Evaluating Enhanced Bioremediation of 1,4-Dioxane with CB1190

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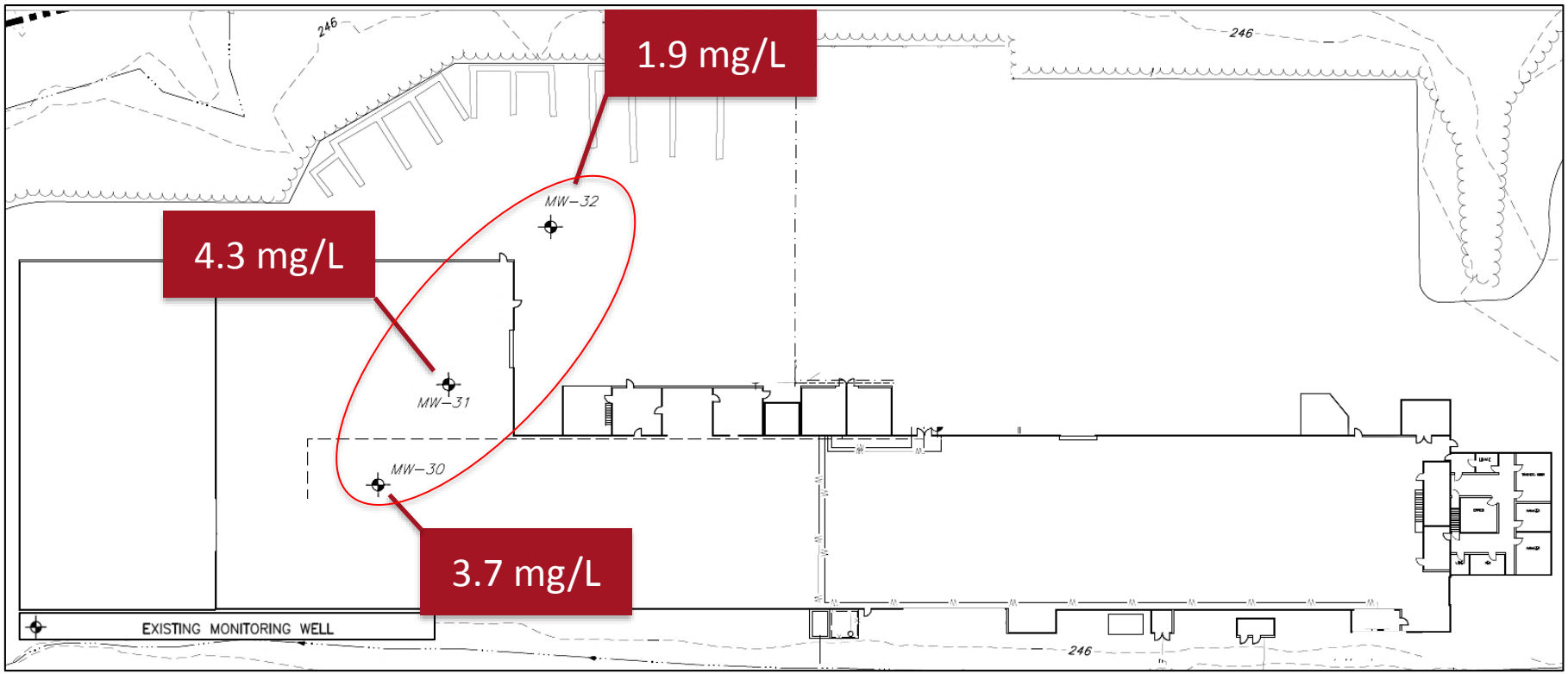
Site History

- Manufacturing company in Pennsylvania – 50 years ago
- Shallow, unconfined aquifer
- Several processing areas, all used chlorinated solvents as degreasers
- Two separate plumes: east and west
  - Eastern plume has very low 1,4-dioxane concentrations
  - Western plume has high 1,4-dioxane concentrations

1,4-Dioxane Concentrations



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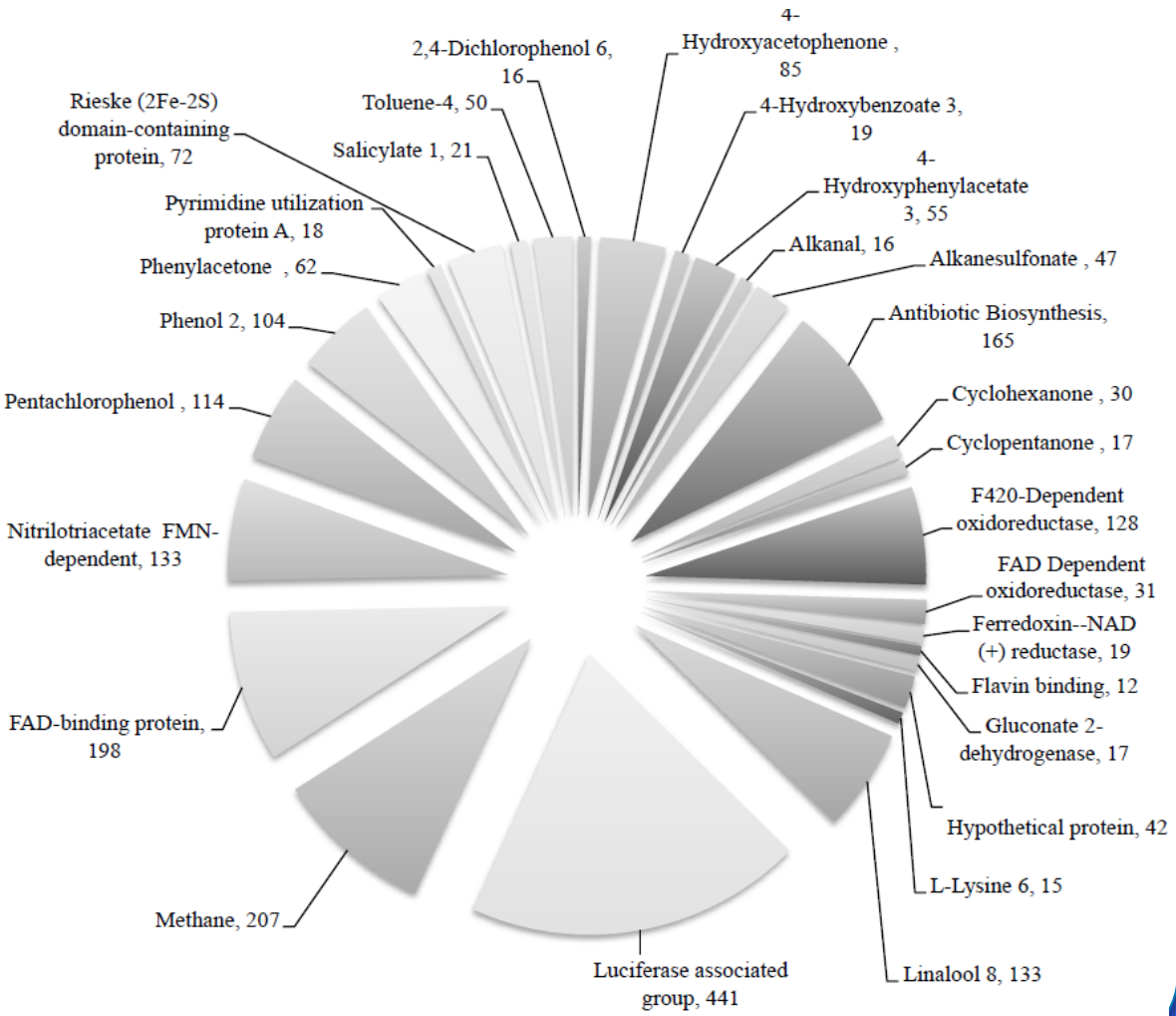
Pseudonocardia dioxanivorans: CB1190

- Biodegrades
  - 1,4-dioxane
  - Benzene
  - Toluene
  - THF
  - Diethyl ether
- Over 2,000 monooxygenase associated genes



P. Gedalanga et al., Appl. Envrion. Microbiol. 2014, 20, S1 – S5

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Site Assessment

- Chemical
  - VOC analysis
- Geochemical
  - Kjeldahl Nitrogen, Phosphorus
  - NO_3^-, NO_2^-
  - TOC

Site Assessment

- Chemical analysis
  - PCE
  - TCE
  - cDCE
  - VC
  - 1,1-DCE
  - 1,1,1-TCA

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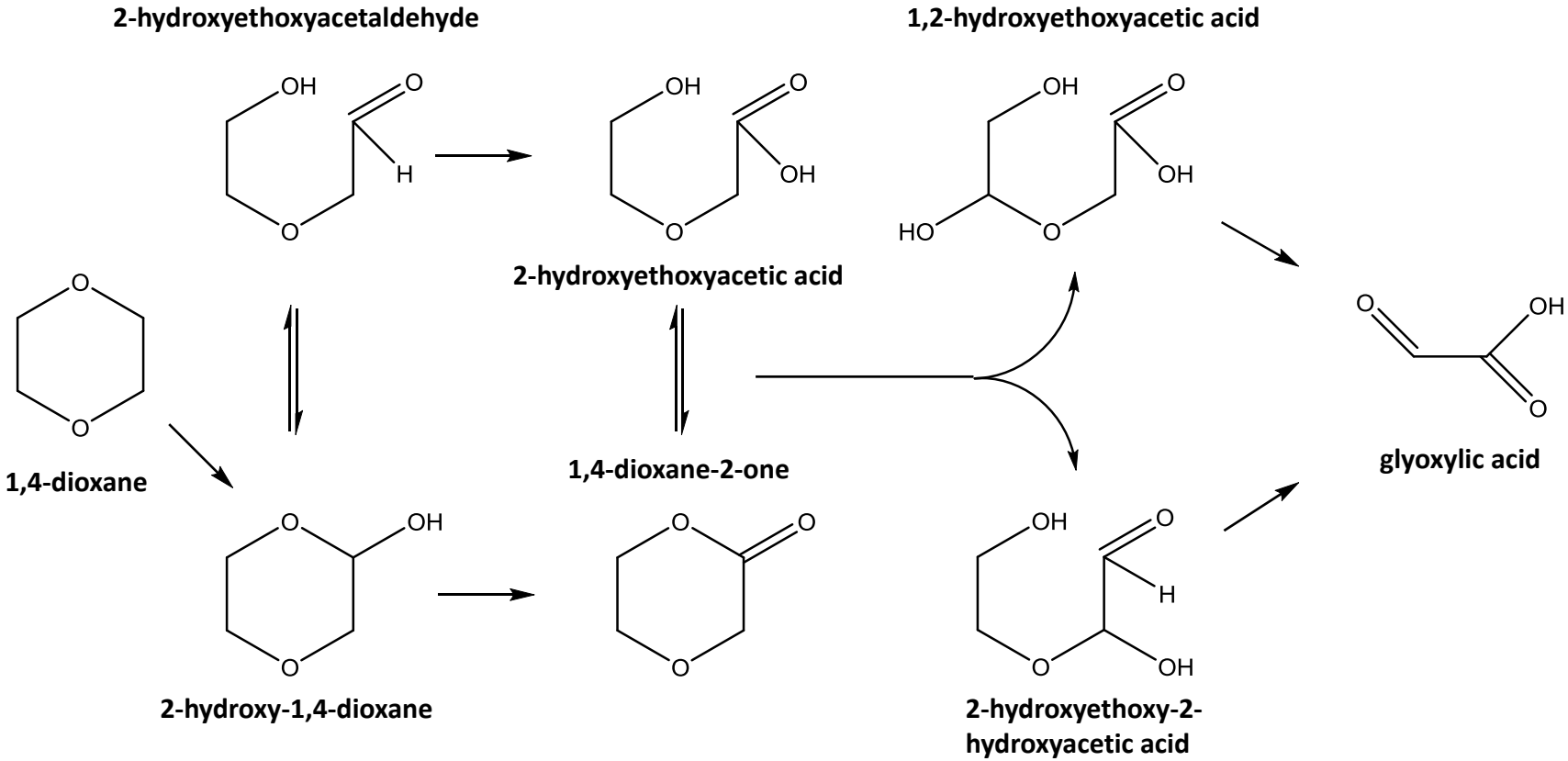
Degradation Pathway



P. Gedalanga et al., Remediation. 2016, 27, 93–114

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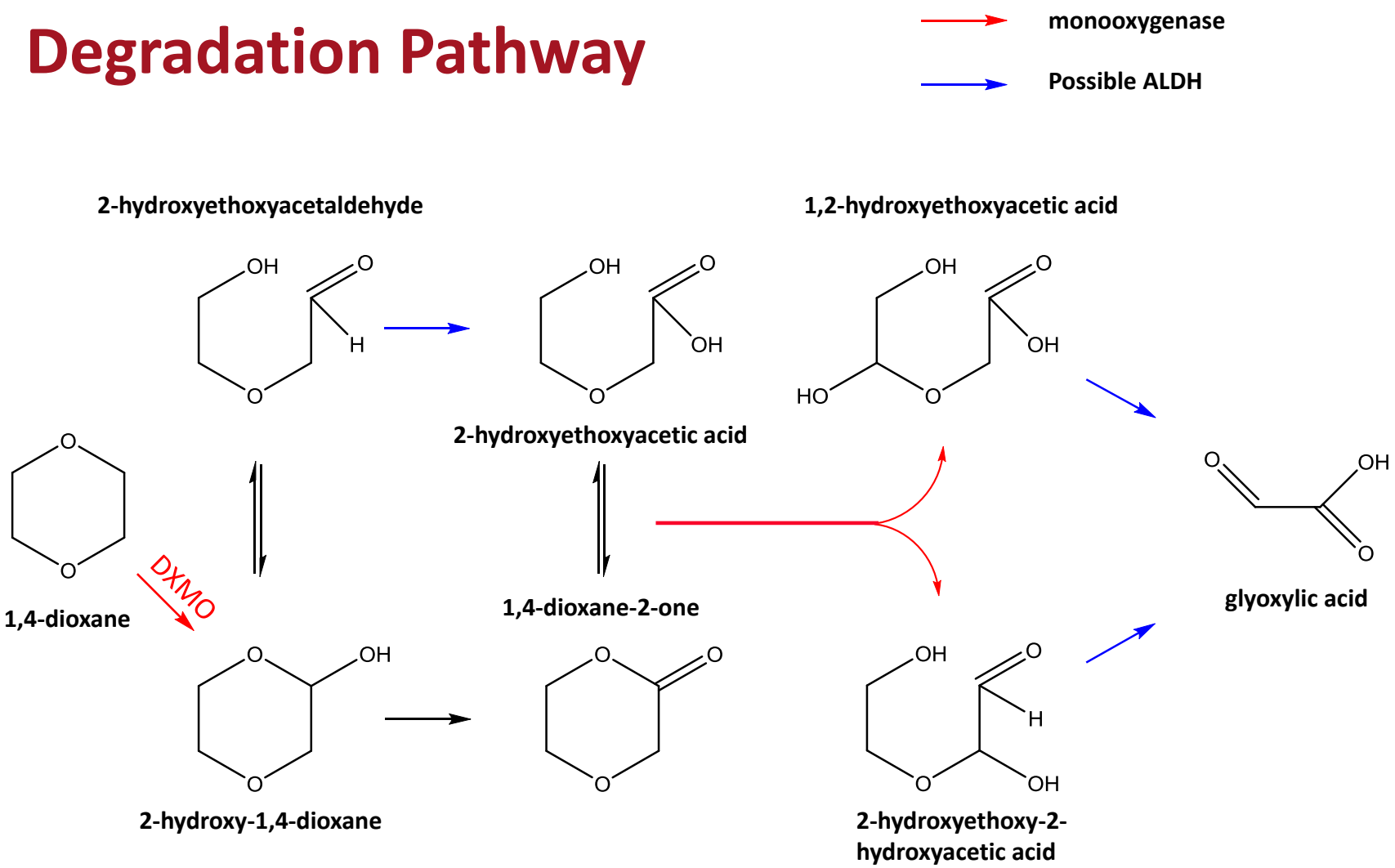
Degradation Pathway



P. Gedalanga et al., Remediation. 2016, 27, 93–114

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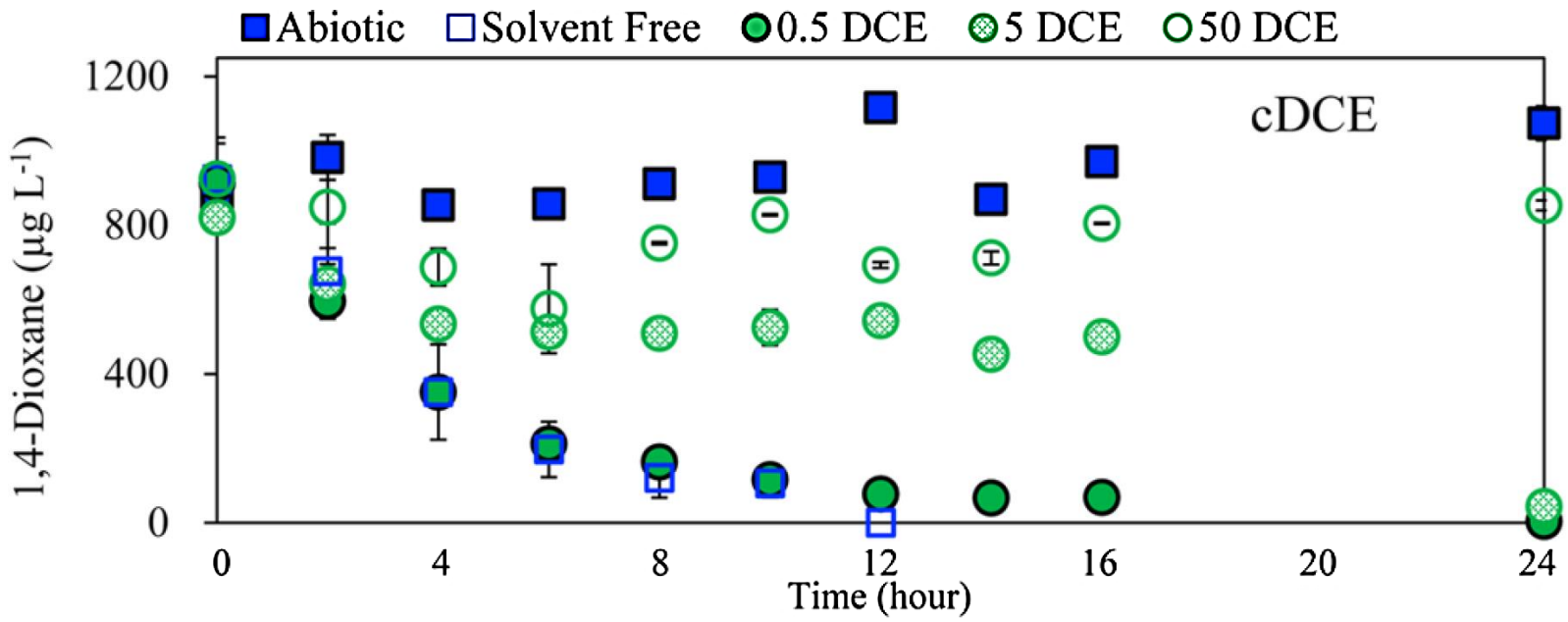
Inhibition by cis-1,2-DCE

- DXMO and ALDH suppressed when exposed to > 5 mg/L (Zhang et al.)



S. Zhang et al., Environ. Sci. Technol. 2016, 50, 9599−9607

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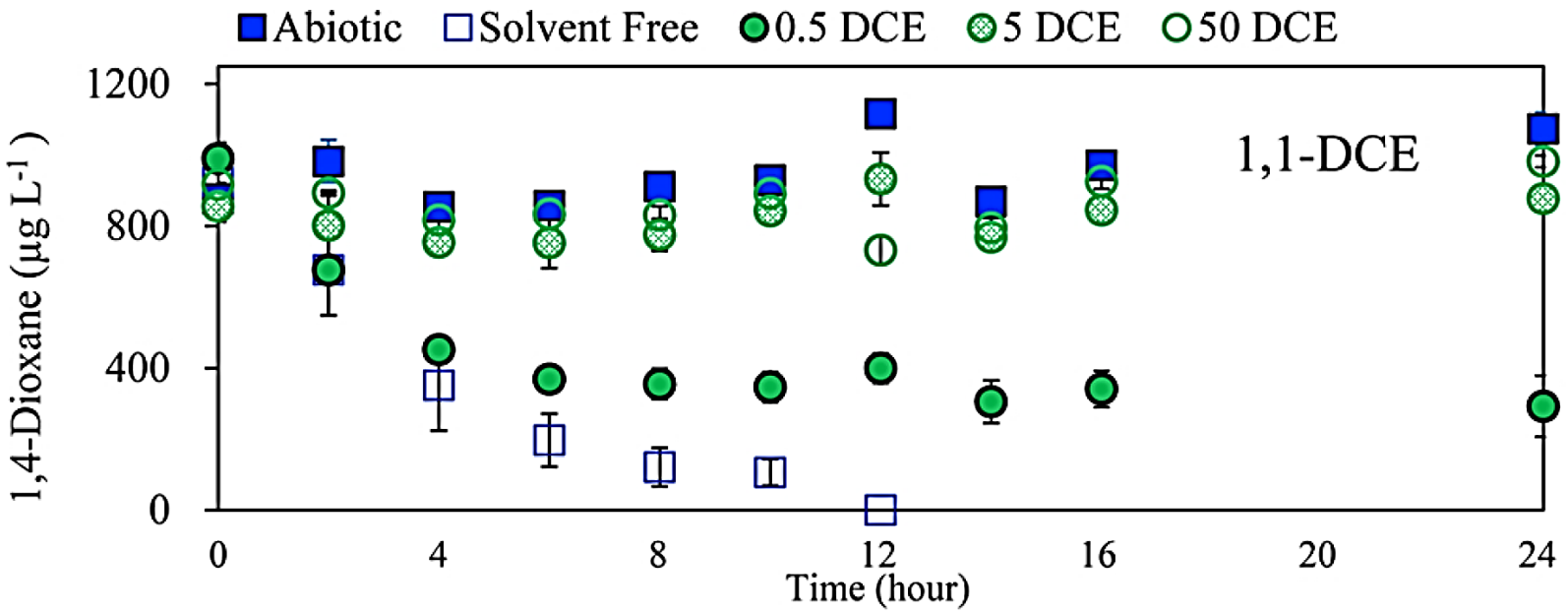
Inhibition by 1,1-DCE

- DXMO and ALDH markedly suppressed when exposed to > 5 mg/L (Zhang et al.)



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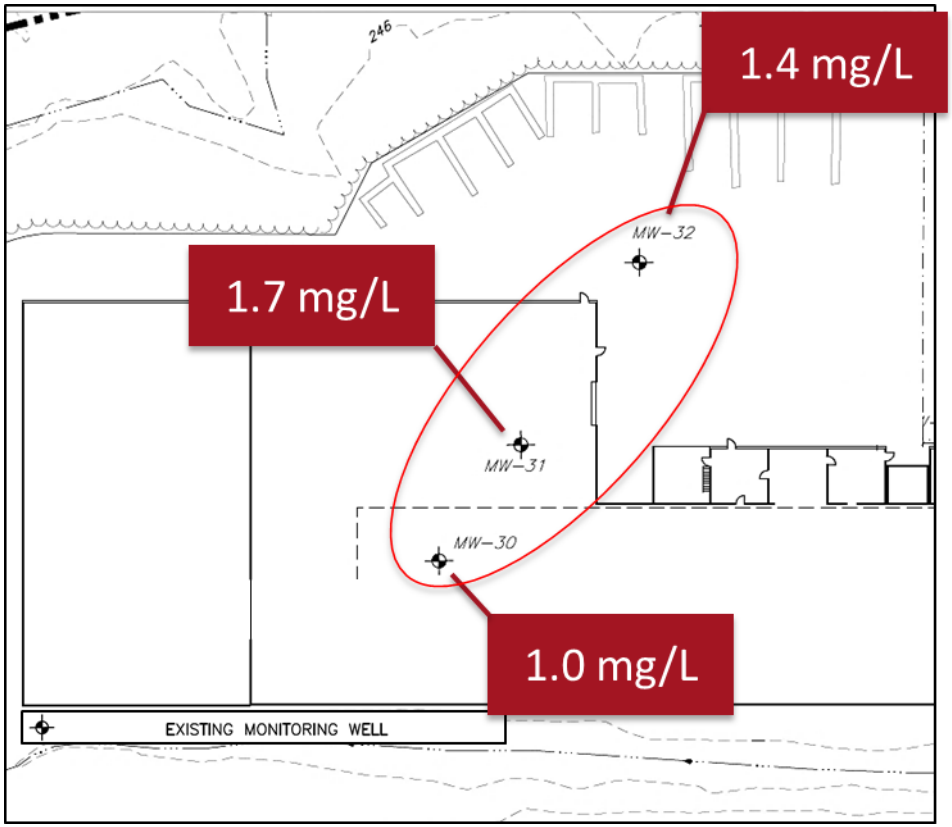
1,1-DCE Concentrations



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Site Assessment

- Geochemical analysis
  - Kjeldahl Nitrogen
  - Phosphorus
  - Nitrate
  - Nitrite
  - TOC

Remediation Challenges

- Mildly anaerobic conditions
- 1,1-DCE present
- Multiple source areas
- Low levels of nutrients

Parameter	Concentration (µg/ L)
NO_3^-	600 - 1000
Organic Nitrogen	<100
Phosphorus	90 - 200
1,1-DCE	1000 - 1700
cis-1,2-DCE	500 - 2100

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Approach

- CENSUS (qPCR)
  - Natural genetic potential
    - Are DXMO and ALDH genes present?
- Compound Specific Isotope Analysis (CSIA)
  - Degradation
    - Is there clear indication of dioxane degradation down-gradient?
- Bio-Trap
  - Culture stability
    - Will a CB1190 culture survive in the environment?

Approach

- CENSUS (qPCR) - **soil and groundwater**
  - **DXMO: ND**
  - **ALDH: ND**
- Compound Specific Isotope Analysis (CSIA)
- Bio-Trap

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Approach

- CENSUS (qPCR)
  - DXMO: ND
  - ALDH: ND
- **Compound Specific Isotope Analysis (CSIA)**
- Bio-Trap

Compound Specific Isotope Analysis (CSIA)

- Measures the ratio of stable isotopes ($^{13}C/^{12}C$, $^{2}H/^{1}H$, $^{37}Cl/^{35}Cl$) of the **contaminant**
- Bonds with the lighter isotope (^{12}C) are slightly weaker and react more quickly resulting in "isotopic fractionation"
- As the contaminant degrades, the $^{13}C/^{12}C$ ratio in the remaining contaminant increases
- Physical processes do not appreciably impact isotopic ratios

Significant Isotopic Fractionation is Conclusive Evidence of Contaminant Degradation

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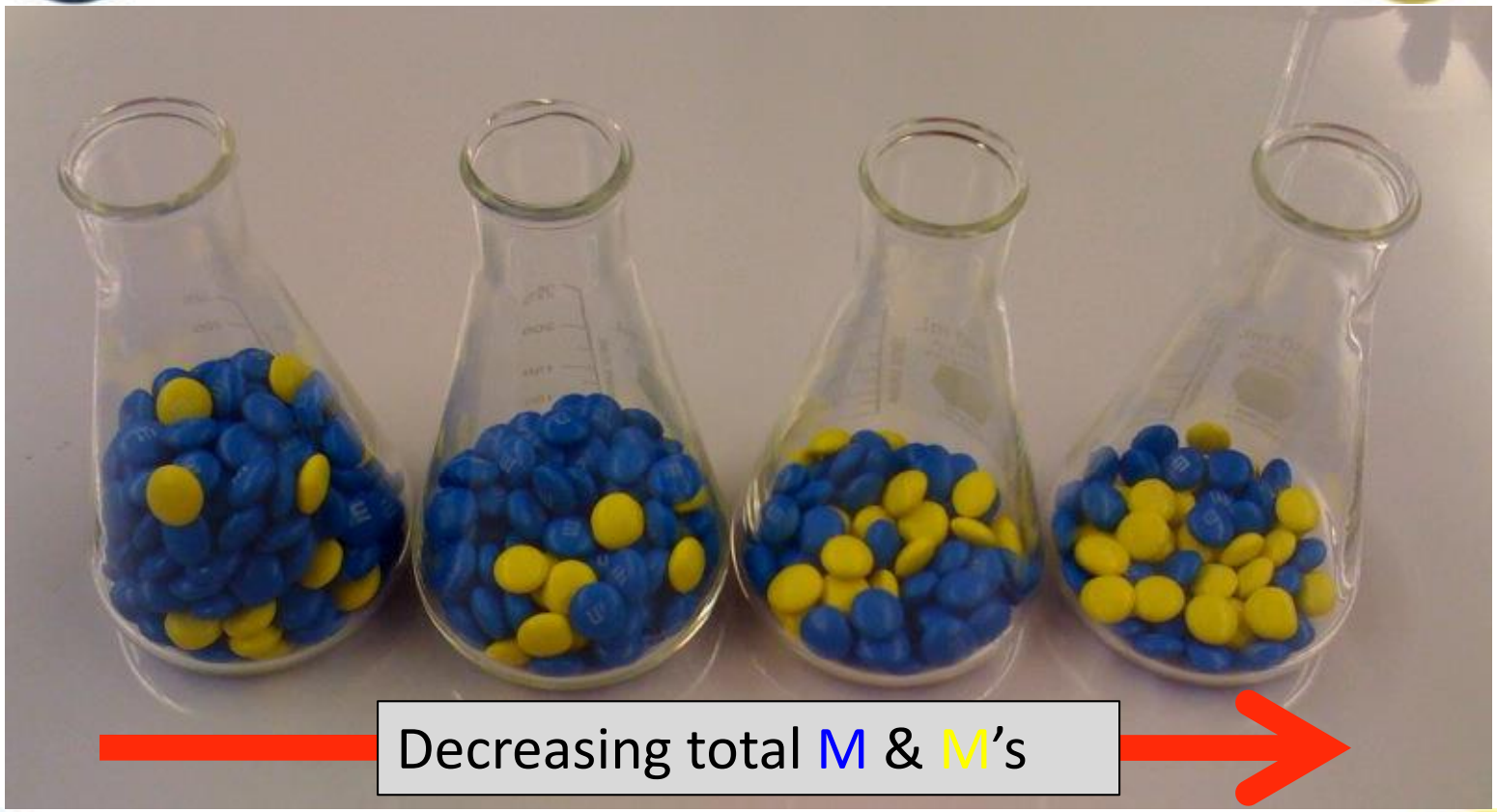
^{13}Chocolate Fractionation



^{12}C

Decreasing ratio M : M

^{13}C

Unit of measure

Amount of ^{13}C relative to ^{12}C is expressed by the $\delta^{13}C$ notation

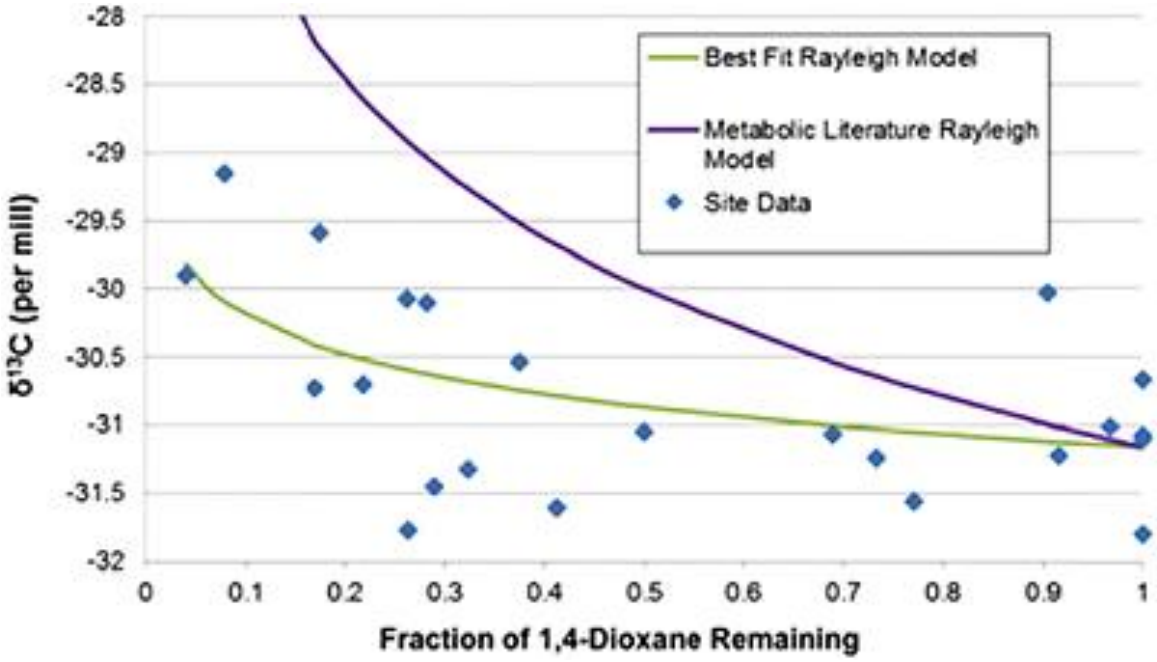
$$\delta^{13}C\,[‰] = \left(\frac{(^{13}C/^{12}C)_{\text{Sample}}}{(^{13}C/^{12}C)_{\text{Standard}}} - 1 \right) \cdot 1000$$

Units of $\delta^{13}C$ are "per mill"

As the $^{13}C/^{12}C$ increases the $\delta^{13}C$ value become more positive

CSIA and 1,4-Dioxane

- ^{13}C CSIA
  - Enrichment factor: −1.73 ± 0.14 (Pornwongthong et al., in review)



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CSIA Results for 1,4-dioxane

- No clear change in values down gradient
  - No clear indication of 1,4-dioxane degradation
  - Plume appears uniform

Isotope	Monitoring Well		
	MW-30	MW-31	MW-32
$\delta^{13}C$ (‰, VPDB)	-31.1	-30.8	-30.6
$\delta^{2}H$ (‰, VSMOW)	-48	-51	-47

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Approach

- CENSUS (qPCR)
  - DXMO: ND
  - ALDH: ND
- **Compound Specific Isotope Analysis (CSIA)**
  - **No indication of degradation**
  - **Isotopically uniform**
- Bio-Trap

Approach

- CENSUS (qPCR)
  - DXMO: ND
  - ALDH: ND
- Compound Specific Isotope Analysis (CSIA)
  - No indication of degradation
  - Isotopically uniform
- **Bio-Trap**

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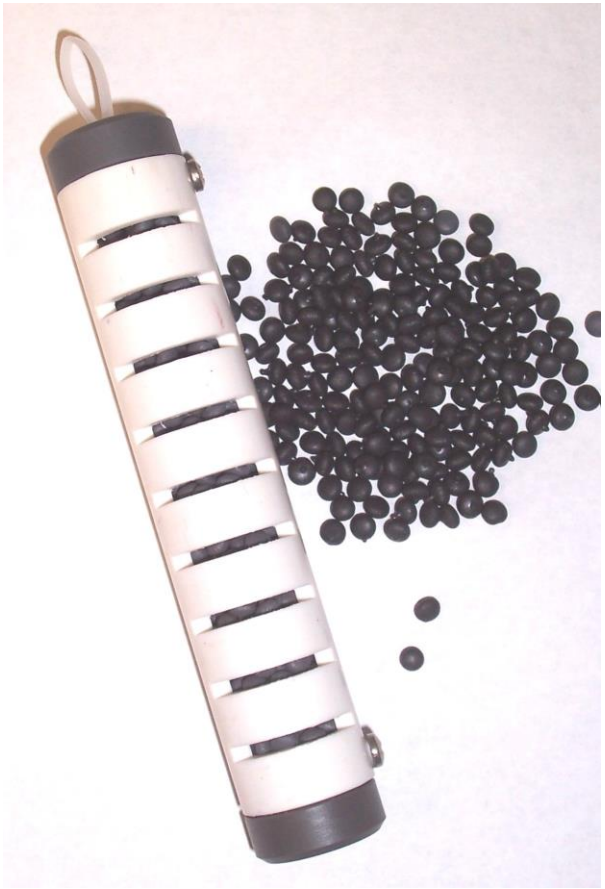
What Are Bio-Trap® Samplers?

- Passive microbial sampling tool
- Colonized by active microbes
  - Useful for bioaugmentation studies
- 25% Nomex and 75% PAC
- Used in conjunction with
  - Stable isotope probing
  - qPCR and QuantArray
  - Other MBTs



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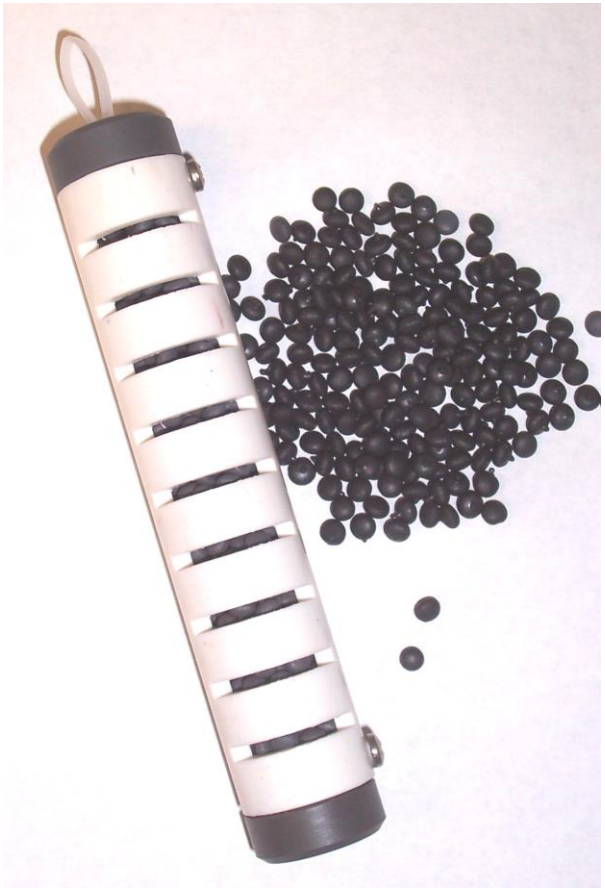
Bio-Trap Study

- Four units
  - MW-30
    - Control Bio-Trap
    - Bioaugmented Bio-Trap
      - 10^4 DXMO gene copies/bead
  - MW-31
    - Control Bio-Trap
    - Bioaugmented Bio-Trap
      - 10^4 DXMO gene copies/bead



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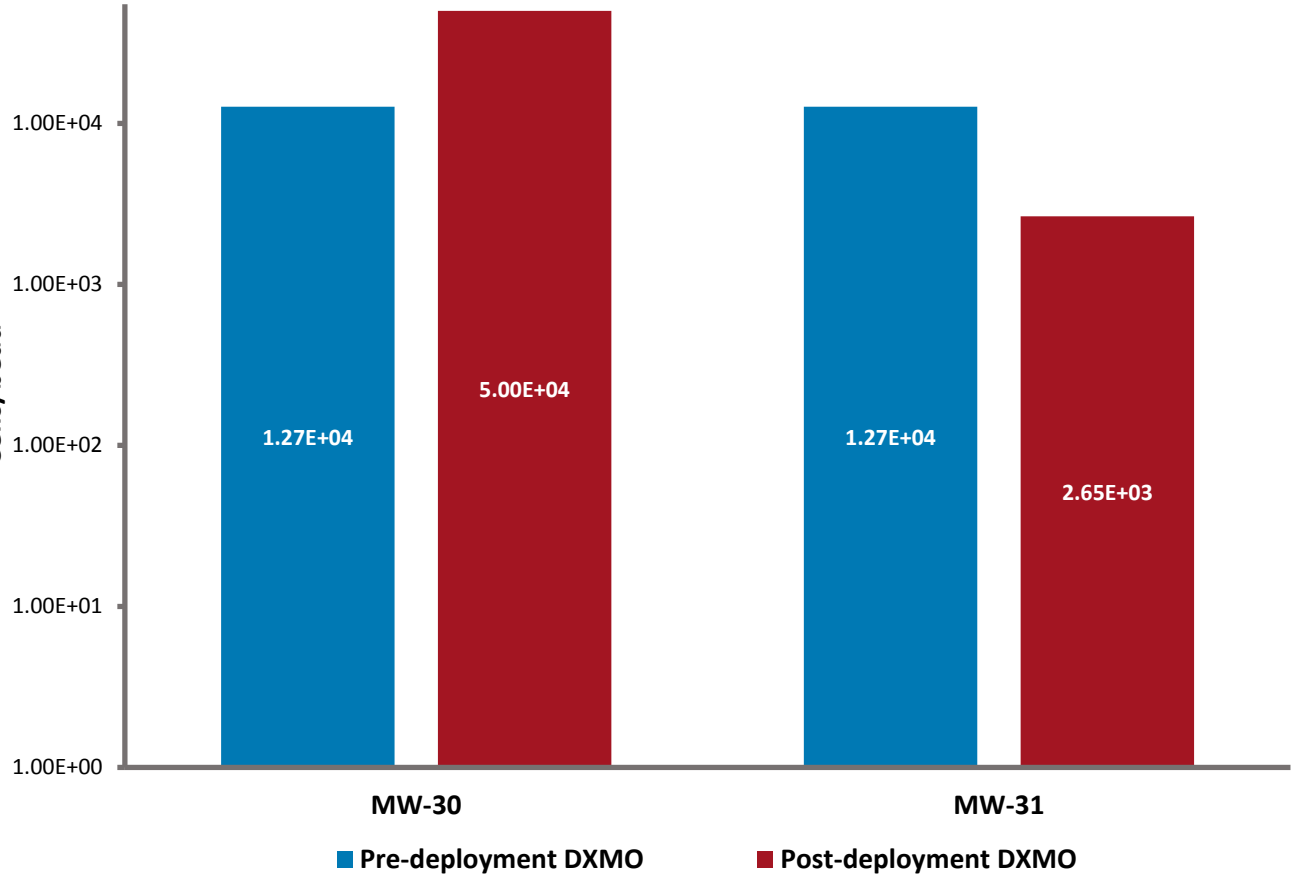
Bio-Trap qPCR Results

- M
- Bi



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Conclusions

- CB1190 is robust; surviving despite
  - Low nutrients
  - Anaerobic environment
  - High concentrations of inhibitor 1,1-DCE
- Bioaugmentation studies to continue
  - In Situ Microcosms

On-going and Future Work

- *In Situ* Microcosm Study
  - Analysis of robustness of CB1190
  - Will bioaugmentation work at this site?
  - Is biostimulation via O_2 required?

- How they work
  - Each unit represent a treatment option
  - Each unit contains passive samplers
  - Deployed for 60 days, recovered, and analyzed

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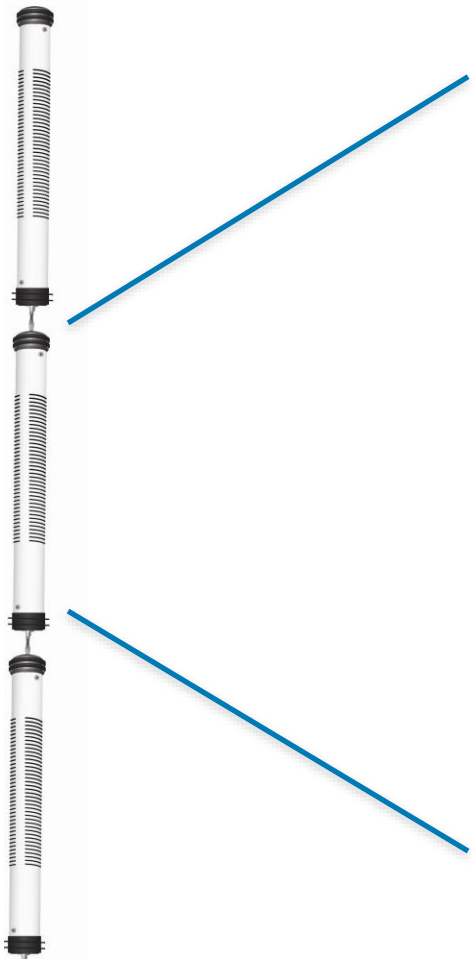
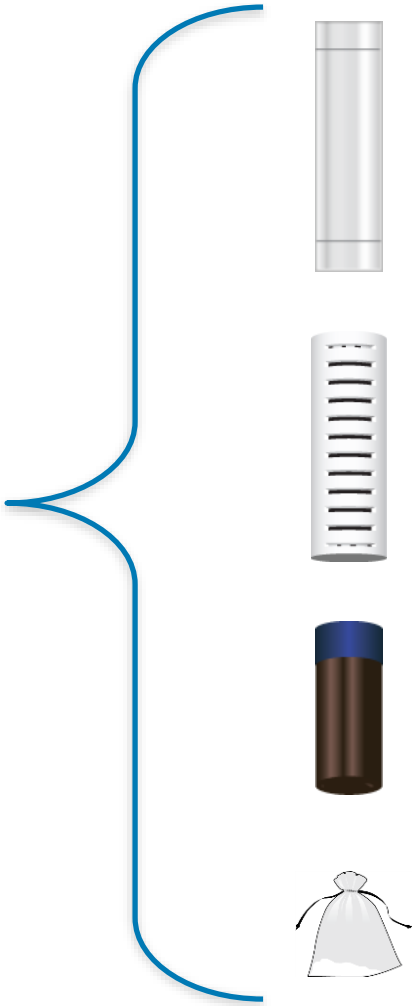
Assembly



Unit

Samplers



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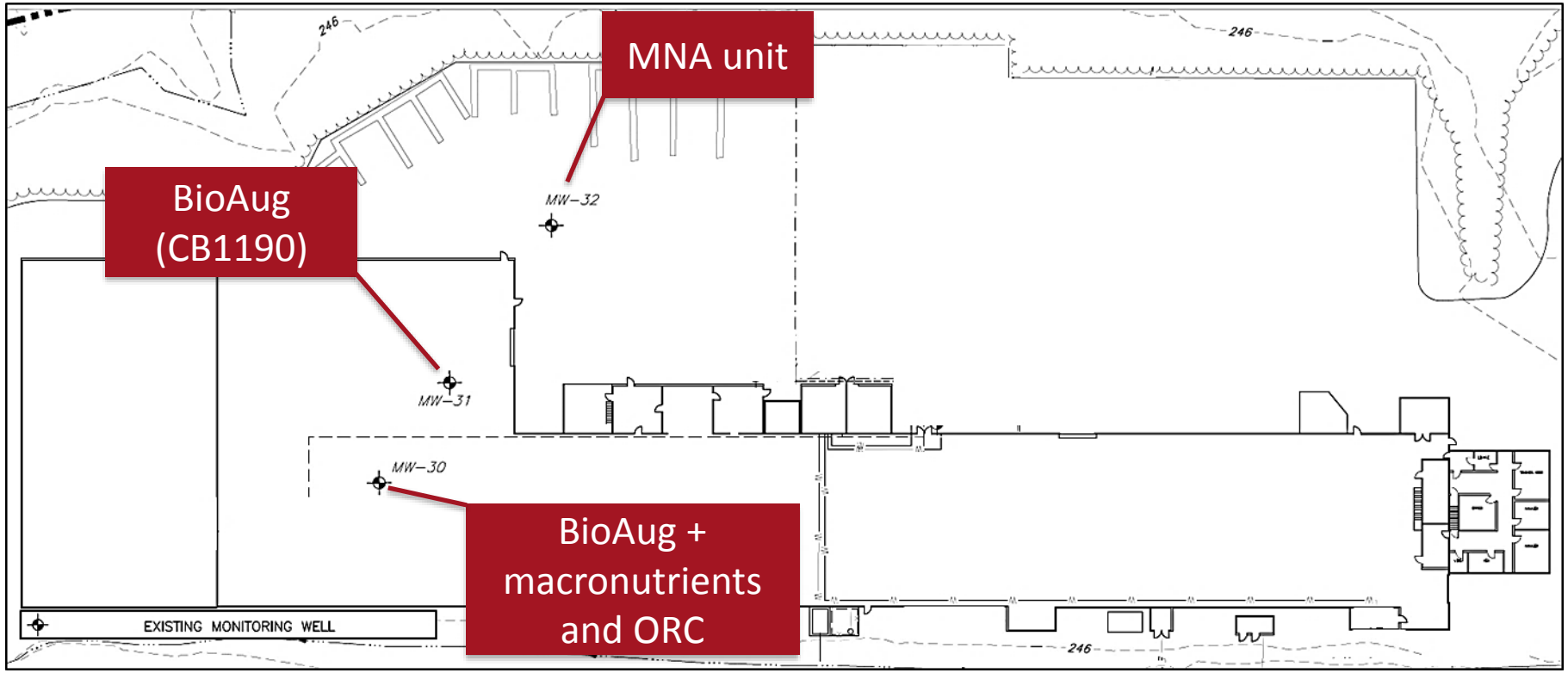
In Situ Microcosm Study



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Questions?