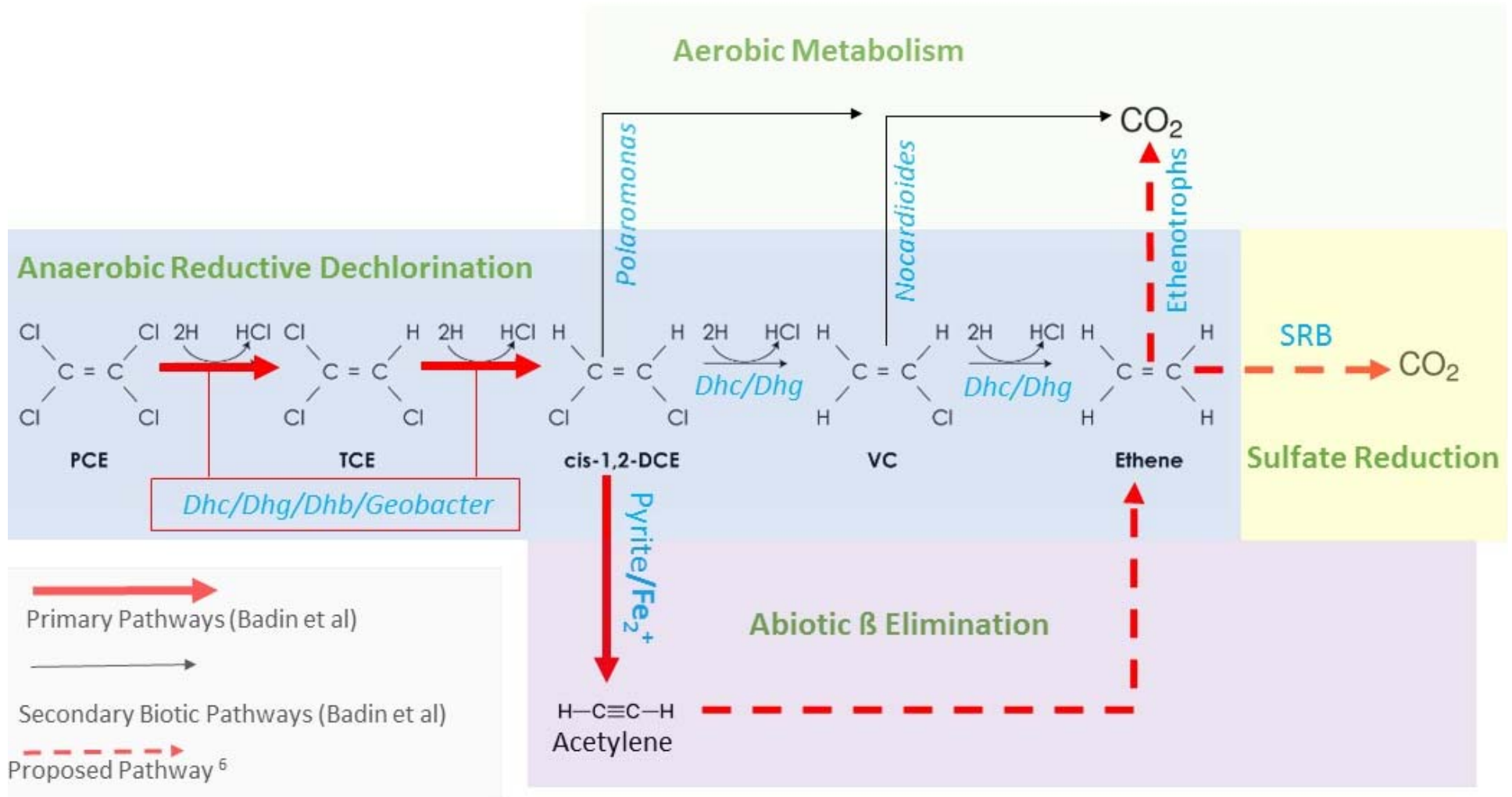
Redox zones observed



Classes of Dechlorinators: Determined by NGS



Classes of Dechlorinators: Determined by NGS

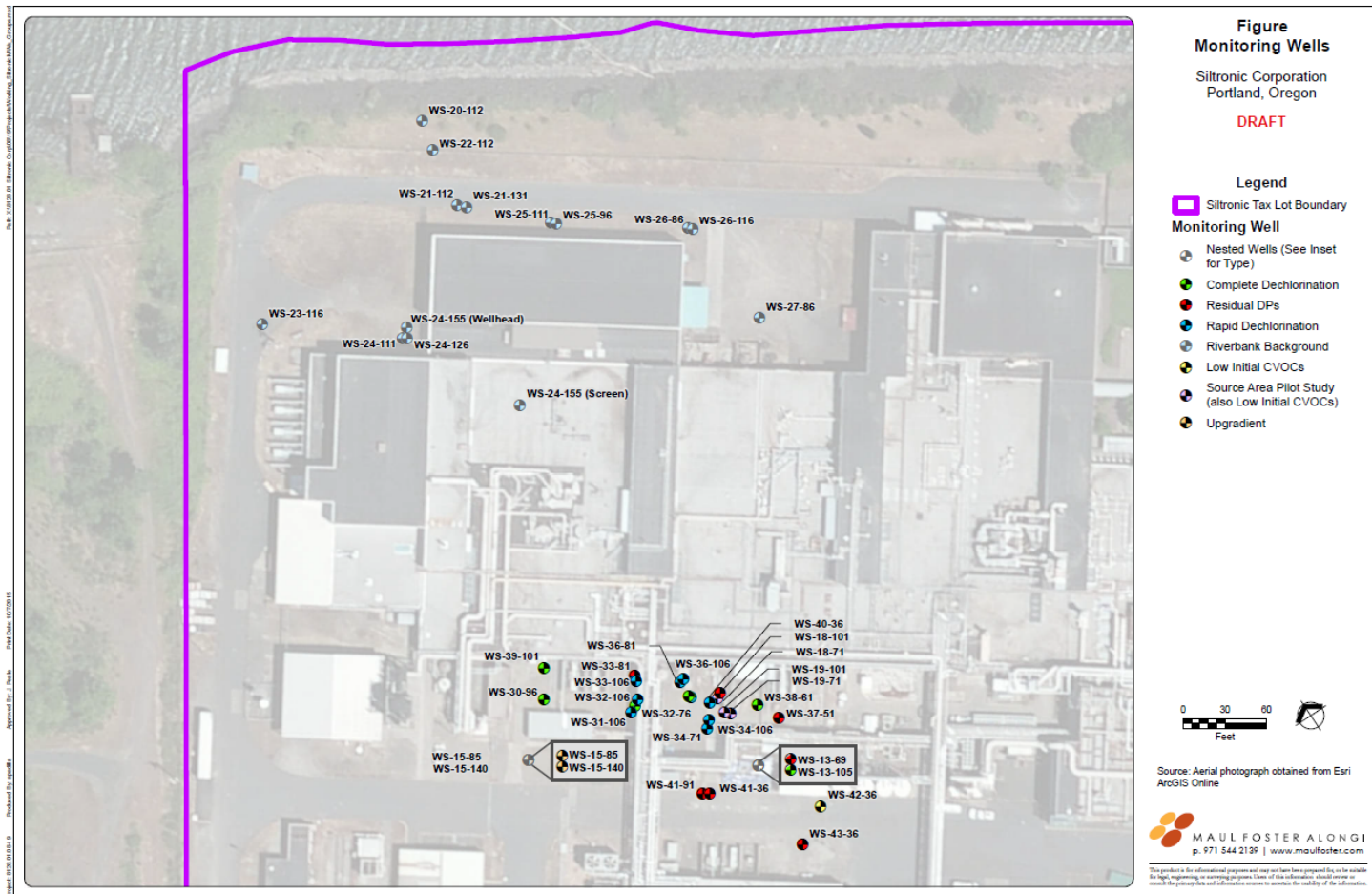




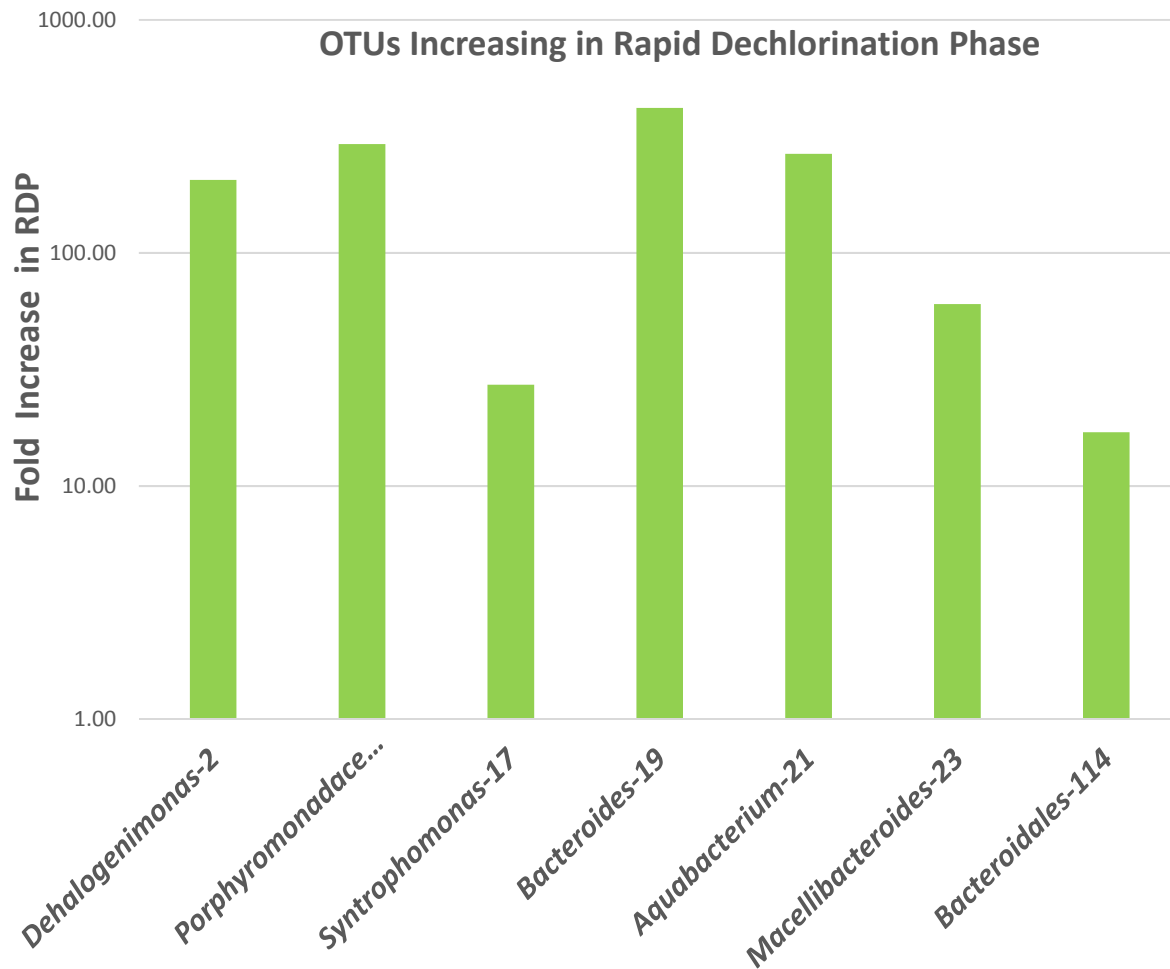
CASE STUDIES : **2) OREGON SITE**



Oregon Site



Organisms with Increasing Abundance at Oregon Site Corelated With Rapid Dechlorination Phase (RDP)



- ***Dehalococcoides*** increased after bioaugmentation

**During RDP
Increases in:**

- ***Dehalogenimonas*** complete dechlorinator
- ***Aquabacterium*** aerobic VC degrader
- ***Bacteroides*** efficient dechlorination to ethene
- ***Syntrophomonas*** symbiotic partner for *Dehalococcoides*

Peale et al., 2017



Conclusions

- Many organisms play important roles in reductive dechlorination;
 - *Geobacter* is important for DNAPL, B12 production, abiotic processes
 - *Dehalogenimonas* is increasingly viewed as significant
 - *Dhc* symbionts such as *Bacterioidetes* are showing increasing evidence as important for optimal performance
 - Aerobic processes may co-occur at sites with reductive dechlorination/biogeochemical processes to enhance site remediation





Conclusions

- We are gaining a better understanding of biologically induced processes through MBTs
 - NGS (16S amplicon sequencing taxonomy) is being used for characterizing microbial systems due to decreasing costs and better interpretability
 - Metagenomics now commercially available and provides even more information including identification of microorganisms to the strain level and functional genes
- Provides insights into:
 - Composition and proportion of microbes
 - Shifts in microbial communities
 - Function of microbial communities





Thank you for attending!

Further Information

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References

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