Open Environmental Restoration Resource (OER2) Webinar

Advances in Microbial Characterization for MNA & Bioremediation

Presented by: Dr. Anthony Danko
NAVFAC Environmental Restoration Program

November 15 2017

Logistics

- **Submit all questions via chat box throughout the presentation**
- **Presentation is being recorded**
- **Complete the webinar survey (main feedback mechanism)**

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Speaker Introduction

Dr. Anthony Danko

(Presenter)

- Environmental Engineer (NAVFAC EXWC)
- 65 Professional Publications
- Specializes in
  - Portfolio optimization
  - Innovative technology development
  - Bio-remediation
- B.S. in Chemical Engineering (Rensselaer Poly Tech)
- M.S. in Civil and Environmental Engineering (U. of Pittsburgh)
- Ph.D. in Environmental Engineering and Science (Clemson University)

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OER2 Webinar Series

- **Why Attend?**
  - Obtain and hear about the latest DOD and DON's policies/guidance, tools, technologies and practices to improve the ERP's efficiency
  - Promote innovation and share lessons learned
  - **FEEDBACK** to the ERP Leadership
- **Who Should Attend?**
  - ERP Community Members: RPMs, RTMs, Contractors, and other remediation practitioners who support and execute the ERP
  - Voluntary participation
- **Schedule and Registration:**
  - Every other month, 4th Wed (can be rescheduled due to holidays)
  - Registration link for each topic (announced via ER T2 email)
- **Topics and Presenters:**
  - **ERP community members** to submit topics (non-marketing and DON ERP-relevant) to POCs (Gunarti Coghlan – gunarti.coghlan@navy.mil or Tara Meyers – tara.meyers@navy.mil)
  - Selected topic will be assigned Champion to work with presenter

Advances in Microbial Characterization for MNA & Bioremediation

Dr. Anthony Danko
NAVFAC EXWC

Survey

- **Placeholder slide for survey**

- **How familiar are you with Molecular Biological Tools?**
  - **Very familiar**
  - **Somewhat familiar**
  - **Not familiar**

- **Why are we interested in microbiology?**
  - **Provide insights into bioprocesses occurring in the subsurface**
    - Biogeochemical
    - Biodegradation
    - etc.

How many different types?

What is going on in the subsurface?

Are microorganisms active?

Are microorganisms present?

What tools can be used?

Molecular Biological Tools

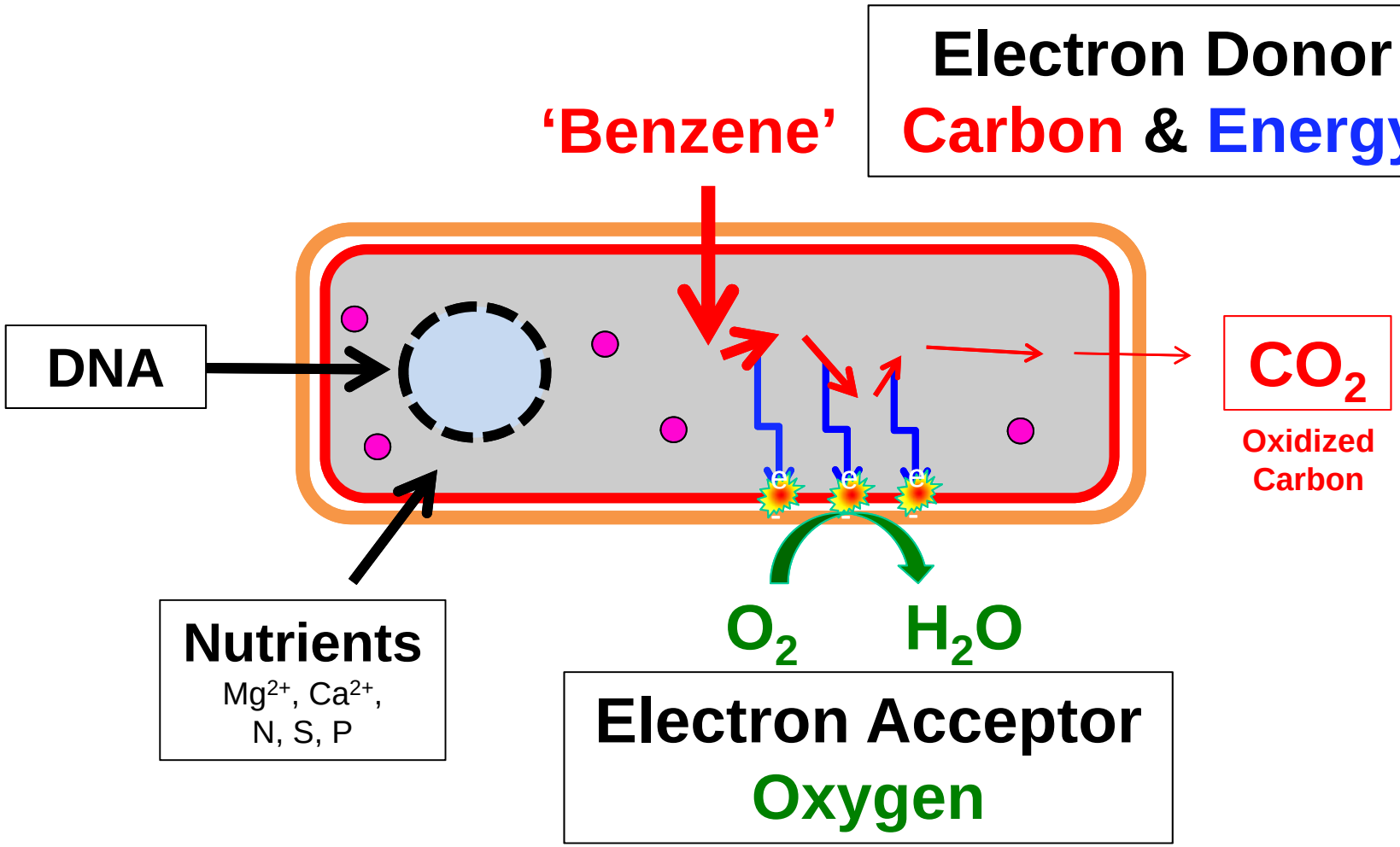
- **Traditional microbial methods may not be able to provide you the information that is needed to assist in site assessment and remediation**
  - **Culturable versus non-culturable microorganisms**

- **Molecular biology studies gene structure and function at the molecular level**

- **Field overlaps with other areas such as:**
  - **Biochemistry: Structures, functions, and interactions of macromolecules**
  - **Genetics: Genes**

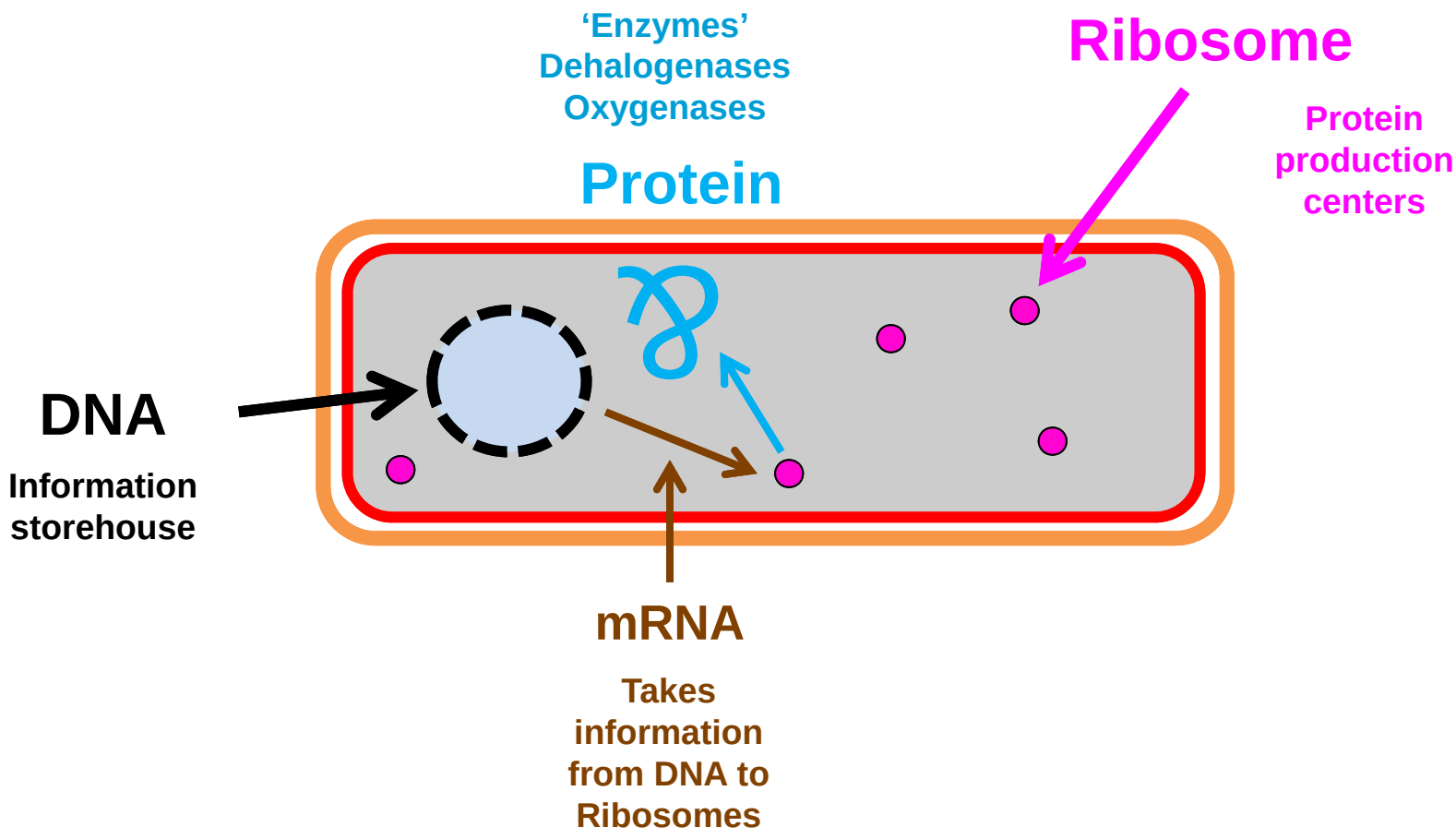
Source: Frank Löffler

Bacterial Cell

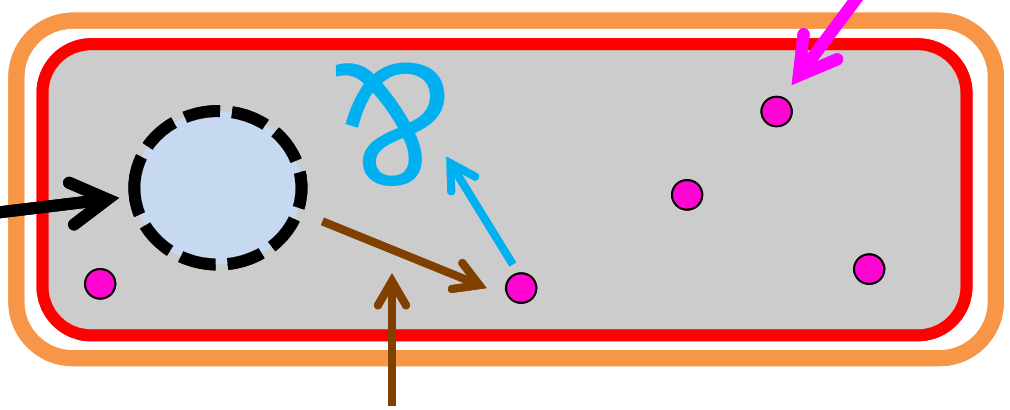


Source: Mike Hyman

Bacterial Cell



Bacterial Cell



Tells you:
Activity

'Enzymes'
Dehalogenases
Oxygenases

Protein

Ribosome

Protein production centers

DNA

Information storehouse

Tells you:
Presence of microbes & metabolic genes

mRNA

Takes information from DNA to Ribosomes

Tells you:
Active microbes and genes being made

Source: Frank Löffler

Molecular Biological Tools (MBTs)

- **Techniques Available**
- **qPCR**
  - **What are the concentrations of specific microorganisms or genes of interest?**
- **Next Generation Sequencing (NGS)**
  - **Which microorganisms are present?**
- **Stable Isotope Probing (SIP) and Compound Specific Isotope Analysis (CSIA)**
  - **Is degradation occurring?**
- **Others**

Molecular Biological Tools (MBTs)

- **Techniques Available**

- **qPCR**

- **Next Generation Sequencing (NGS)**

- **Stable Isotope Probing (SIP) and Compound Specific Isotope Analysis (CSIA)**

- **Others**

Survey

- **Placeholder slide for survey**

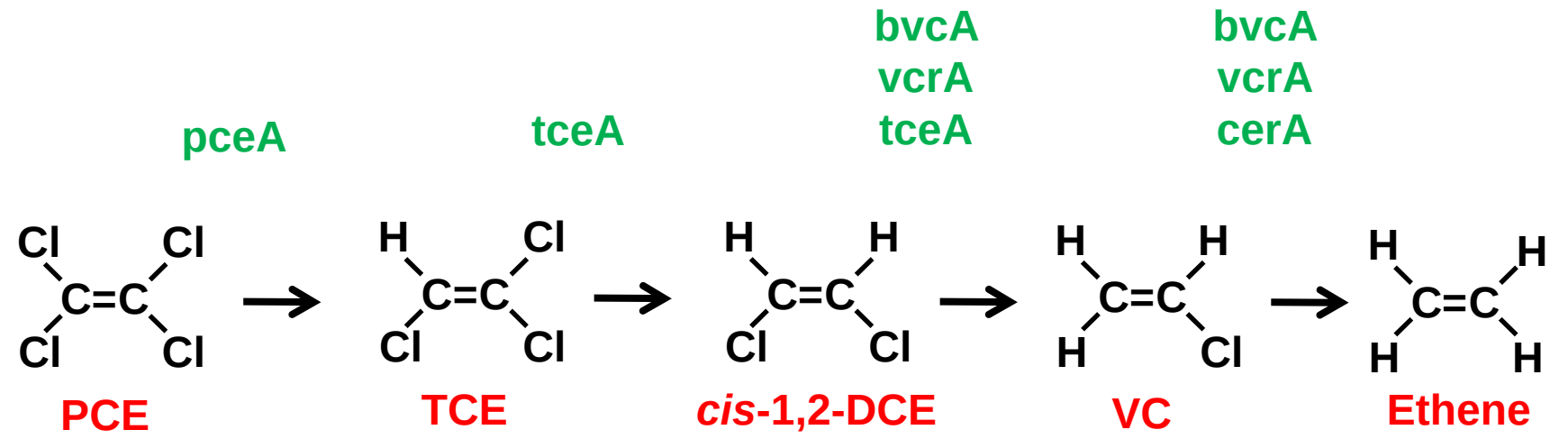
- **Have you used qPCR as part of your Environmental Management Strategy?**

qPCR and QuantArray

- **Determines the concentration of a specific microorganism or particular functional gene of interest**
  - **How much Dehalococcoides is present?**
    - 10^4 cells/mL is generally required for effective bioremediation
  - **How much TOD (toluene/benzene diooxygenase) is present?**
- **Technique requires knowledge beforehand of the microorganisms and/or genes of interest and specifically targets them!**
  - **Established link between function of gene or microorganism to the process**

What genes are we interested in? – Anaerobic Processes

Genes



Dehalobacter
Dehalospirillum
Desulfitobacterium
Desulfuromonas
Dehalococcoides
Etc.

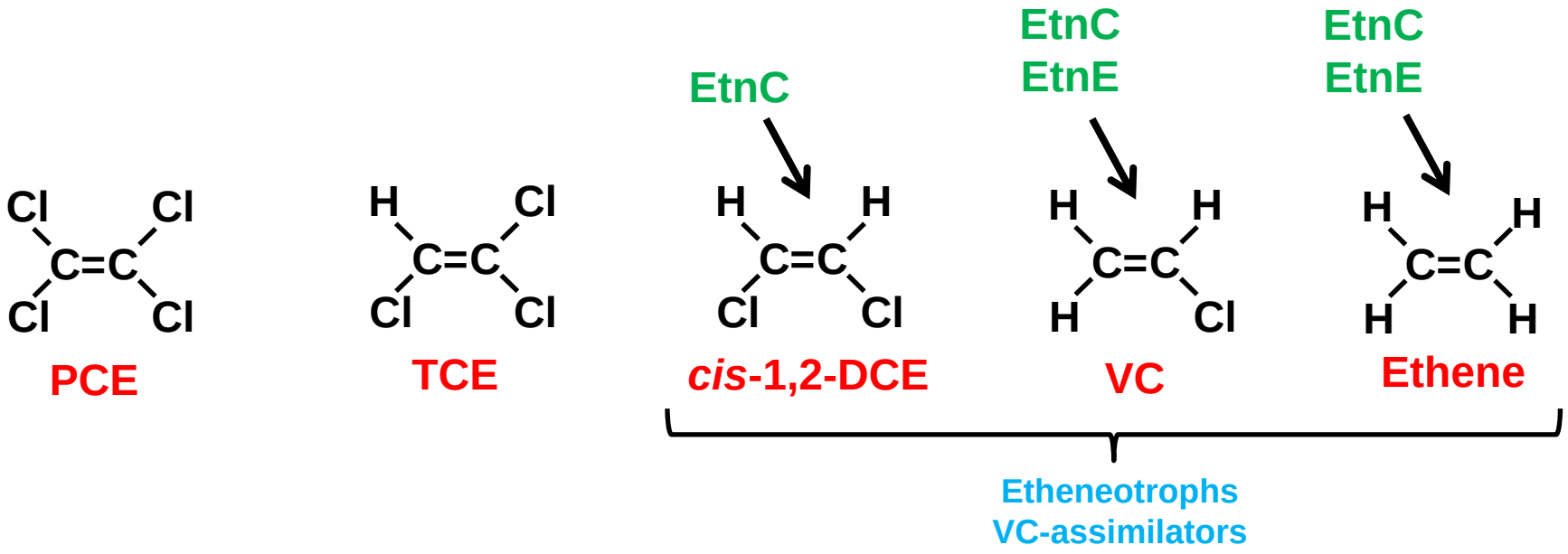
Dehalococcoides
Dehalogenimonas

Microorganisms

Source: Frank Löffler

What genes are we interested in? – Aerobic Processes

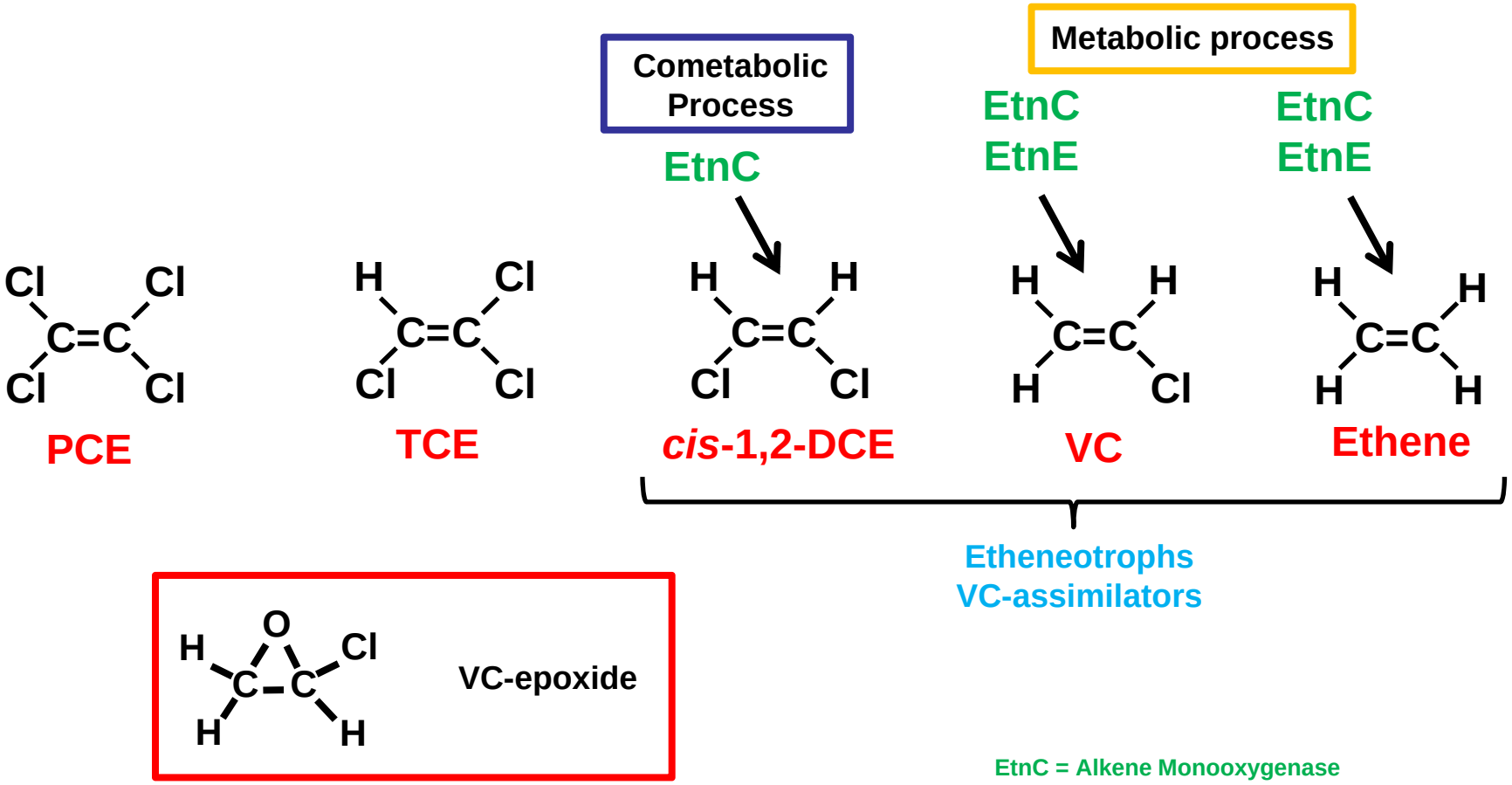
Genes



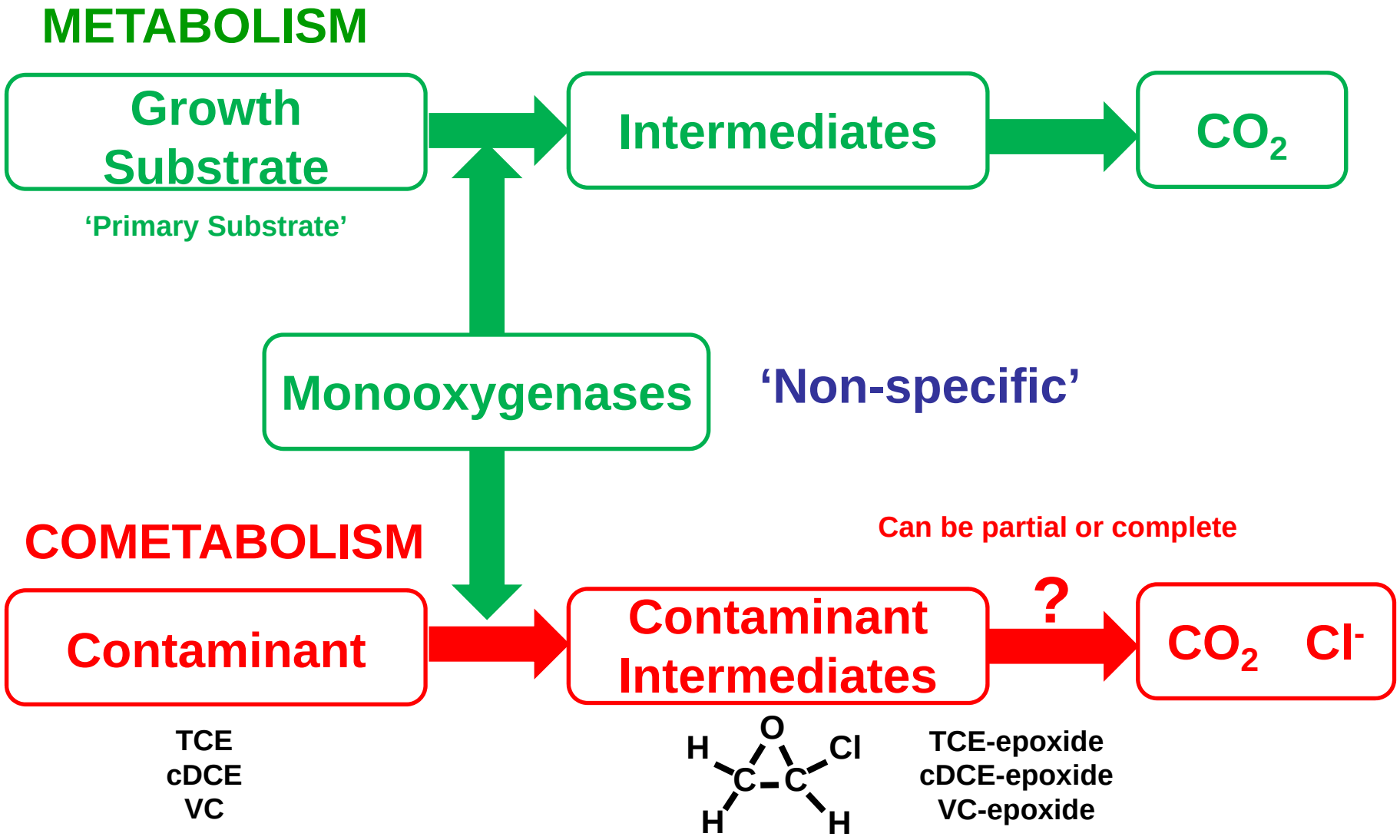
EtnC = Alkene Monooxygenase
EtnE = Epoxyalkane CoM Transferase

What genes are we interested in? – Aerobic Processes

Genes

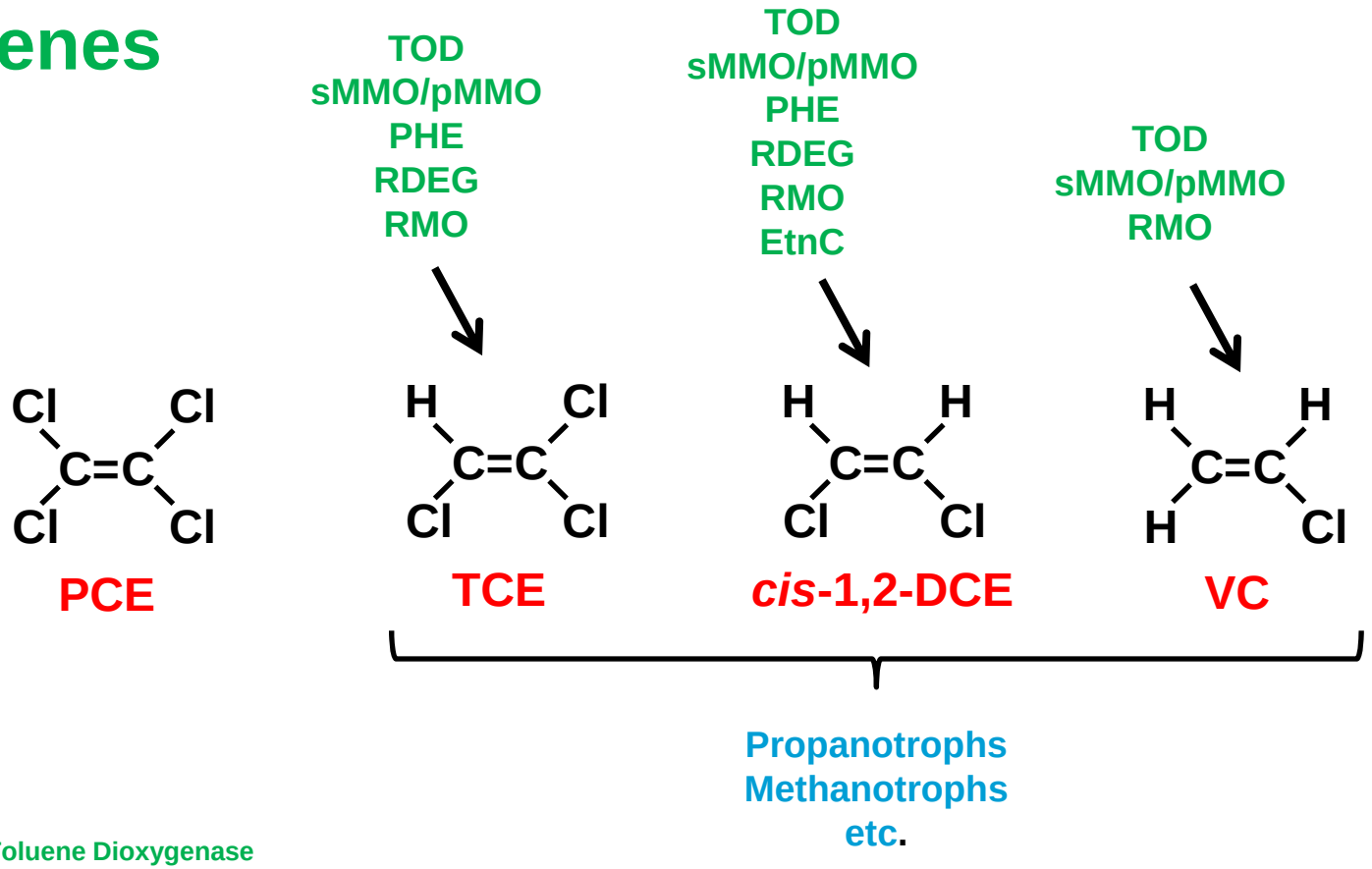


Metabolism versus Cometabolism



What genes are we interested in? – Aerobic Cometabolic Processes

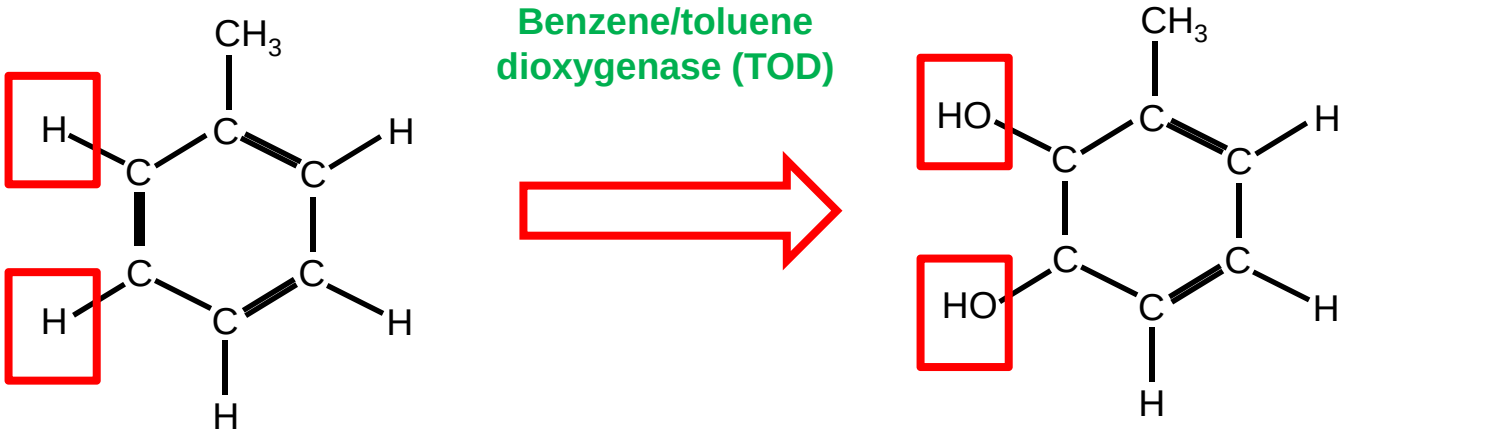
Genes



TOD = Toluene Dioxygenase
sMMO/pMMO = soluble/particulate Methane Monooxygenase
PHE = Phenol Hydroxylase
RMO = Toluene Monooxygenase
RDEG 2 = Toluene Monooxygenase
EtnC = Alkene Monooxygenase

Microorganisms

What genes are we interested in? – Monoaromatic Hydrocarbons



Toluene

cis-cyclohexa-3,5-diene-1,2-diol

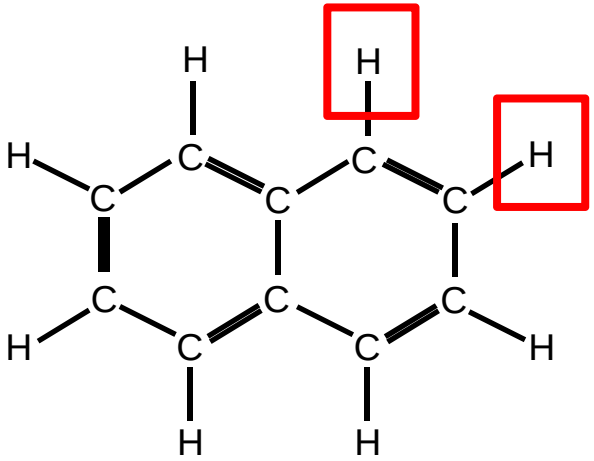
TOD = Toluene Dioxygenase
RMO = Toluene Monooxygenase
RDEG = Toluene Monooxygenase
PHE = Phenol Hydroxylase
TOL = Xylene/Toluene Monooxygenase
EDO = Ethylbenzene/Isopropylbenzene Diooxygenase
BPH4 = Biphenyl/Isopropylbenzene Diooxygenase

Aerobic

BCR = Benzoyl Coenzyme A Reductase
BSS = Benzylsuccinate Synthase
ABC = Benzene Carboxylase

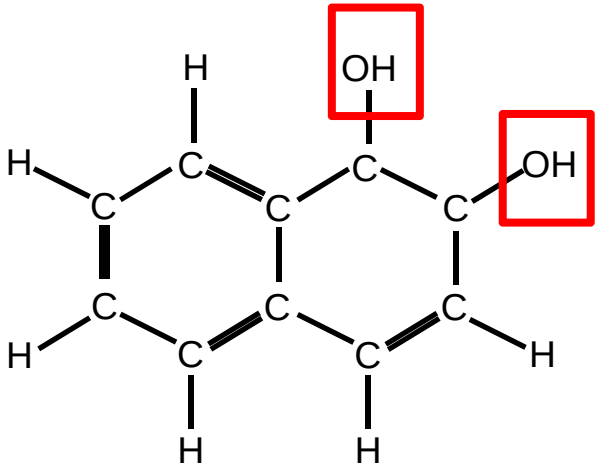
Anaerobic

What genes are we interested in? – Polyaromatic Hydrocarbons



Naphthalene

Naphthalene dioxygenase (NAH)



cis-1,2-dihydroxy-1,2-dihydronaphthalene

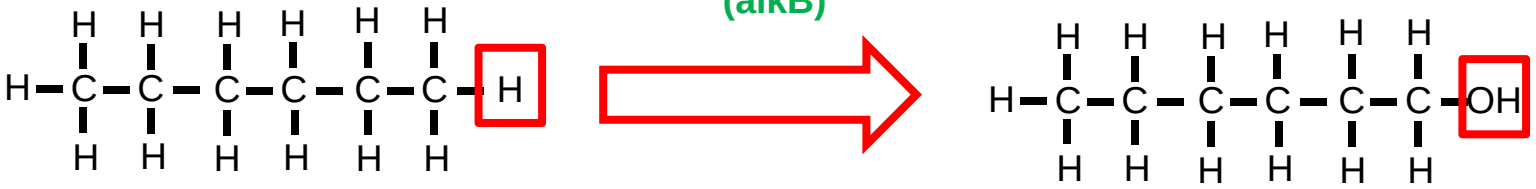
NAH = Naphthalene Dioxygenase
PHN = Phenanthrene Dioxygenase

BCR = Benzoyl Coenzyme A Reductase
NMS = Naphthylmethylsuccinate Synthase
ANC = Alkylsuccinate Synthase

Aerobic

Anaerobic

What genes are we interested in? – Alkanes



Alkane hydroxylase (alkB)

Hexane

Hexanol

ALKB = Alkane hydroxylase

ASSA = Alkylsuccinate Synthase

Aerobic

Anaerobic

Molecular Biological Tools (MBTs)

- **Techniques Available**
- **qPCR**
- **Next Generation Sequencing (NGS)**
- **Stable Isotope Probing (SIP) and Compound Specific Isotope Analysis (CSIA)**
- **Others**

Survey

- **Placeholder slide for survey**

- **Have you used NGS as part of your Environmental Management Strategy?**

Next Generation Sequencing (NGS)

- **Information about the entire community**
  - **Not targeting a specific functional gene or microorganism as in qPCR**
  - **No prior knowledge of the genes or microorganisms are needed**
- **Not quantitative but relative amount**
- **Information on community shifts over time**
- **Function?**
- **May not detect low concentrations of microorganisms**

Molecular Biological Tools (MBTs)

- **Techniques Available**
- **qPCR**
- **Next Generation Sequencing (NGS)**
- **Stable Isotope Probing (SIP) and Compound Specific Isotope Analysis (CSIA)**
- **Others**

Survey

- **Placeholder slide for survey**

- **Have you used SIP or CSIA as part of your Environmental Management Strategy?**

- **Variety of isotopes in the environment that can be used**
  - **Naturally occurring isotopes (% varies)**
  - **Same protons, more neutrons**
  - **Stable**
  - **Tracking the isotope**

$^{13}C/^{12}C$
$^{15}N/^{14}N$ **S, H, Cl, etc**

$$\delta^{13}C = \frac{R_x - R_{std}}{R_{std}} \times 1000$$

Both SIP and CSIA look for enrichment in the $\delta^{13}C$ values!

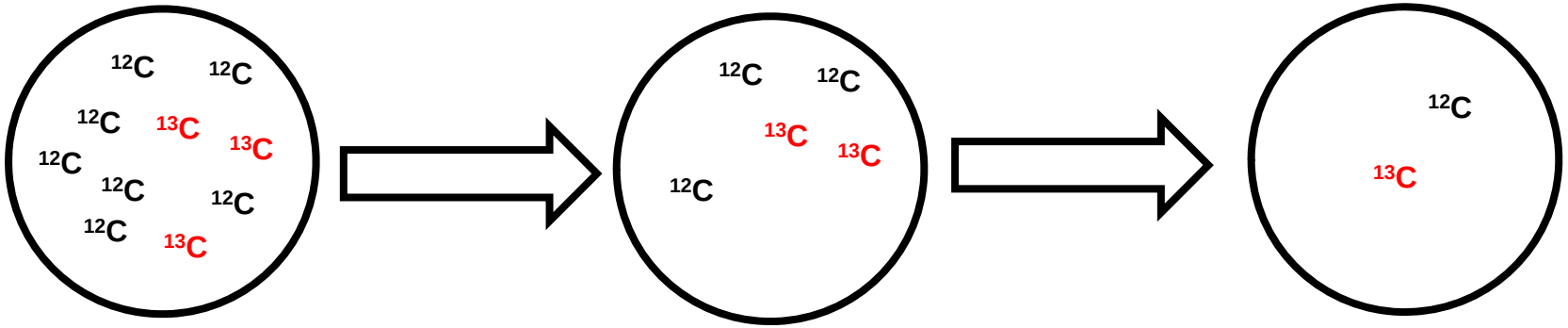
Units are per mil, ‰ (if $\delta^{13}C$ = +10, that means 10 per thousand)

Is degradation occurring?

CSIA

- Looking for enrichment (like SIP)
- Not added (unlike SIP), naturally present
- Can be used for electron donors or acceptors (unlike SIP)
- ^{12}C bonds are slightly weaker and easier to break
- ^{13}C bonds are slightly stronger and harder to break

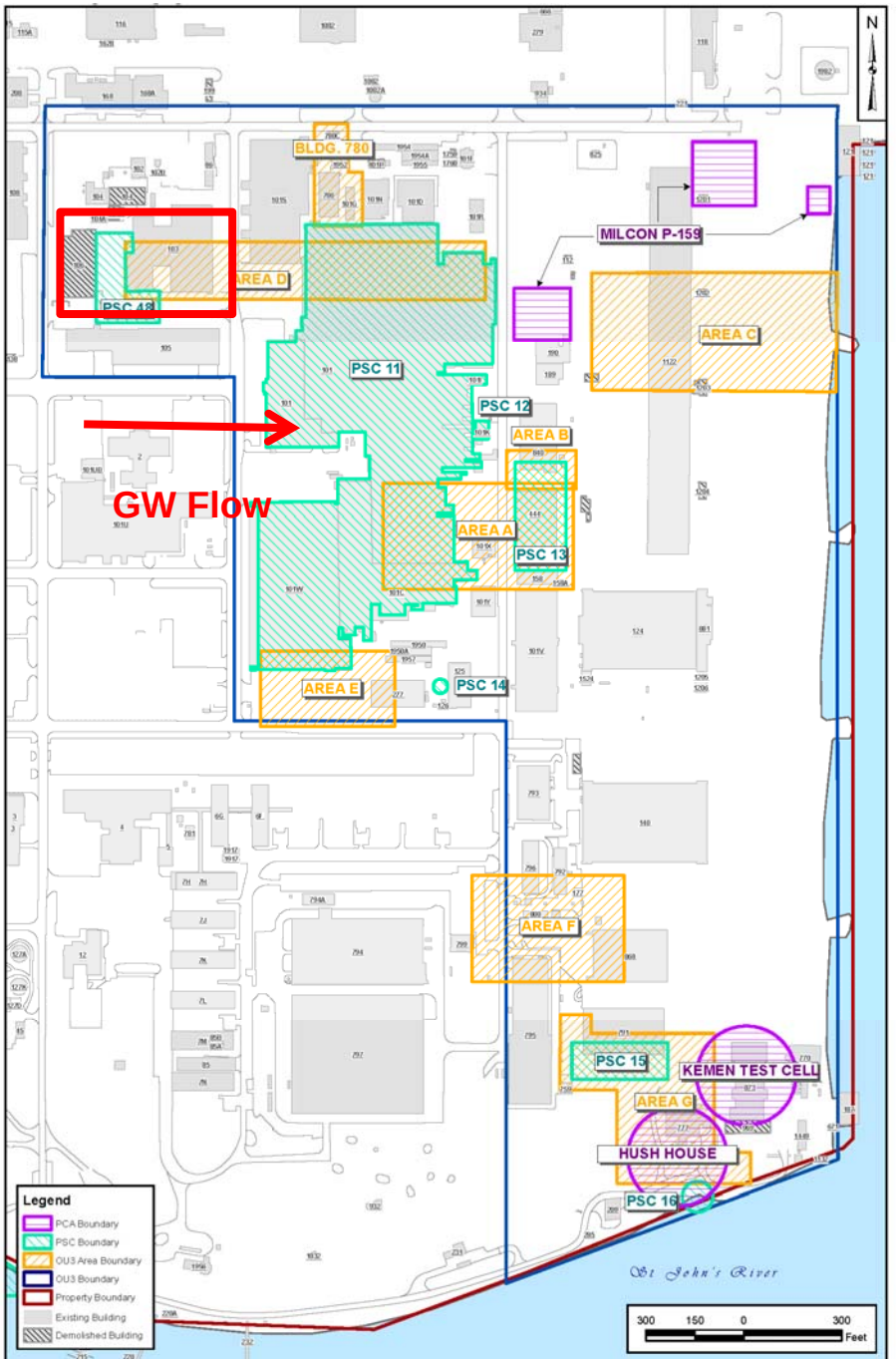
$k_{12} > k_{13}$



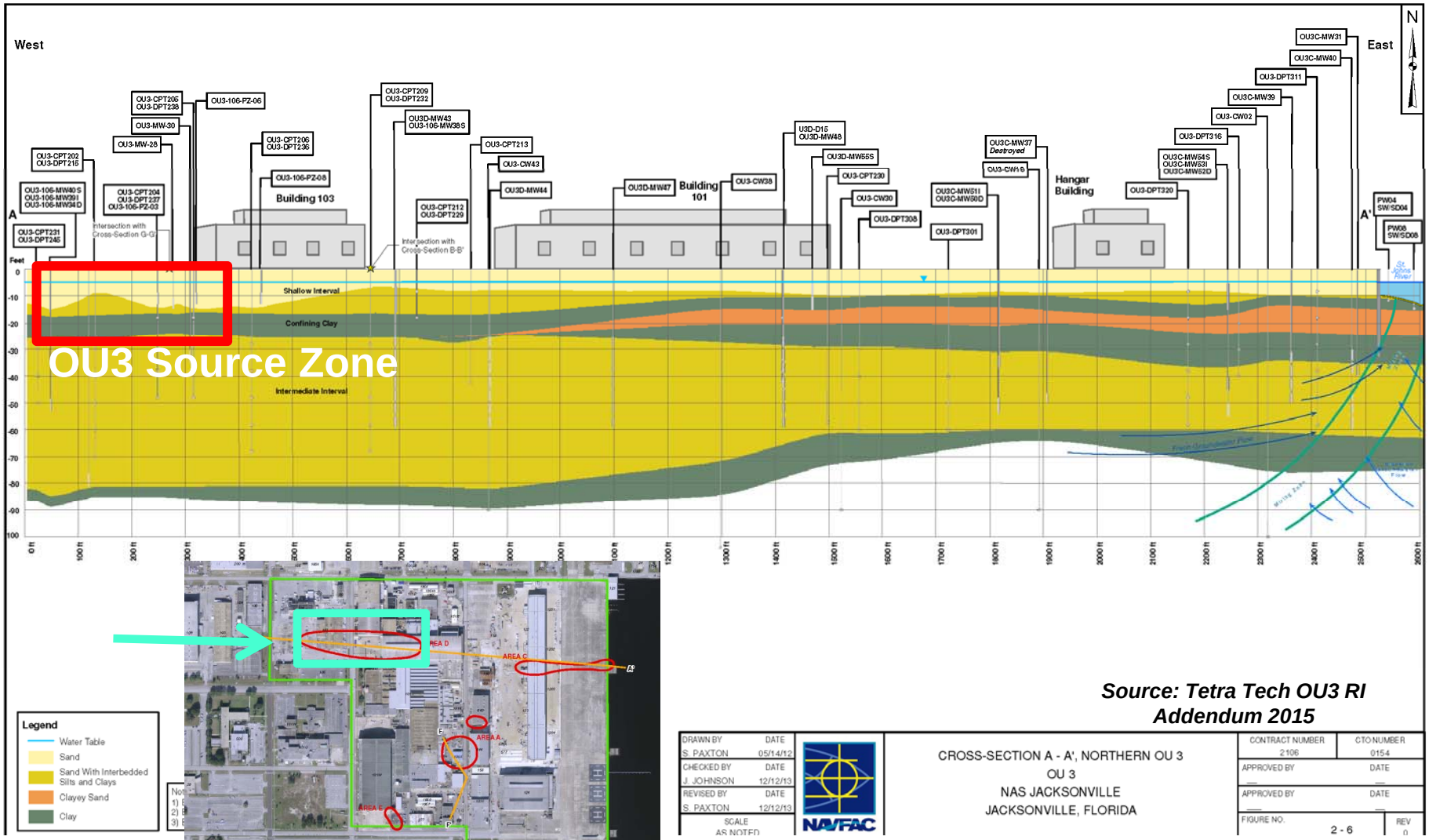
^{13}C and ^{12}C are decreasing with time
Increasing ratio of ^{13}C

OU3, NAS Jacksonville

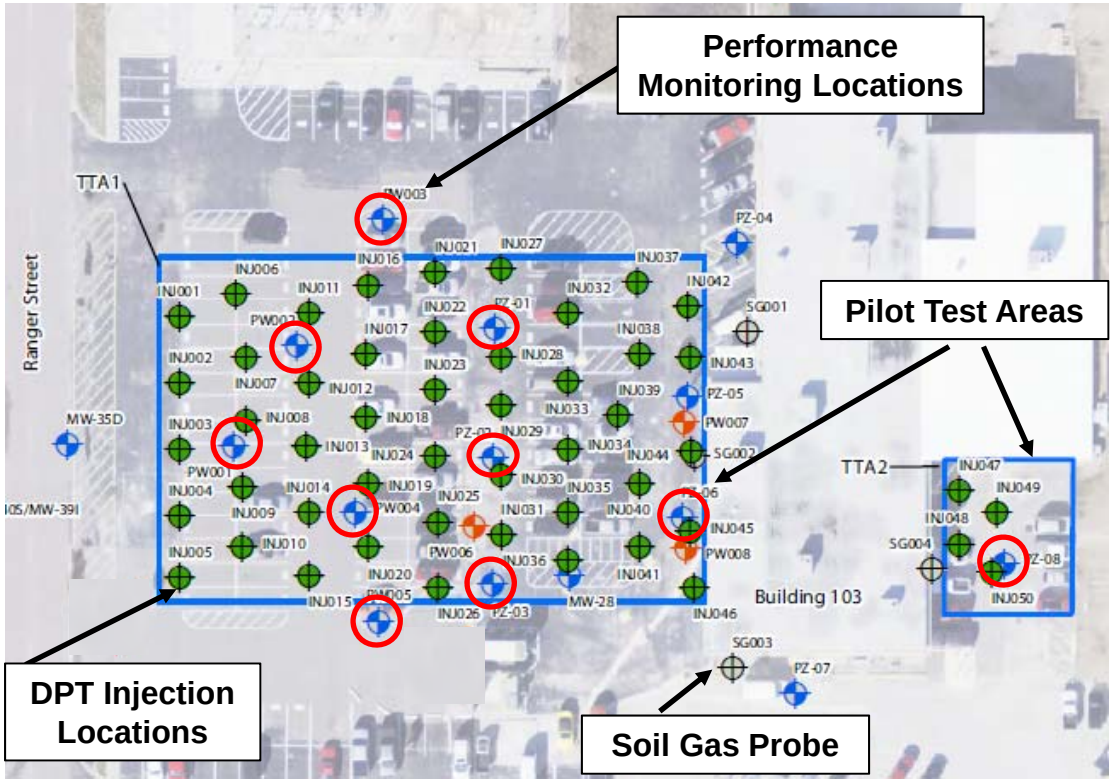
- Occupies 134 acres on eastern side of installation
  - –Industrial/commercial land use
- Fleet Readiness Command (FRC) (formerly NADEP) primary tenant on installation since 1940s
- Former dry cleaner facility located within OU3 property
- 7 identified groundwater plumes (Areas A – G)
- Buildings 780 and 106 also sources of contamination



OU3 Cross-Section - Northern Plume

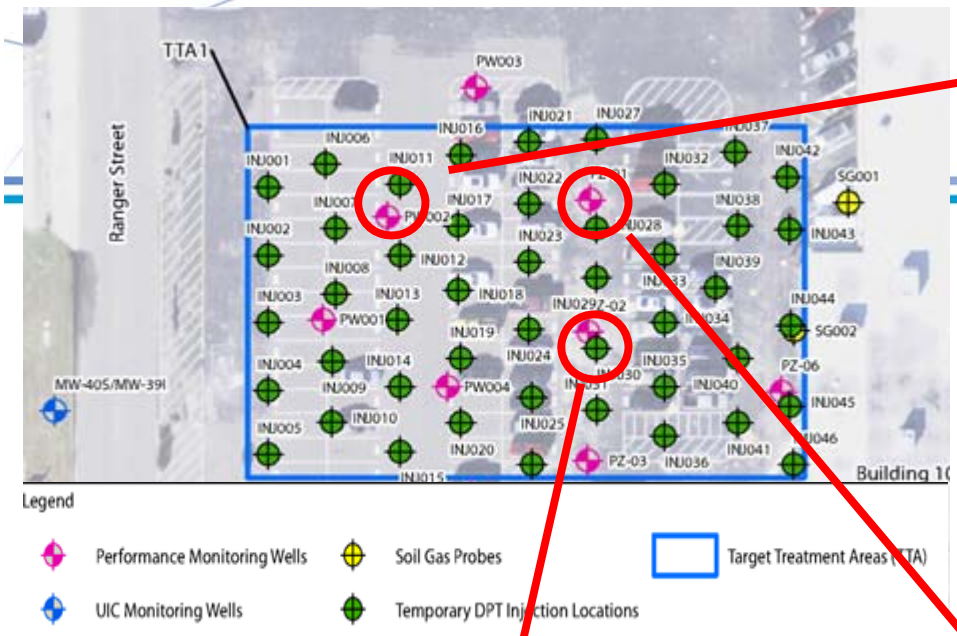


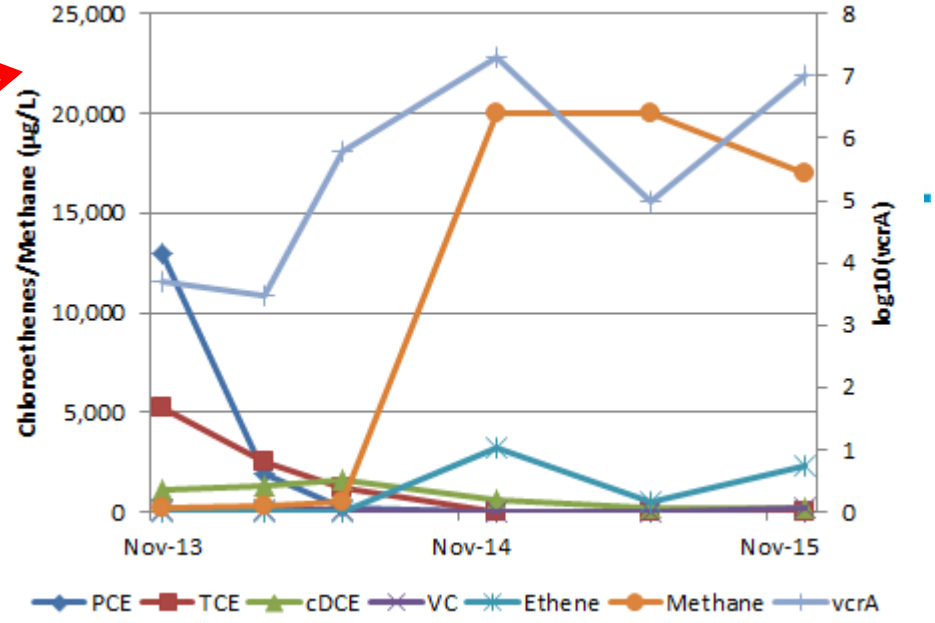
Pilot Test Summary

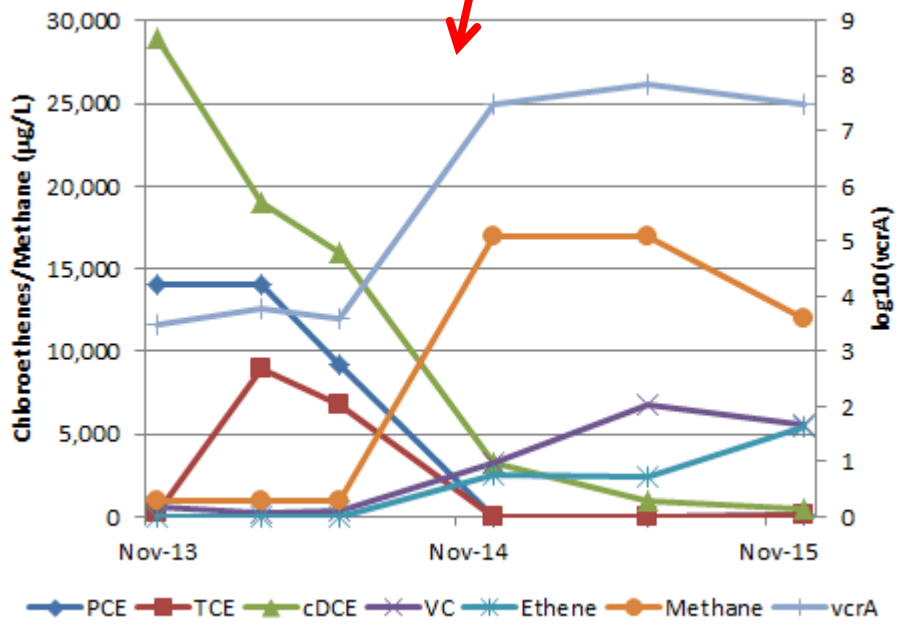


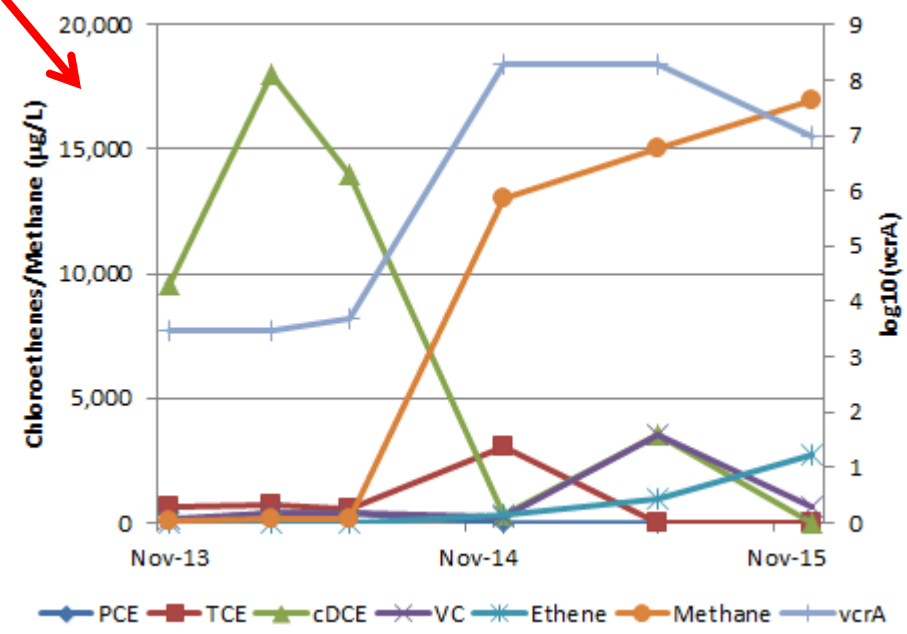
- 50 DPT injection locations
- December 2013
- 2 injection intervals per location
- 145,000 gallons of an 0.7% emulsified vegetable oil solution (EDS-ER™)
- Average flow rate of 1.8 gpm
- 100 liters of KB-1® and KB-1® Plus injected
- Bromide tracer used
- 10 performance monitoring wells
- 4 soil gas probes

Source: Geosyntec 2016


TTA1
Ranger Street
PW003
INJ021
INJ027
INJ016
INJ006
INJ001
INJ011
INJ032
INJ042
SG001
INJ022
INJ017
INJ038
INJ002
INJ023
INJ043
INJ012
INJ039
INJ008
INJ003
INJ013
INJ018
INJ044
PW001
INJ019
SG002
INJ004
INJ014
INJ024
INJ035
PZ-06
MW-40S/MW-39I
INJ009
PW004
INJ040
INJ045
INJ005
INJ010
INJ025
INJ020
PZ-03
INJ036
INJ041
INJ046
INJ015
Building 1
Legend
Performance Monitoring Wells
Soil Gas Probes
Target Treatment Areas (TTA)
UIC Monitoring Wells
Temporary DPT Injection Locations


Chloroethenes/Methane (µg/L)
log10(vcrA)
25,000
20,000
15,000
10,000
5,000
0
8
7
6
5
4
3
2
1
0
Nov-13
Nov-14
Nov-15
PCE
TCE
cDCE
VC
Ethene
Methane
vcrA


Chloroethenes/Methane (µg/L)
log10(vcrA)
30,000
25,000
20,000
15,000
10,000
5,000
0
9
8
7
6
5
4
3
2
1
0
Nov-13
Nov-14
Nov-15
PCE
TCE
cDCE
VC
Ethene
Methane
vcrA

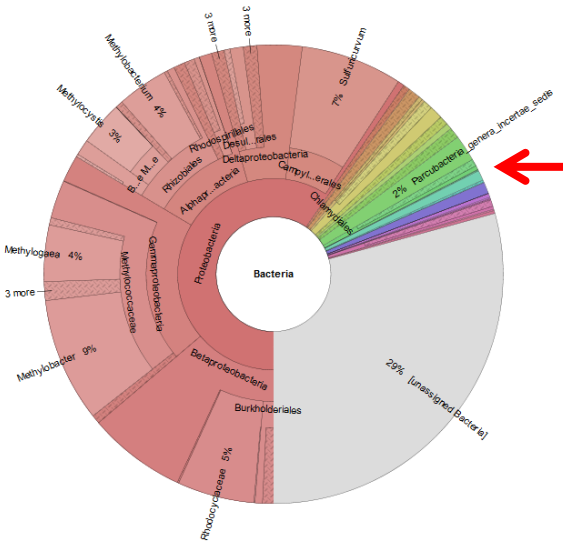

Chloroethenes/Methane (µg/L)
log10(vcrA)
20,000
15,000
10,000
5,000
0
9
8
7
6
5
4
3
2
1
0
Nov-13
Nov-14
Nov-15
PCE
TCE
cDCE
VC
Ethene
Methane
vcrA

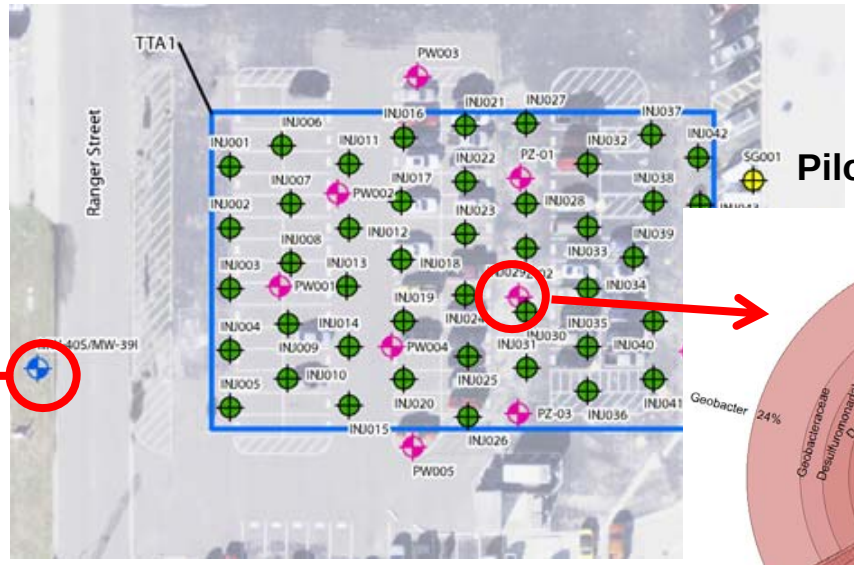
Microbial Composition

Battelle
The Business of Innovation

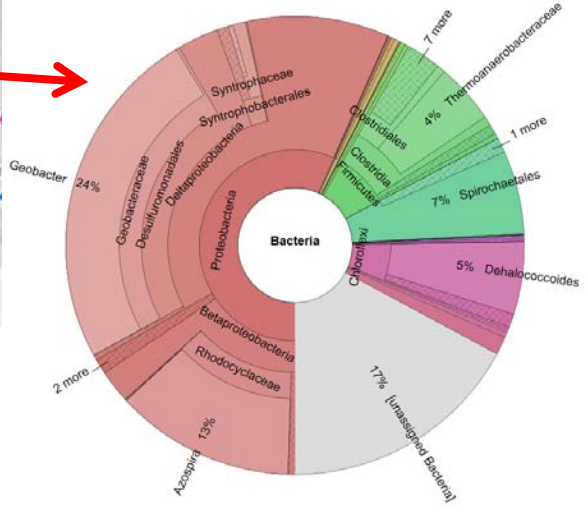
NAVFAC

Upgradient Well MW-40S





Pilot Test Well PZ-02



	% of Bacteria
Dehalococcoides	**0.01%**
Dehalogenimonas	**0.2%**
Methylobacter	**9%**
Sulfuricurvum	**7%**

	% of Bacteria
Dehalococcoides	**5%**
Geobacter	**24%**
Methylobacter	**9%**
Desulfuromonadales	**27%**

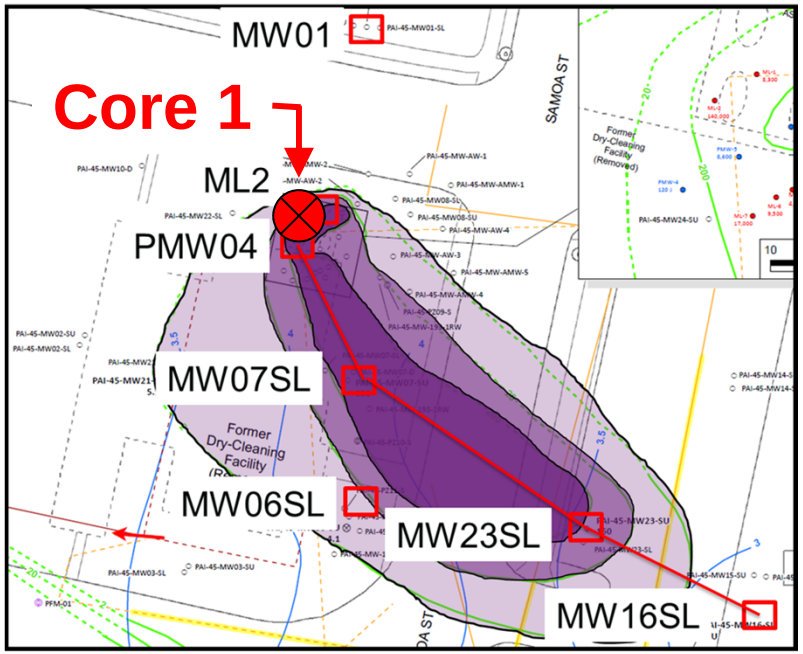
Source: Battelle 2015

Aerobic and Anaerobic VC degraders

- **Previous work at 5 different cVOC sites with different concentrations and biodegradation rates but generally low in DO and ORP**
  - **Found high concentrations of aerobic VC genes/transcripts (EtnC/EtnE) + anaerobic VC genes/transcripts (vcrA/bvcA)**
  - **Not true with methanotrophs**

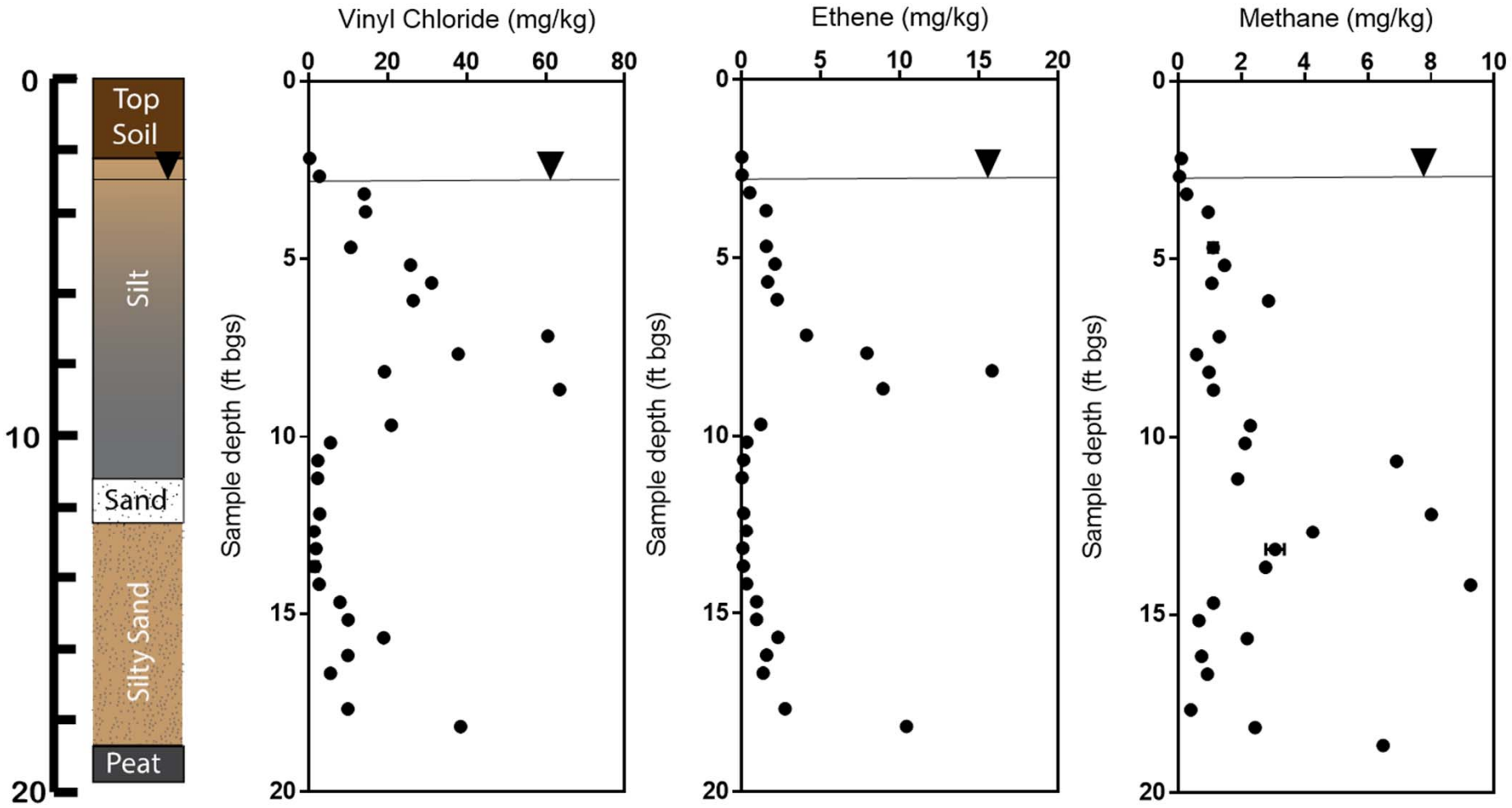
Parris Island, SC

Parameter	Avg. sample (39) concentrations
PCE + TCE	**292 µg/L**
cDCE	**2651 µg/L**
VC	**1938 µg/L**
Ethene	**218 µg/L**
Methane	**2621 µg/L**
DO	**0.74 mg/L**
ORP	**15 mV**



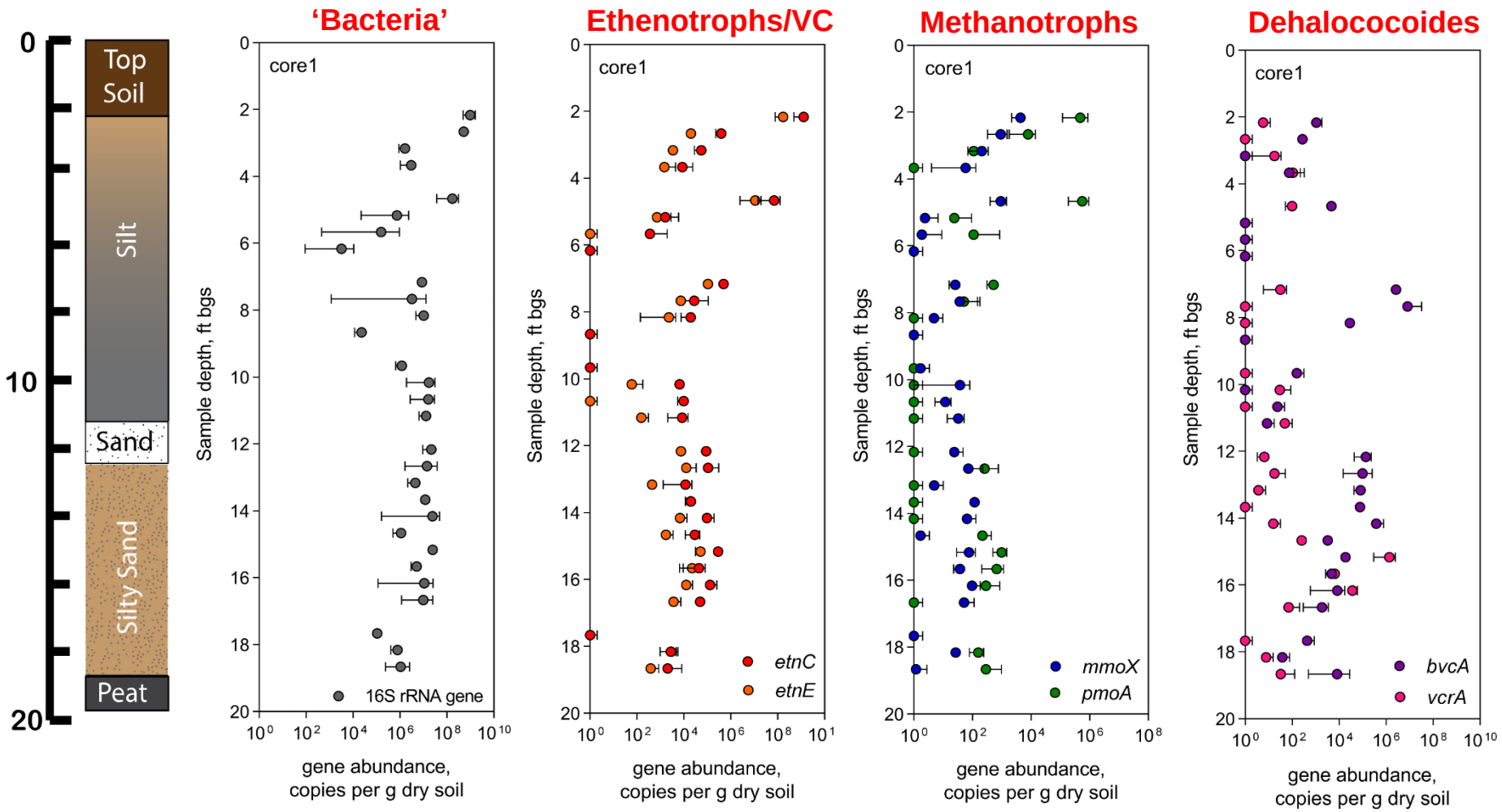
Source: Tim Mattes

Geochemical data



Source: Tim Mattes

qPCR data



Source: Tim Mattes

Introduction to MBTs - Key Points

- A variety of MBTs are available to assist in the following:
  - Determine if the remediation is working
  - Selection of the appropriate remediation strategy
  - Transition from active treatment to MNA
  - Additional lines of evidence for the above

- However, the application of which MBT to use must be carefully be considered taking into account the objectives
  - What are your goals?
  - What type of data will you need?
  - Which phase is the site?
  - What is the geochemical data saying (spatially and versus time)?
  - What are your other lines of evidence telling you?

Acknowledgements

- Tim Mattes
- Kerry Sublette
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- Frank Löffler
- Aaron Peacock
- Brady Lee
- Kate Kucharzyk

Navy Points of Contact

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arun.gavaskar@navy.mil

Questions

Wrap Up

- **Please complete the feedback questionnaire at the end of this webinar. We are counting on your feedback to make this webinar series relevant!**

- **Next OER2 Webinar Info….**

Title: Rehabilitation and Maintenance of Pump and Treat Systems

Presenter: Jennifer Segura (NAVFAC) and Fred Payne (ARCADIS)

Date: 31 January 2018

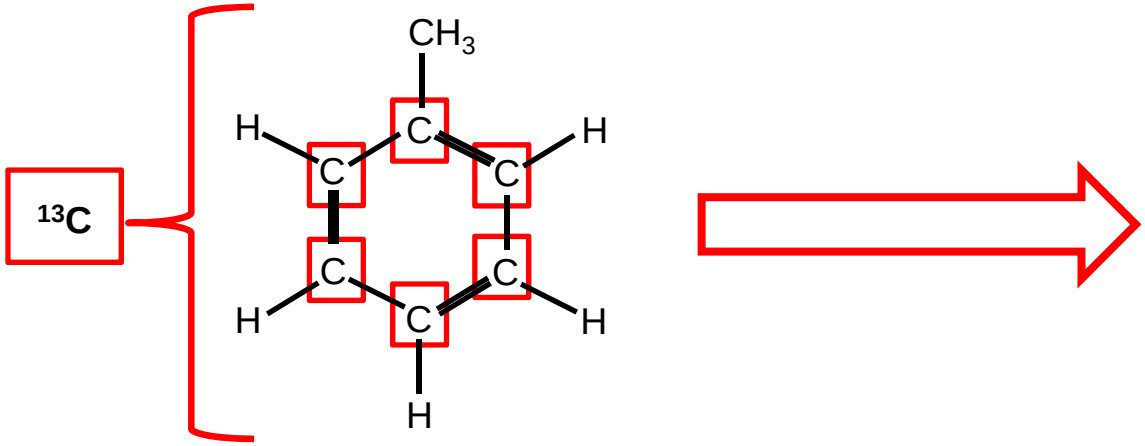
Time:1100-1200 PDT

- **Thank you for participating!**

Backup Material

- **Is biodegradation occurring?**
- **^{13}C label compound added to the 'environment'**

- **Knowledge of microbes or pathways involved is not required**
- **Only the active cells will incorporate the ^{13}C**



^{13}C incorporated into biomass!

$^{13}CO_2$

- **Cannot be used for electron acceptors**

Notes for CSIA

- **Isotope ratio shifts for COCs**
  - **Larger for chemical or biological processes**
  - **Smaller for physical processes**
  - **Differentiates between them (destructive and non-destructive)**

- **Can be used with both electron donors and acceptors**
  - **MTBE, cVOCs, etc.**
  - **2-D (C-H, C-Cl, etc) or even 3-D may be appropriate (C-H-Cl)**
  - **Can be used to discriminate between abiotic/biotic and also different degradation pathways (aerobic versus anaerobic)**

- **Forensics**
  - **Perchlorate - 2-D plot of $^{37}Cl/^{18}O$ clustering from natural versus artificial**

- **Applications to Vapor Intrusion**