



Sustained *In Situ* Detoxification of Priority Chloroorganic Pollutants

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Center for Environmental Microbiology

Department of Microbiology

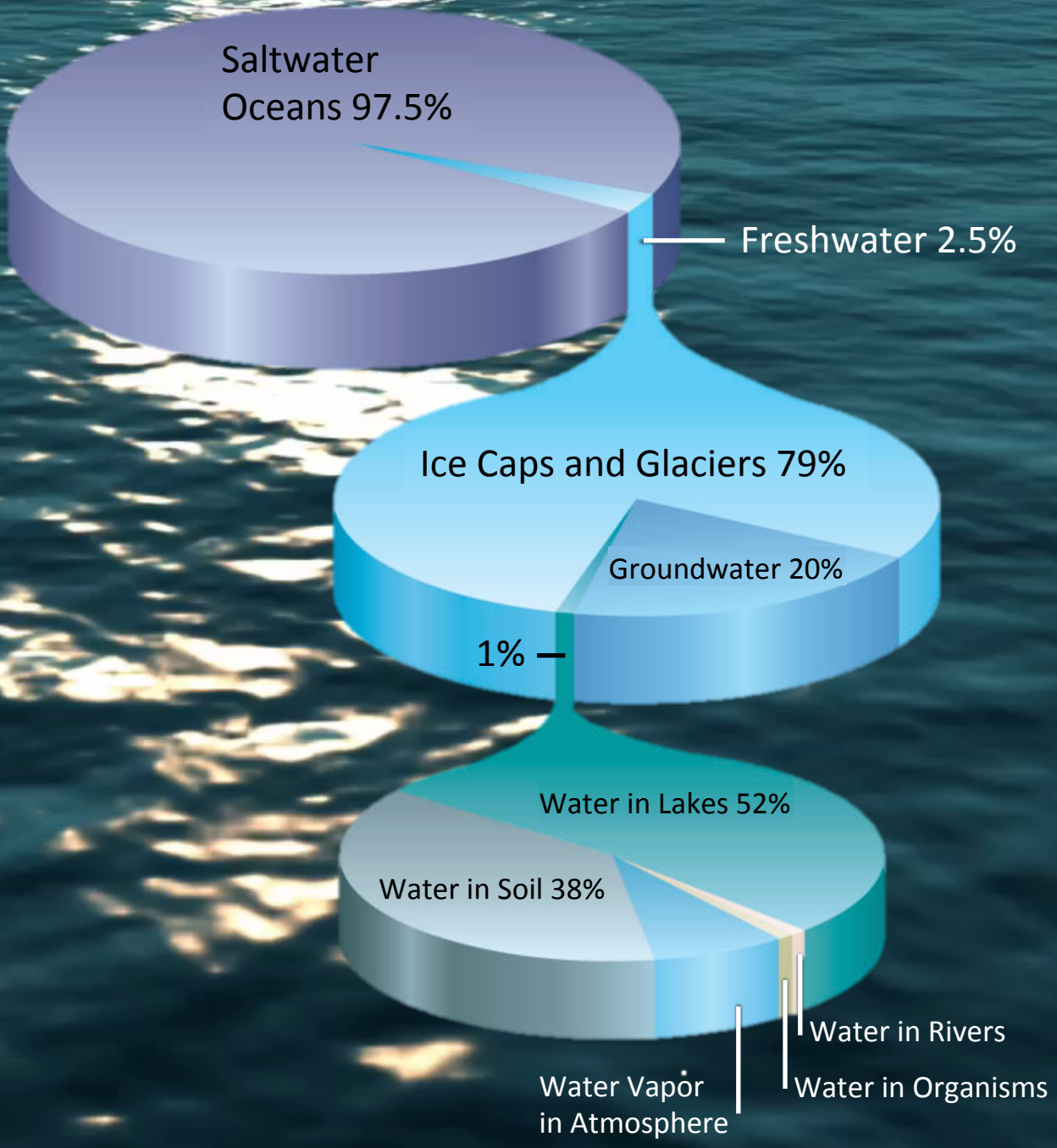
Department of Civil & Environmental Engineering

Bioscience Division, Oak Ridge National Laboratory



Oak Ridge National Laboratory





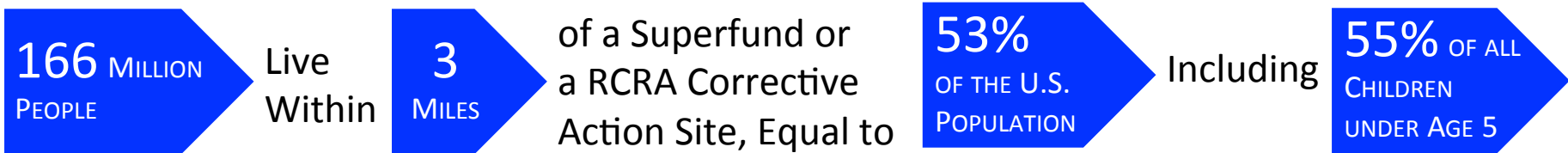
Contaminated Sites in the U.S.



1,322 Superfund sites ●

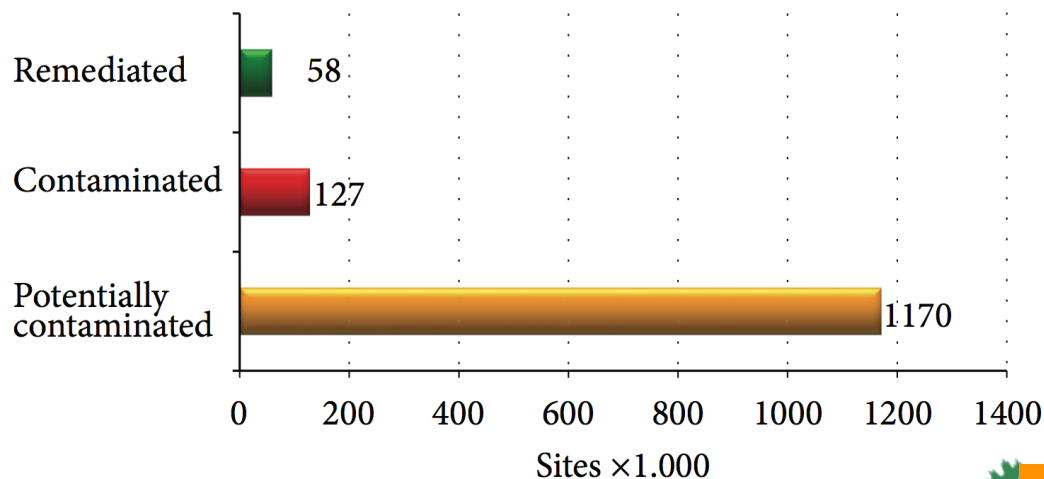
3,747 RCRA sites ●

>450,000 Brownfields ●

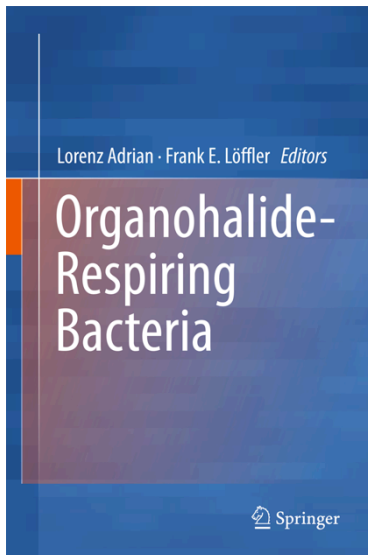
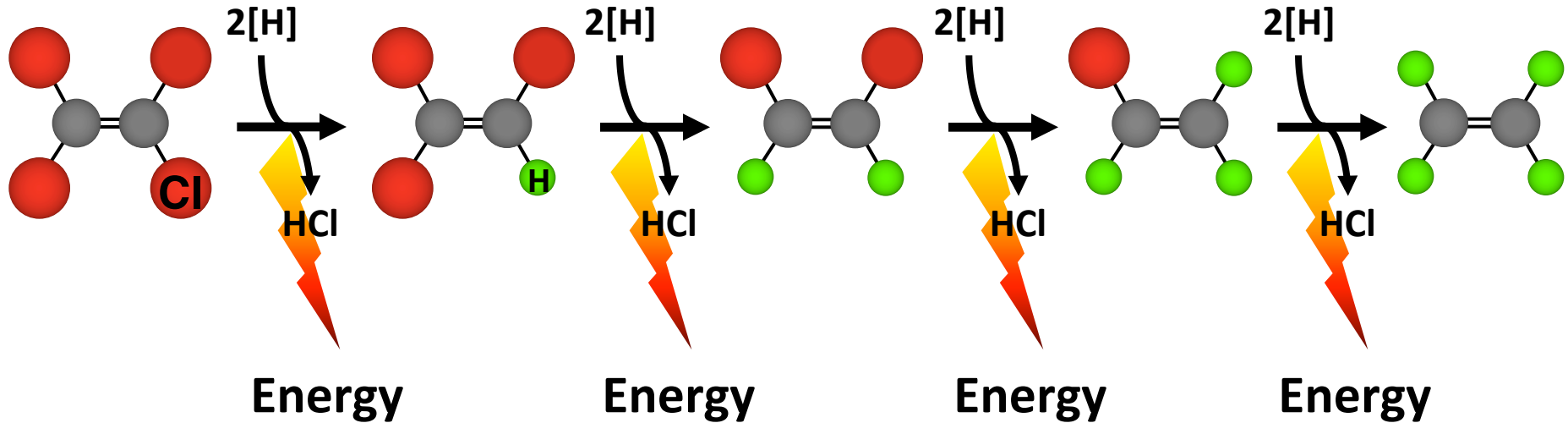


... and in Europe

Majority of sites impacted with chlorinated compounds



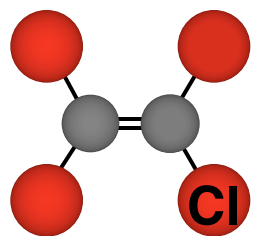
Chlorinated Ethenes as Respiratory Electron Acceptors



Organohalide Respiration
(Respiratory Reductive Dechlorination)



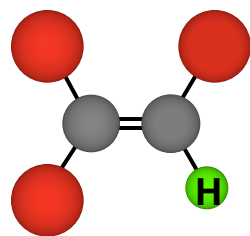
Chlorinated Ethenes are Major Risk Drivers



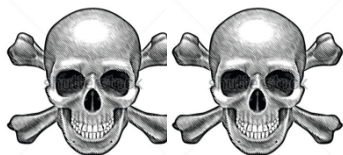
PCE



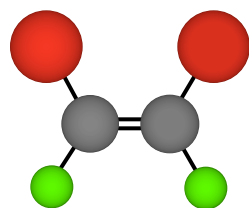
5 ppb



TCE



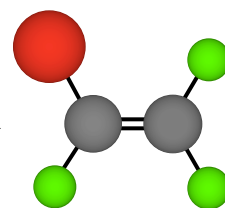
5 ppb



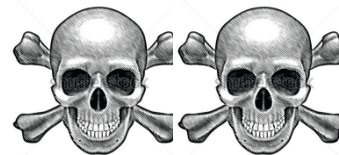
cis-DCE



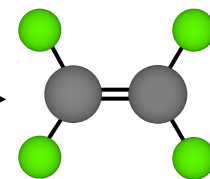
7 ppb



VC



2 ppb



Ethene

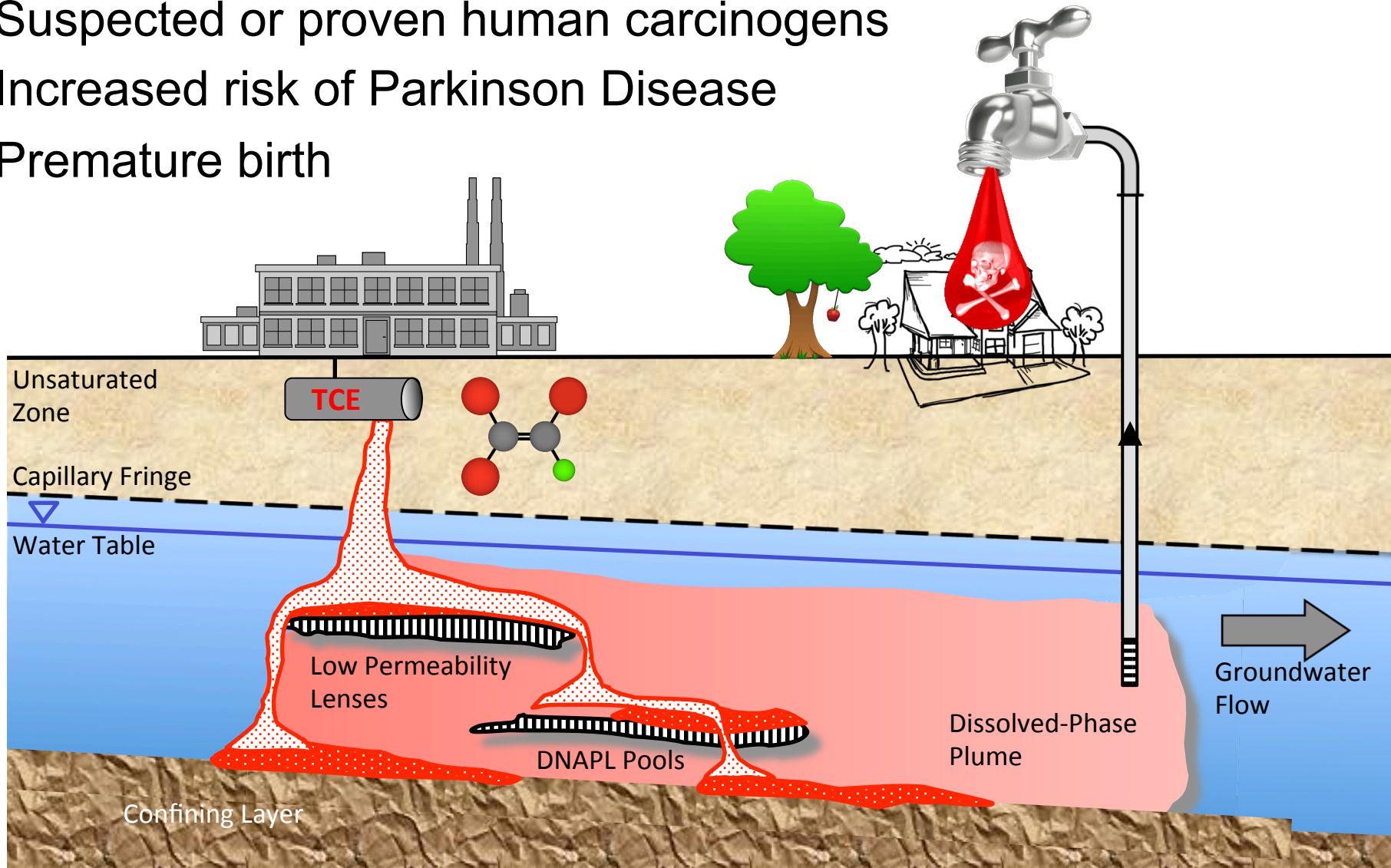


Human carcinogen
Parkinson's disease
Premature birth

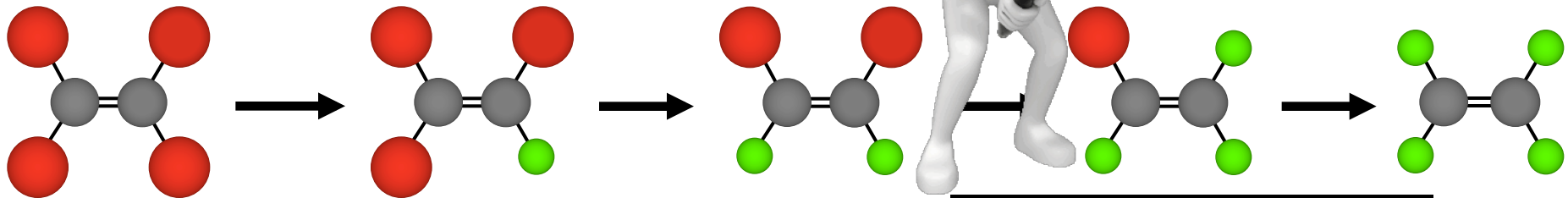
Human carcinogen

Contaminant Source Zones and Plumes

- Suspected or proven human carcinogens
- Increased risk of Parkinson Disease
- Premature birth

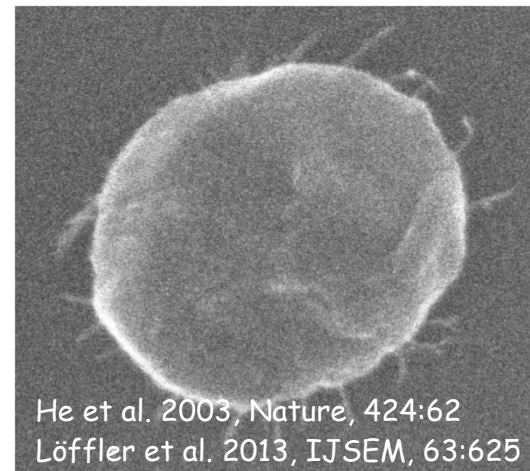
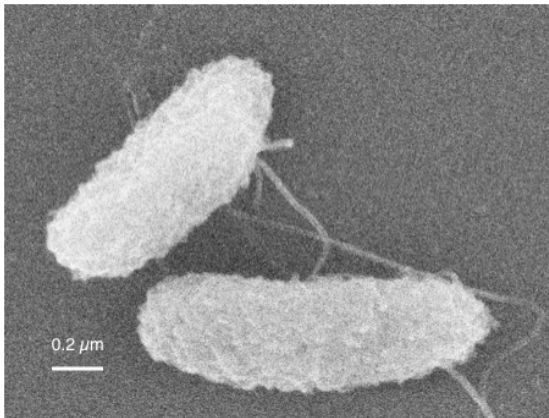


Populations Involved in Reductive Dechlorination of Chlorinated Benzenes



Geobacter lovleyi, *Dehalobacter*, *Sulfurospirillum*,
Desulfuromonas, *Desulfitobacterium*

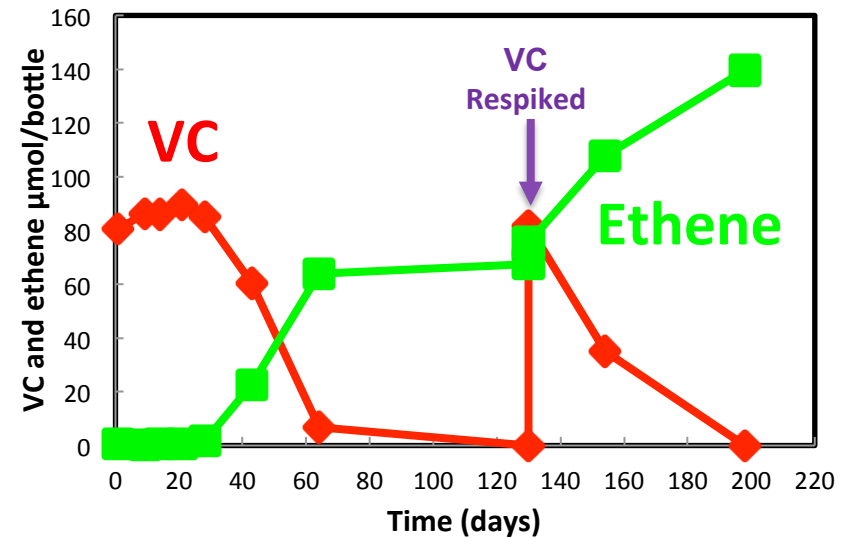
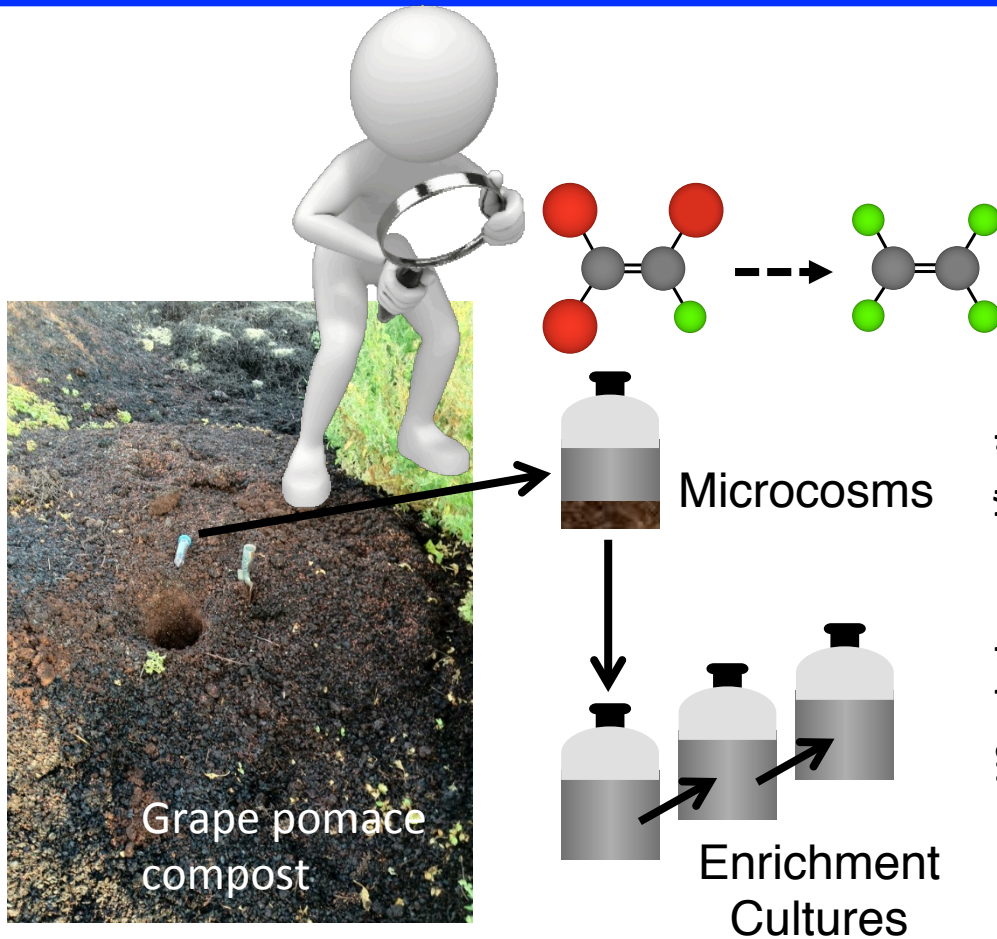
***Dehalococcoides
mccartyi***



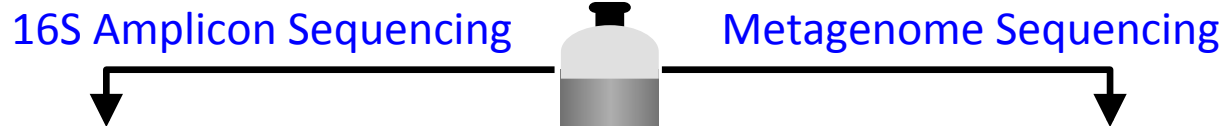
He et al. 2003, Nature, 424:62
Löffler et al. 2013, IJSEM, 63:625



Novel Dechlorinators from Pristine Sources

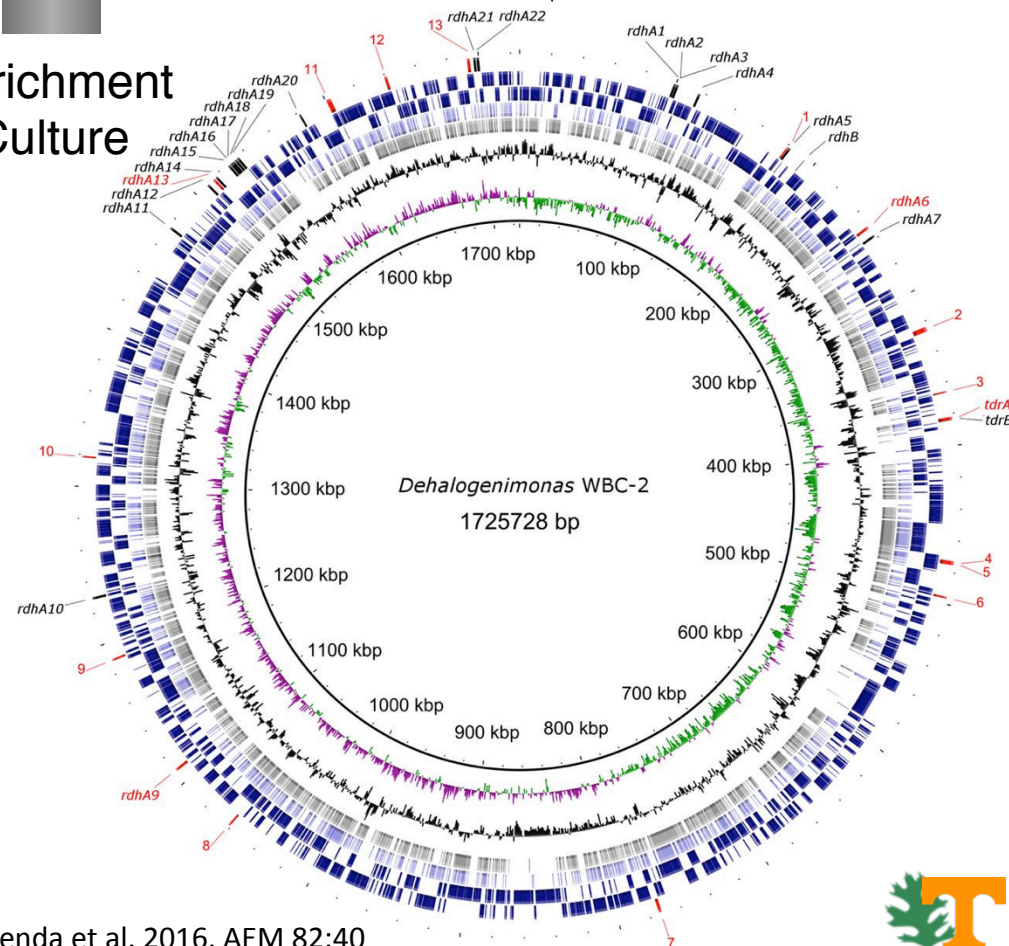


Pristine Environments are Reservoirs for Novel RDase Genes



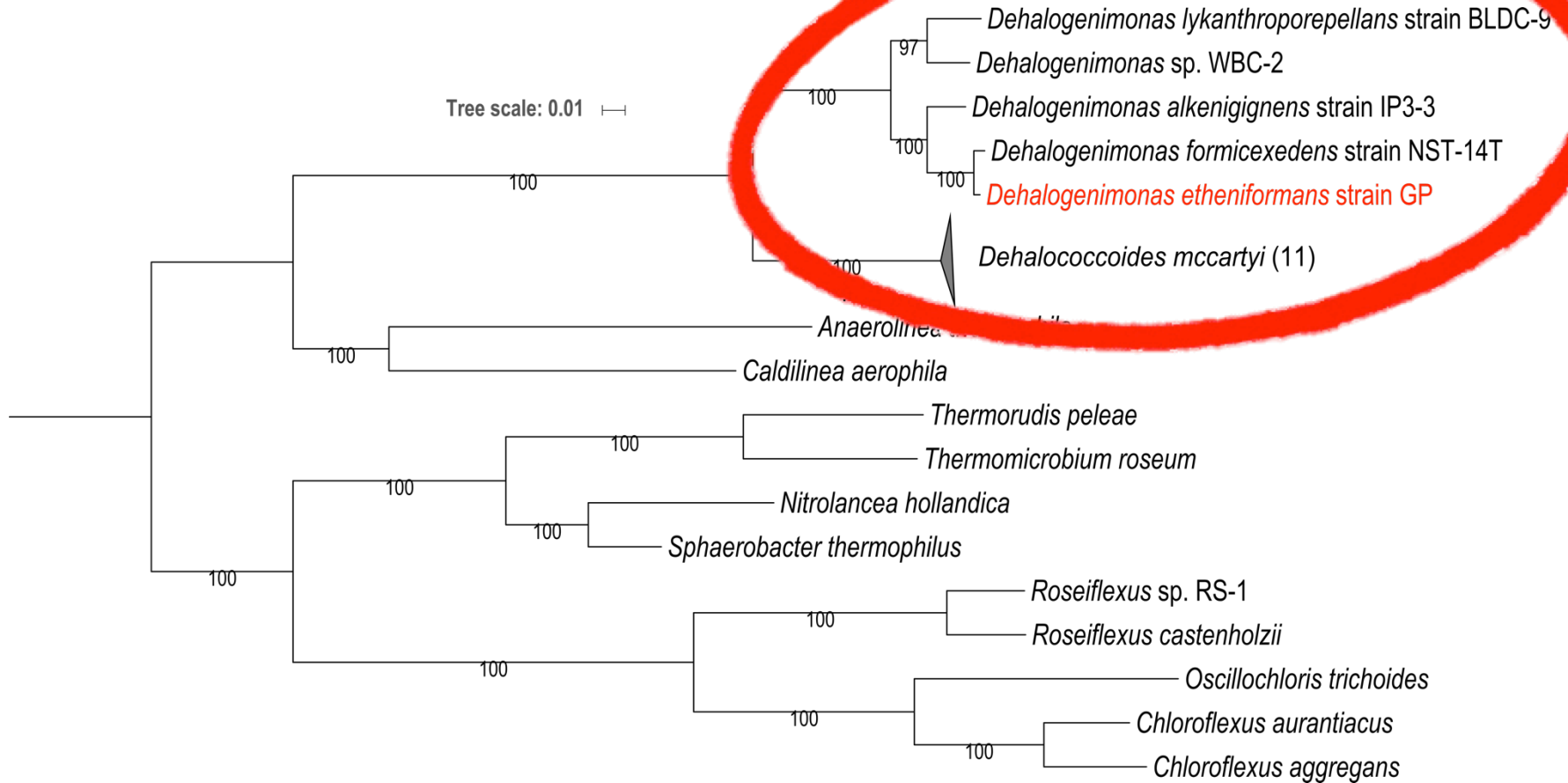
	1 st VC Addition	2 nd VC Addition
Others	32.1	16.5
VadinCA02	1.3	2.8
<i>Clostridium</i>	10.1	13.6
<i>Pelotomaculum</i>	1	1.6
<i>Bacteroidetes</i>	4.5	6.8
Unclassified WPS-2	1.8	9.4
<i>Dehalogenimonas</i>	47.4	46.1
<i>Oscillospira</i>	0.1	0.08
<i>Geobacter</i>	0.1	0.22
<i>Treponema</i>	0.7	2.6
<i>Dehalococcoides</i>	0	0
<i>Acetobacterium</i>	0.9	0.3

Enrichment Culture

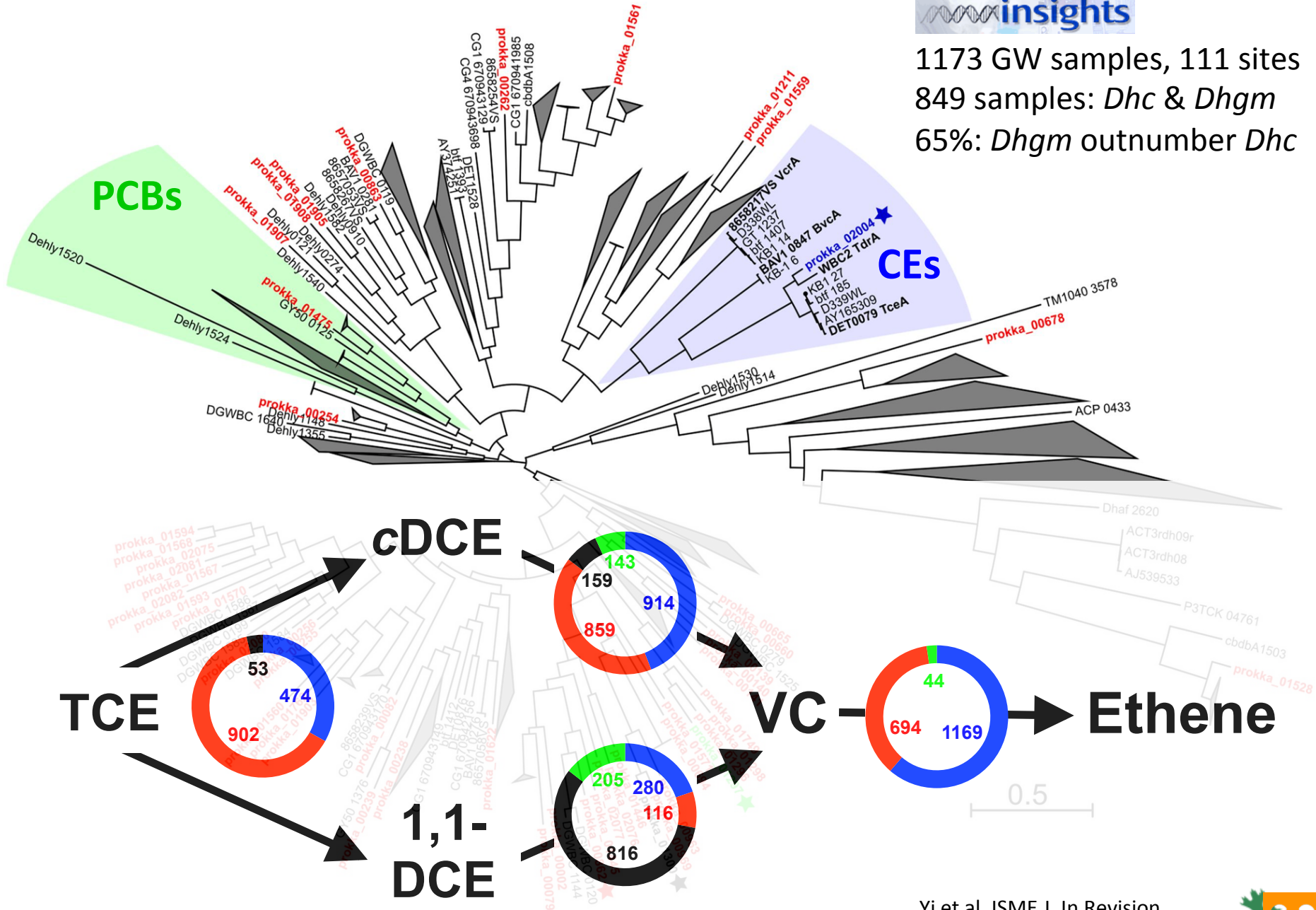


Phylogeny of Novel VC Dechlorinator

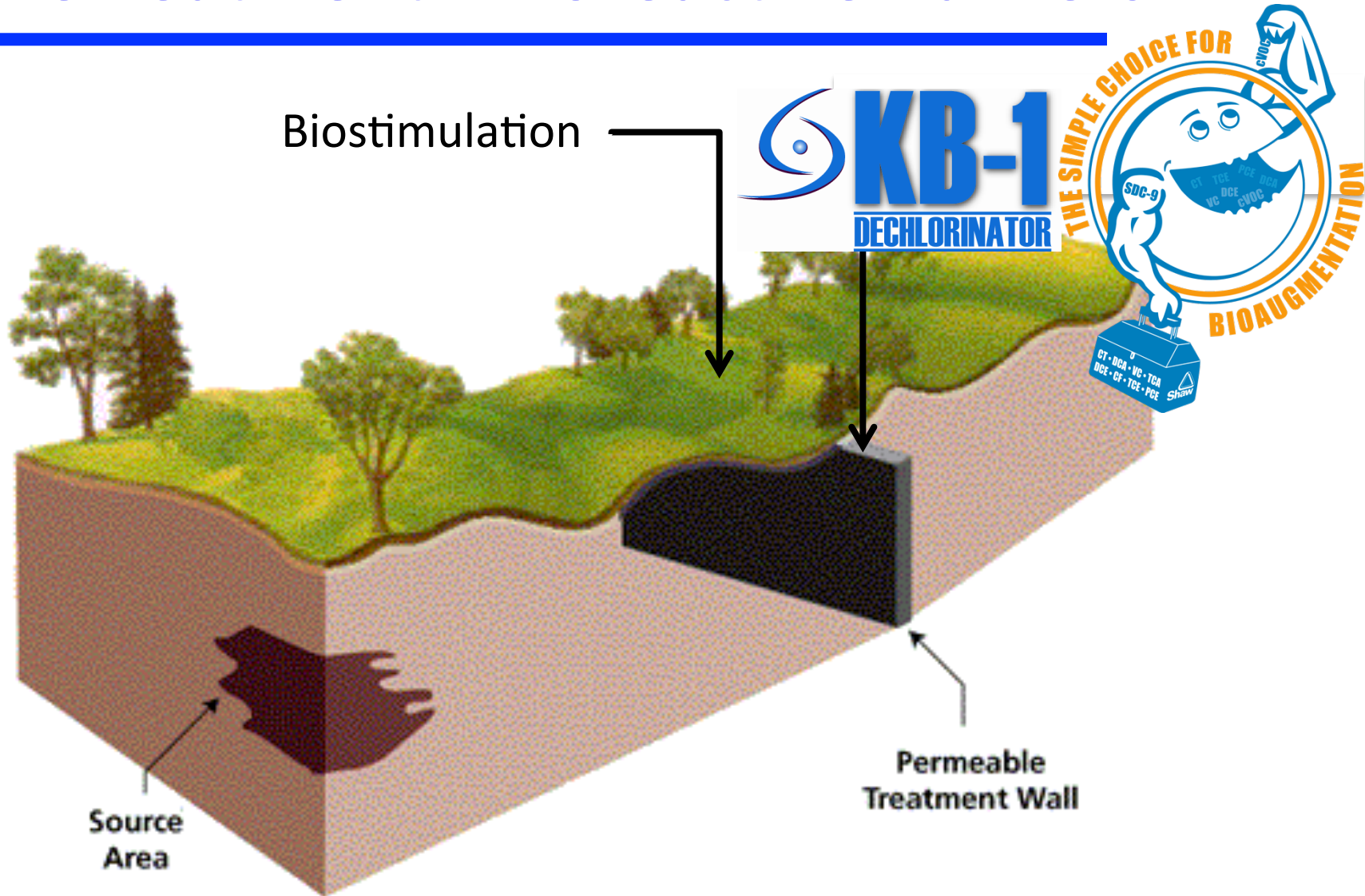
Organohalide-Respiring Chloroflexi



1173 GW samples, 111 sites
 849 samples: *Dhc* & *Dhgm*
 65%: *Dhgm* outnumber *Dhc*

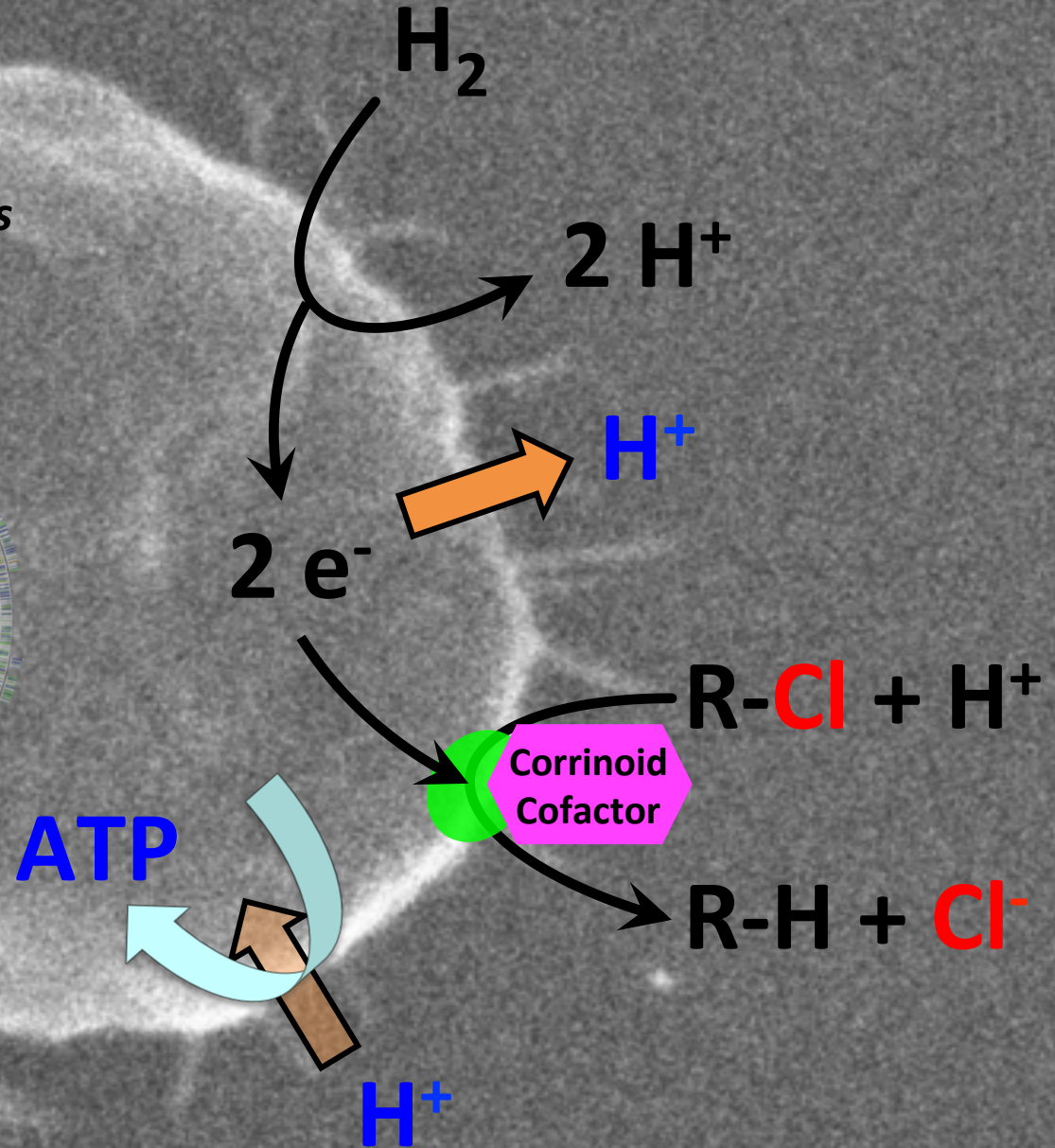
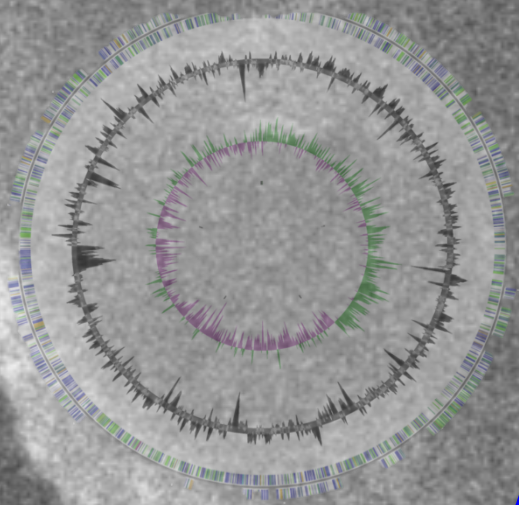


Plume Treatment - Bioreactive Barriers



Simplified Model of Organohalide Respiration

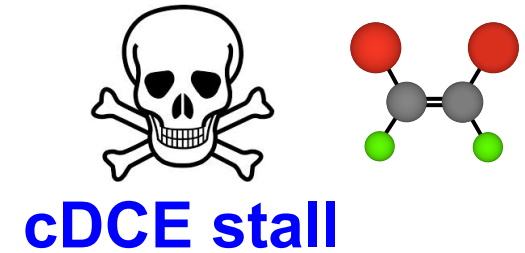
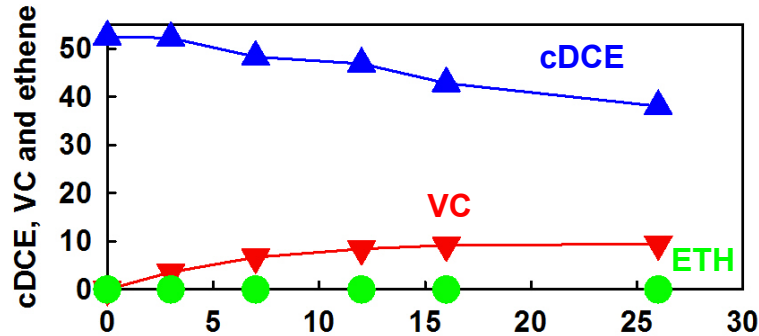
Dehalococcoides
mccartyi
strain BAV1



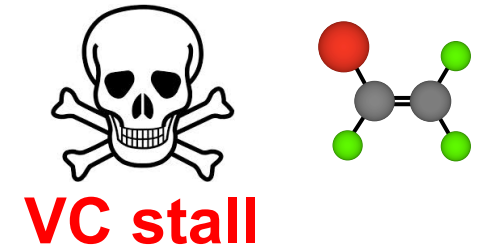
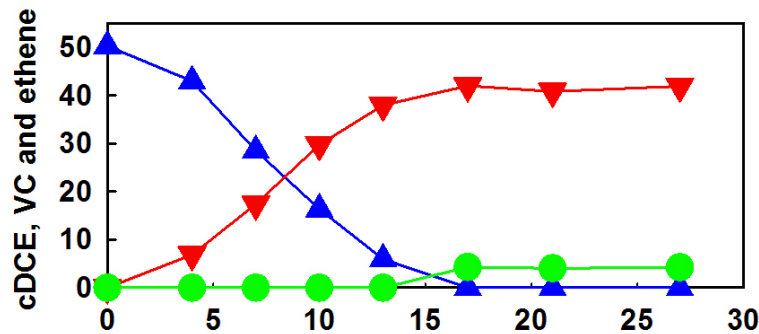
Dhc: Strict Requirement for Corrinoid

Dhc strain BAV1

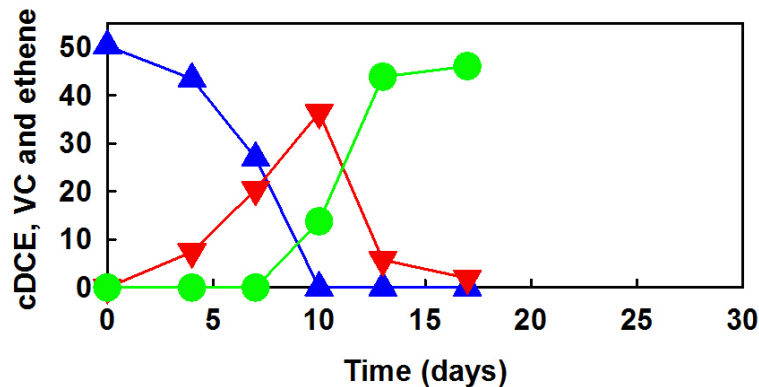
No B₁₂

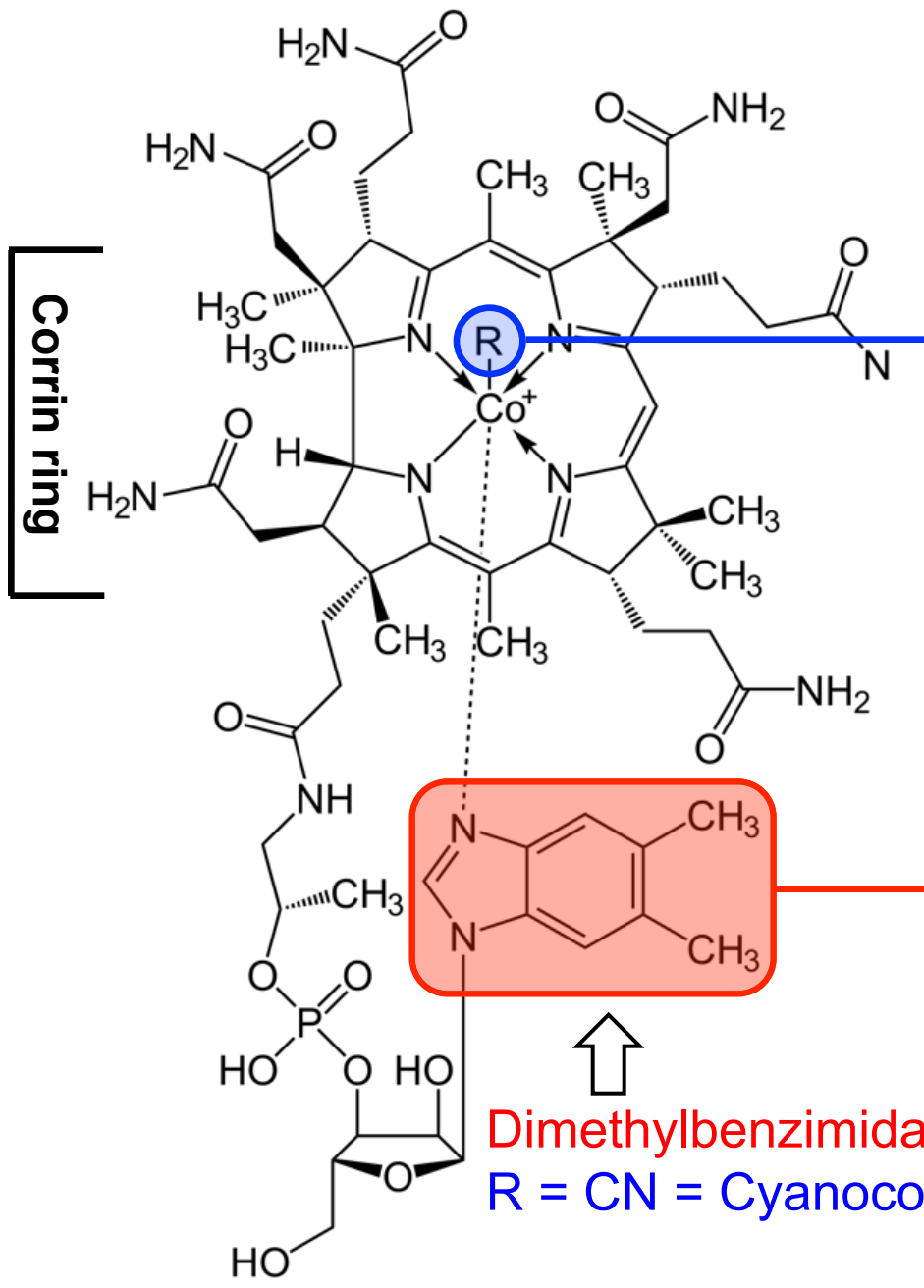


Limited B₁₂
[1 µg/L]



Sufficient B₁₂
[25 µg/L]



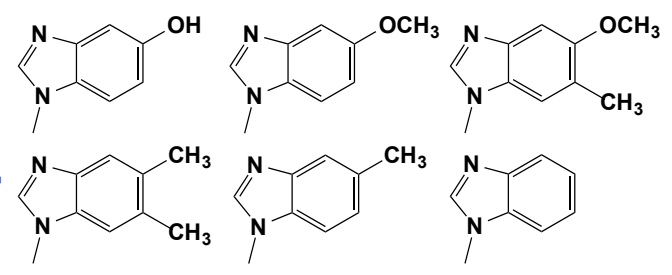


Dimethylbenzimidazole (DMB)
R = CN = Cyanocobalamin (Vitamin B₁₂)

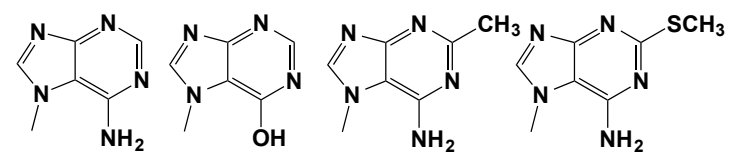
Lower Bases

Upper ligand

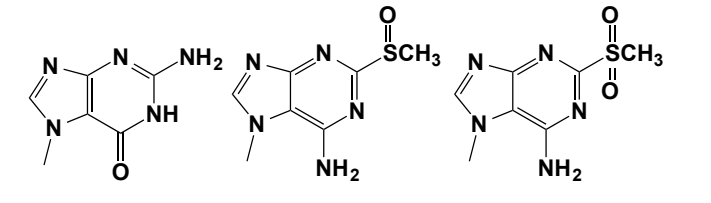
Benzimidazole (Bza) type



Nucleobase type

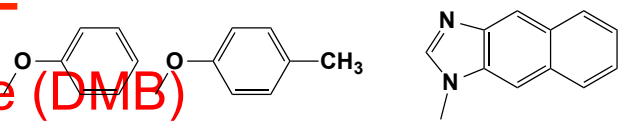


Lower base

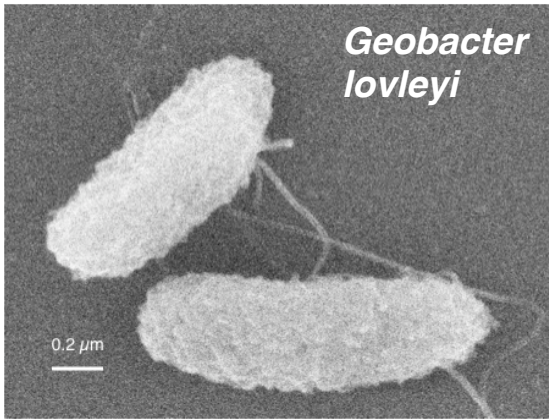
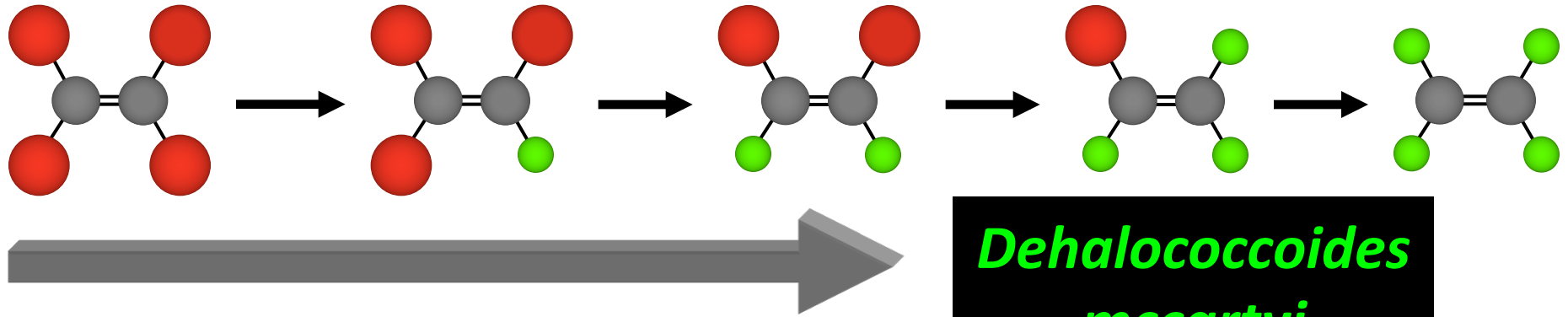


Phenol type

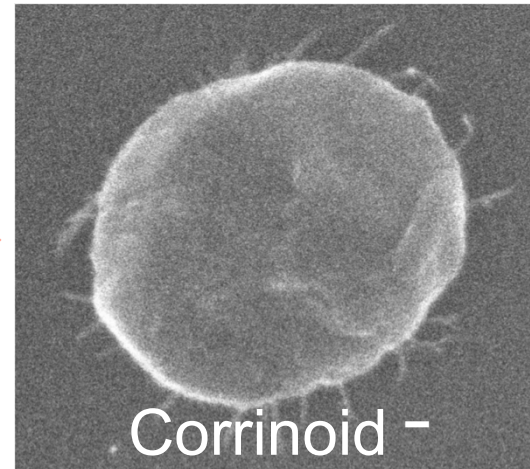
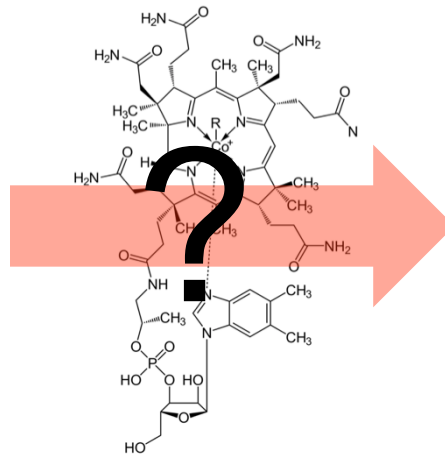
Naphthimidazole



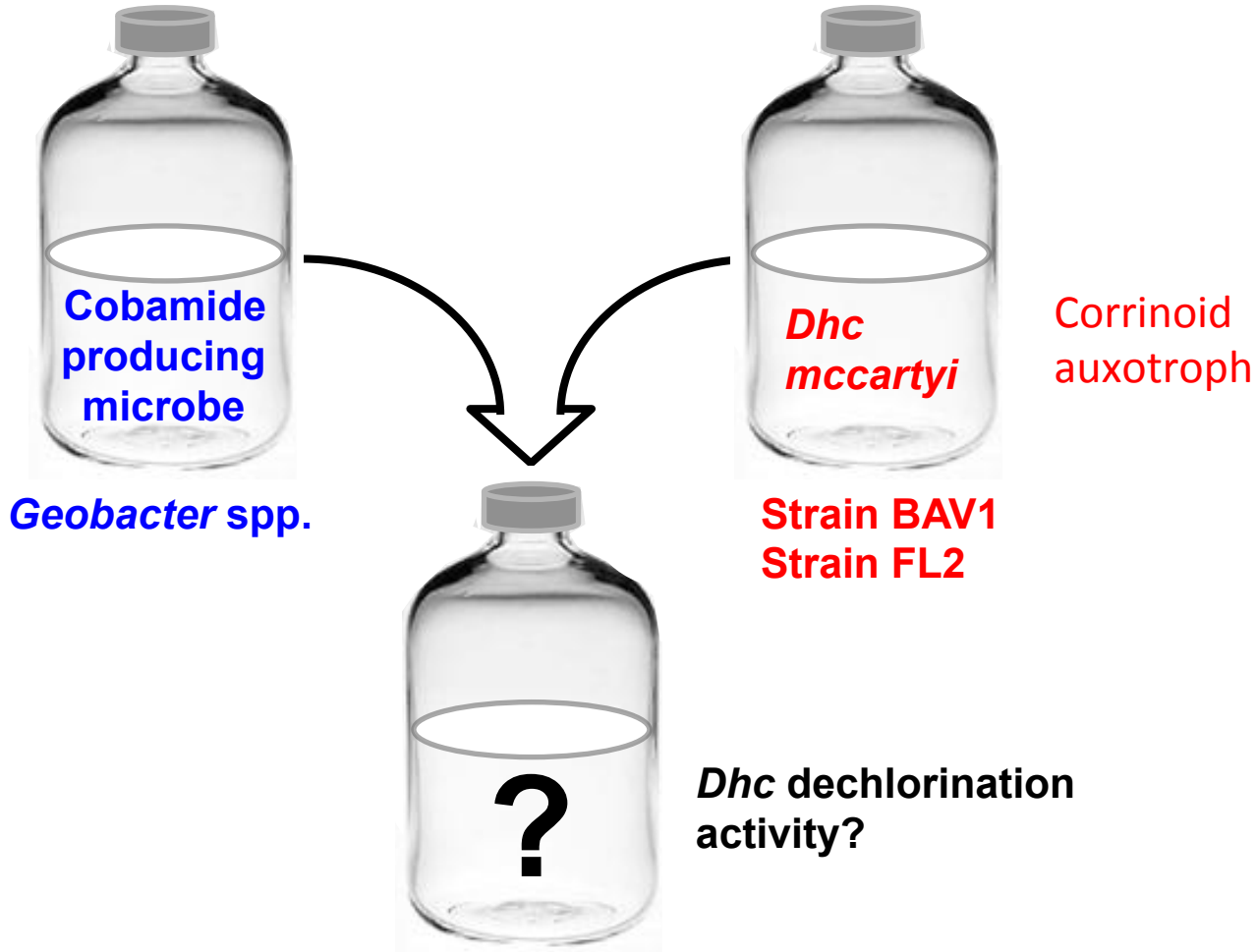
Who Supplies the Corrinoid?



Corrinoid +



Co-Culture Experiments: Corrinoid Producer / *Dhc mccartyi*



Summary of Co-Culture Experiments

Co-Cultures		<i>Dhc</i> Growth
Corrinoid Producer	<i>Dhc</i> Strains	
<i>Geobacter lovleyi</i>	BAV1, FL2	+



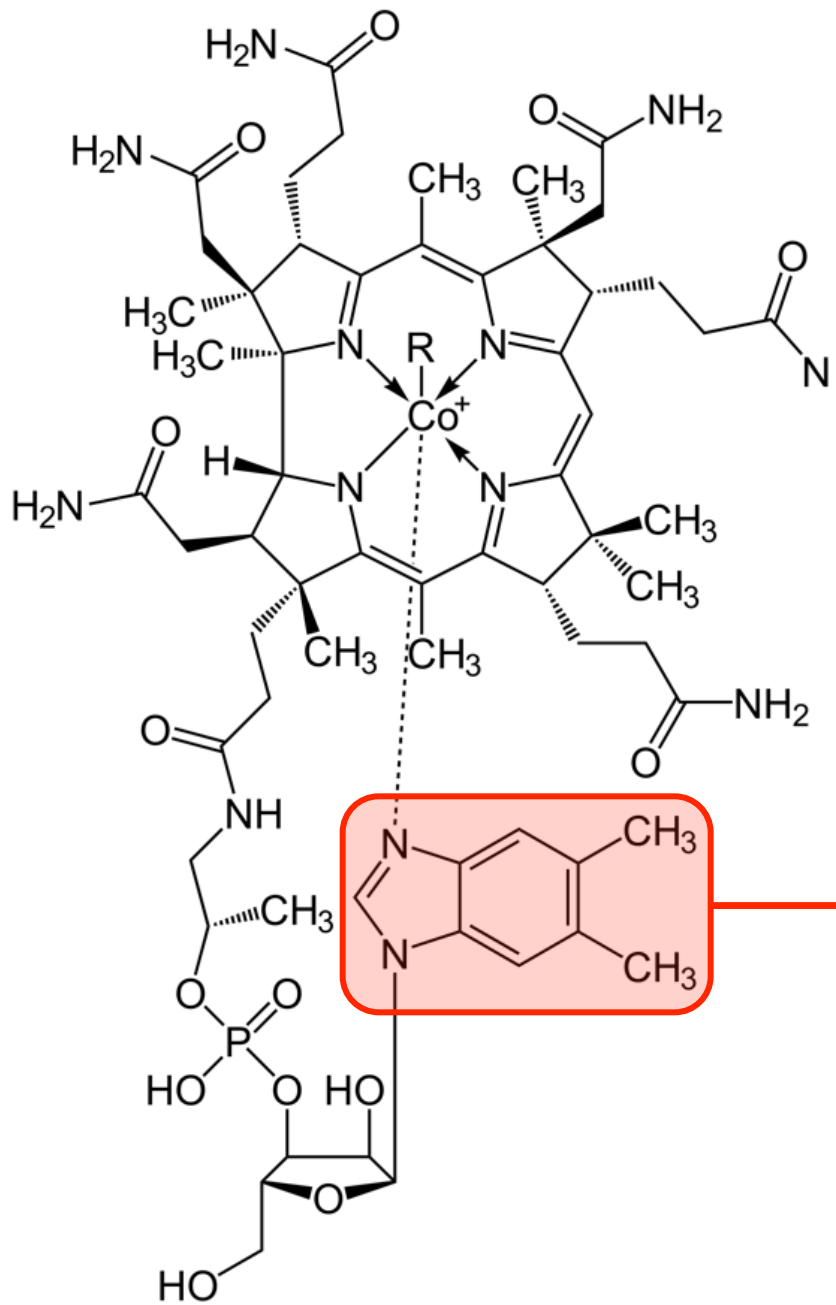
Summary of Co-Culture Experiments

Co-Cultures		<i>Dhc</i> Growth	<i>Dhc</i> Growth with DMB
Corrinoid Producer	<i>Dhc</i> Strains		
<i>Geobacter lovleyi</i>	BAV1, FL2	+	+
<i>Geobacter sulfurreducens</i>	BAV1, FL2	-	+
<i>Sporomusa</i> sp.	BAV1, FL2, GT	-	+
<i>Acetobacterium</i> sp.	BAV1, FL2	-	+
<i>Clostridium aceticum</i>	BAV1, FL2	-	ND
<i>Methanosarcina barkeri</i>	BAV1, FL2, GT	-	+

Yan et al. 2012. Appl. Environ. Microbiol. 78:6630-6636

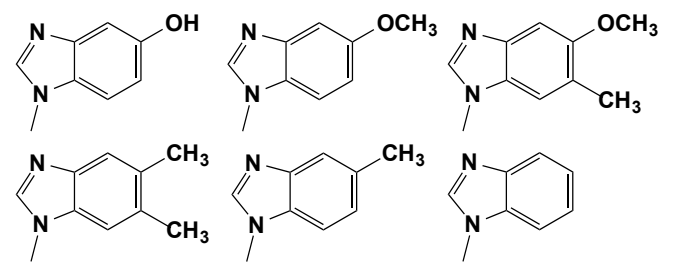
Yan et al. 2013. Phil. Trans. R. Soc. B. 368, 20120320



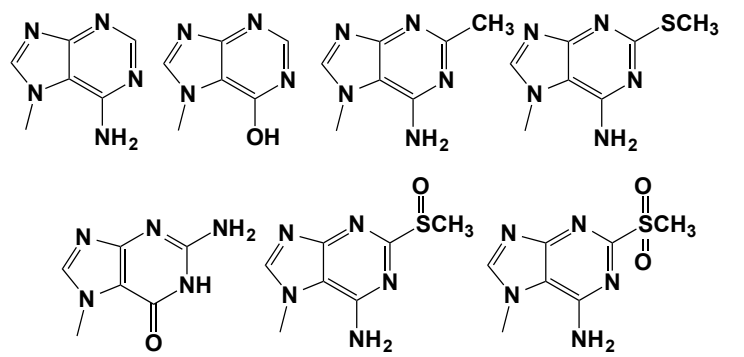


Lower Bases

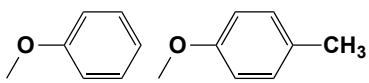
Benzimidazole (Bza) type



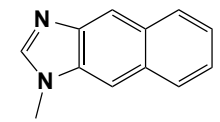
Nucleobase type



Phenol type



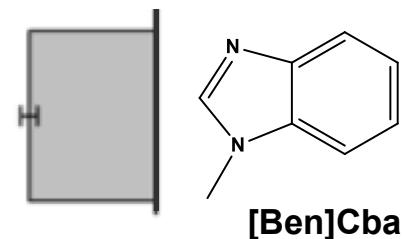
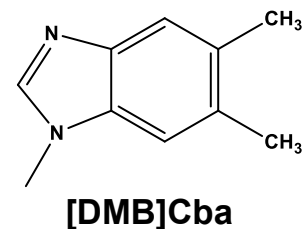
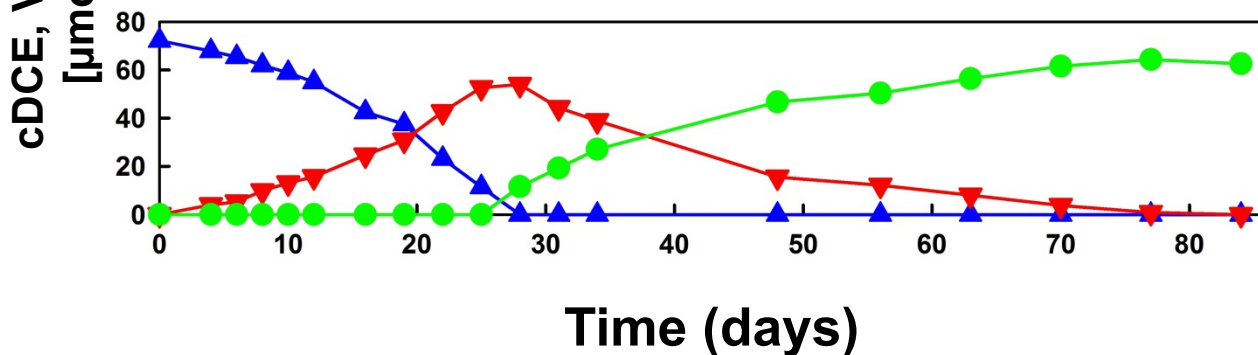
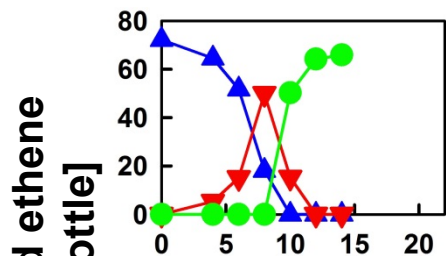
Napththimidazole



Lower Base Affects Dechlorination Rates

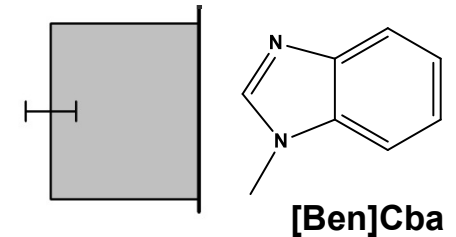
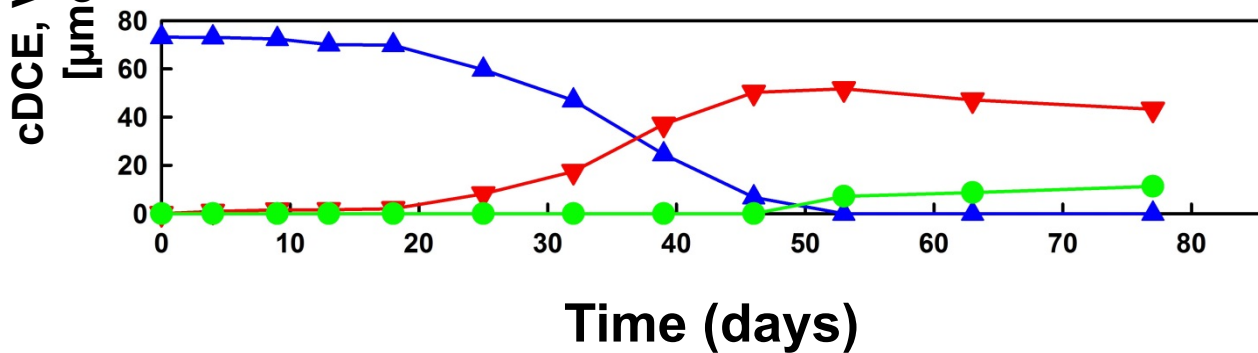
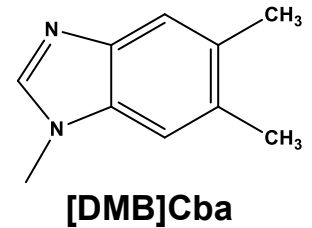
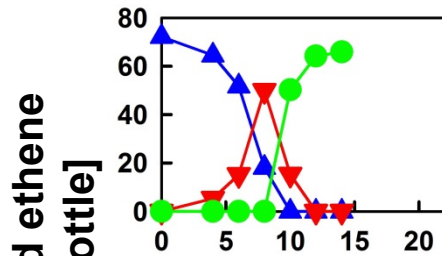
Strain BAV1 (BvcA)

Dechlorination rates [$\text{mmoles Cl}^- \text{L}^{-1} \text{d}^{-1}$]



Lower Base Affects Dechlorination Extent

Strain GT (VcrA)

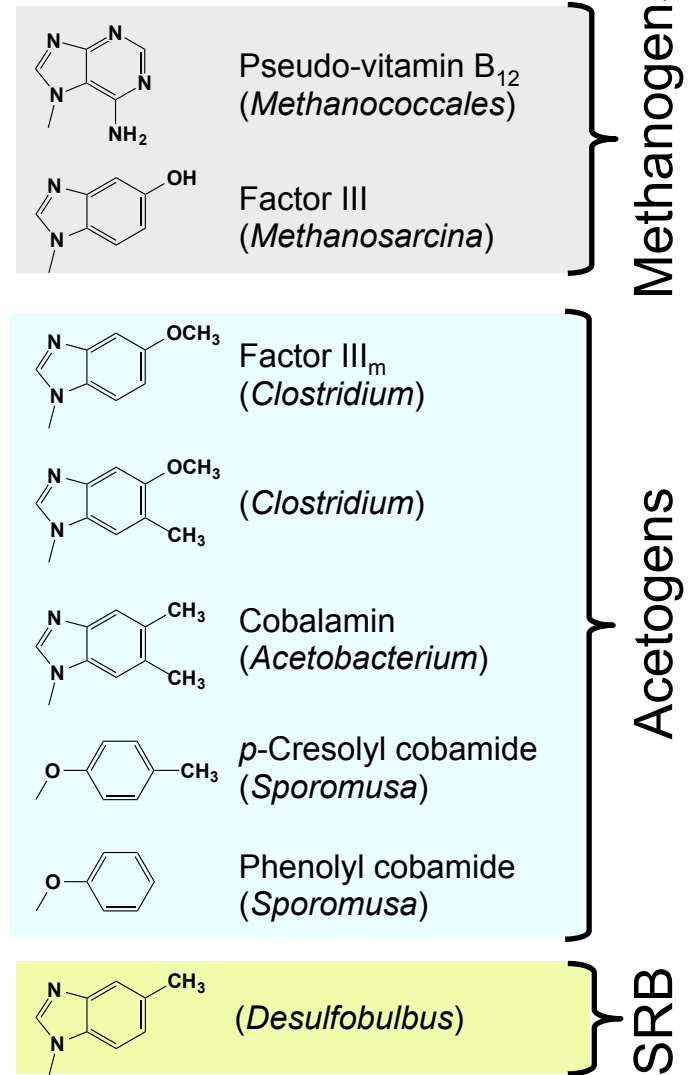
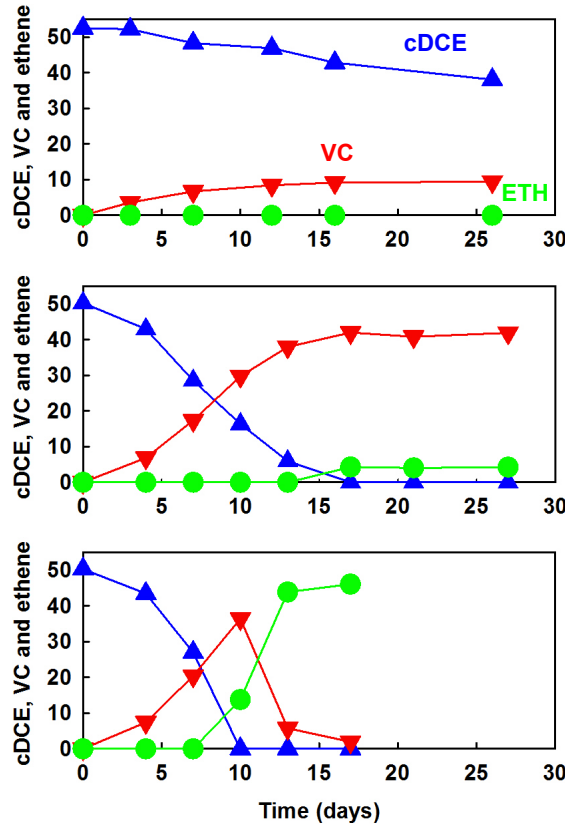


Corrinoid Quantity and Quality Matter

No B₁₂

Limited B₁₂
[1 µg/L]

Sufficient B₁₂
[25 µg/L]



Corrinoid Production Under Different Redox Conditions

Third Creek Site

Knoxville, TN

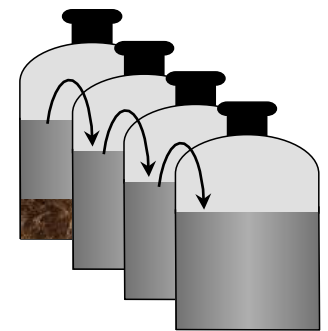
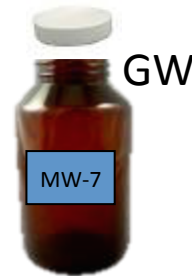
- Metal-manufacturing factory
- Third Creek sediment receiving chlorinated solvents (predominantly TCE) from underlying fractures



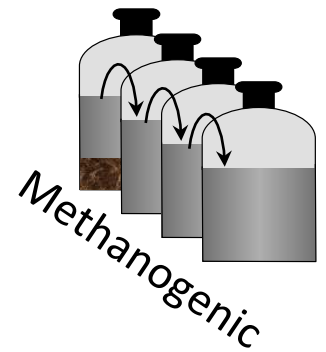
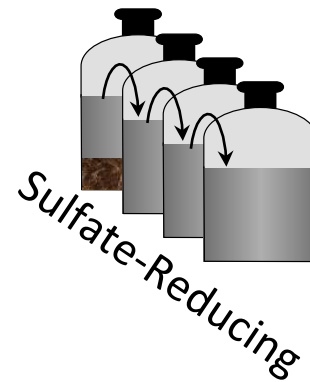
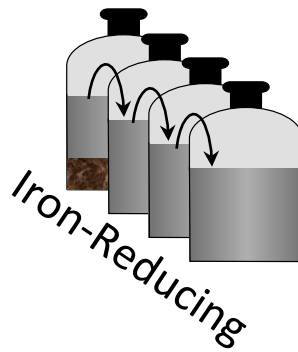
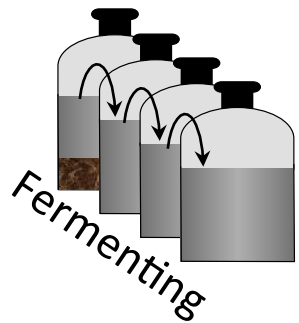
Commerce Street Superfund Site

Williston, Vermont (outside Burlington)

- Multi-tenant industrial park
- Soil and groundwater contaminants include TCE, *cis*-DCE, petroleum hydrocarbons and metals (chromium, cadmium, and nickel)

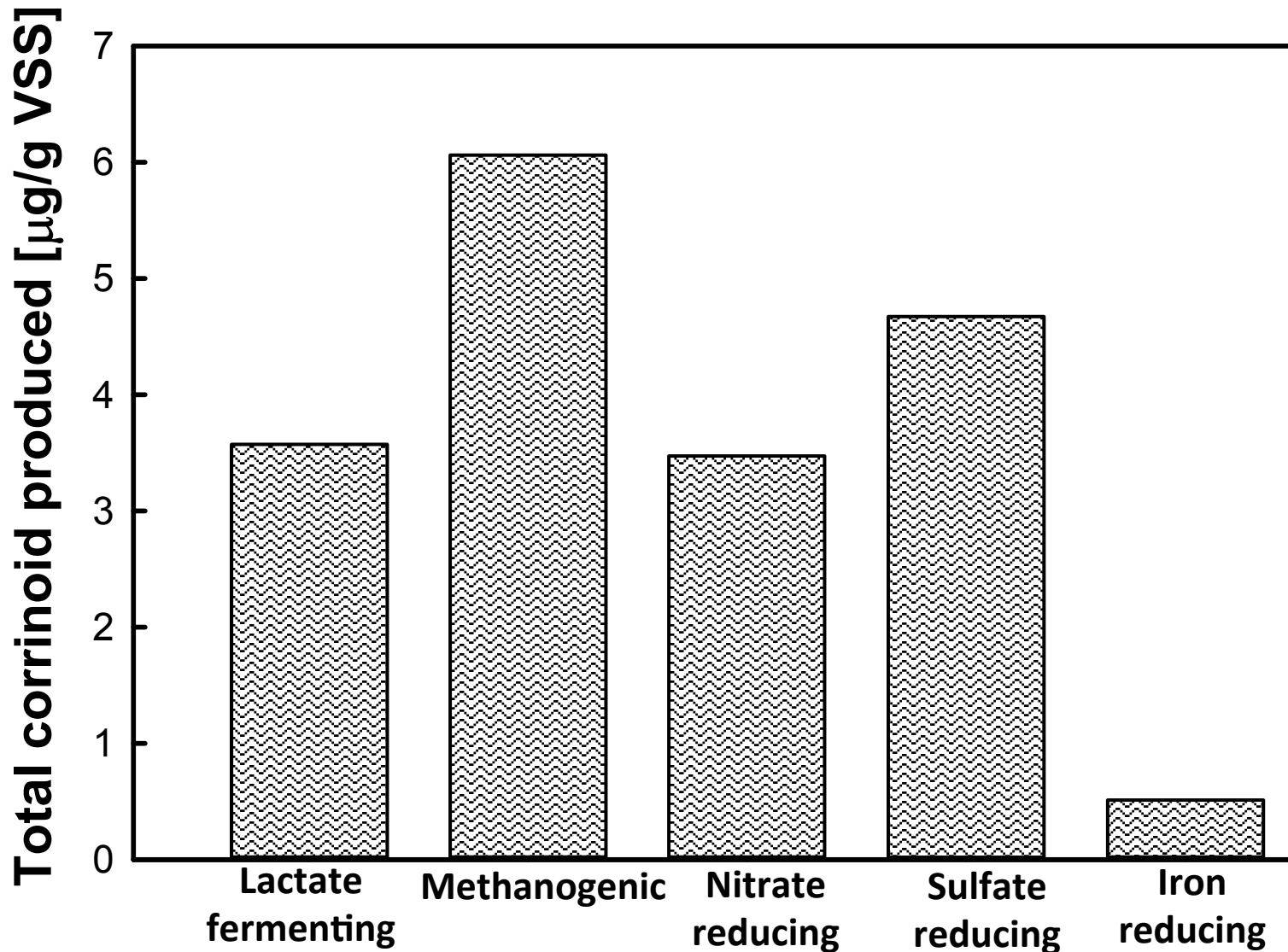


Corrinoid Production Under Different Redox Conditions

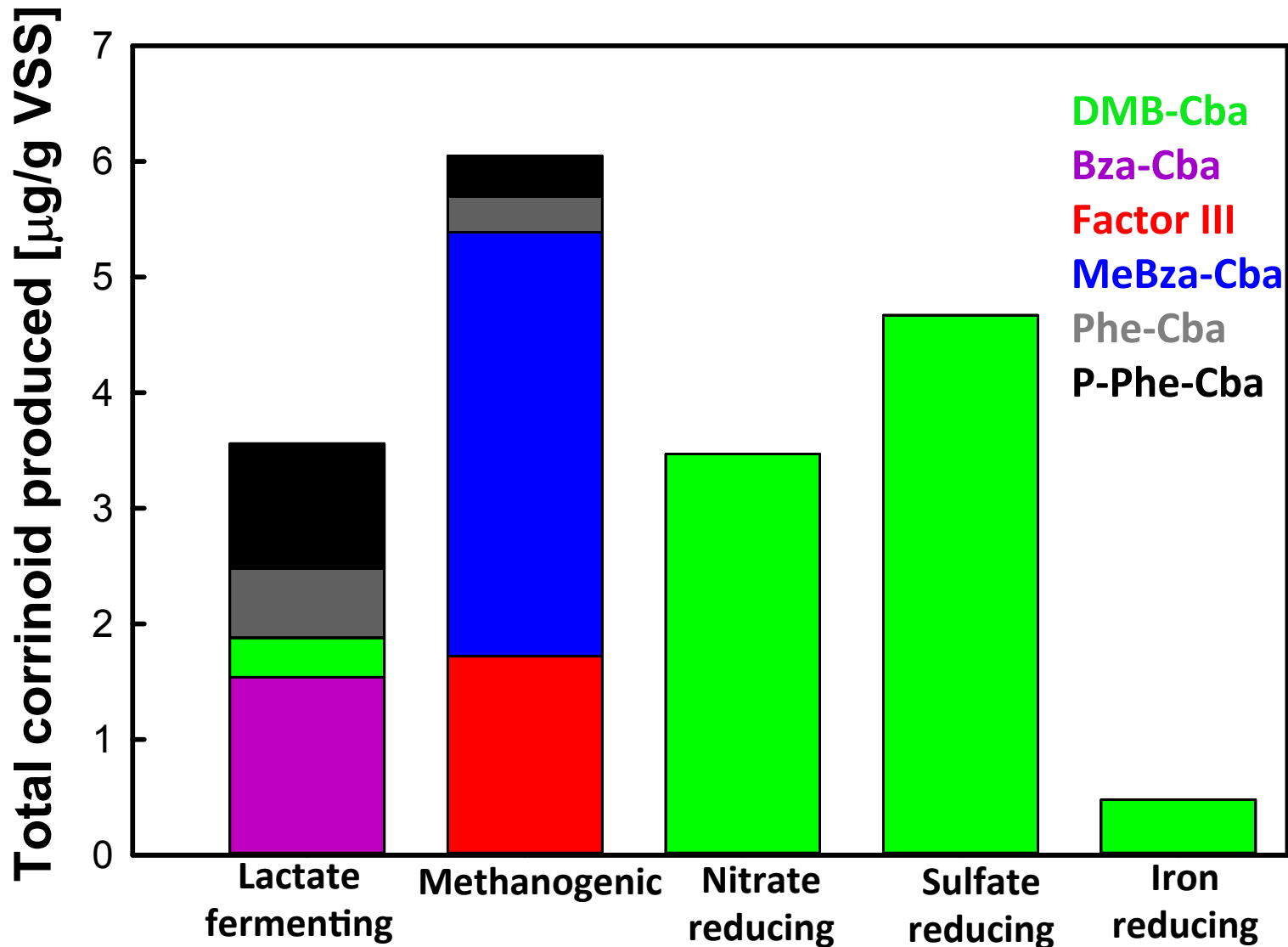


Corrinoid Extraction and Identification

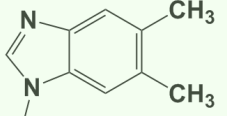
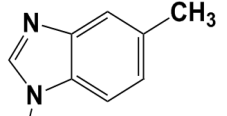
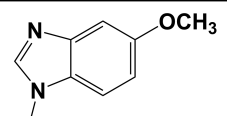
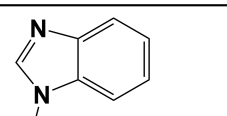
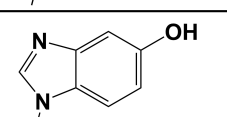
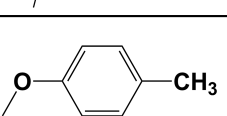
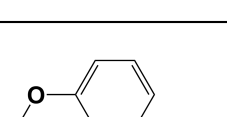
The Redox Condition Affects Corrinoid Quantity



The Redox Condition Affects Corrinoid Quality

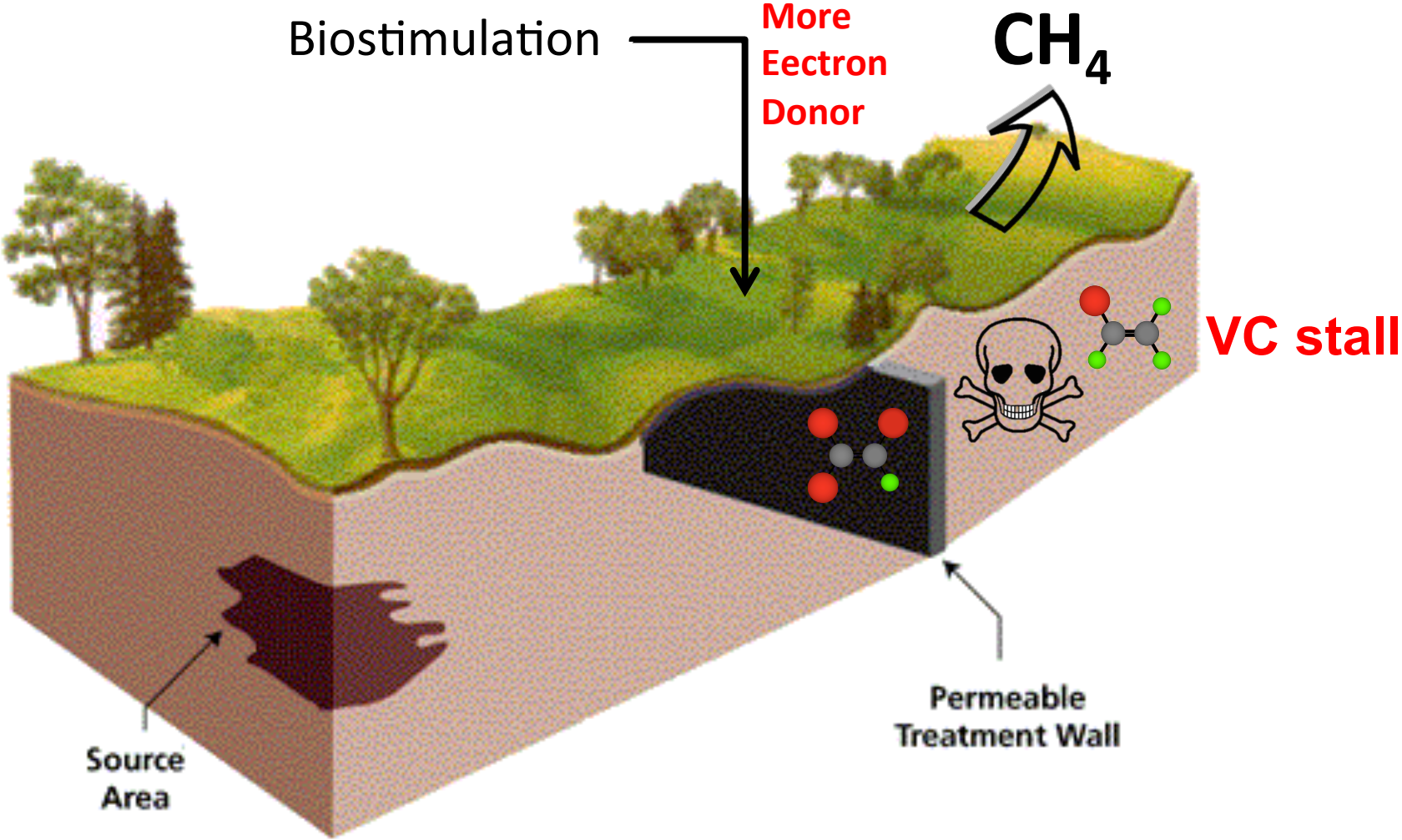


Corrinoids Produced by the Community Under Different Redox Conditions

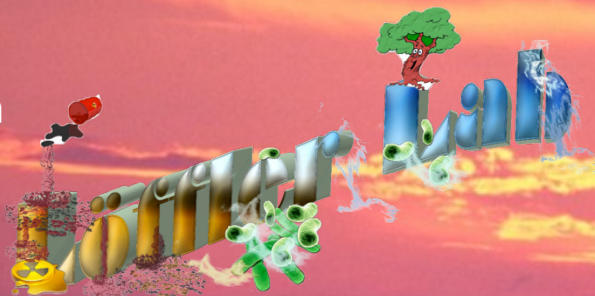
Corrinoids produced	Lactate fermenting	Methanogenic	Iron reducing	Sulfate reducing	Nitrate reducing
	+	-	+	+	+
	-	+	-	-	-
	-	-	-	-	+
	+	-	-	-	-
	-	+	-	-	-
	+	+	-	-	-
	+	+	-	-	-




Plume Treatment - Bioreactive Barriers



Team Dechlorination



Dr. Jun Yan
Dr. Fadime Kara Murdoch
Dr. Devrim Kaya
Yongchao Yin
Yongchao Xie
Steve Higgins
Cindy Swift
Burcu Şimşir, Meng Bi, Yi Yang,

Microbial Insights, Inc. 
Dora Ogles-Taggart, Brett Baldwin

UTK Chemistry
Shawn Campagna

Tufts University
Natalie Capiro, Kurt Pennell

Oak Ridge National Laboratory
Bob Hettich

University of Toronto
Elizabeth Edwards

Georgia Tech
Kostas Konstantinidis

 National Institute of Environmental Health Sciences
Your Environment. Your Health.

 **SERDP**
DOD • EPA • DOE

ESTCP
Environmental Security
Technology Certification Program


 **OAK
RIDGE**
National Laboratory



