THE UNIVERSITY of TENNESSEE KNOXVILLE

Biogeochemical Controls Over Organohalide-Respiring Chloroflexi

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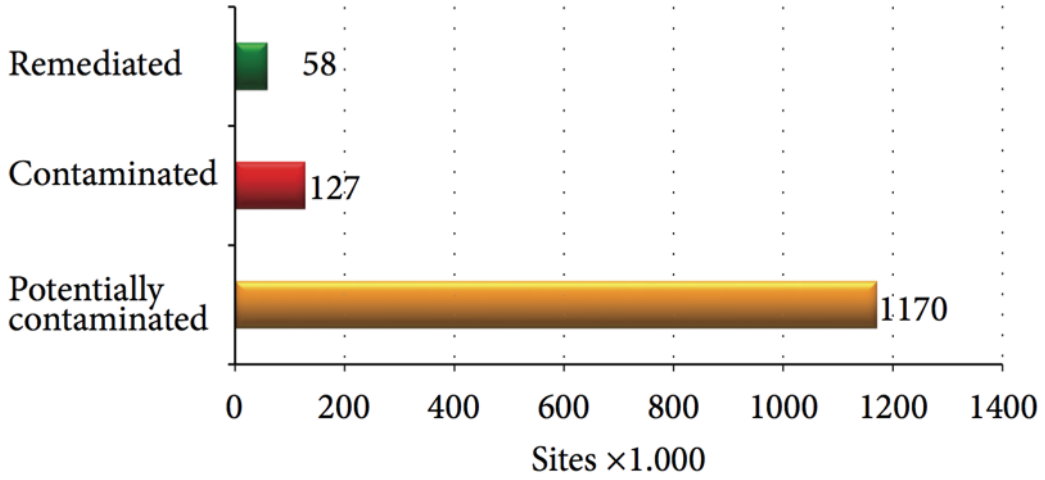
Contaminated Sites in the U.S.

- 1,322 Superfund sites
- 3,747 RCRA sites
- >450,000 Brownfields

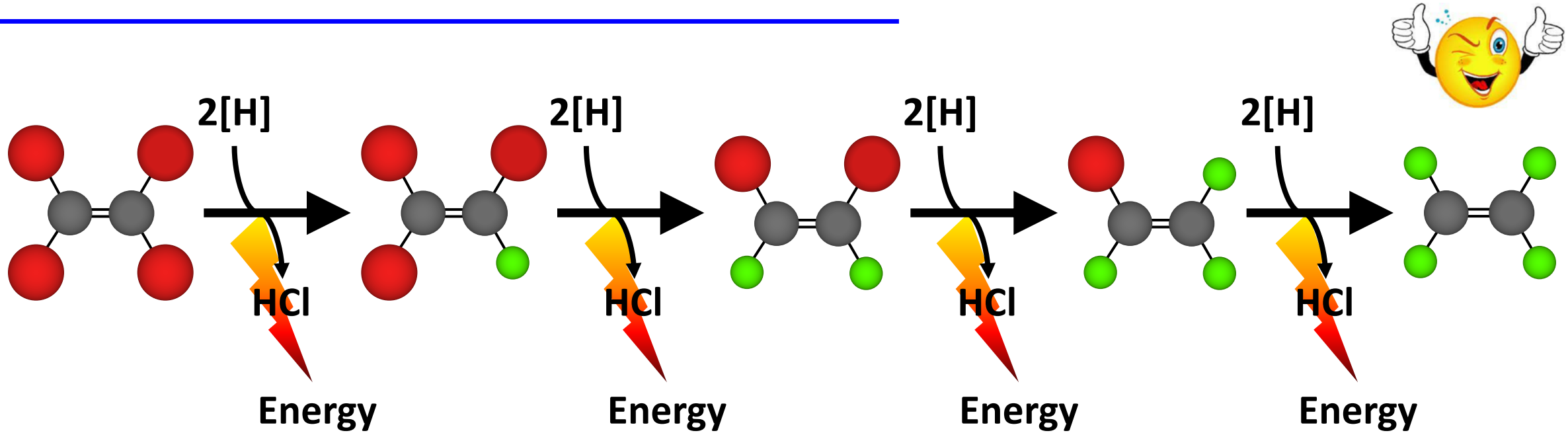
166 Million People Live Within **3 Miles** of a Superfund or a RCRA Corrective Action Site, Equal to **53% of the U.S. Population** Including **55% of all Children under Age 5**

… and in Europe

Majority of sites impacted with chlorinated compounds



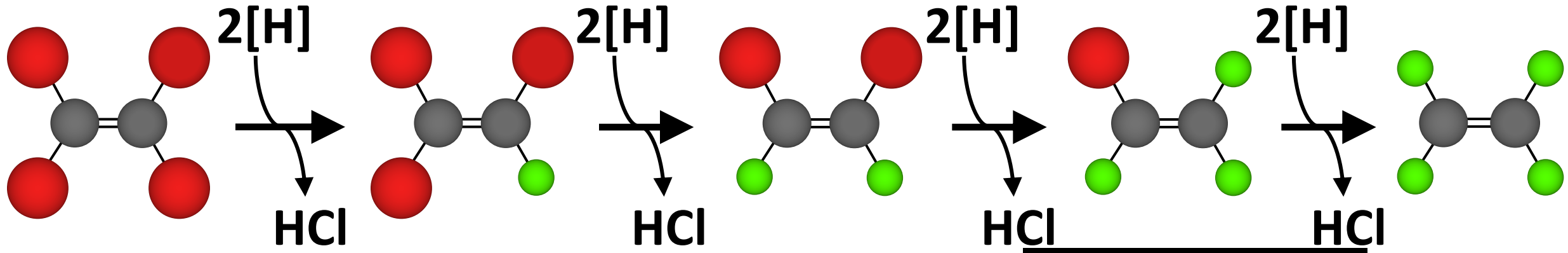
Reductive Dechlorination: A Process that Leads to Contaminant Detoxification *In Situ*



Organohalide Respiration

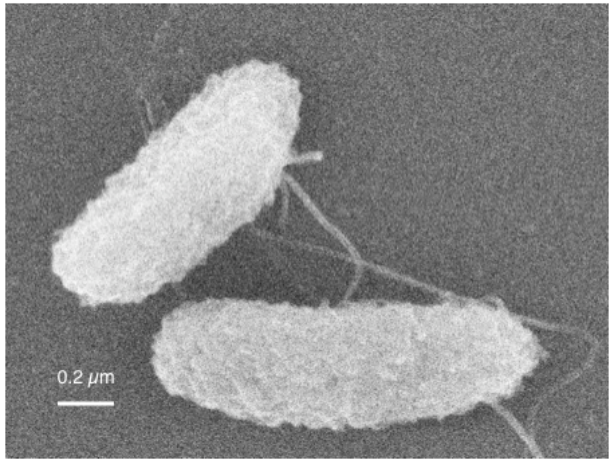
Freedman, D. L., and J. M. Gossett. 1989. Appl. Environ. Microbiol. 55:2144-2151
He et al. 2003. Nature. 424:62-65

Populations Involved in Reductive Dechlorination of CEs

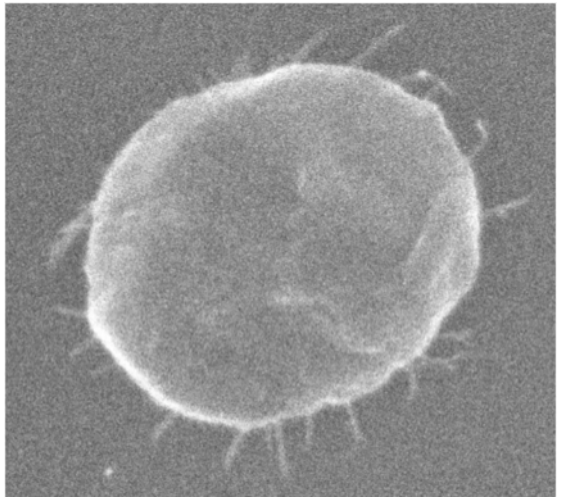


Geobacter lovleyi, Dehalobacter, Sulfurospirillum, Desulfuromonas, Desulfitobacterium

Dehalococcoides mccartyi

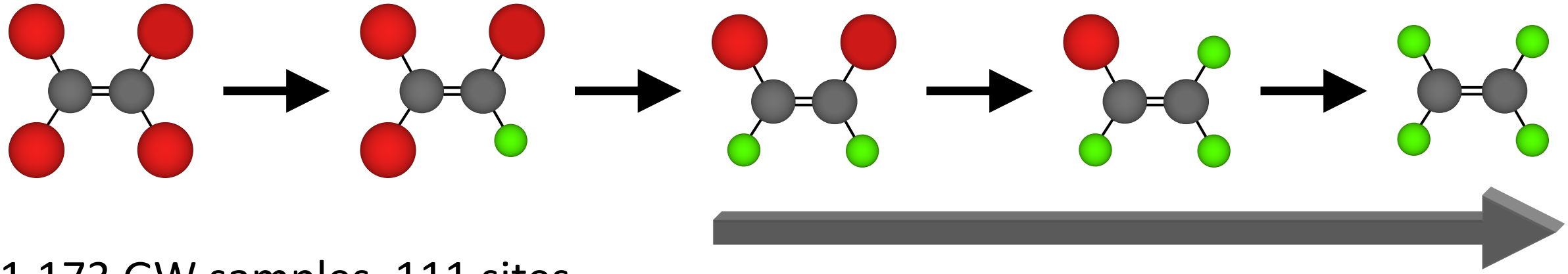


Sung et al. 2006
AEM, 72:2775



Löffler et al. 2013
IJSEM, 63:625

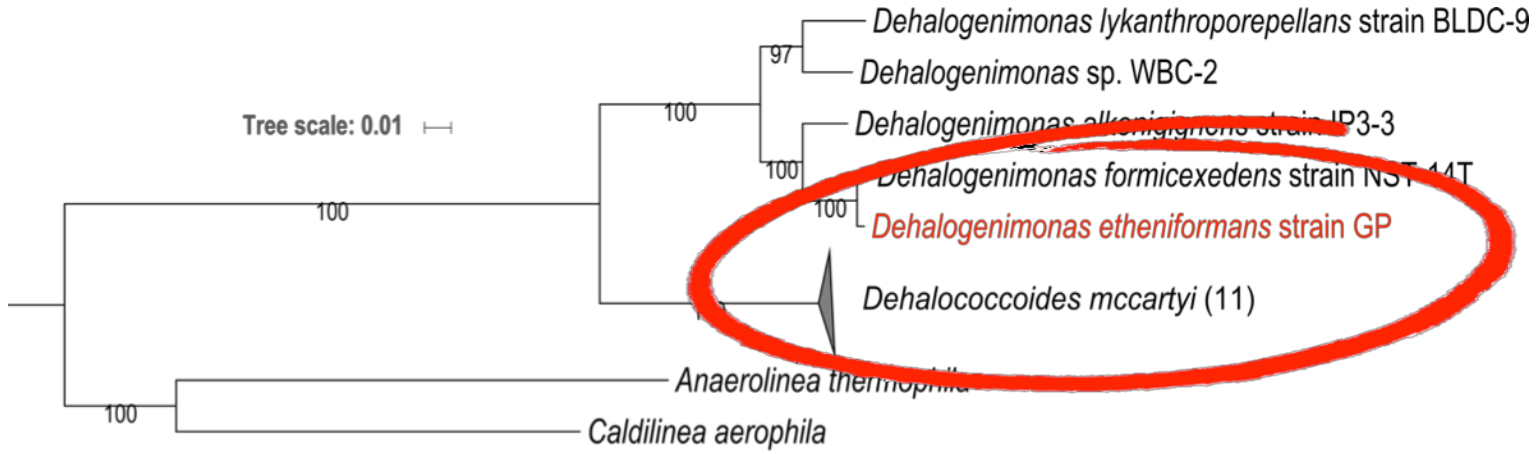
Populations Involved in Reductive Dechlorination of Chlorinated Ethenes



1,173 GW samples, 111 sites

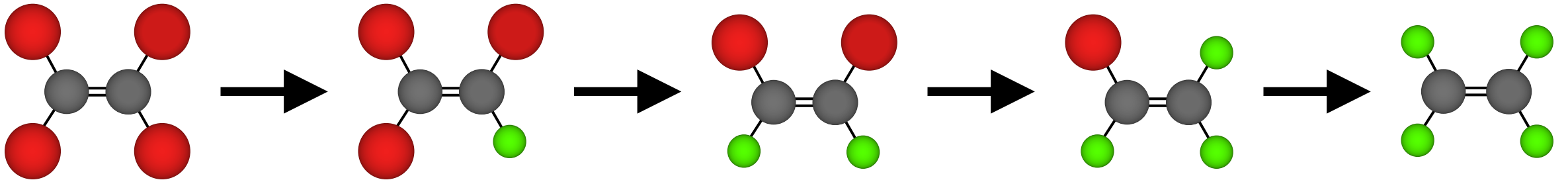
849 samples: *Dhc* & *Dhgm*

65%: *Dhgm* outnumber *Dhc*



Yang et al. ISME J. 2017. 11:2767-2780

Populations Involved in Reductive Dechlorination of Chlorinated Ethenes



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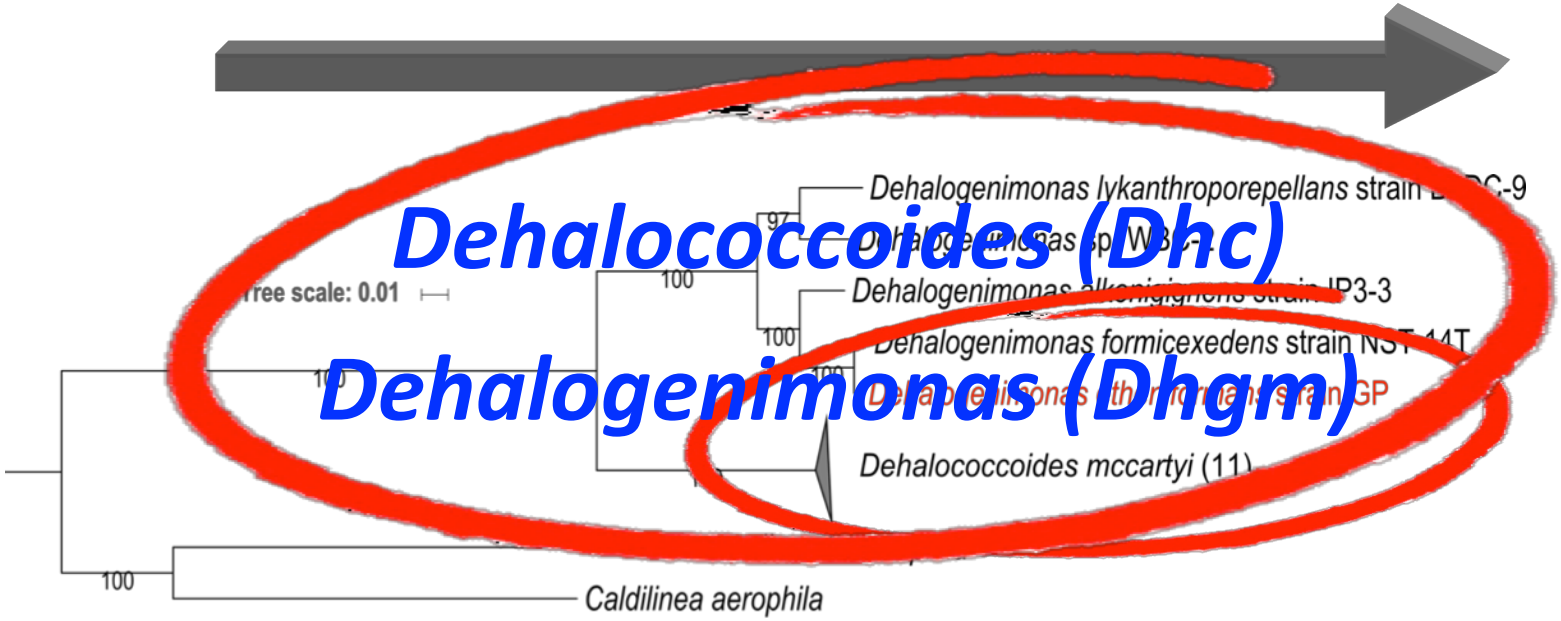
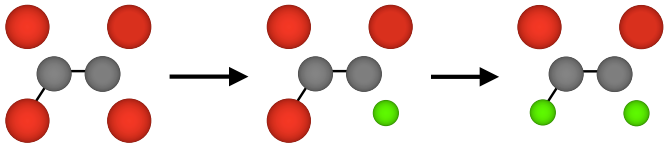


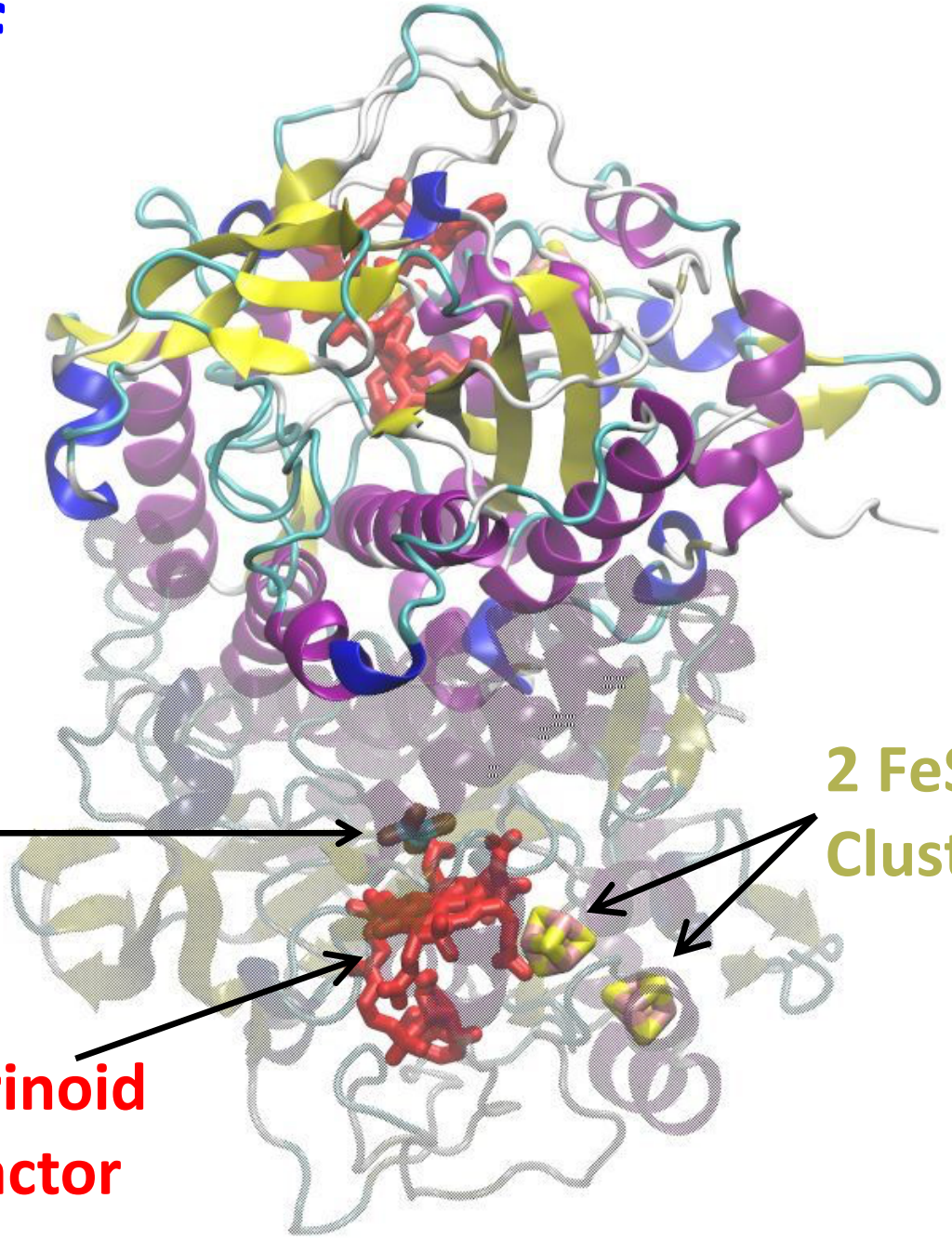
Yang et al. ISME J. 2017. 11:2767-2780

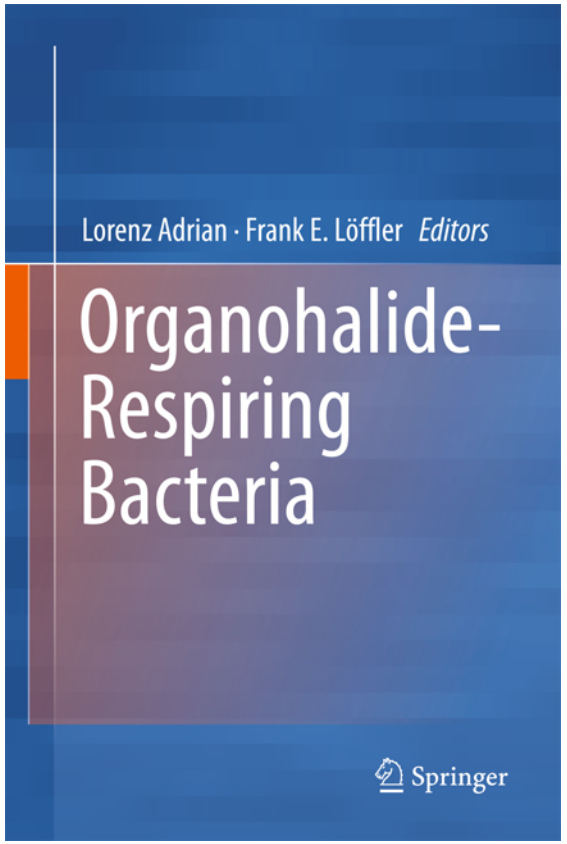
Structural Basis of Organohalide Respiration

Dimer of PceA

Sulfurospirillum multivorans

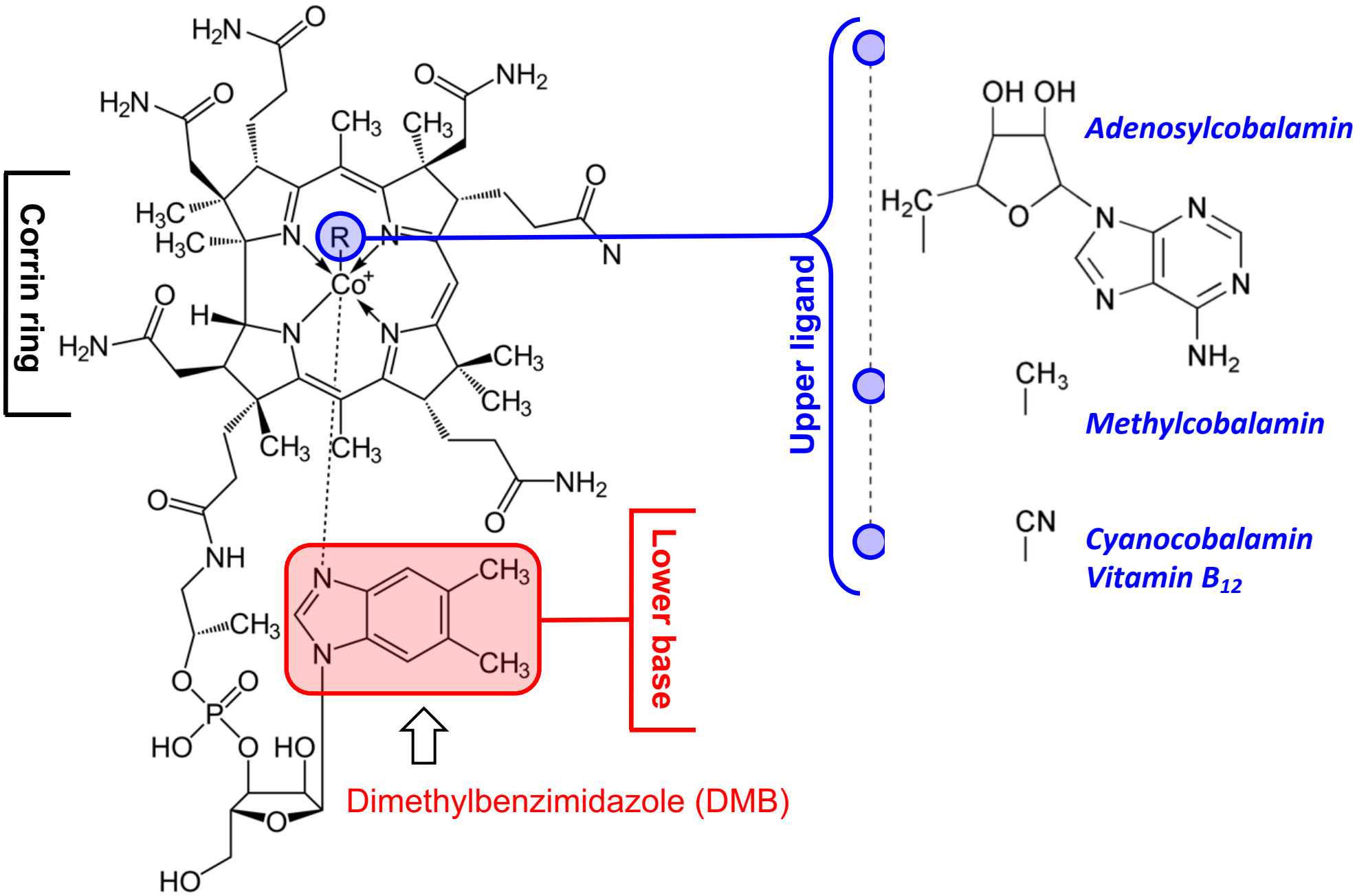




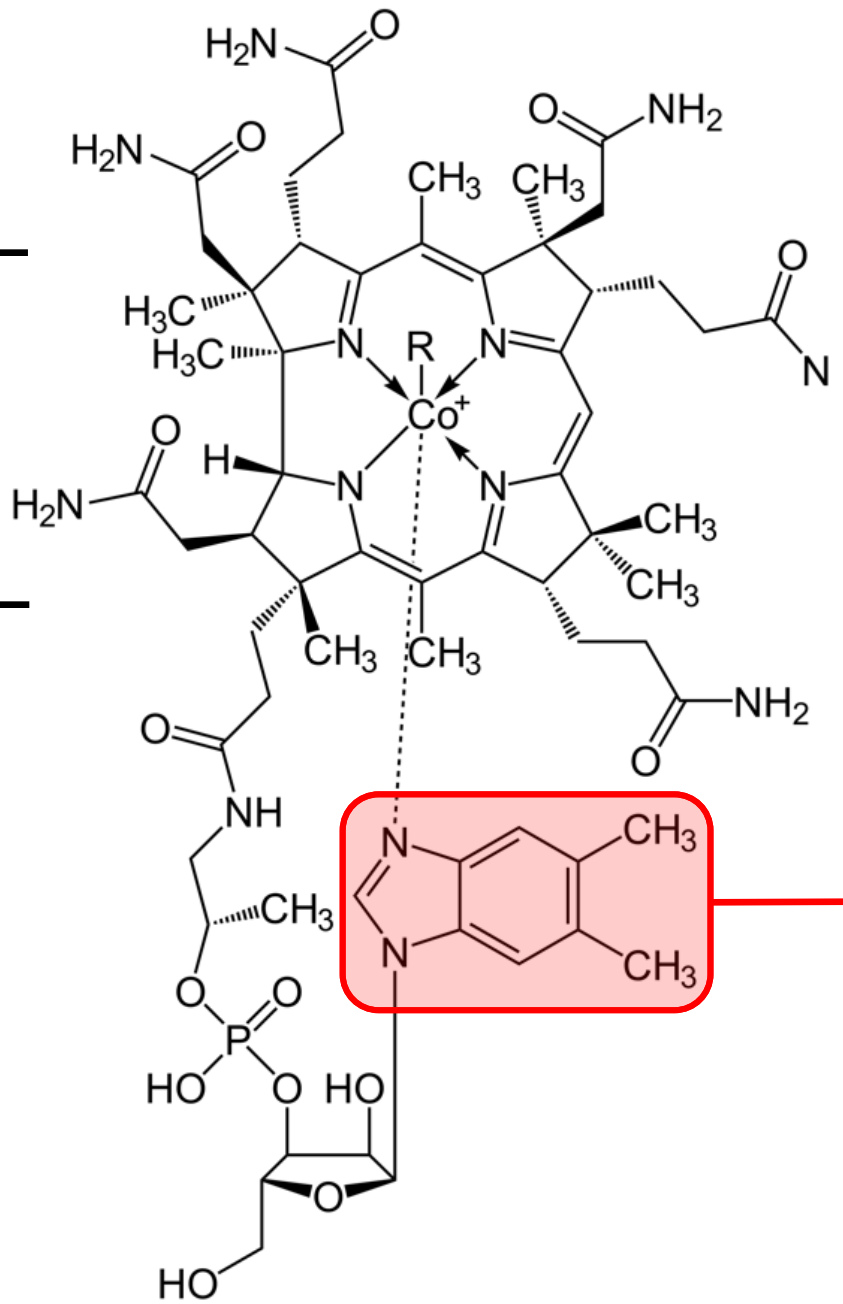


Bommer et al. 2014. Science, 346:455

Payne et al. 2015. Nature, 517:513

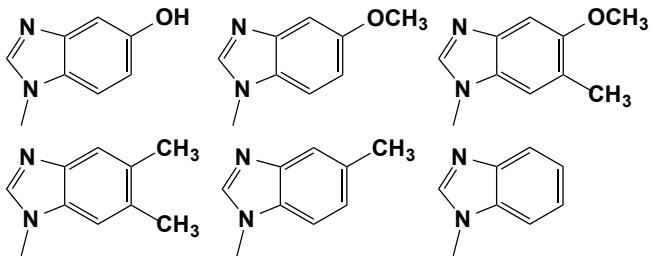

Corrin ring
Upper ligand
Lower base
R
Co+
Adenosylcobalamin
Methylcobalamin
Cyanocobalamin
Vitamin B12
Dimethylbenzimidazole (DMB)

Corrin ring

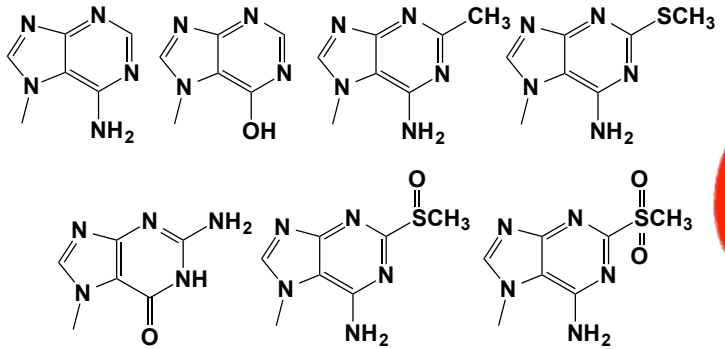


Lower Bases

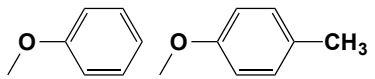
Benzimidazole (Bza) type



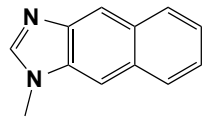
Nucleobase type

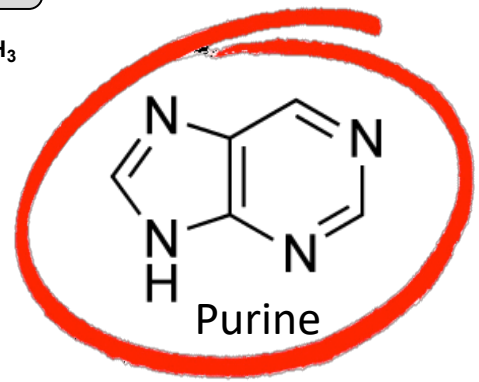


Phenol type



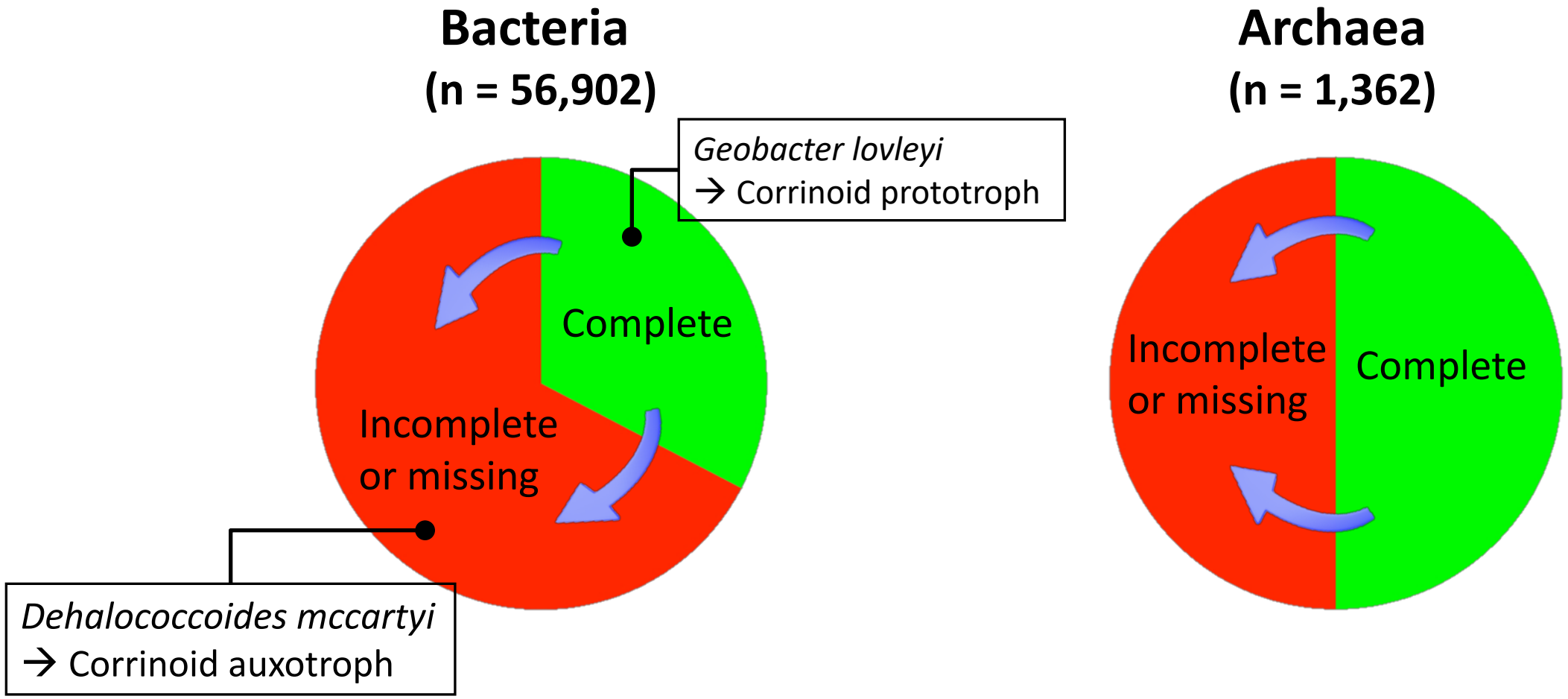
Naphthimidazole





Yan et al. 2018. Nat. Chem. Biol. 14:8-14.

De novo Biosynthesis of Corrinoids



Dhc & *Dhgm*: Strict Requirement for Corrinoid

Dhc strain BAV1

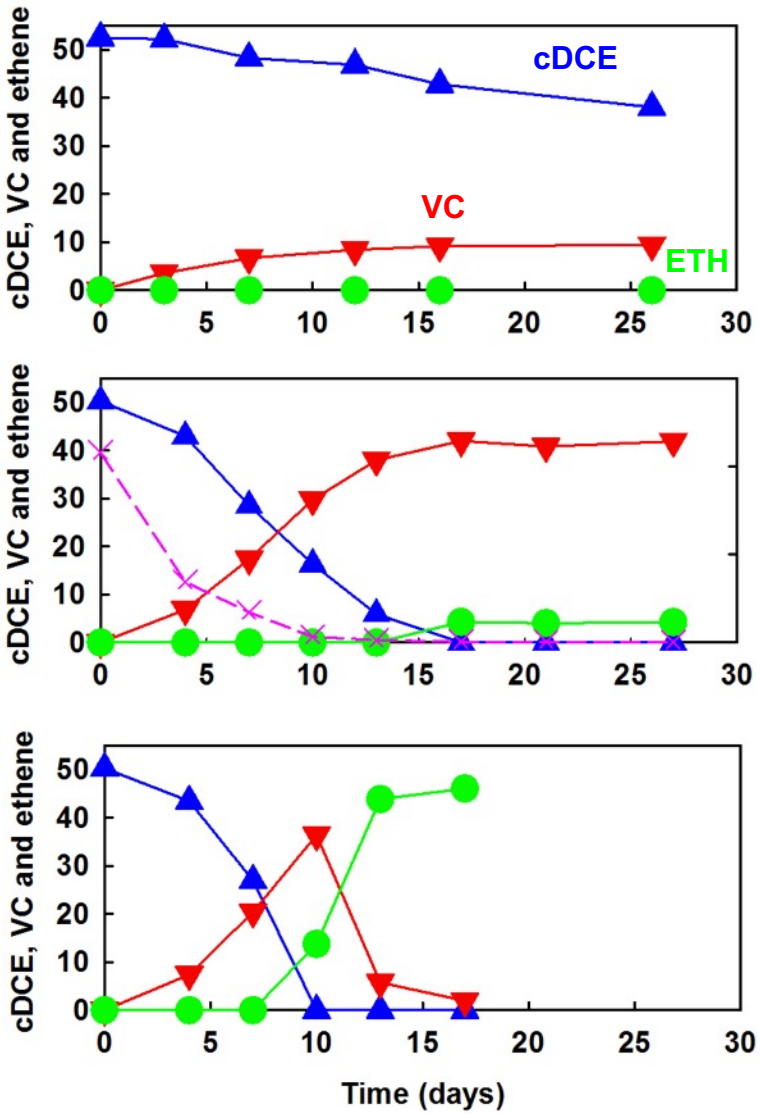
No B_{12}

cDCE

VC

Limited B_{12} [1 µg/L]

Sufficient B_{12} [25 µg/L]



No dechlorination

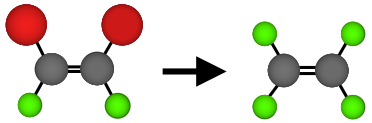
VC stall

Complete Dechlorination (Detoxification)

Specific Aims

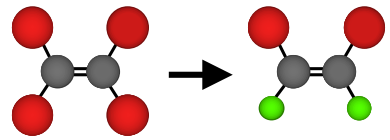
- **Aim 1:** Explore the specific cobamide requirements of organohalide-respiring *Dhc* relevant for detoxification of chlorinated ethenes
- **Aim 2:** Demonstrate that geochemical conditions affect the specific cobamide pool, and hence *Dhc* activity
- **Aim 3:** Identify community and *Dhc* biomarkers that indicate when cobamide and/or lower base bioavailability limit *Dhc* reductive dechlorination activity

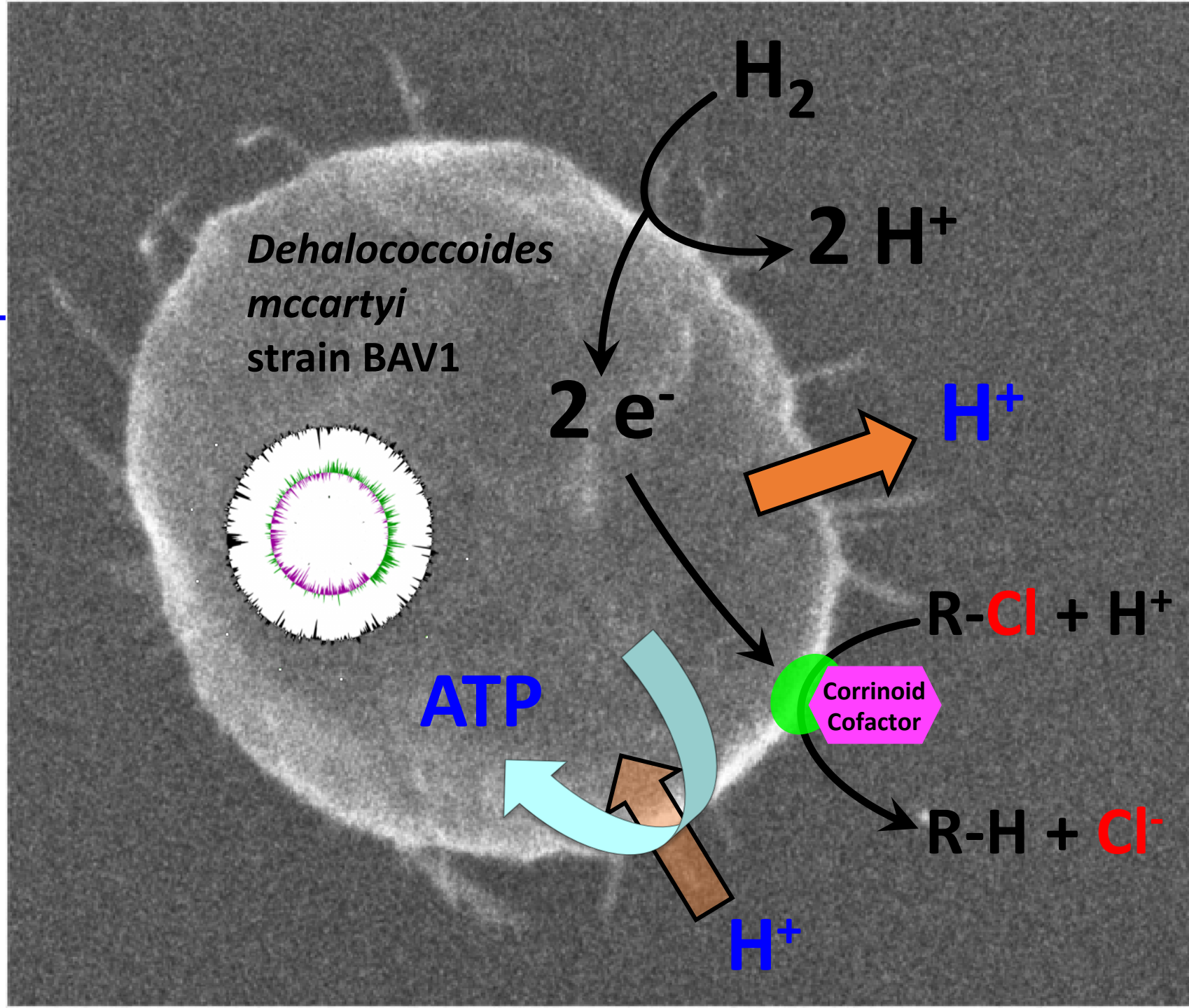
Simplified Model of Organohalide Respiration



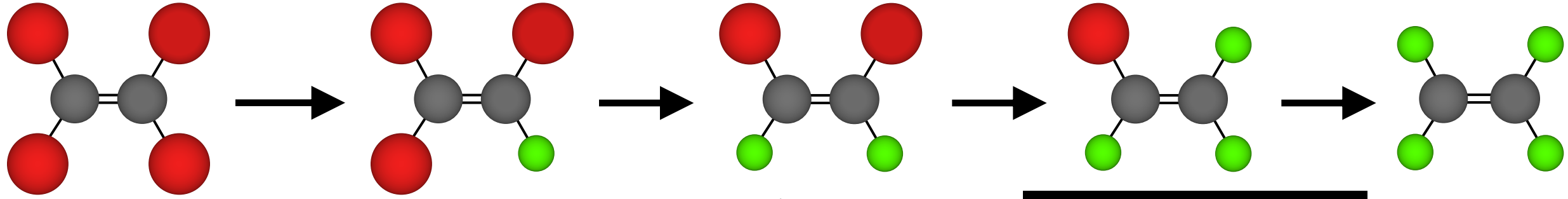
Dehalococcoides mccartyi
→ Corrinoid auxotroph

Geobacter lovleyi
→ Corrinoid prototroph

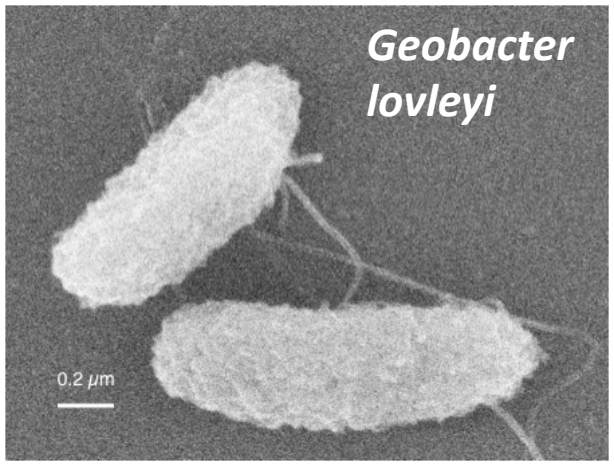




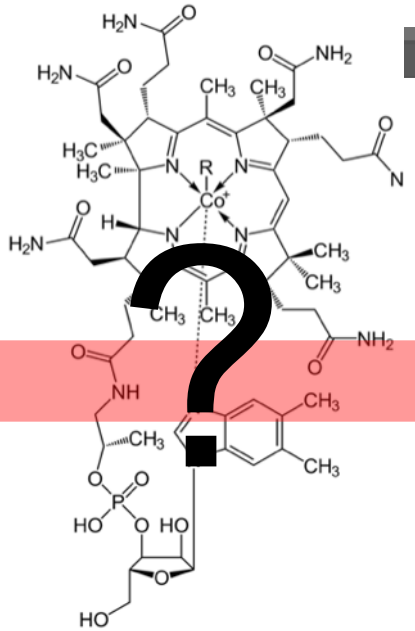
Who Supplies Corrinoid to *Dhc* and *Dhgm*?

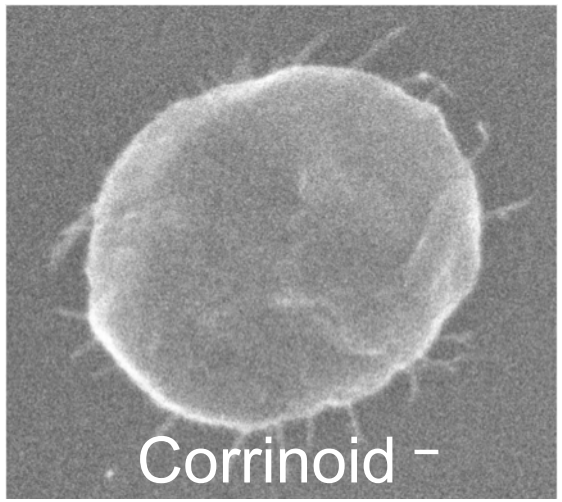


Dehalococcoides mccartyi

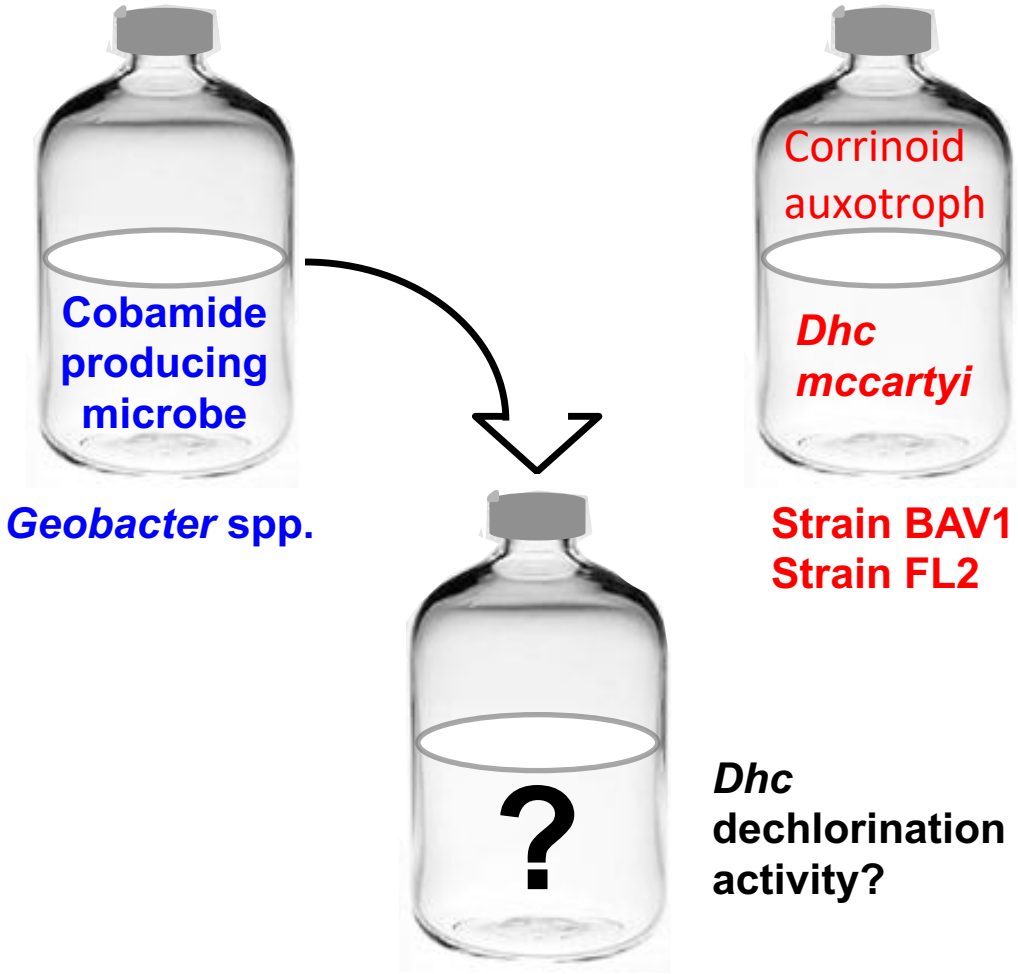


Corrinoid $^{+}$





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



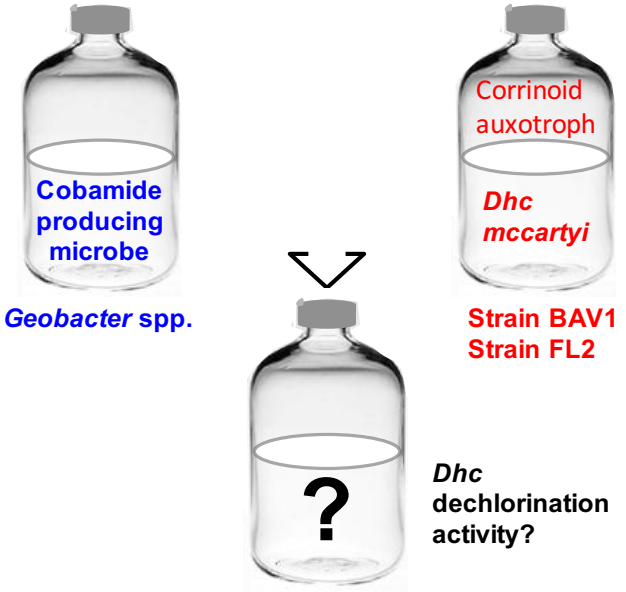
***Geobacter* spp.**

Strain BAV1
Strain FL2

***Dhc* dechlorination activity?**

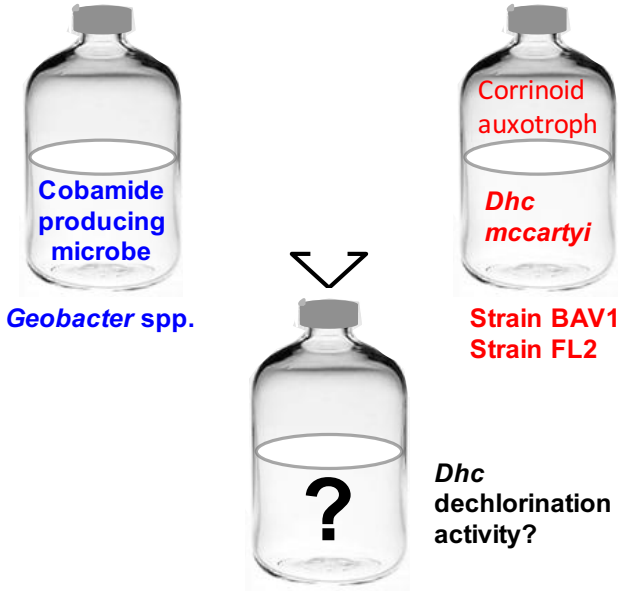
Summary of Co-Culture Experiments

Co-Cultures	*Dhc* Strains	*Dhc* Growth
	BAV1, FL2	+



Summary of Co-Culture Experiments

Co-Cultures		*Dhc* Growth
Corrinoid Producer	*Dhc* Strains	
	BAV1, FL2	+
	BAV1, FL2	-
	BAV1, FL2, GT	-
	BAV1, FL2	-
	BAV1, FL2	-
	BAV1, FL2, GT	-

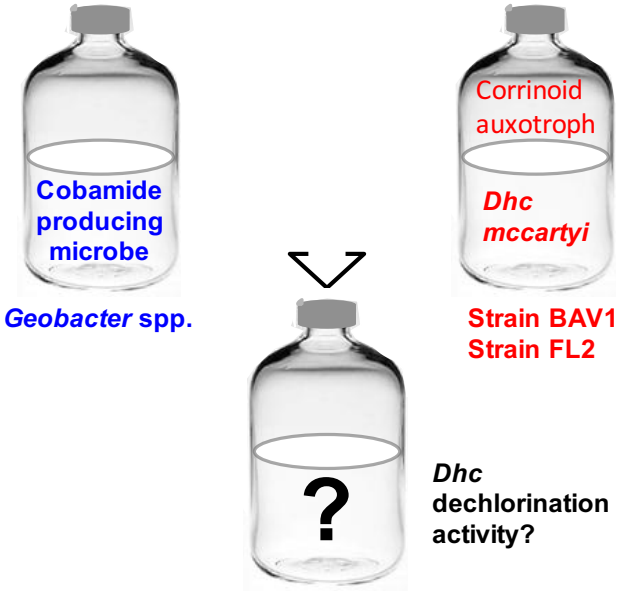


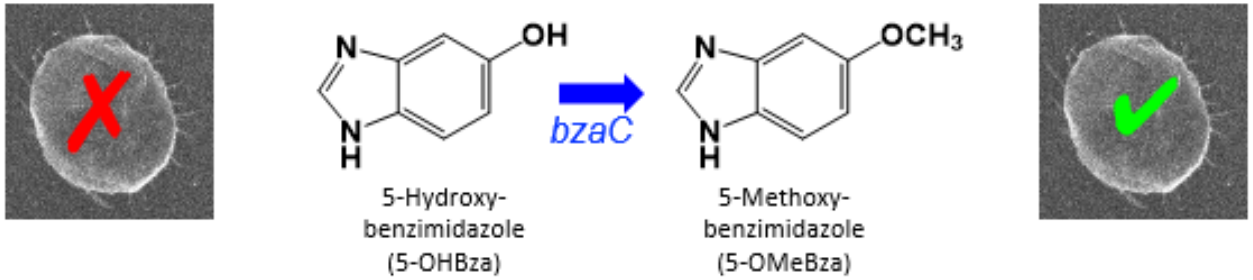
Yan et al. 2012. Appl. Environ. Microbiol. 78:6630-6636

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

Co-Culture Experiments

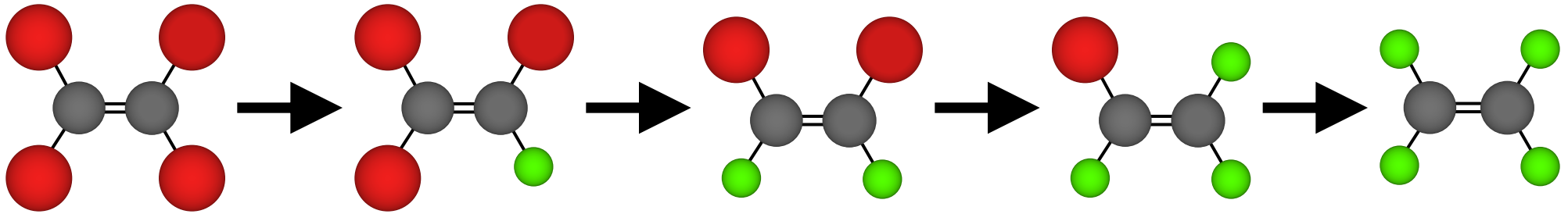
Co-Cultures		*Dhc* Growth
Corrinoid Producer	***Dhc* Strains**	
Geobacter lovleyi	BAV1, FL2	+
Geobacter sulfurreducens Wildtype	BAV1, FL2	-
Geobacter sulfurreducens + pNJ052	BAV1, FL2	+





Jiang et al. 2019. In Preparation

Key *Dhc* RDases



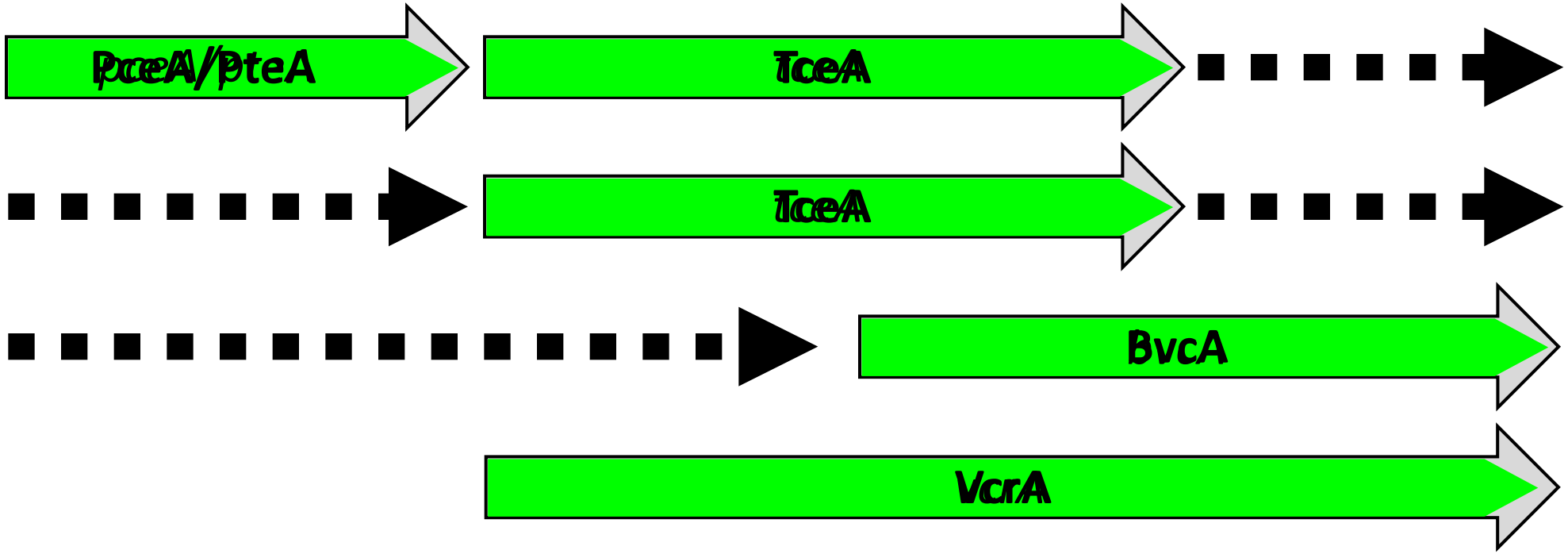
Dhc str. 195

Dhc str. FL2

Dhc str. BAV1

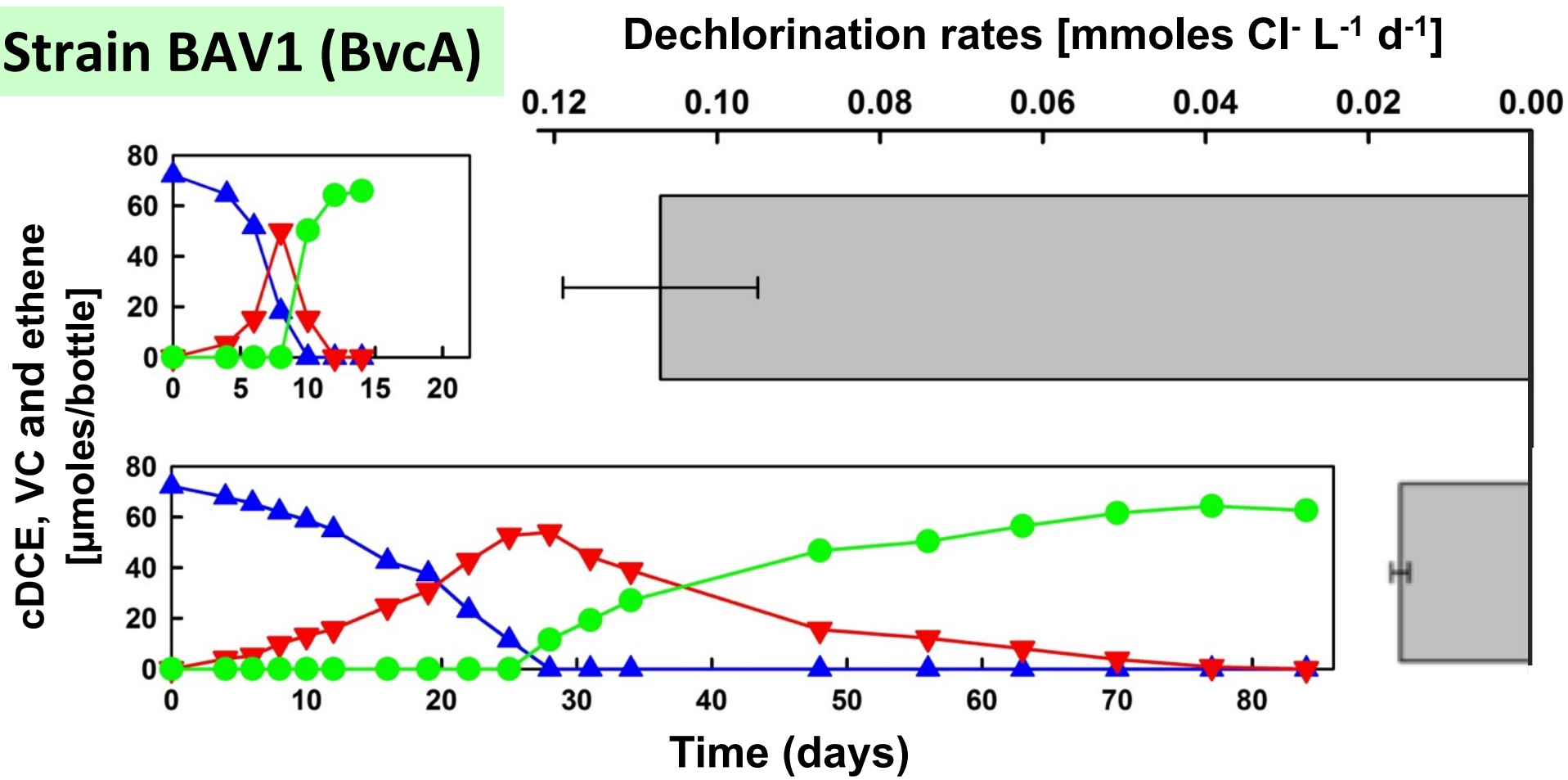
Dhc str. VS

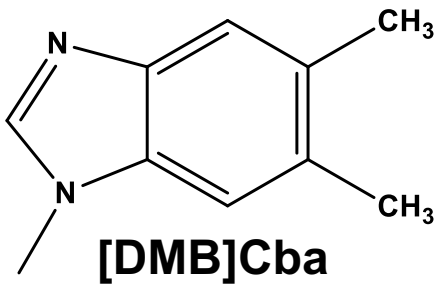
Dhc str. GT



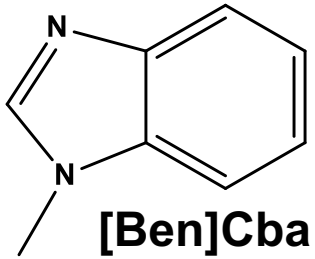
Lower Base Affects Dechlorination Activity

Strain BAV1 (BvcA)





[DMB]Cba

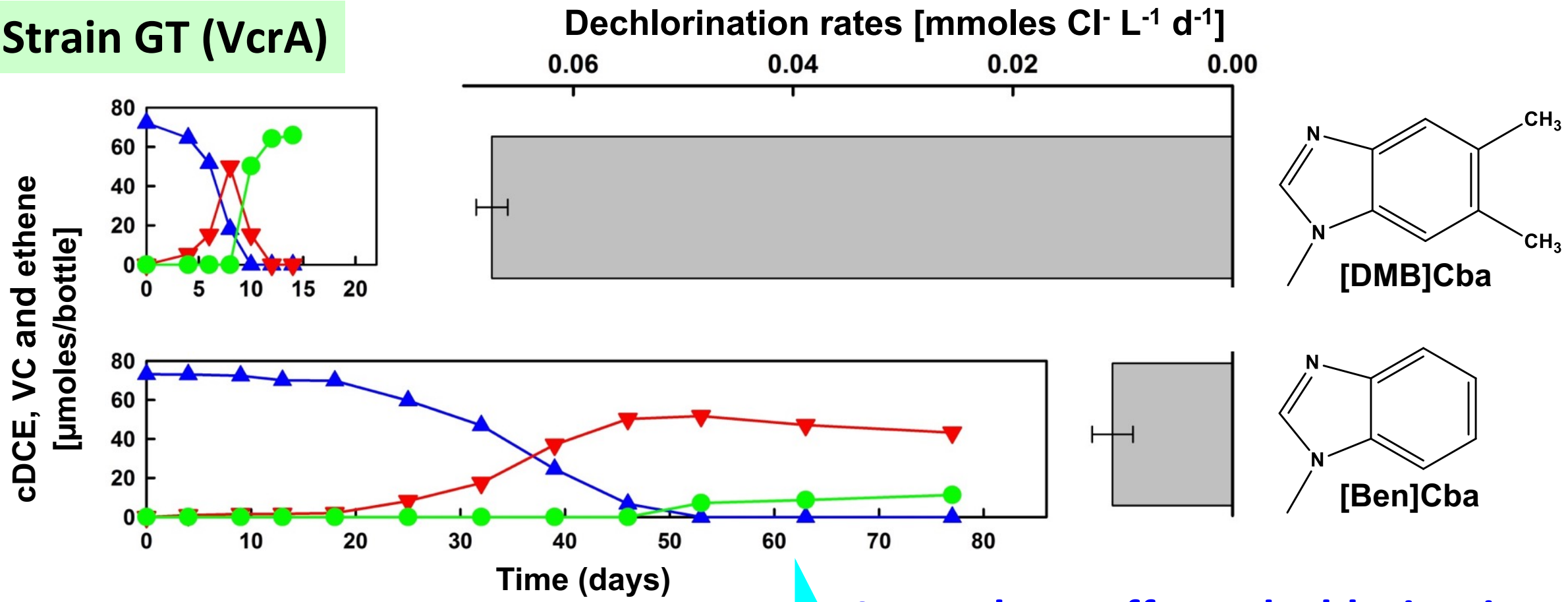


[Ben]Cba

Yan et al. 2016. ISME J. 10:1092–1101

Lower Base Affects Dechlorination Activity

Strain GT (VcrA)



Yan et al. 2016. ISME J. 10:1092–1101

Lower base affects dechlorination rates and endpoints

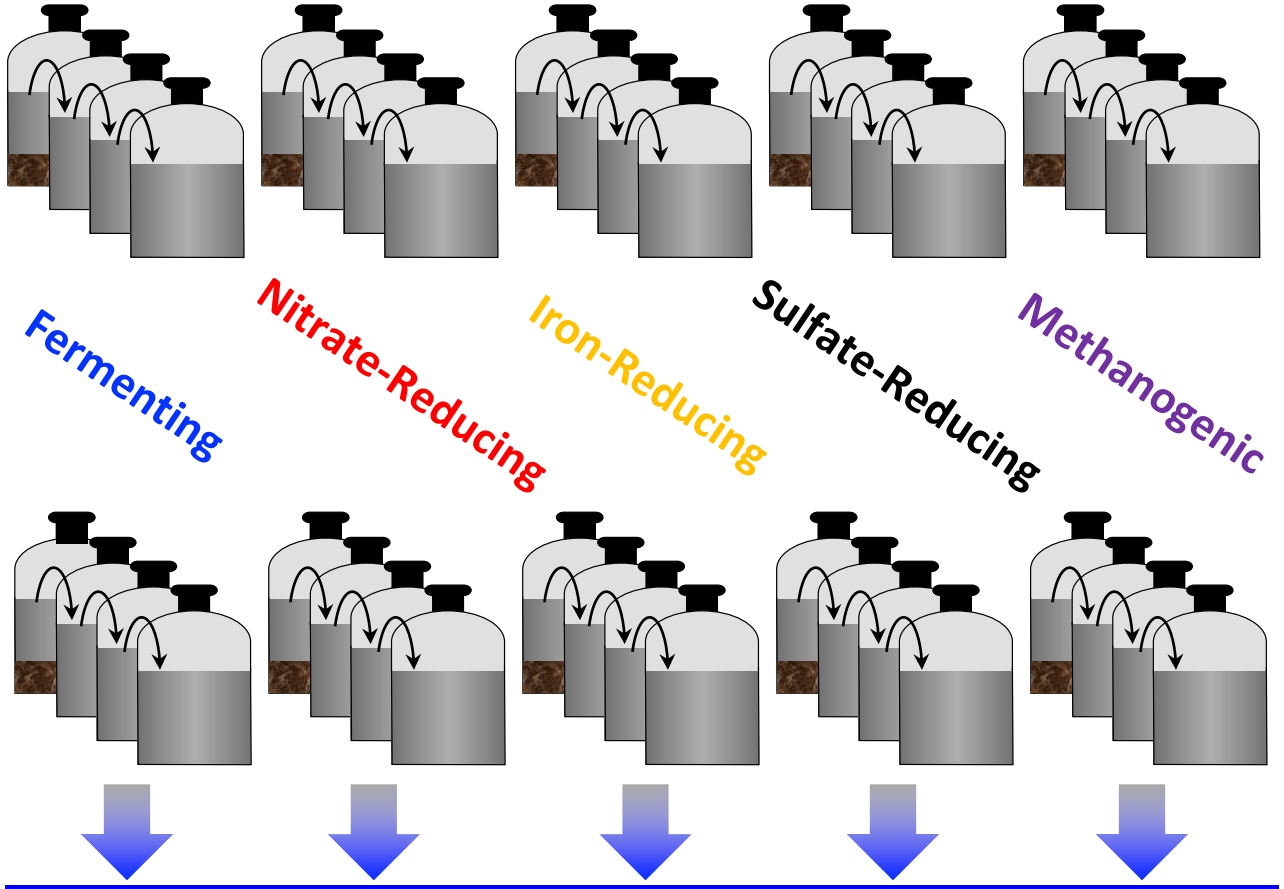
Corrinoid Production Under Different Redox Conditions

Third Creek Site
Knoxville, TN

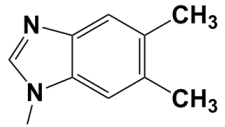
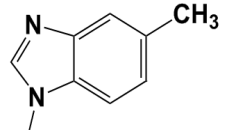
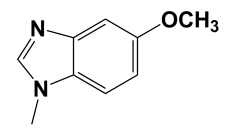
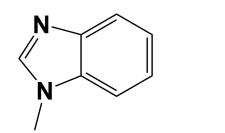
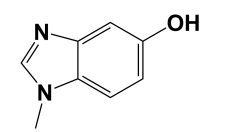
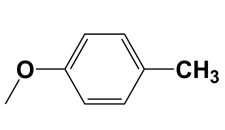
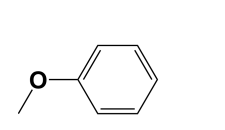
- Metal-manufacturing
- Chlorinated solvents

Commerce Street Superfund Site
Williston, Vermont

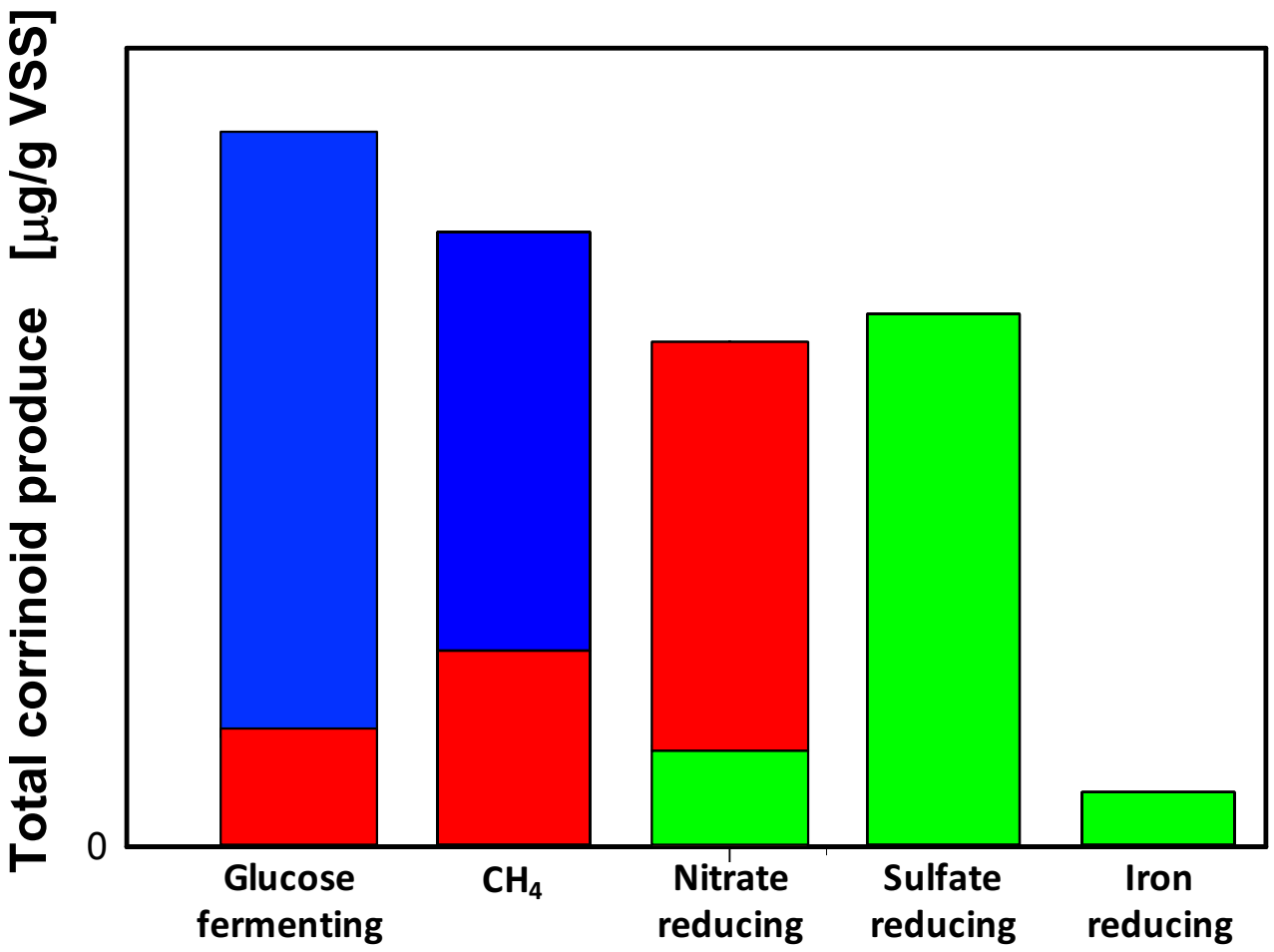
- Multi-tenant industrial park
- TCE, *cis*-DCE, petroleum hydrocarbons, metals (chromium, cadmium, nickel)

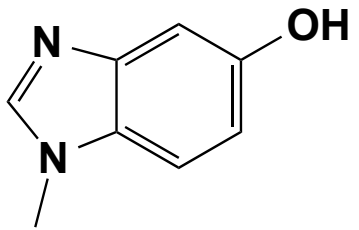


Corrinoids Produced by the Community Under Different Redox Conditions

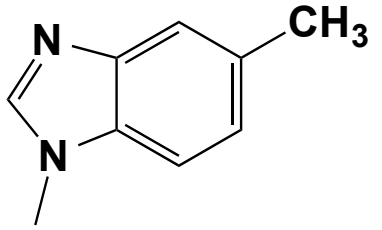
	Corrinoids produced	Glucose fermenting	Methanogenic	Iron reducing	Sulfate reducing	Nitrate reducing
✔		-	-	+	+	+
✔		+	+	-	-	-
✔		-	-	-	-	+
✔		-	-	-	-	-
✗		+	+	-	-	-
✗		-	+	-	-	-
✗		-	+	-	-	-

Redox Conditions Affect Corrinoid Type(s) and Quantity

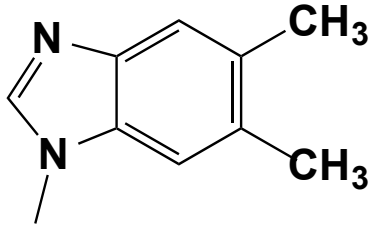




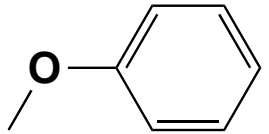
5-OHBza-Cba



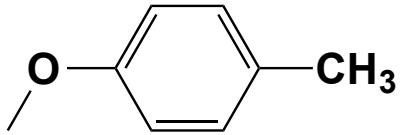
MeBza-Cba



DMB-Cba



Phe-Cba



Cre-Cba

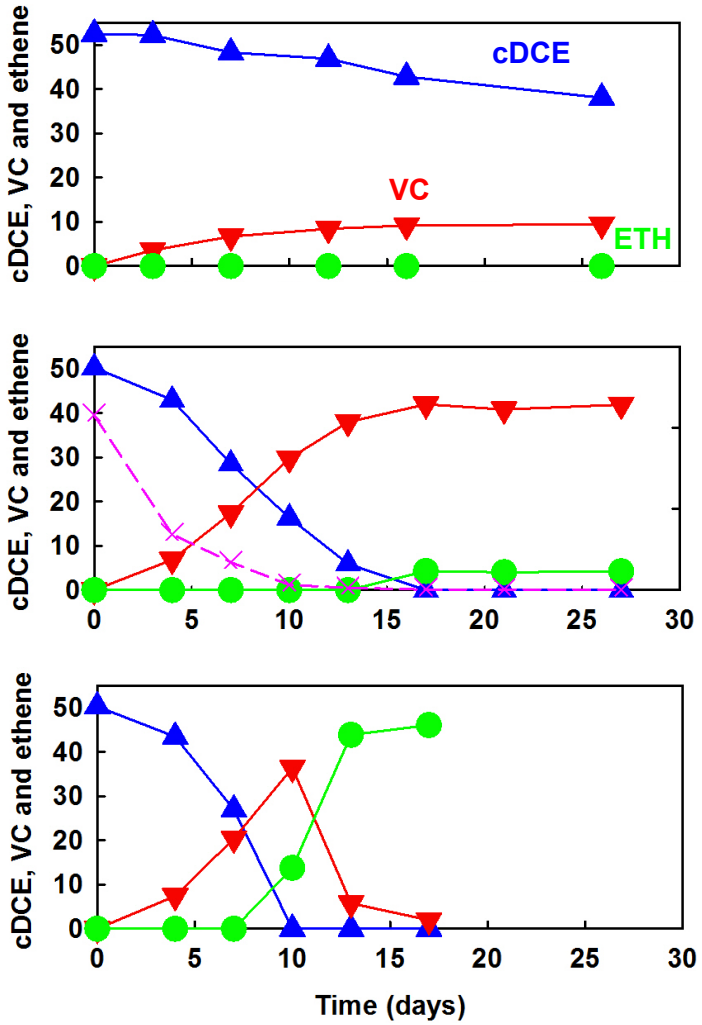
Seus et al. 2019. In Preparation

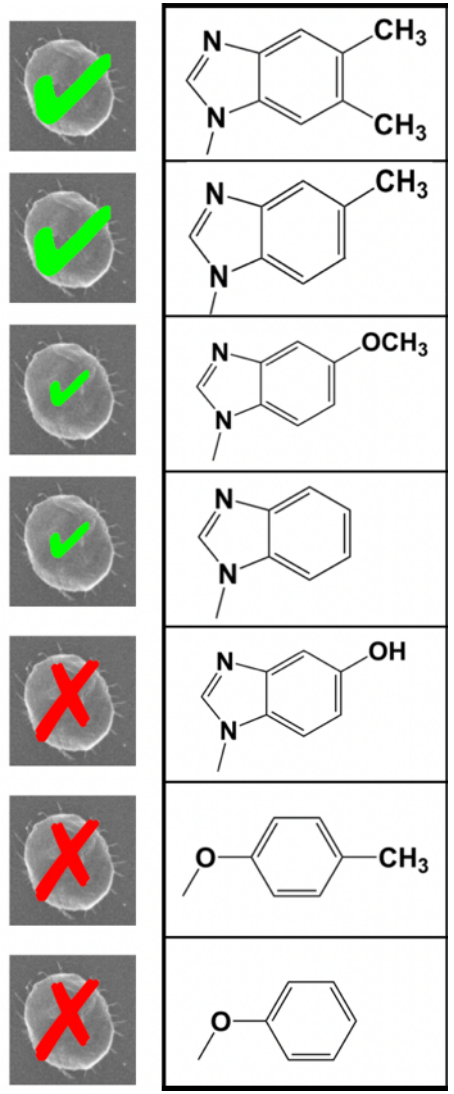
Corrinoid Quantity and Quality Determine *Dhc* Activity

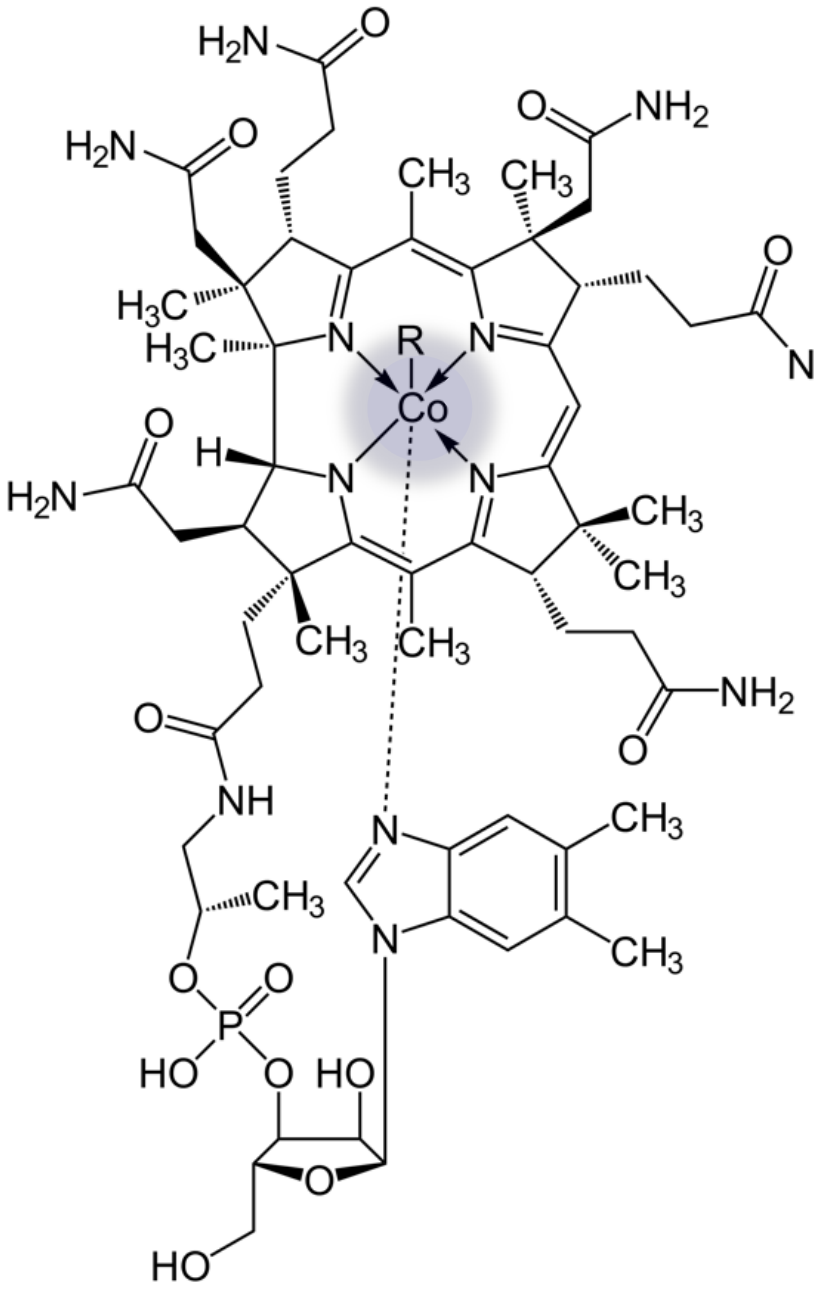
No B_{12}

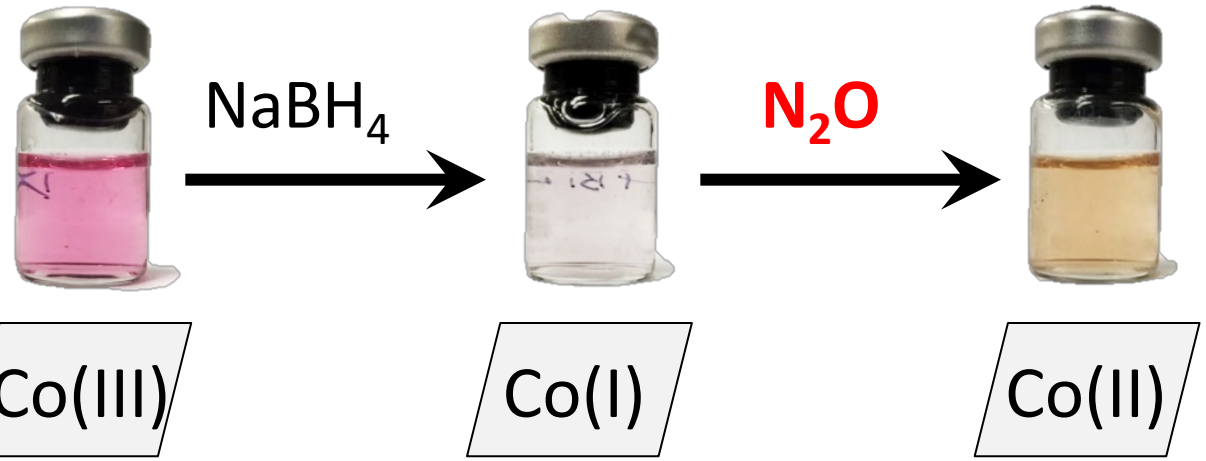
Limited B_{12} [1 µg/L]

Sufficient B_{12} [25 µg/L]

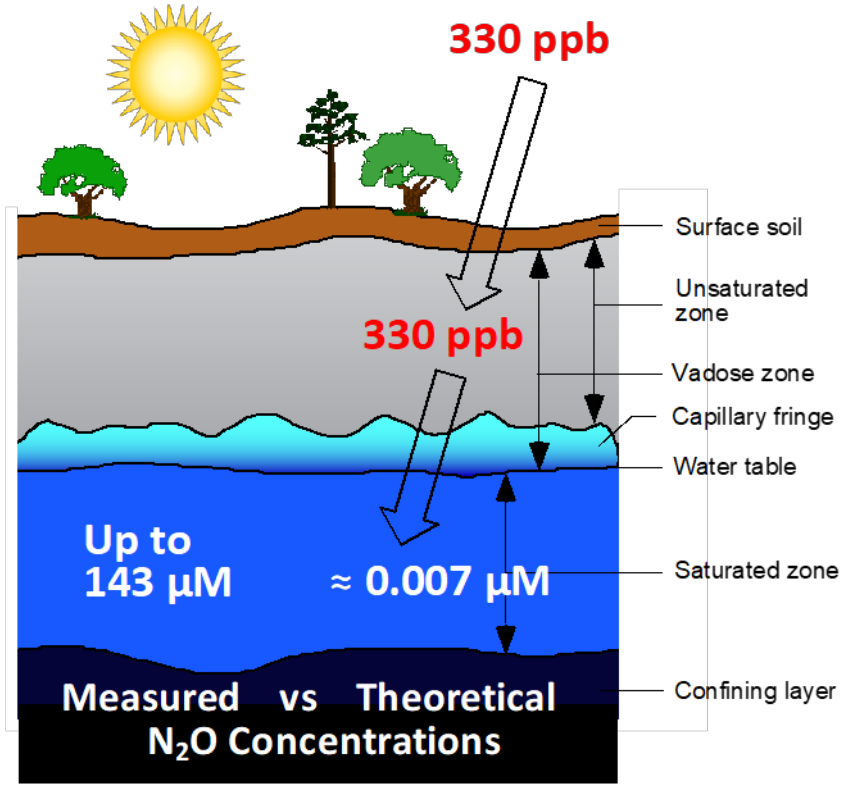








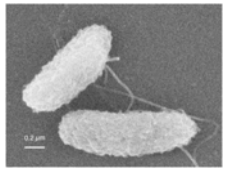
Banks et al. 1968. J. Chem. Soc. A, 2886

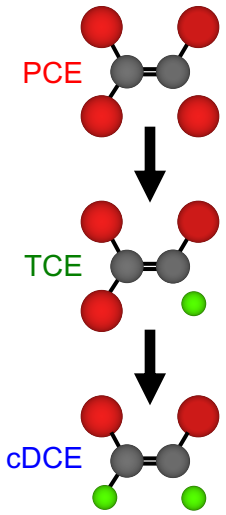


Jurado et al. 2017, Sci. Total Environ. 584–585:207-218

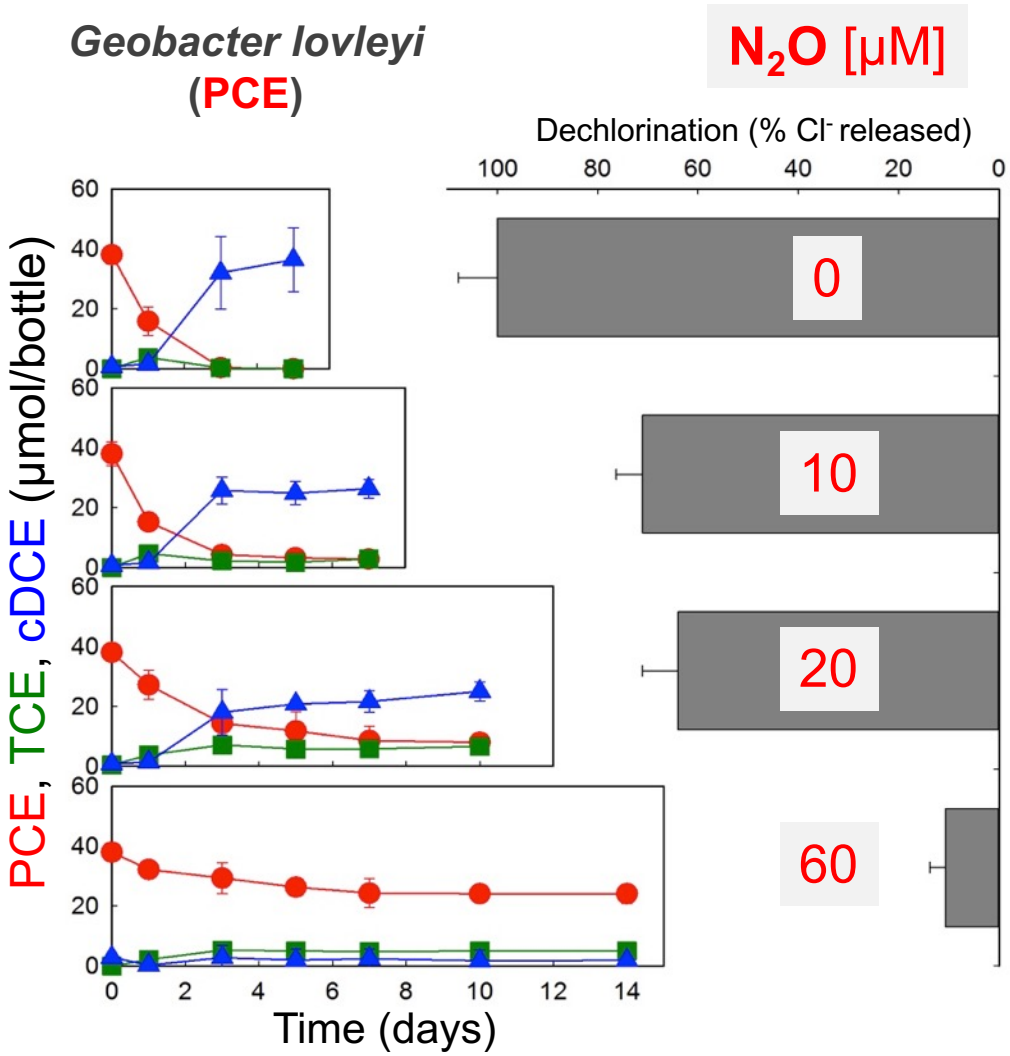
Impact of N_2O on corrinoid-dependent reductive dechlorination?

N_2O Inhibits Corrinoid-Dependent Reductive Dechlorination

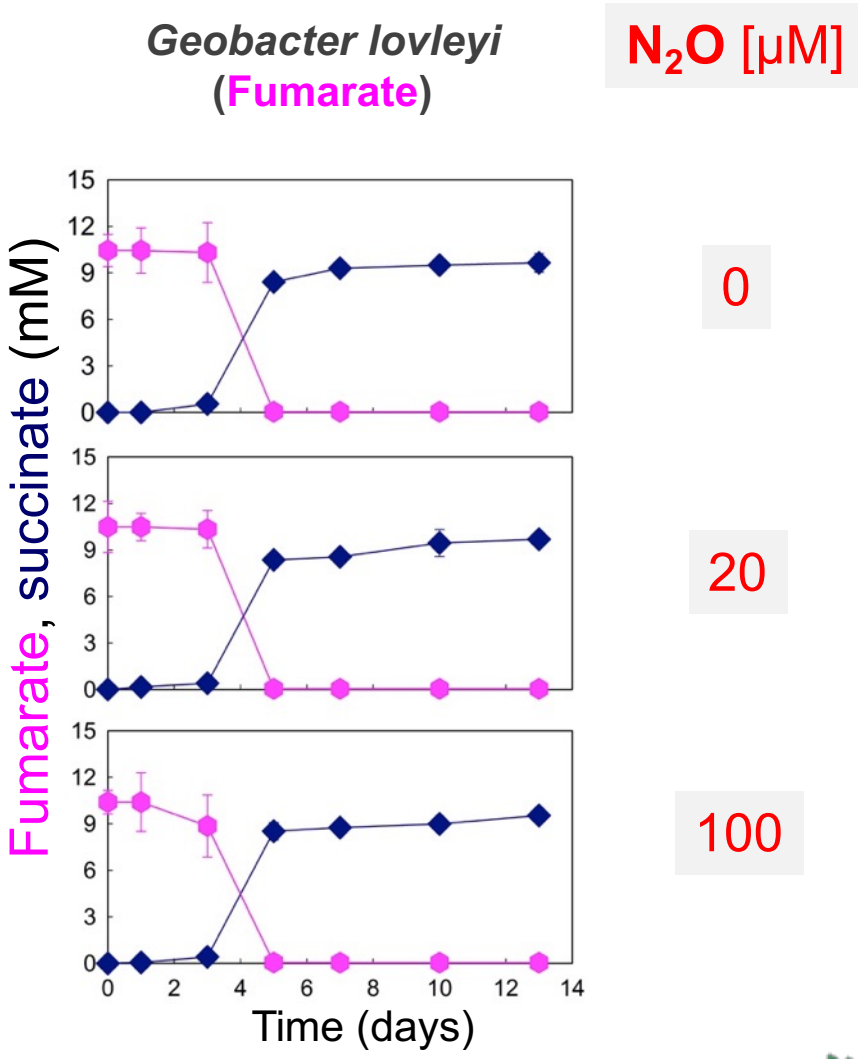




Geobacter lovleyi (PCE)



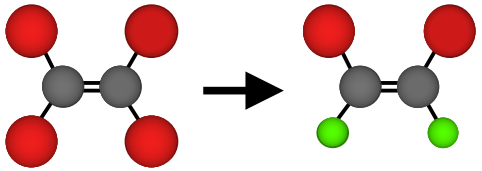
Geobacter lovleyi (Fumarate)



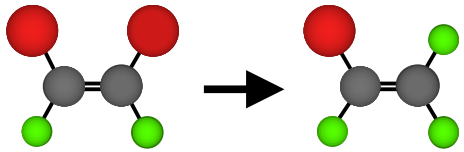
Impact of Increased N_2O in Environmental Systems

N_2O (µM)
0.3
12.5
37.4
75
84
143
65.7

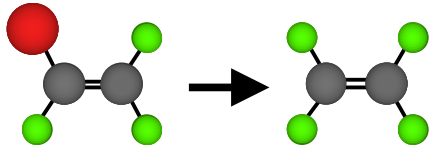
Jurado et al. 2017, Sci. Total Environ. 584–585:207-218



$K_{i,\ N2O}$ = 40.8 ± 3.8 µM

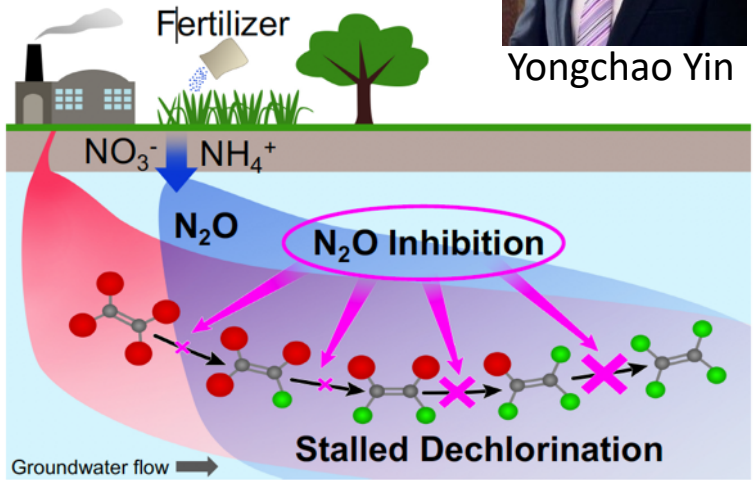


$K_{i,\ N2O}$ = 21.2 ± 3.5 µM



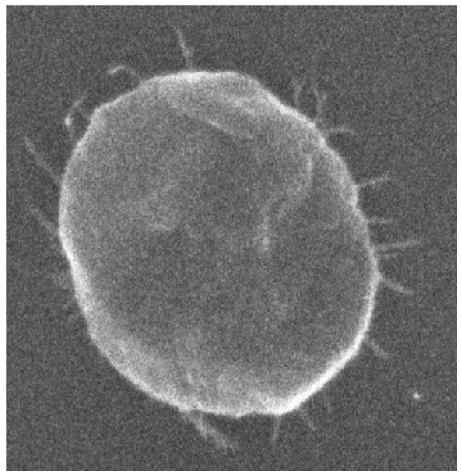
$K_{i,\ N2O}$ = 9.6 ± 0.4 µM

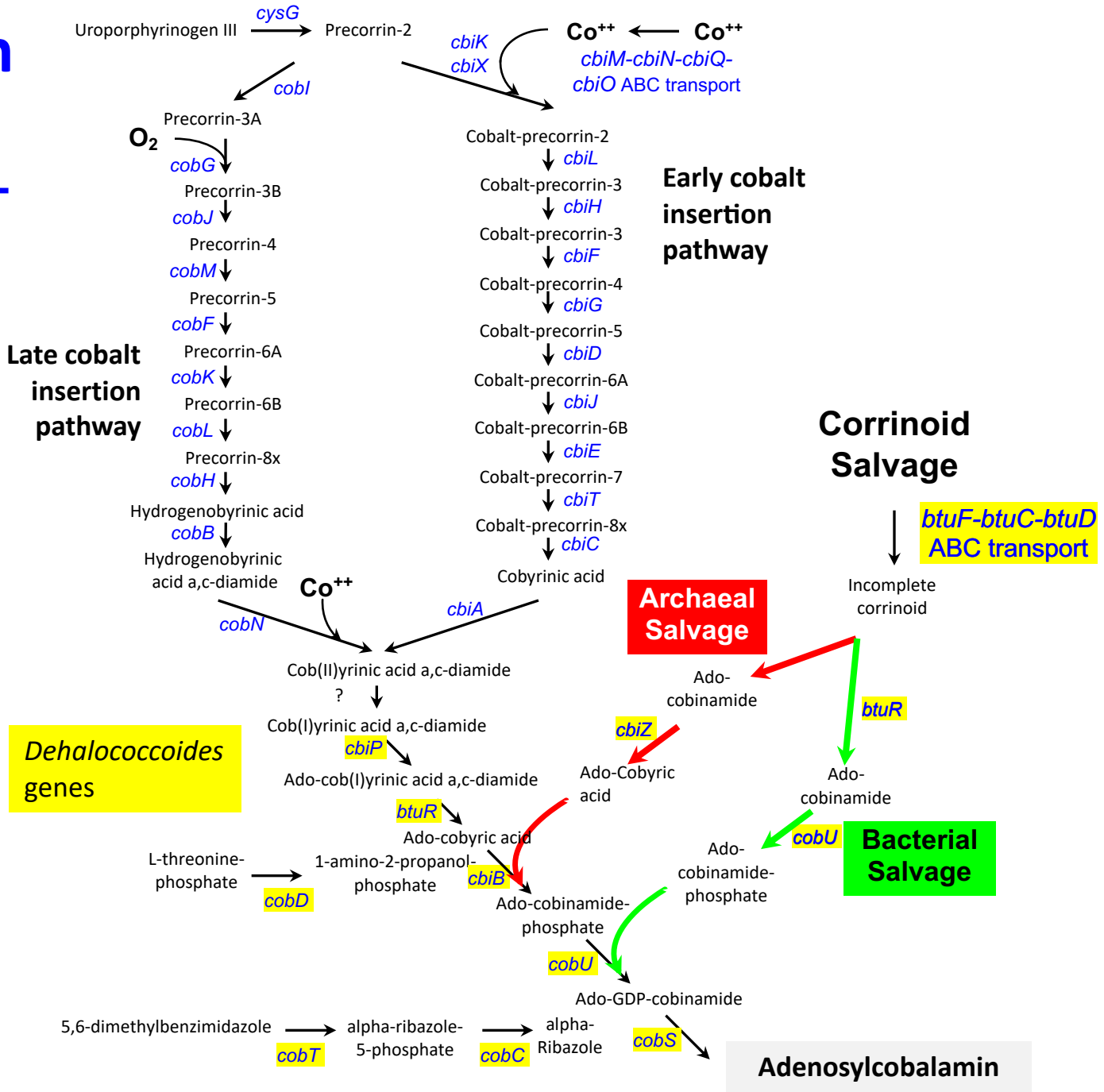
Yongchao Yin



Yin et al. 2019. Environ. Sci. Technol. Nitrous Oxide is a Potent Inhibitor of Bacterial Reductive Dechlorination. 53:692-701

Adenosylcobalamin Biosynthesis





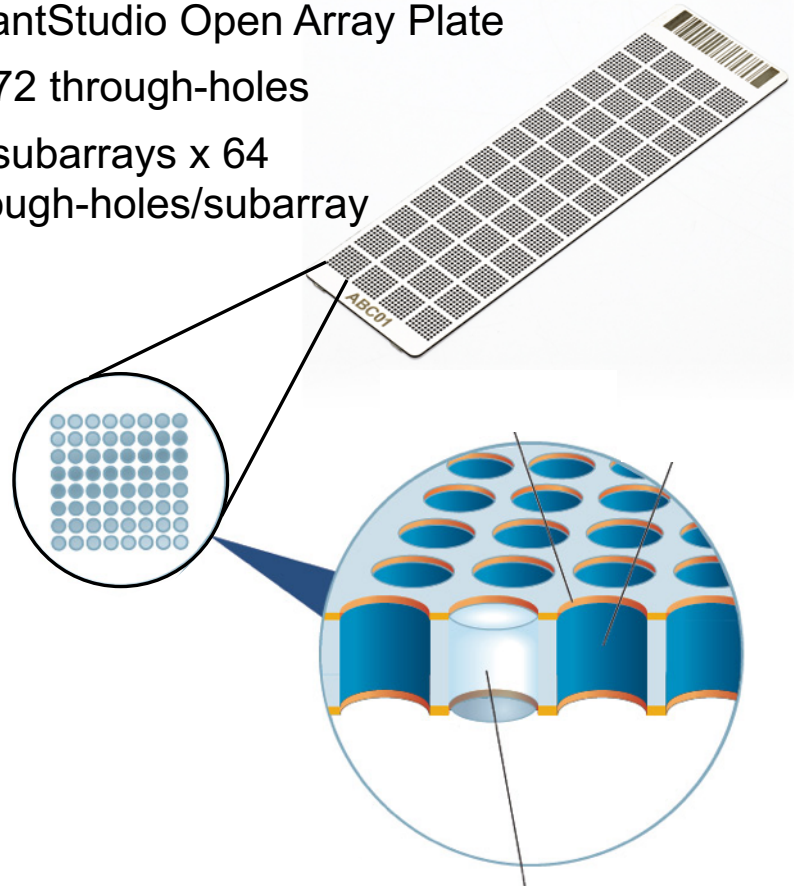
High-Throughput qPCR

- **Monitor many biomarker genes simultaneously**

QuantStudio Open Array Plate

3.072 through-holes

48 subarrays x 64 through-holes/subarray

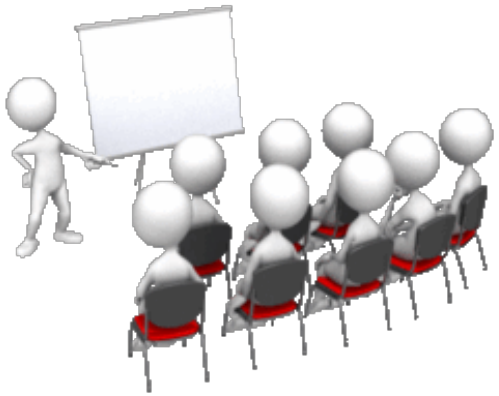


- Scalable platform (224x12; 112x24; 56x48)
- Cost per reaction reduced from $3.0 to $0.30
- Four plates can be cycled simultaneously, producing up to 12,288 qPCR data points per run

Robot reduces pipetting errors

Kara Murdoch et al. 2019. In Preparation

Take Home Messages



- Corrinoids are essential for most organisms
- Corrinoid-auxotrophic OHRBs (e.g., *Dhc*, *Dhgm*) are ideal systems to study corrinoid effects on metabolism
- Corrinoid quantity (flux) affects dechlorination activity
- Corrinoid type (lower base) determines reductive dechlorination rates and end points (i.e., function) in *Dhc*
- Geochemistry affects corrinoid pool
- Purine is a naturally occurring lower base
- New avenues to manipulate microbal metabolism (function, ecology → biotechnology, medicine)

Peer-reviewed Manuscripts

Yin, Y., Yan, G. Chen, F. Kara Murdoch, N. Pfisterer, and F.E. Löffler. 2019. Nitrous oxide is a potent inhibitor of bacterial reductive dechlorination. Environ. Sci. Technol. 53:692-701 | doi: 10.1021/acs.est.8b05871

Yan, J., M. Bi, A.K. Bourdon, A.T. Farmer, P.-H. Wang, O. Molenda, A. Quaile, N. Jiang, Y. Yang, Y. Yin, B. Şimşir, S.R. Campagna, E.A. Edwards, and F.E. Löffler. 2018. Purinyl-cobamide is a native prosthetic group of reductive dehalogenases. Nat. Chem. Biol. 14:8-14. | doi:10.1038/nchembio.2512

Clark, K., D.M. Taggart, B.R. Baldwin, K.M. Ritalahti, R.W. Murdoch, J.K. Hatt, and F.E. Löffler. 2018. Normalized quantitative PCR measurements as predictors for ethene formation at sites impacted with chlorinated ethenes. Environ. Sci. Technol. 52:13410-13420 | doi: 10.1021/acs.est.8b04373

Yang, Y., S.A. Higgins, J. Yan, B. Şimşir, K. Chourey, R. Iyer, R.L. Hettich, B. Baldwin, D.M. Ogles, and F.E. Löffler. 2017. Grape pomace compost harbors organohalide-respiring *Dehalogenimonas* species with novel reductive dehalogenase genes. The ISME Journal. 11:2767-2780. | doi: 10.1038/ismej.2017.127

Yan, J., B. Şimşir, A.T. Farmer, M. Bi, Y. Yang, S.R. Campagna, and F.E. Löffler. 2016. The corrinoid cofactor of reductive dehalogenases affects dechlorination rates and extents in organohalide-respiring *Dehalococcoides mccartyi*. ISME J. 10:1092-1101. | doi: 10.1038/ismej.2015.197

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

SERDP DOD • EPA • DOE

ESTCP Environmental Security Technology Certification Program

OAK RIDGE National Laboratory