Remediation Test Panel: Collecting and Interpreting Contaminant, Geochemical, Isotopic and Molecular Biology Data

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Pace Analytical Energy Services

Making analytical data work for you

- Pace created Pace Energy by bringing together:
  - Zymax (isotopic and forensic abilities)
  - Microseeps (geochemistry and isotopic)
  - Microbiology (Aaron Peacock's addition)
- Interpretation Services – working with you to make it meaningful
- Remediation Test Panel (RTP) – tying it all together

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Multiple Lines of Evidence (MLE)

- Based on:
  - EPA's 1998 "Technical Protocol …".
  - OSWER 9200.5-17P
- Each line of evidence systematically narrows the field of plausible interpretations.
- Steers the investigation toward a more accurate representation of the ground water system.
- MLE argument is made from 4 types of measurements:
  - Contaminant (from routine concentration monitoring)
  - Geochemical
  - Isotopic (via Compound Specific Isotope Analysis)
  - Microbiology
- These are the 4 components of the RTP.

Contaminant

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Rates

- Can generate attenuation rates from routine concentration monitoring.
- Attenuation is a lot more acceptable as a remedial strategy if it can be shown to be mostly degradation.
- Claiming measured attenuation rates are plausible degradation rates is goal of RTP.
  - Must show
    - Degradation has occurred
    - "Stage is set" for degradation to continue
  - Use all 4 lines of evidence.
  - Interpretation is best if evidence is considered both
    - As individual pieces
    - Holistically (*i.e.*, not separately).
- Idea behind ESTCP BioPic project (ER 201129)
  - Project of the year 2015

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Case Study

- Large site impacted with benzene, chlorobenzene and dichlorobenzenes
- Part of site underlain by peat layer, but more oxic areas not underlain by peat.
- Monitoring showed attenuation along a flow path.
- Regulators thought a large pump & treat system was needed.
- On site consultant decided to use RTP first to see if engineered biodegradation or even monitored natural attenuation (MNA) were a viable remedial strategies.

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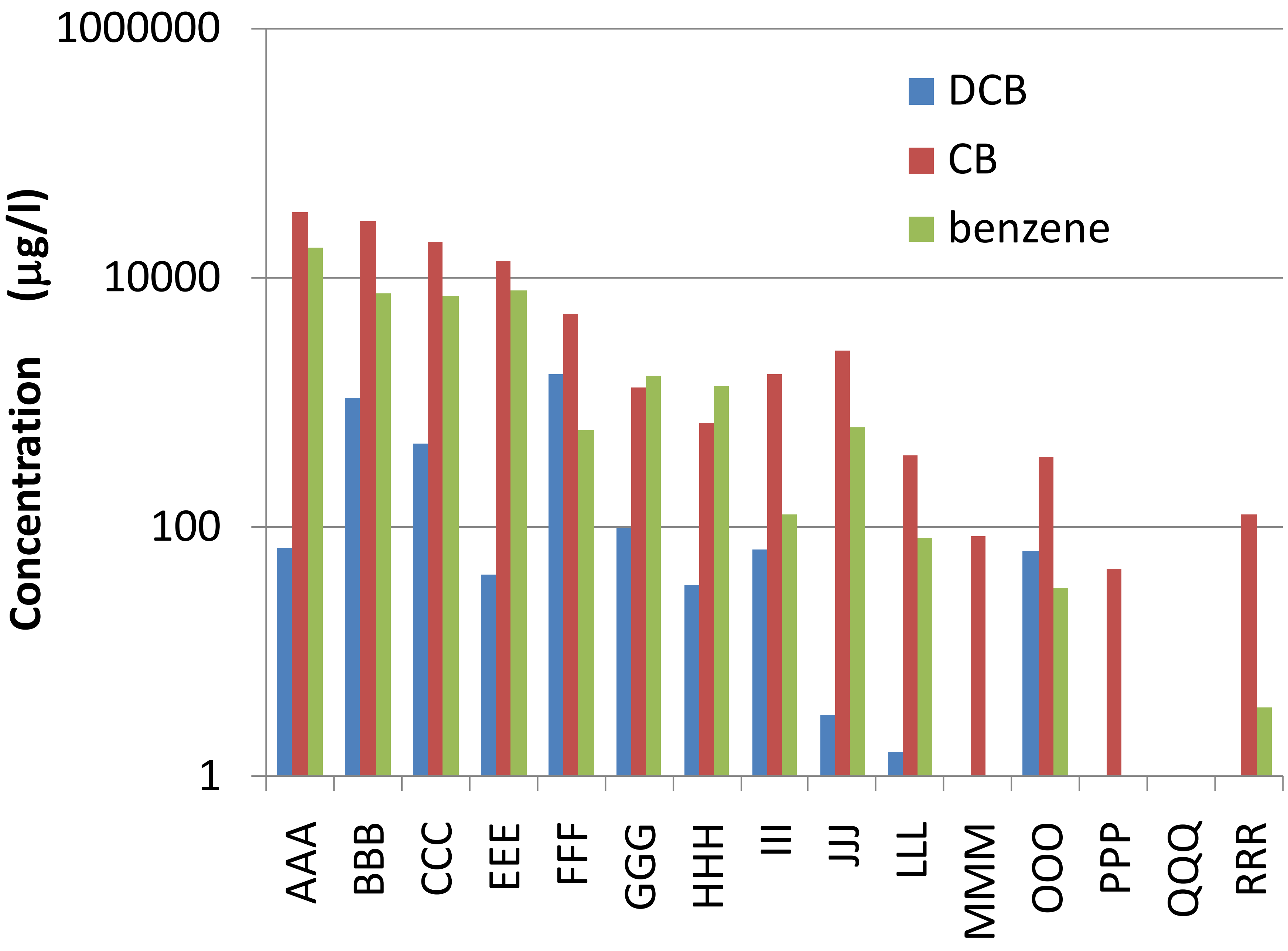
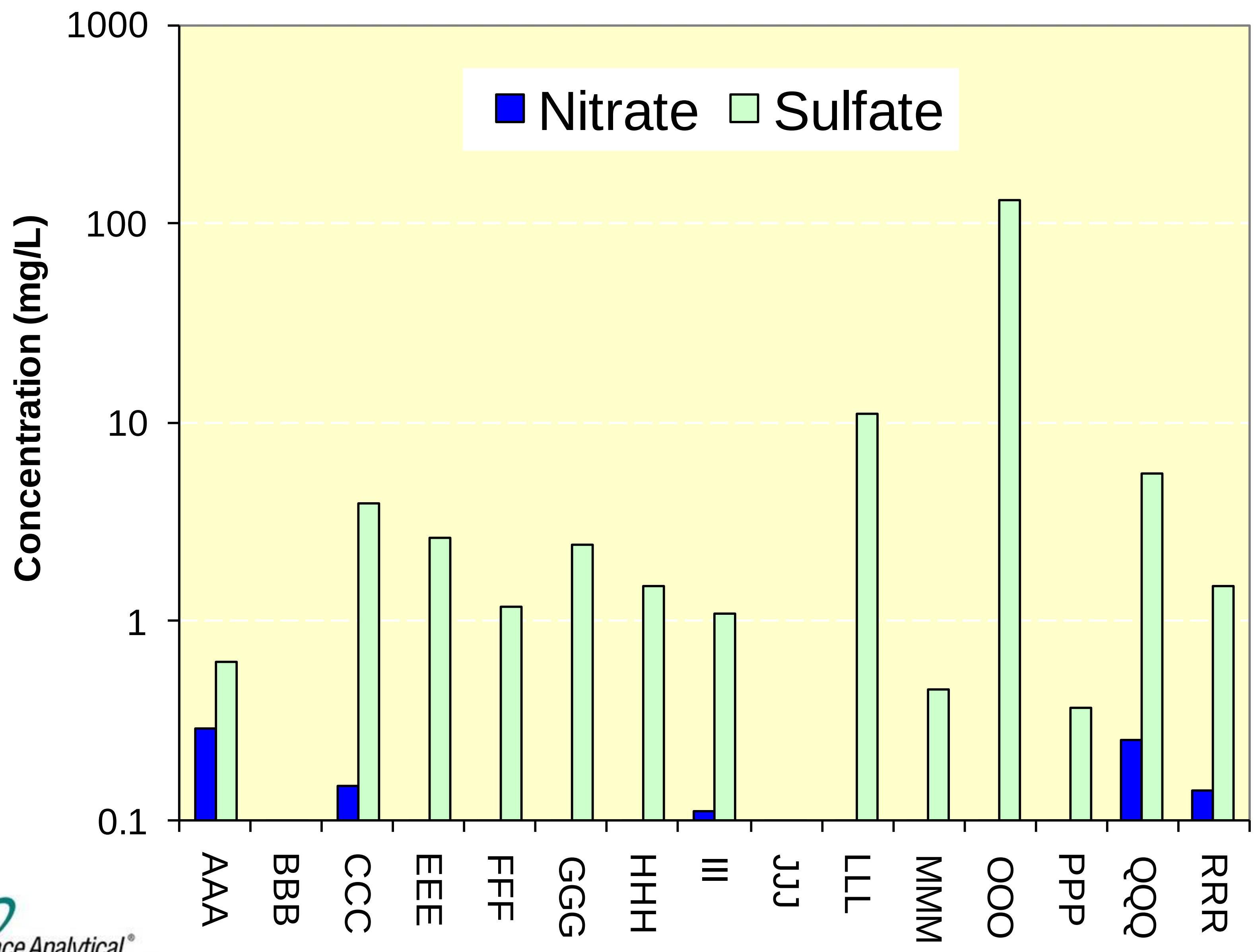


1000000
10000
100
1
Concentration (µg/l)
DCB
CB
benzene
AAA
BBB
CCC
EEE
FFF
GGG
HHH
III
JJJ
LLL
MMM
OOO
PPP
QQQ
RRR

Soluble Ionic Reactants



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Case study One: Goals

- Evaluate whether the attenuation of the contaminants was due to biodegradation or physical processes.
- Determine whether biodegradation was occurring for each of the analyzed locations and describe the likely attenuation mechanism active at each of those locations.
- Compare degradation pathways and rates under various conditions to evaluate whether inhibiting or limiting factors to biodegradation were present.
- Evaluate if the predominant attenuation pathways were site-wide.

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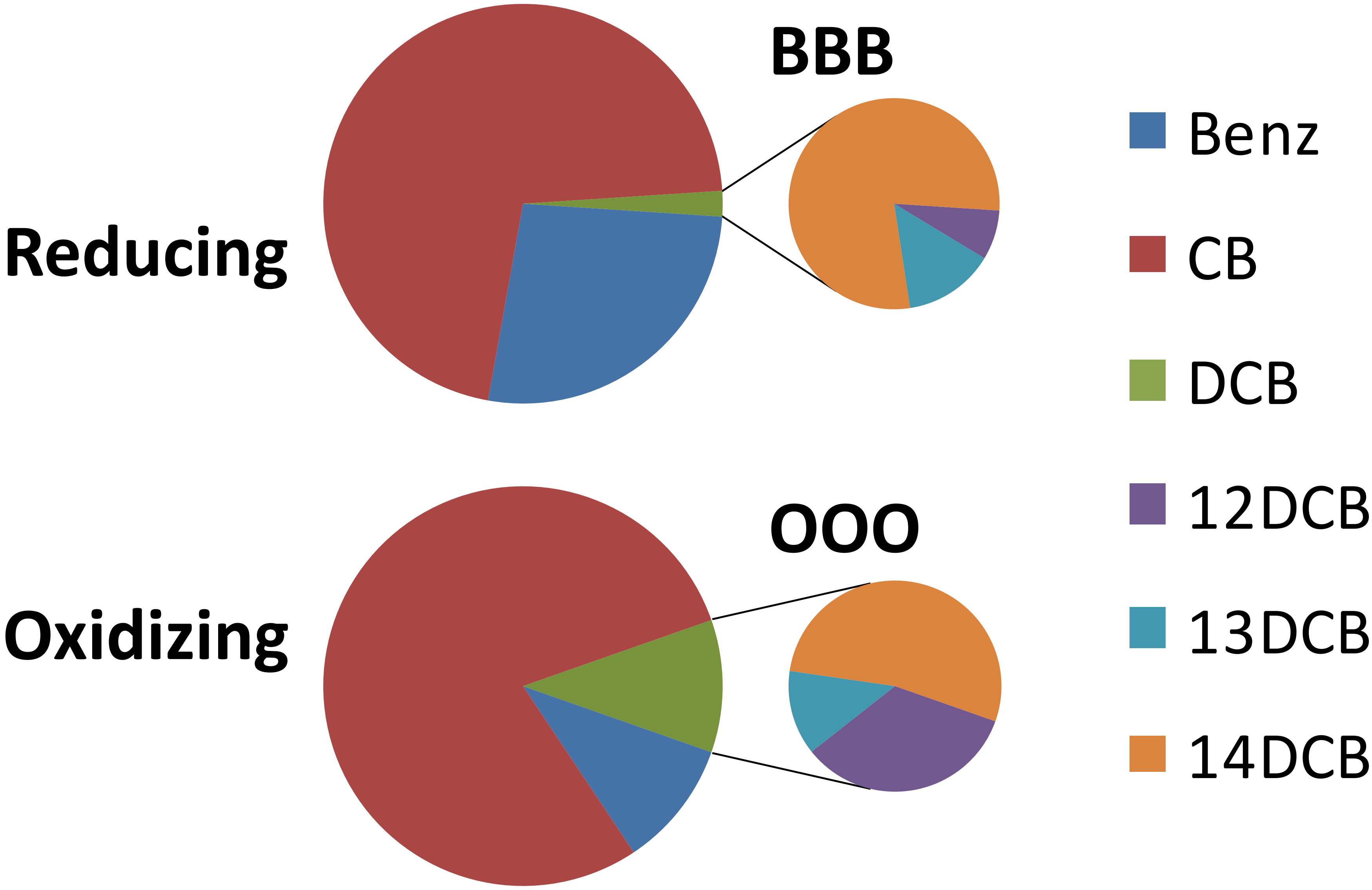

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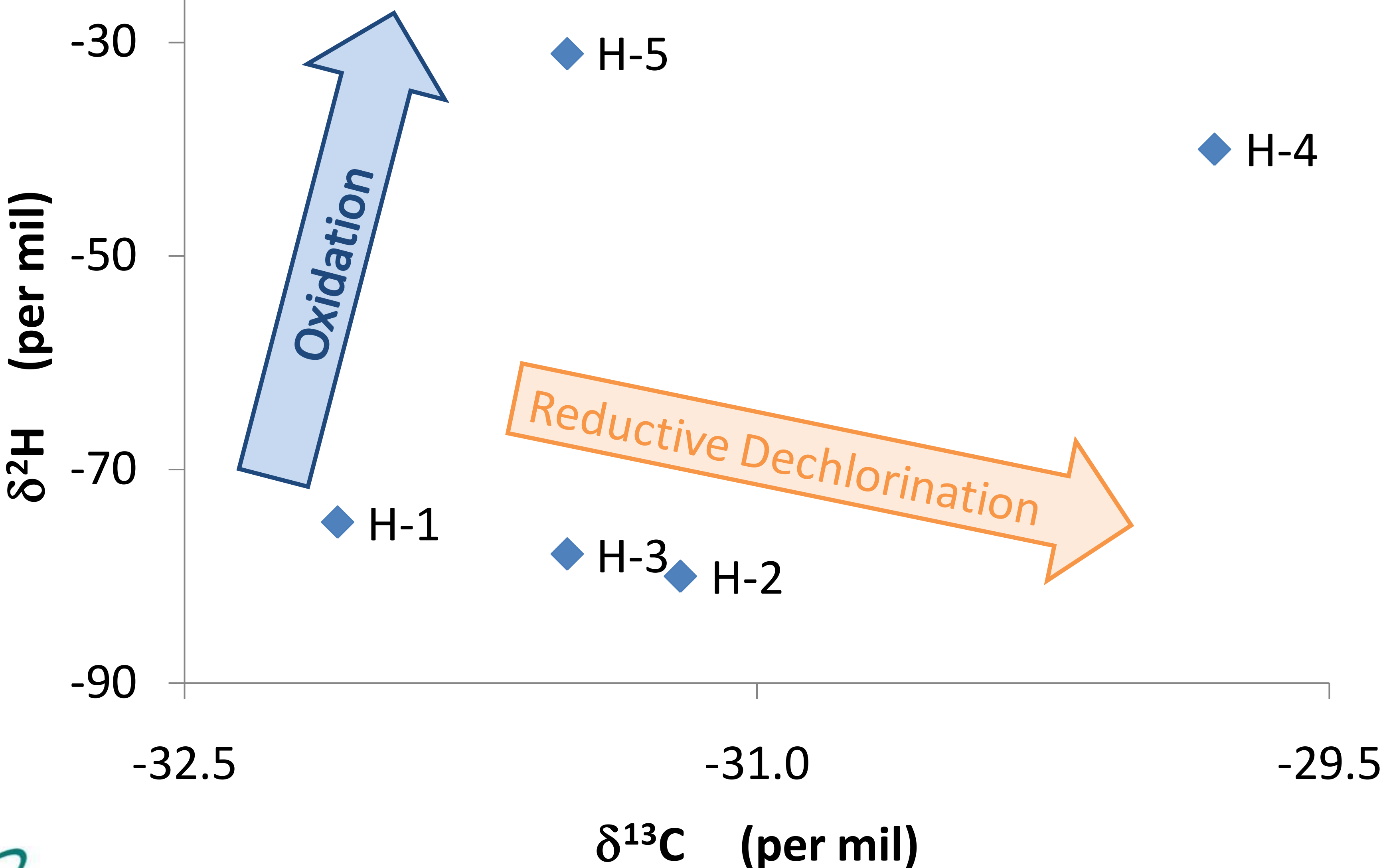
“Textbook” vs. Observed

- Goal of interpretation is that you can SHOW what the data means.
- That is a lot easier if you have a point of reference.
- RTP is set up to show a text book graph alongside graphs of the observed data.

DCB (Ideal)



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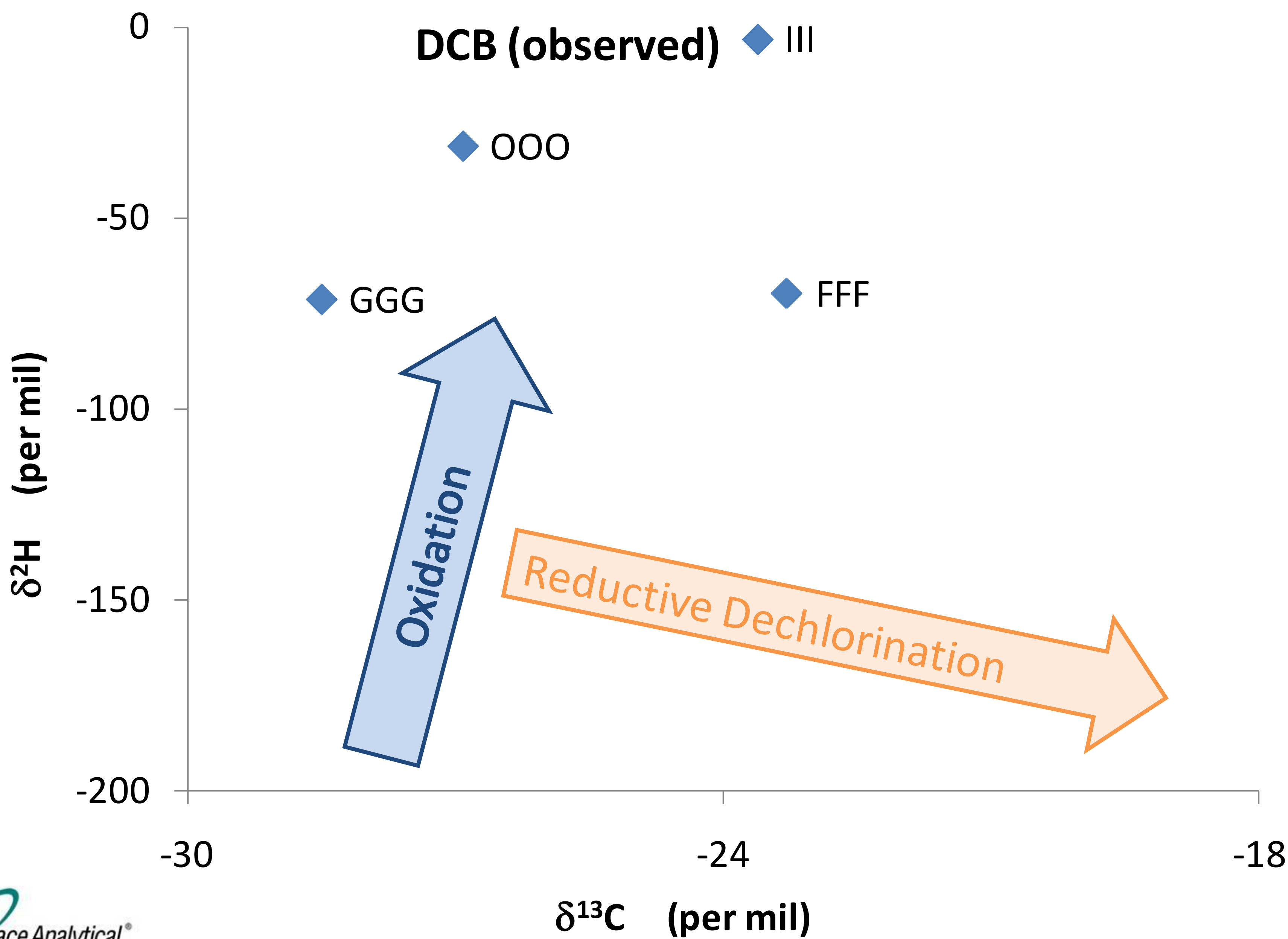
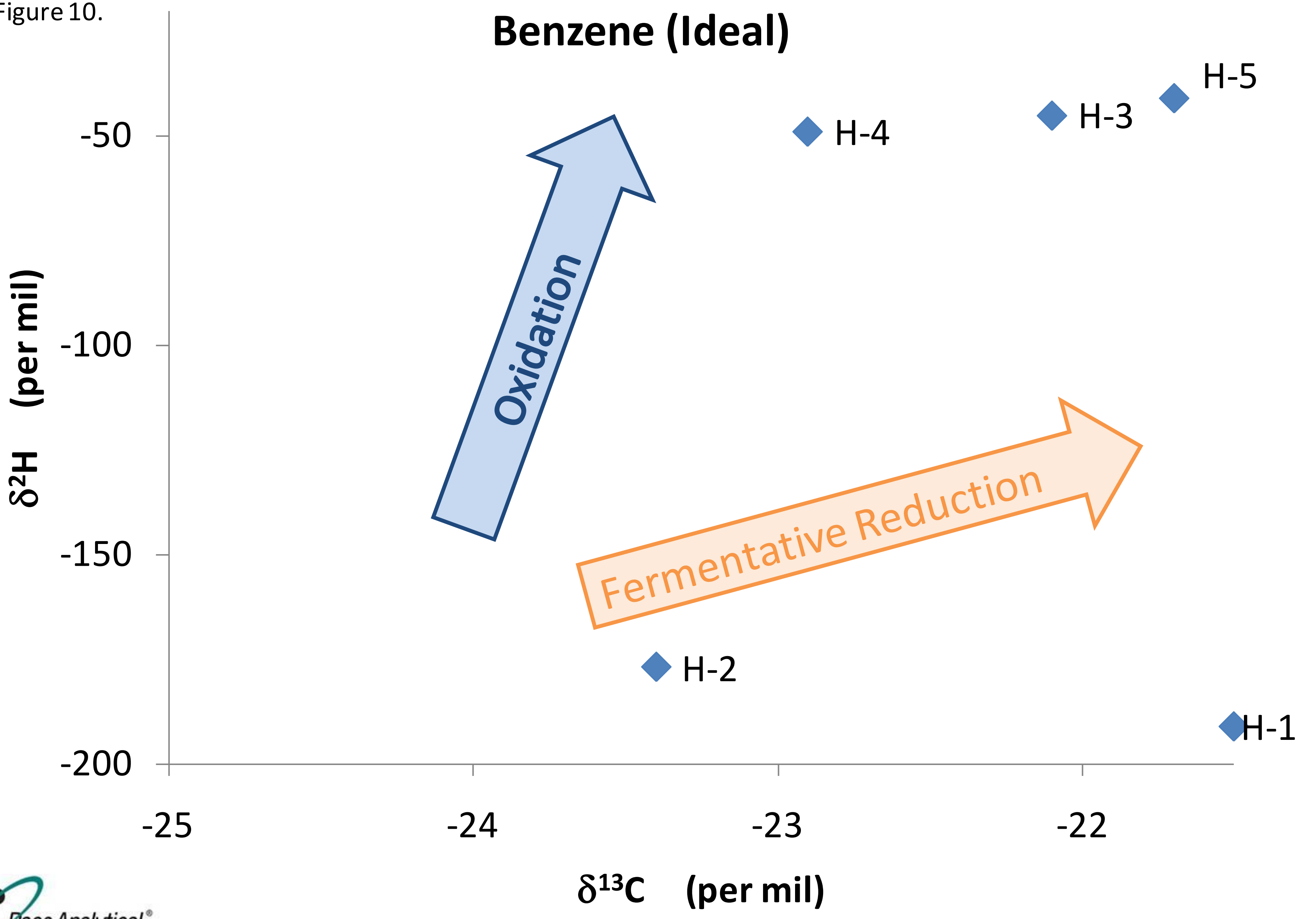
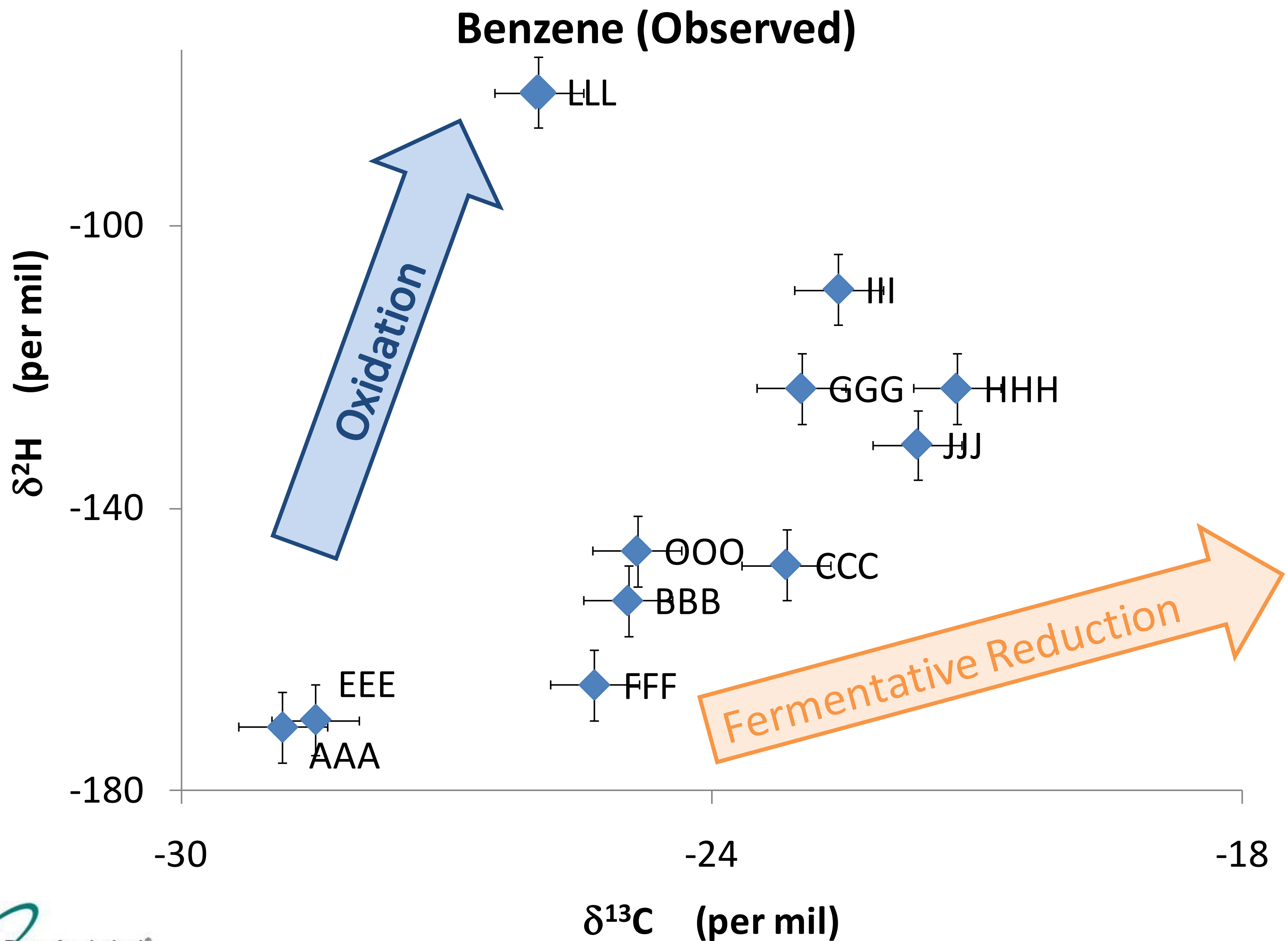


DCB (observed)
III
OOO
GGG
FFF
Oxidation
Reductive Dechlorination
0
-50
-100
-150
-200
-30
-24
-18
δ²H (per mil)
δ¹³C (per mil)

Figure 10.



Benzene (Ideal)
-50
-100
-150
-200
-25
-24
-23
-22
δ13C (per mil)
δ2H (per mil)
Oxidation
Fermentative Reduction
H-1
H-2
H-3
H-4
H-5



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RTP: Microbiology and Biogeochemical Degradation Pathway Analysis

- A combination of techniques employed depending on the contaminant or application
  - qPCR for specific groups of interest
  - DNA arrays for broader coverage
  - 16s sequencing
- Data is used to show the metabolic capabilities of the microbial community
  - General geochemical cycling and contaminant degrading potential

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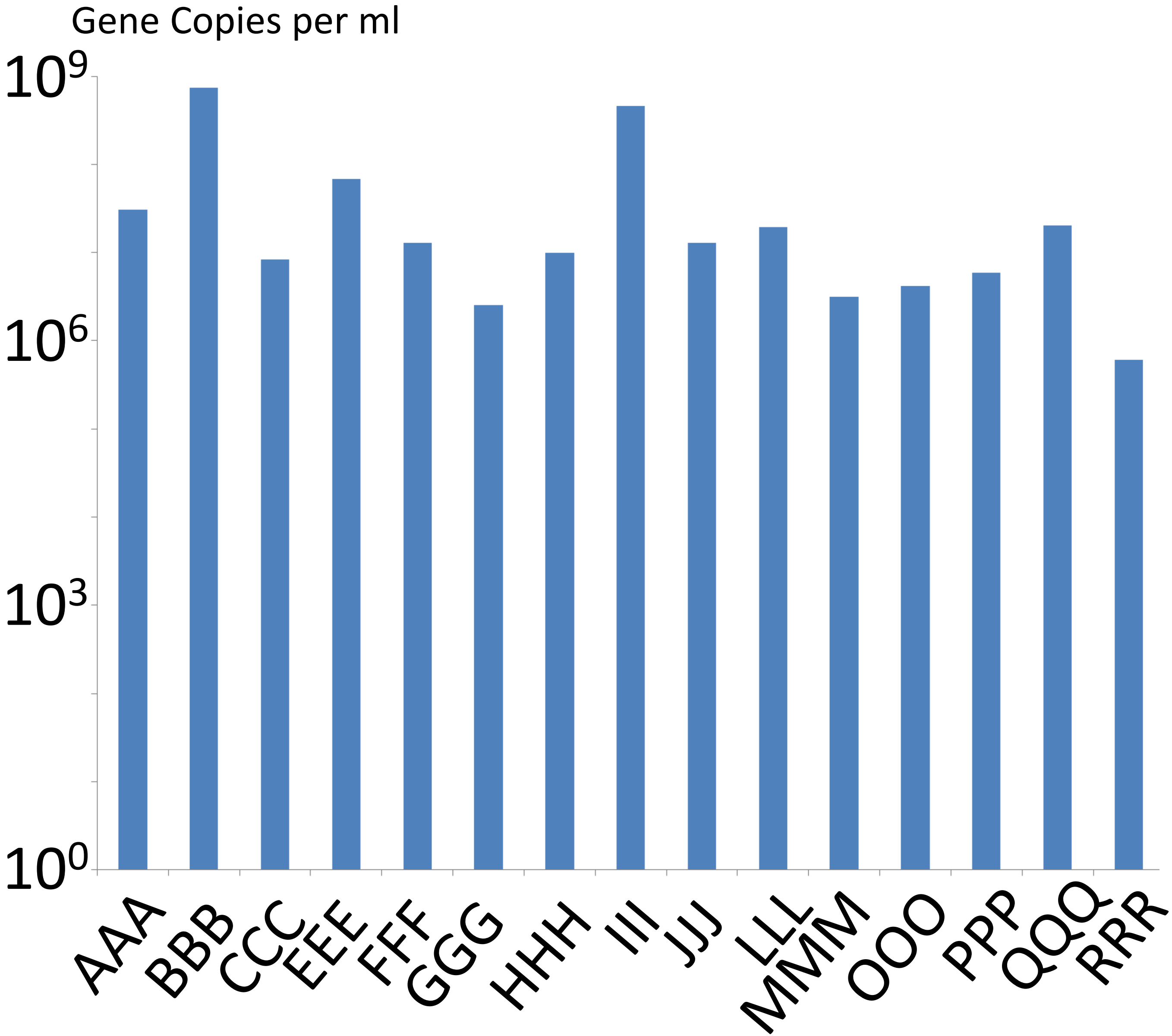
Dehalococcoides spp. (qPCR)



Compliments genetic sequencing .

Shows robust dechlorinating community.

Bacteria Known to Degrade Chlorobenzene(s)

Aerobic

Aerobic bacterial strains that utilize chlorinated benzenes as a sole source of carbon and energy.

Bacterial Strain	Congener	Reference
Burkholderia sp. strain PS12	CB	(Sander et al., 1991)
Burkholderia sp. strain PS14	CB	(Sander et al., 1991)
Escherichia hermanii	CB	(Kiernicka et al., 1999)
Hydrid strain WR1313	CB	(Oltmanns et al., 1988)
Pseudomonas aeruginosa RHO1	CB	(Brunsbach & Reineke, 1994)
Pseudomonas putida GJ31	CB	(Oldenhuis et al., 1989; Mars et al., 1997)
Pseudomonas sp. strain JS100	CB	(Haigler et al., 1988)
Pseudomonas sp. strain JS150	CB	(Haigler et al., 1992)
Pseudomonas sp. strain JS6	CB	(Pettigrew et al., 1991)
Ralstonia sp. strain JS705	CB	(van der Meer et al., 1998)
Rhodococcus phenolicus	CB	(Rehfuss & Urban, 2005)
Rhodococcus sp.	CB	(Vogt et al., 2004a)
Pseudomonas sp.	CB	(Vogt et al., 2004a)
Xanthobacter sp.	CB	(Vogt et al., 2004a)
Paenibacillus sp.	CB	(Vogt et al., 2004a)
Kocuria sp.	CB	(Vogt et al., 2004a)
Stenotrophomonas sp.	CB	(Vogt et al., 2004a)
Unidentified strain 1469	CB	(Nishino et al., 1992)
Unidentified strain 1474	CB	(Nishino et al., 1994)
Unidentified strain WR1306	CB	(Reineke & Knackmuss, 1984)
Planococcus sp strain ZD22	CB	(Li et al., 2006)
Acidovorax facilis 13517	CB	(Vogt et al., 2004a; Vogt et al., 2004b)
Cellulomonas turbata B529	CB	(Vogt et al., 2004b)
Pseudomonas veronii 13547	CB	(Vogt et al., 2004b)
Pseudomonas veronii B549	CB	(Vogt et al., 2004b)
Paenibacillus polymyxa B550	CB	(Vogt et al., 2004b)
Burkholderia sp. strain PS12	1,2-DCB	(Sander et al., 1991)
Burkholderia sp. strain PS14	1,2-DCB	(Sander et al., 1991; Rapp & Timmis, 1999)
Pseudomonas sp. strain GJ60	1,2-DCB	(Oldenhuis et al., 1989)
Pseudomonas sp. strain P51	1,3-DCB	(Van Der Meer et al., 1987)
Alcaligenes sp. strain OBB65	1,3-DCB	(Debont et al., 1986)
Burkholderia sp. strain PS12	1,3-DCB	(Sander et al., 1991)
Burkholderia sp. strain PS14	1,3-DCB	(Sander et al., 1991; Rapp & Timmis, 1999)
Pseudomonas sp. strain JS100	1,2-DCB	(Haigler et al., 1988)
Pseudomonas sp. strain P5	1,2-DCB	(Van Der Meer et al., 1987)
Acidovorax avenae	1,2-DCB	(Monferran et al., 2005)
Alcaligenes sp. strain R3	1,4-DCB	(Oltmanns et al., 1988)
Burkholderia sp. strain PS12	1,4-DCB	(Sander et al., 1991)
Burkholderia sp. strain PS14	1,4-DCB	(Sander et al., 1991; Rapp & Timmis, 1999)
Hydrid strain WR1323	1,4-DCB	(Oltmanns et al., 1988)
Pseudomonas aeruginosa RHO1	1,4-DCB	(Oltmanns et al., 1988;Brunsbach &Reineke, 1994)
Pseudomonas sp. B1	1,4-DCB	(Oltmanns et al., 1988)
Pseudomonas sp. strain JS150	1,4-DCB	(Haigler et al., 1992)
Pseudomonas sp. strain JS6	1,4-DCB	(Spain and Nishino, 1987)
Pseudomonas sp. strain P51	1,4-DCB	(Van Der Meer et al., 1987)
Sphingomonas (Alcaligenes) A175	1,4-DCB	(Schraa et al., 1986)
Unidentified strain 1474	1,4-DCB	(Nishino et al., 1994)
Xanthobacter flavus 14p1	1,4-DCB	(Sommer and Gorisch, 1997)
Rhodococcus phenolicus	1,4-DCB	(Rehfuss and Urban, 2005)
Burkholderia sp. strain PS12	1,2,4-TCB	(Sander et al., 1991)
Burkholderia sp. strain PS14	1,2,4-TCB	(Sander et al., 1991; Rapp & Timmis, 1999; Rapp, 2001)
Pseudomonas sp. strain P51	1,2,4-TCB	(Van Der Meer et al., 1987)
Pseudomonas chlororaphis RW71	1,2,3,4-TeCB	(Potrawfke et al., 1998a)
Burkholderia (Pseudomonas) PS12	1,2,4,5-TeCB	(Beil et al., 1997; Beil et al., 1998)
Burkholderia sp. strain PS14	1,2,4,5-TeCB	(Sander et al., 1991; Rapp & Timmis, 1999

Anaerobic

Examples of anaerobic reductive dechlorination of CBs.

Congener	Reference
Trichlorobenzenes	Holliger et al., 1992
Trichlorobenzenes	Jayachandran et al., 2004
Trichlorobenzenes	Adrian et al., 2000
Dichlorbenzenes	Fung et al., 2009
Dichlorbenzenes	Bosma et al., 1988
Monochlorinated Benzene	Fung et al., 2009
Monochlorinated Benzene	Nowak et al., 1996
Monochlorinated Benzene	Masunaga et al., 1996
Monochlorinated Benzene	Liang et al., 2013
Benzene	Liang et al., 2013

Bacteria identified in CB anaerobic dechlorination.

Bacterial Genus	Reference
Dehalococcoides sp.	Jayachandran et al., 2003
Dehalococcoides sp.	Adrian et al., 2000
Dehalococcoides sp.	Fennell et al., 2004
Dehalococcoides sp.	Zhou et al., 2015
*Geobacter sp.	Zhou et al., 2015
Dehalococcoides sp.	Vandermeeren et al., 2014
Desulfitobacterium sp.	Vandermeeren et al., 2014
Dehalobacter sp.	Vandermeeren et al., 2014
Sedimentibacter sp.	Vandermeeren et al., 2014
Dehalobacter sp.	Nelson et al., 2014

Cometabolic

Example bacterial genus with members capable of chlorinated benzene cometabolism

Genus	Contaminant	Cometabolite	Reference
Pseudomonas	1,2-DCB, 1,3-DCB	CB	Haigler et al., 1992
Pseudomonas	1,2-DCB, 1,2,4-TCB	CB	Brunsbach and Reineke, 1994
Methylosinus	1,2,3-TCB	Methane/Formate	Sullivan and Chase, 1996
Methylocystis	CB	Methane	Jechorek et al., 2003
Mycobacterium	CB	Propane	Burback and Perry, 1993
Pseudomonas	CB	Benzene	Bestetti et al., 1992
Pseudomonas	1,2-DCB	Benzene	Ballschmiter and Scholz, 1981

Genetic Sequencing Bacterial Profile

Monitoring Well LLL				
Genus (closest match)	% of profile	Metabolism	Gram	Putative COC Capability
sulfuritalea	6.33	Falculative/SOX	Negative	
chlamydia	5.39		Negative	
nitrosovibrio	4.45	Aerobic	Negative	
streptomyces	4.45	Aerobic	Positive	
candidatus protochlamydia	3.79	Aerobic		
methylobacter	3.59	Aerobic/MOB	Negative	Cometabolic Potential
eubacterium	3.27	Anaerobic	Variable	
geobacter	1.79	Anaerobic/MRB	Negative	Produces Cofactor/AB
methylocystis	1.70	Aerobic/MOB		Cometabolic Potential
verrucomicrobium	1.50	Anaerobic/Fermenter	Negative	
aquicella	1.49	Aerobic	Negative	
arthrobacter	1.48	Aerobic	Positive	
nitrospira	1.47	Aerobic/NOB	Negative	
candidatus metachlamydia	1.46	Aerobic	Negative	
candidatus rhabdochlamydia	1.25	Aerobic	Negative	
opitutus	1.23	Anaerobic	Negative	
dehalococcoides	1.21	Anaerobic	Positive	Reductive Dechlorination
pelotomaculum	1.19	Anaerobic	Positive	Anearobic Benzene
clostridium	1.16	Anaerobic/Fermenter	Positive	Produces Cofactors
salinibacter	1.16			
dechloromonas	1.12	Anaerobic/NRB	Negative	Anearobic Benzene
candidatus babela	1.11			
planktothricoides	1.07			
prochlorococcus	1.02	Aerobic	Negative	
desulfomonile	0.99	Anaerobic/SRB	Negative	Reductive Dechlorination
anaeromyxobacter	0.93	Anaerobic/MRB	Negative	
acidobacterium	0.91	Aerobic	Negative	

Bacterial phylotypes capable of :

- Reductive dechlorination of CBs
- Production of cofactors for reductive dechlor.
- Cometabolism of CBs
- Anaerobic benzene degradation

Demonstrates large presence of contaminant degraders.

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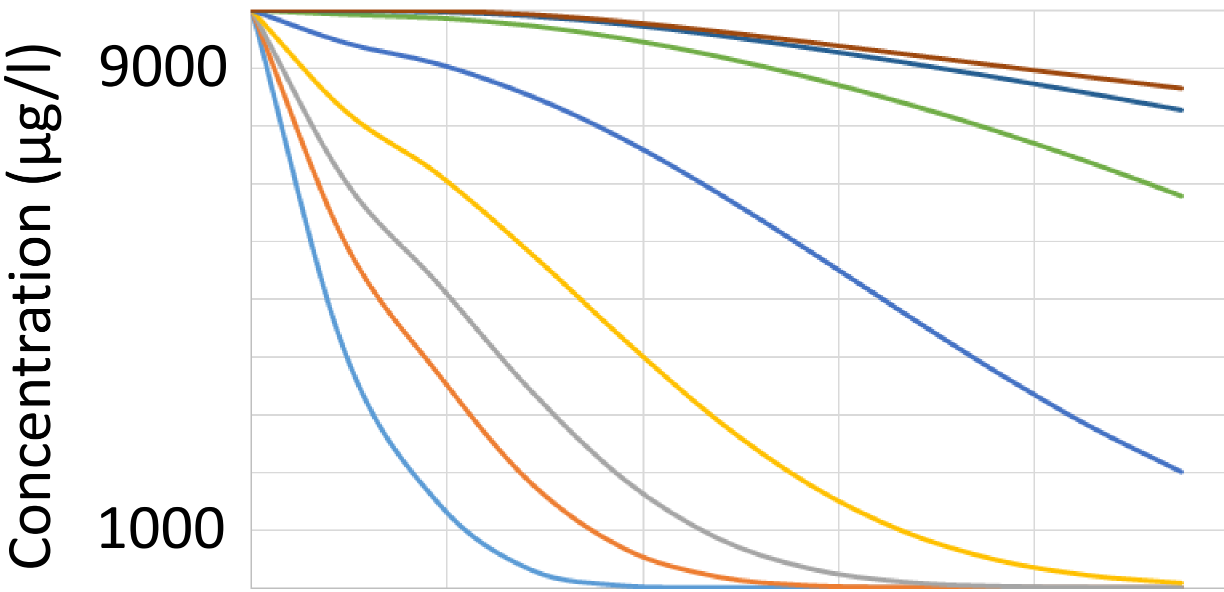
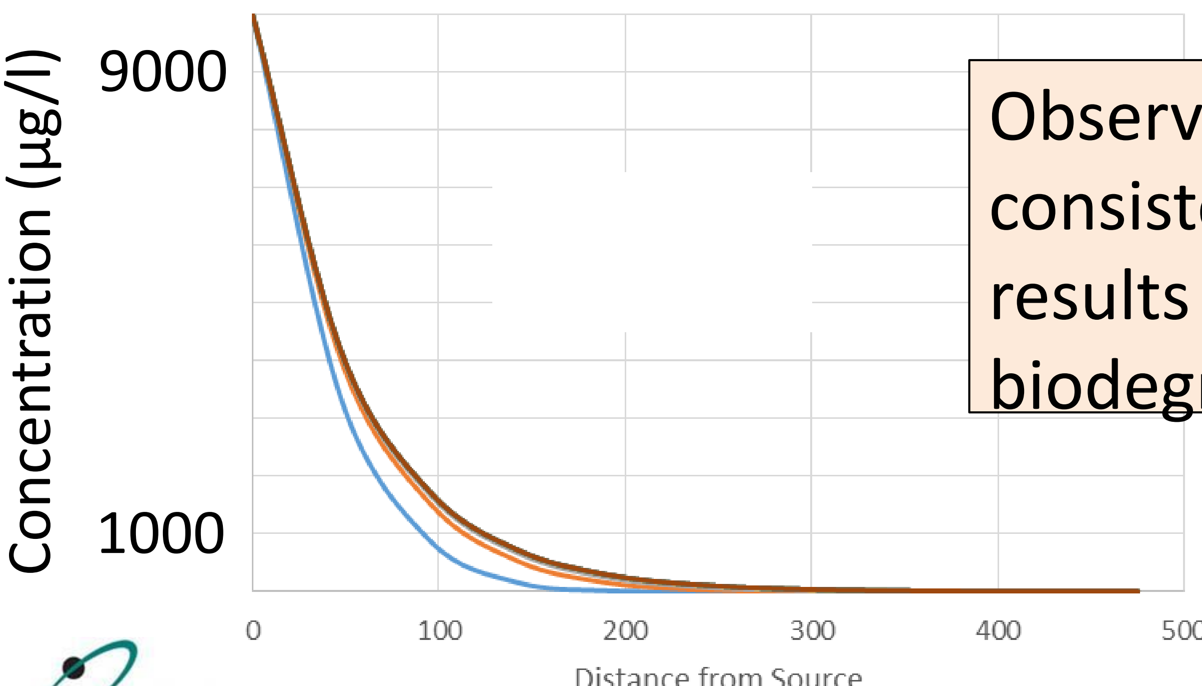
Comparison Without and With Biodegradation





Observed data are consistent with simulated results that include biodegradation.

Summary

- RTP puts together multiple lines of evidence, using 4 types of measurements:
  - Contaminant (from routine concentration monitoring)
  - Geochemical
  - Isotopic (via Compound Specific Isotope Analysis)
  - Microbiology
- Ideal for sites with multiple degradation mechanisms.
- RTP lowers remedial costs:
  - RTP could be used to precisely target remedial actions for maximum efficiency and effect with minimal footprint.
  - Can be used to eliminate both capital and O&M costs.

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