EVALUATION OF RDX BIODEGRADATION USING COMPOUND SPECIFIC ISOTOPE ANALYSIS AND STABLE ISOTOPE PROBING

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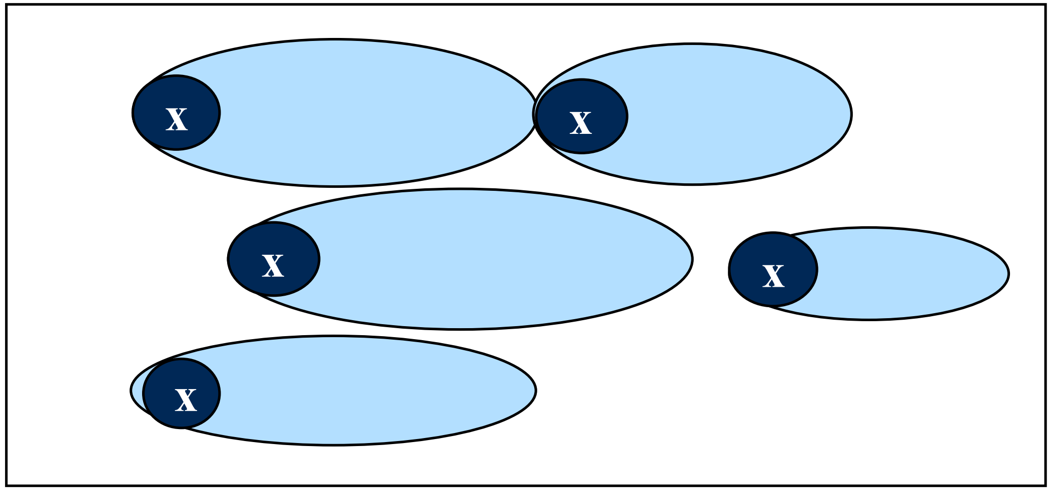
BACKGROUND

Degradation of RDX can be difficult to document in the field

- Point source contamination (rather than traditional "plume")
- Variety of degrading organisms and conditions
- Aerobic and multiple anaerobic pathways
- Biotic and abiotic mechanisms
- Degradation products are common in environment (e.g., NH_4^+, HCHO, NO_2^-, NO_3^-) and/or transient & somewhat difficult to analyze (MEDINA, NDAB)

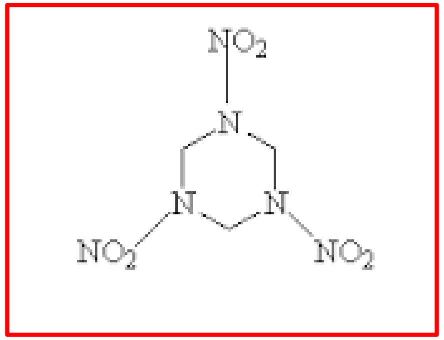


OBJECTIVES

- Evaluate Compound-Specific Isotope Analysis (CSIA) as a technique to identify and quantify aerobic and anaerobic biodegradation of RDX at field sites

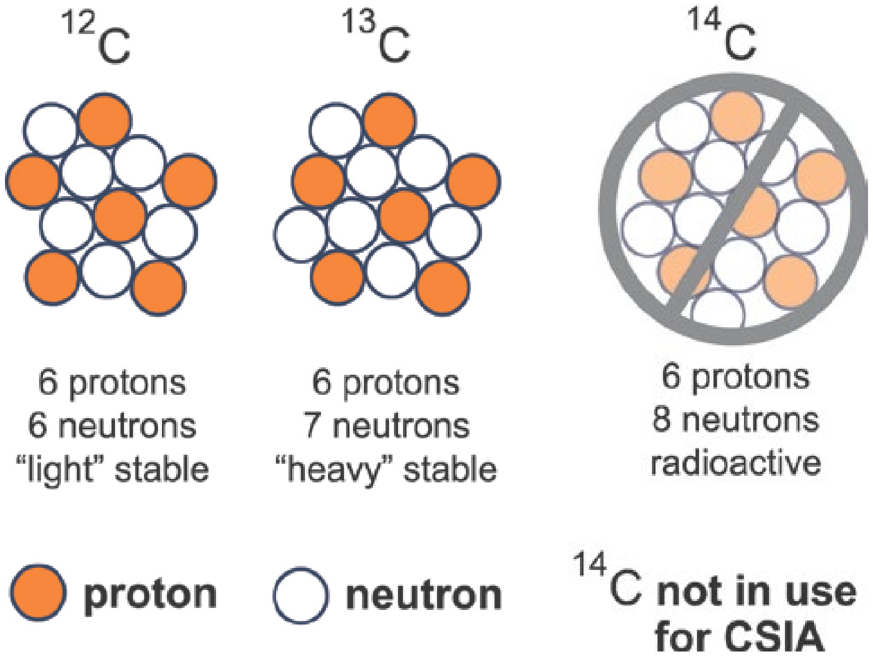
- Utilize Stable Isotope Probing (SIP) to identify organisms degrading RDX in the field

FUNDAMENTALS: RELEVANT ISOTOPES FOR RDX?



Isotopes of an element have the same number of protons and electrons but a different number of neutrons

Isotopes of Carbon

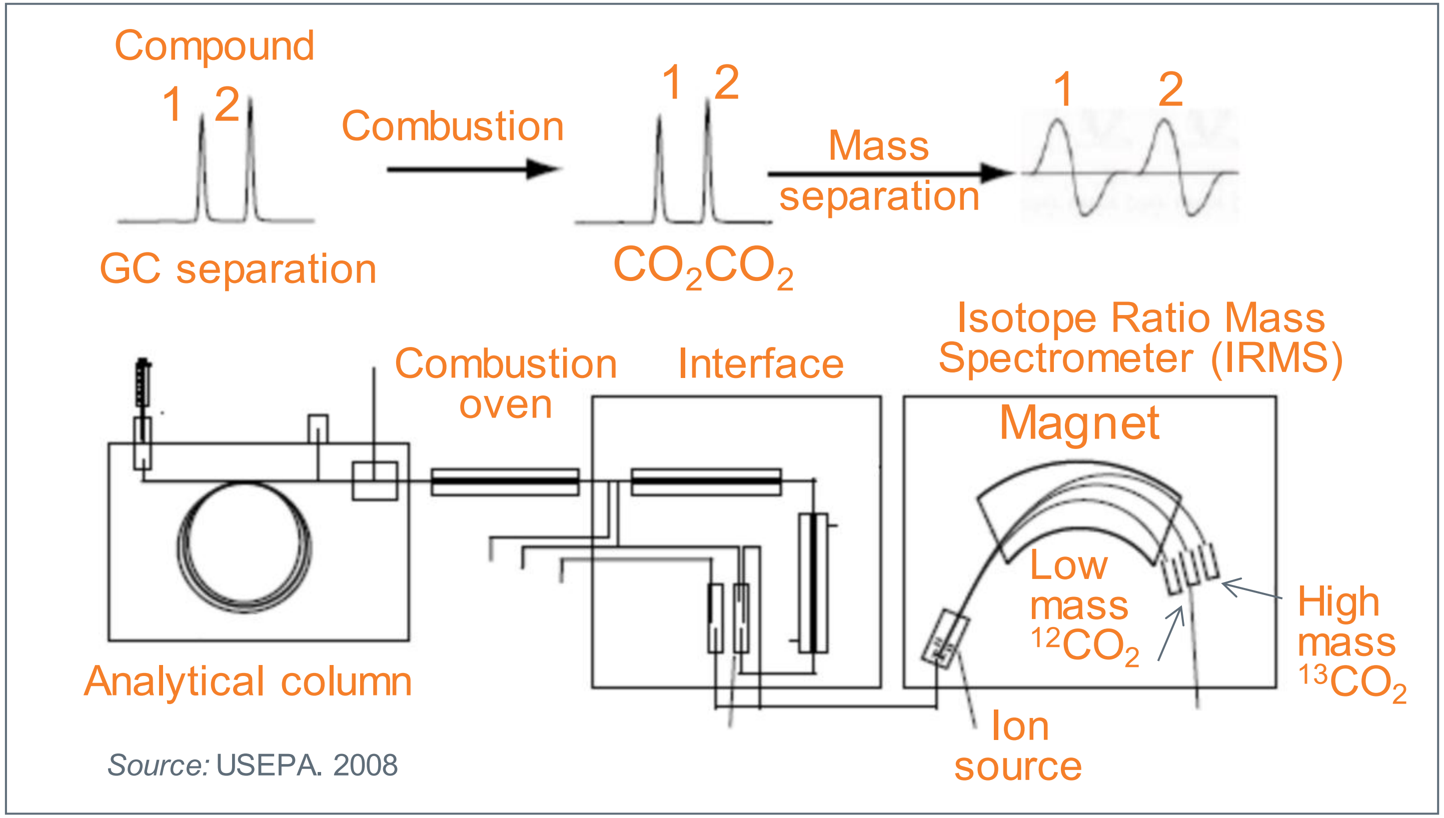


Relevant Stable Isotopes for RDX

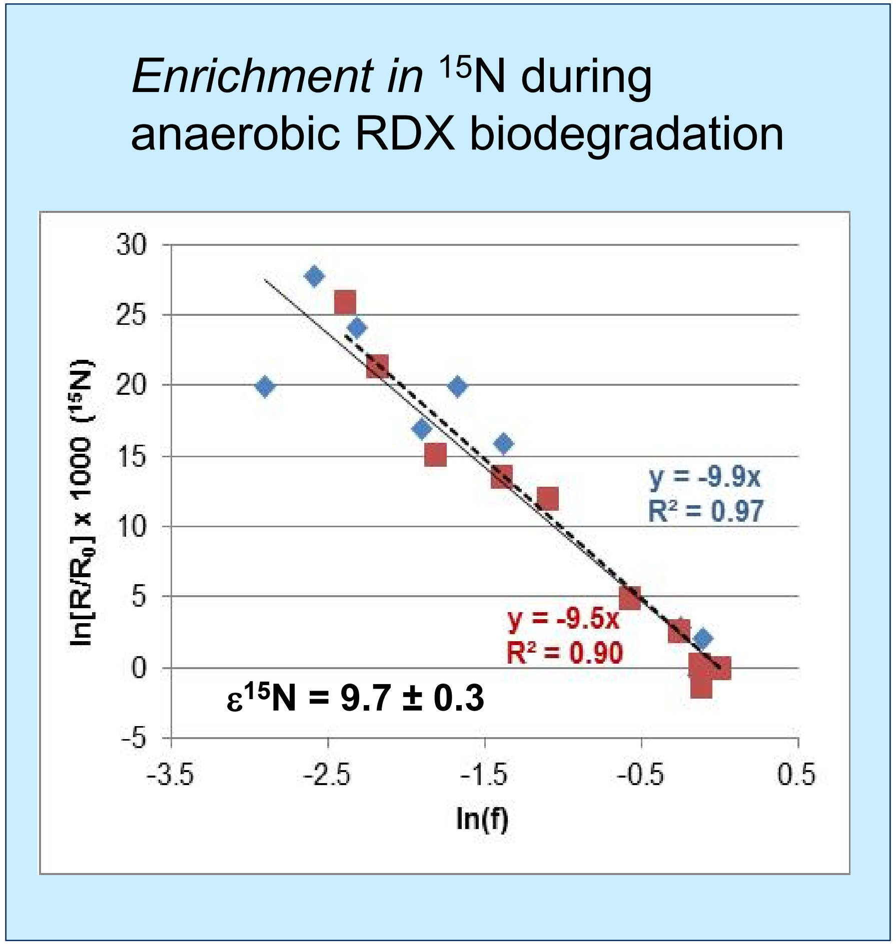
Hydrogen	^{1}H, ^{2}H	99.99 %
Oxygen	^{16}O, ^{17}O, ^{18}O	99.76 %
Carbon	^{12}C, ^{13}C	98.89 %
Chlorine	^{35}Cl, ^{37}Cl	75.78 %
Nitrogen	^{14}N, ^{15}N	99.63 %
Sulfur	^{32}S, ^{34}S	95.01 %

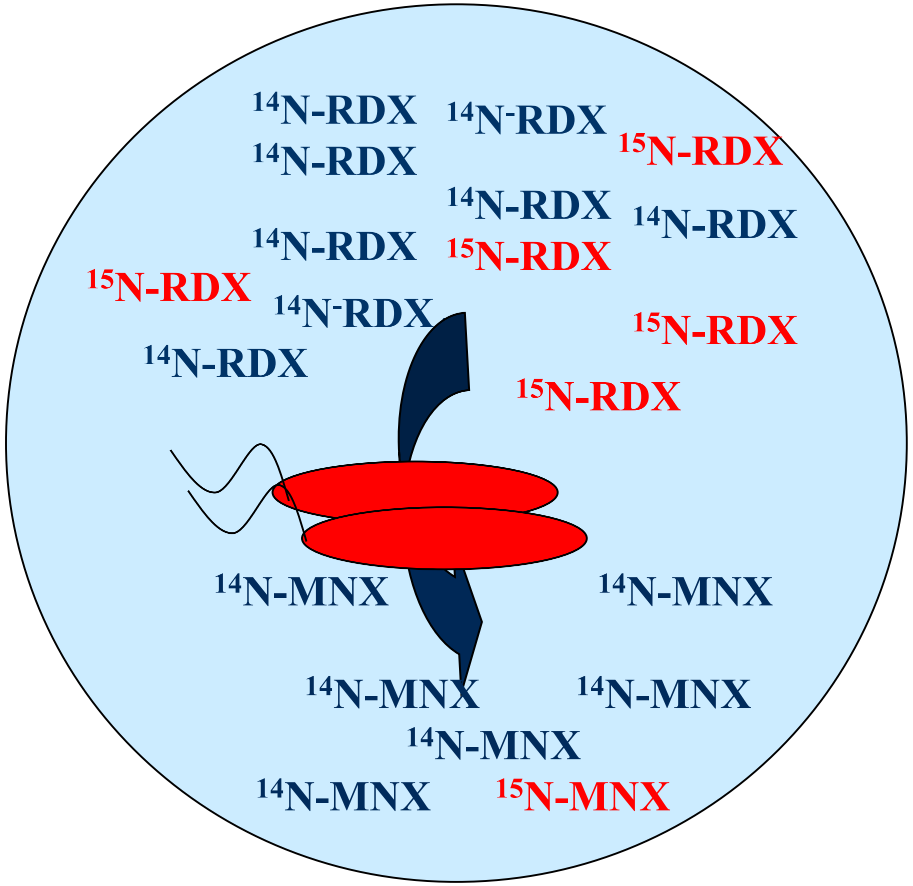
SOME FUNDAMENTALS – HOW ARE ISOTOPE RATIOS MEASURED AND REPORTED?

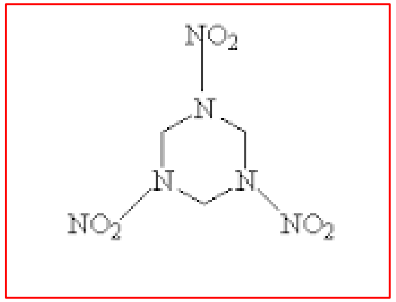
- Stable isotope ratios are typically measured using Isotope Ratio Mass Spectrometers (IRMS).
- If chemicals are analyzed individually (e.g., separated first by GC) then the process is termed *"Compound Specific Isotope Analysis (CSIA)"*



CONTAMINANT DEGRADATION - ISOTOPE FRACTIONATION MAKES CSIA USEFUL!



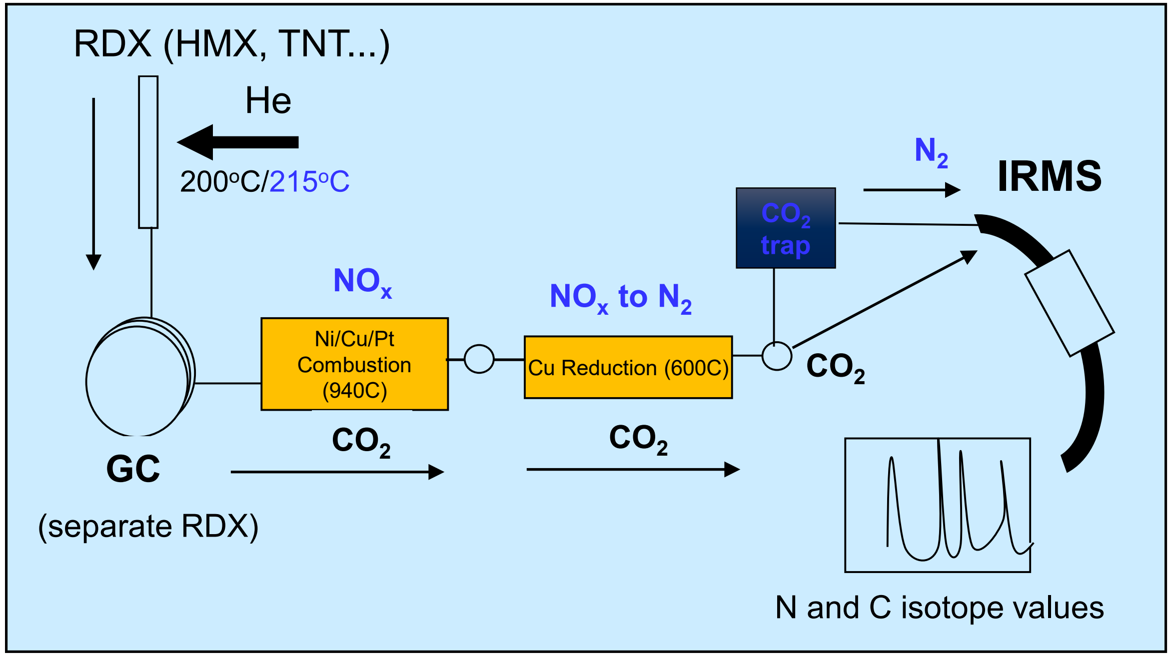




CSIA METHOD FOR RDX – MEASURE $\delta^{13}C$ AND $\delta^{15}N$

1. Collect RDX from cultures/groundwater
2. Concentrate RDX via SPE - acetonitrile
3. Separate RDX (GC) and Quantify Stable Isotope Ratios (IRMS)

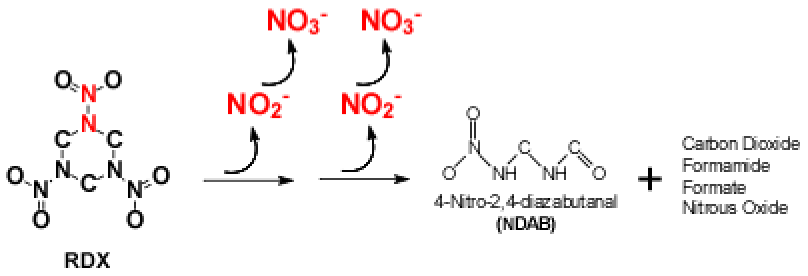
Continuous Flow GC-IRMS



CO_2 and N_2 sent in continuous-flow gas stream to isotope ratio mass spectrometer (IRMS) - 2 different runs

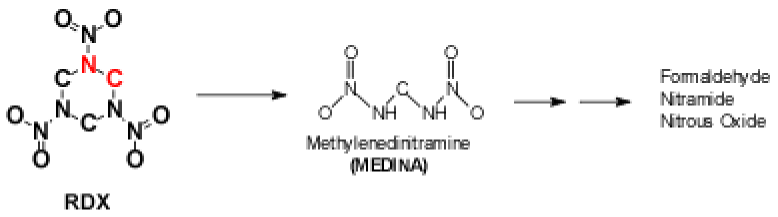
* 10 µg RDX per analysis

PURE CULTURE STUDIES – DIFFERENT PATHWAYS & STRAINS



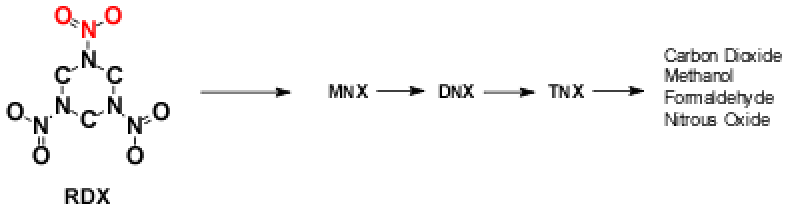
Pathway 1: Aerobic

Rhodococcus sp. DN22
Rhodococcus rhodocrous 11Y
Rhodococcus sp. Strain A
Gordonia sp. KTR9



Pathway 2: Anaerobic (Ring cleavage)

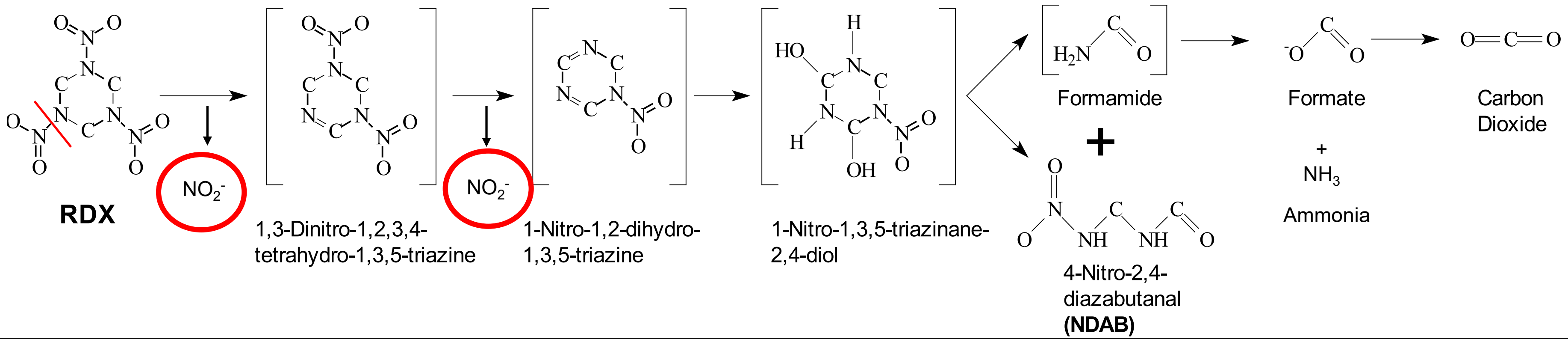
Pseudomonas putida II-B
Pseudomonas fluorescens I-C

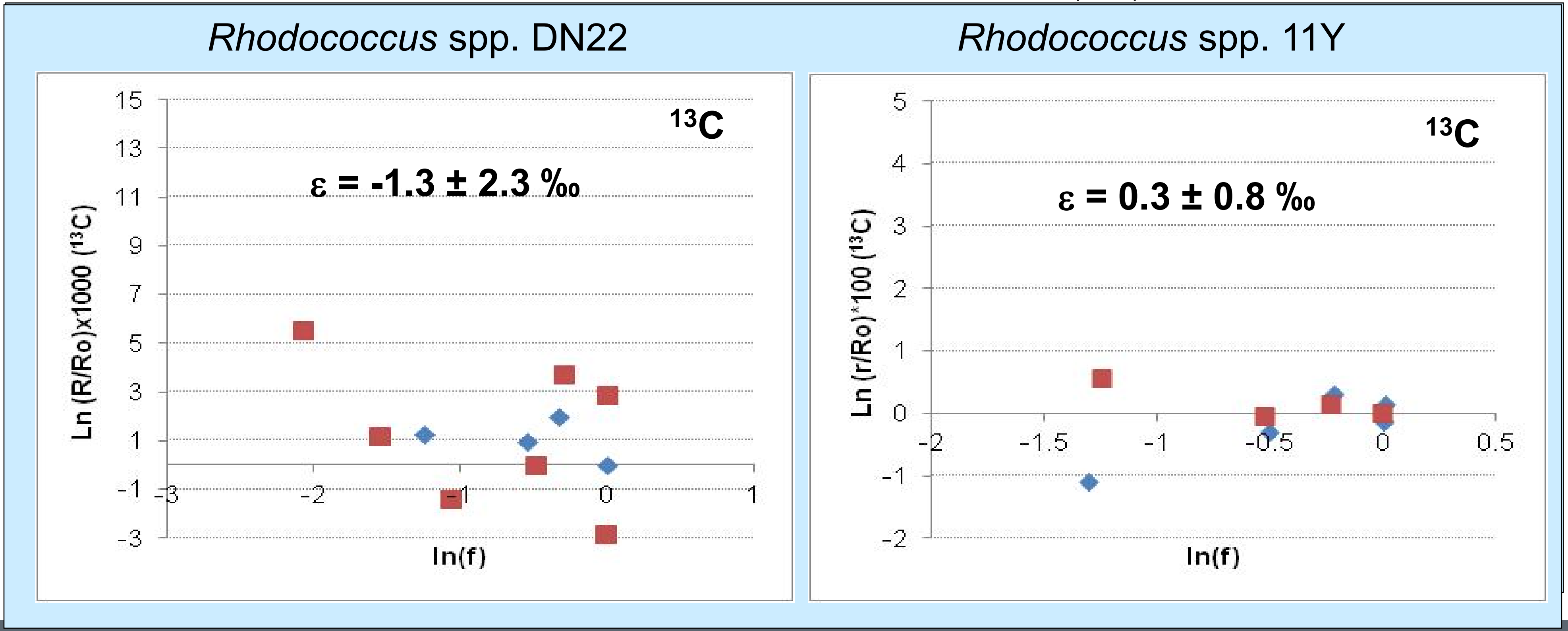


Pathway 3: Anaerobic (MNX)

Shewanella sp. MR-1
Klebsiella sp. SCZ-1
Clostridium acetobutylicum ATCC824
Desulfovibrio spp. Strain EFX-DES

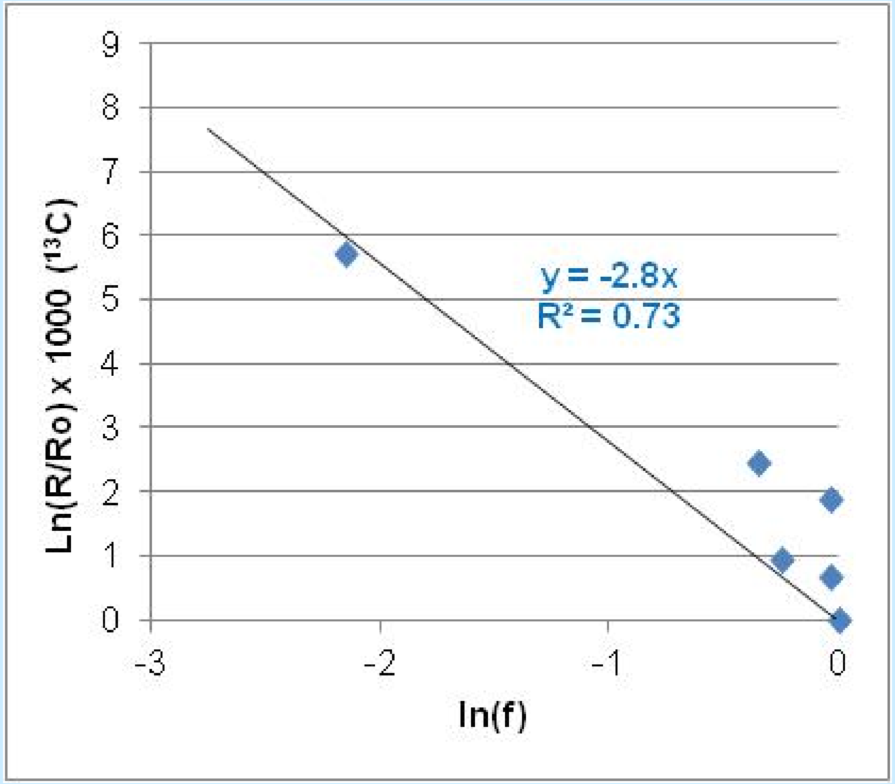
AEROBIC BIODEGRADATION OF RDX - *XPLA*



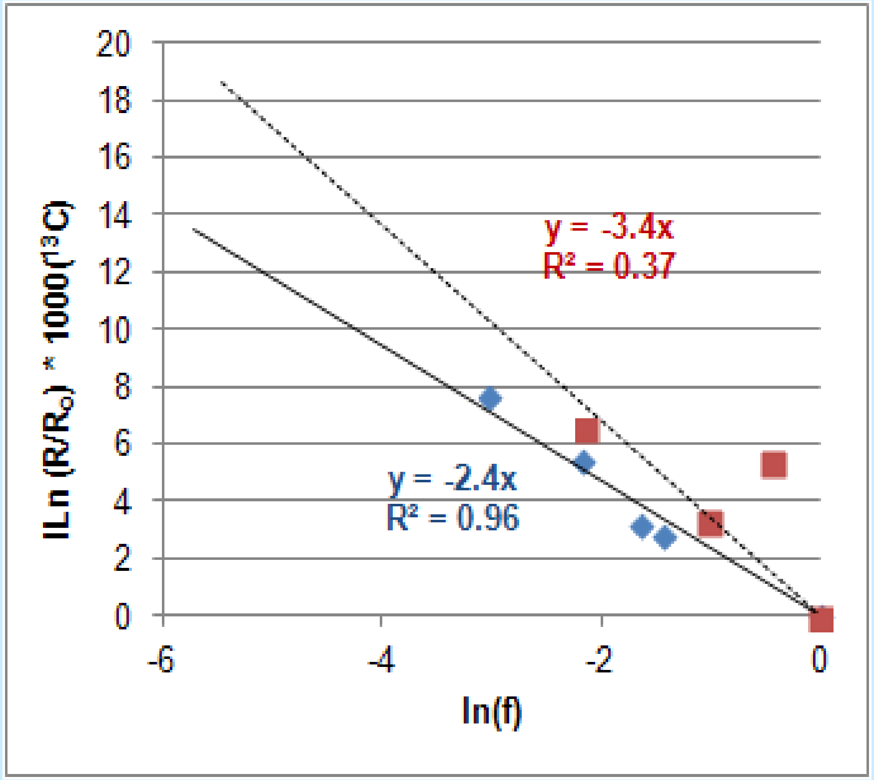


RING-CLEAVAGE PATHWAY - XENA/XENB

Pseudomonas putida II-B (^{13}C)

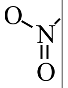


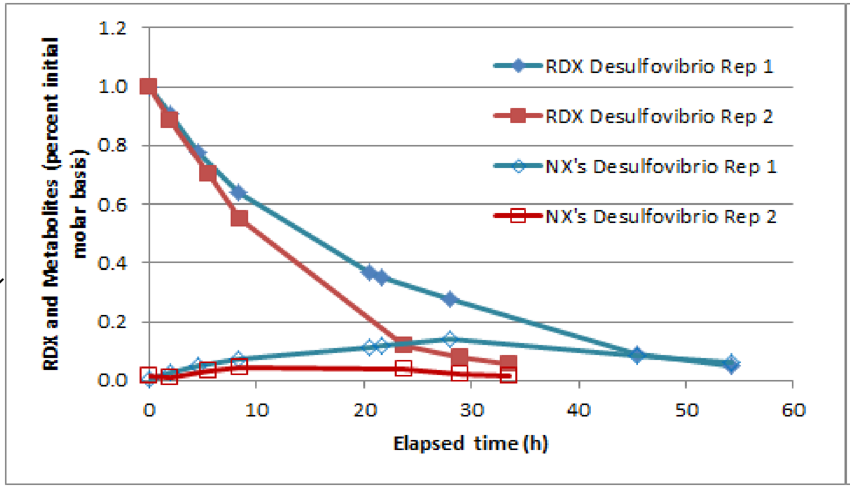
Pseudomonas fluorescens I-C (^{13}C)

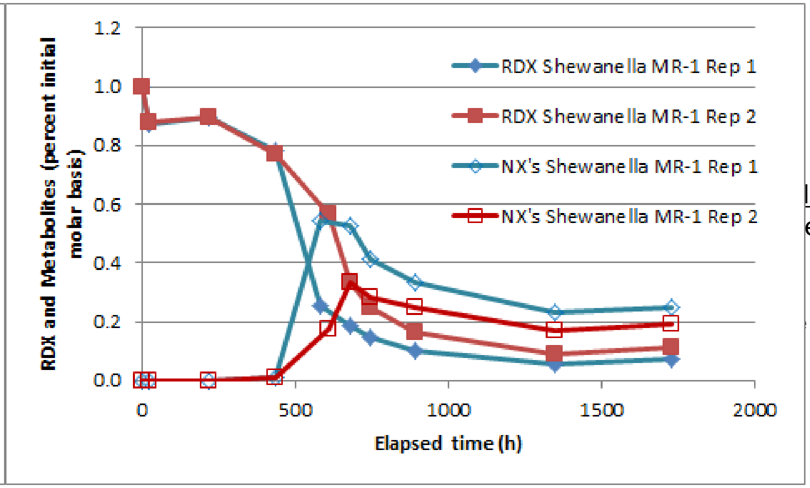


ε ^{13}C = -2.7 ± 0.7‰

NITRO-REDUCTION PATHWAY - MNX, DNX, TNX







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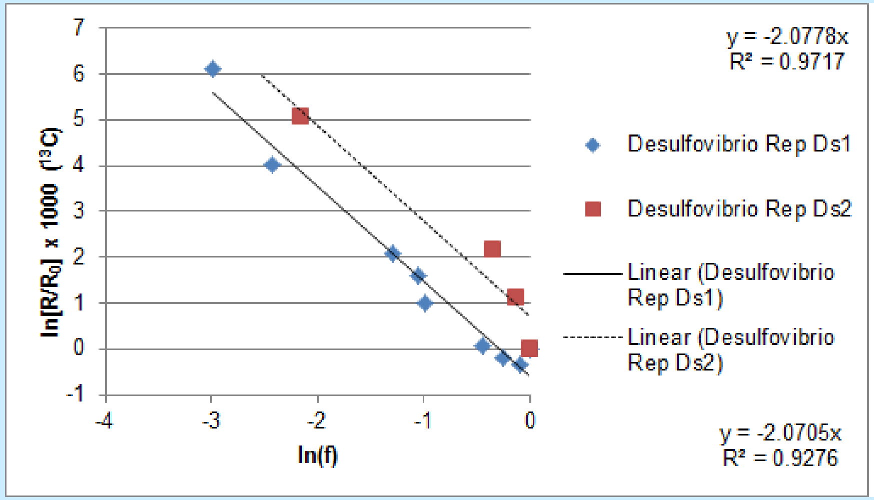
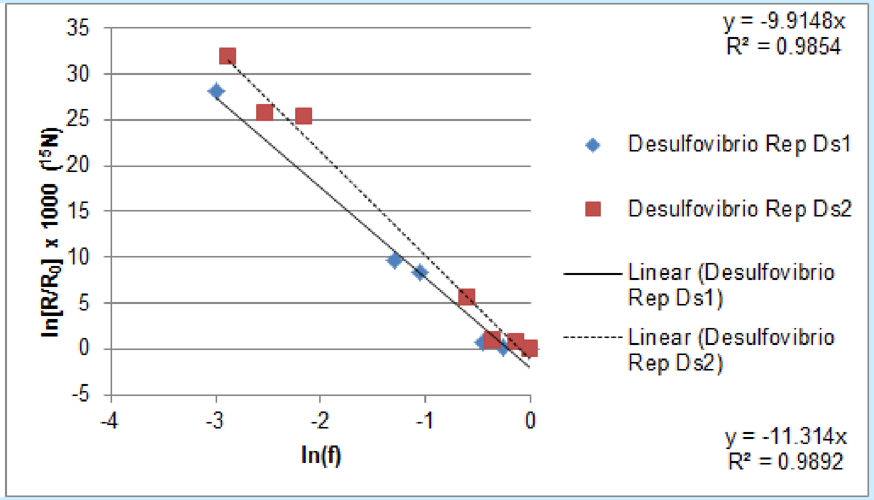
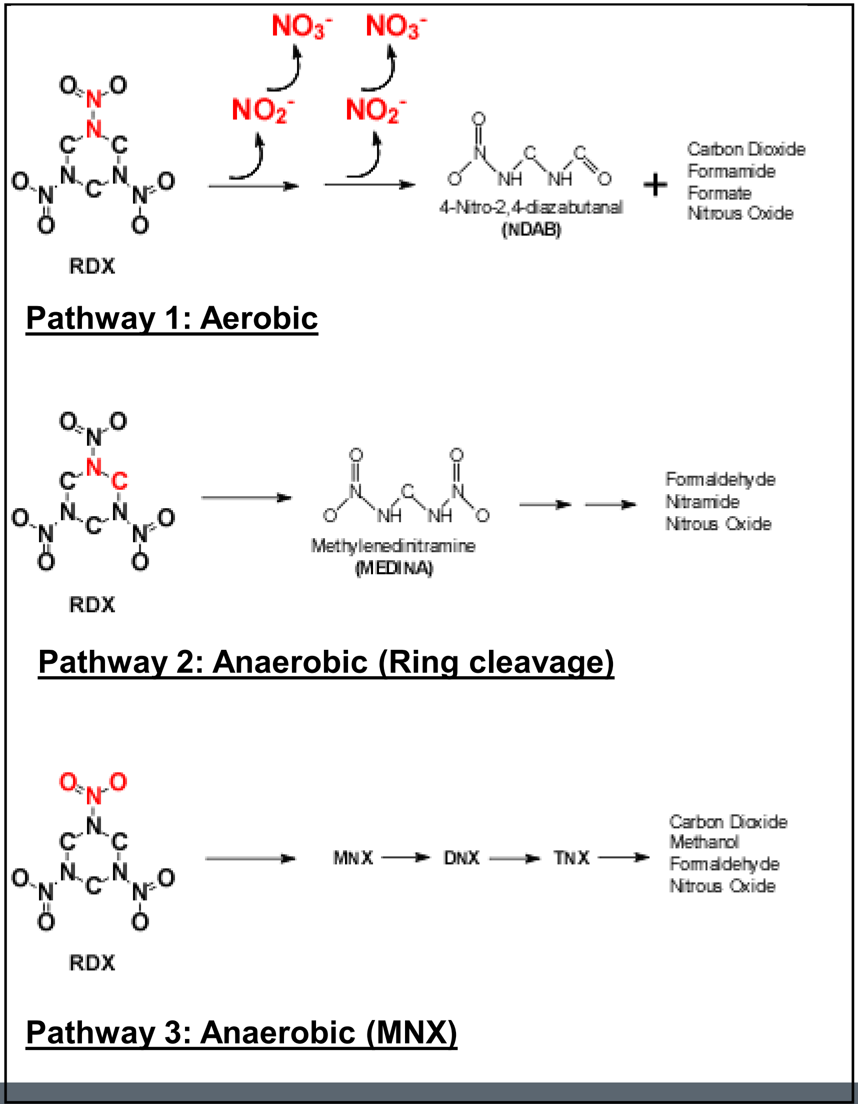




$\varepsilon^{13}C$ = -2.1 ± 0.5 ‰ **(4 Strains -2.0 to -6.0 ‰)** **$\varepsilon^{15}N$ = -10.9 ± 1.2 ‰**

SUMMARY OF PURE CULTURE CSIA RESULTS



Rhodococcus sp. DN22
Rhodococcus rhodocrous 11Y
Rhodococcus sp. Strain A
Gordonia sp. KTR9

$\varepsilon^{15}N$	$\varepsilon^{13}C$
-2.4 ± 0.2 ‰	-0.8 ± 0.5 ‰

Pseudomonas putida II-B
Pseudomonas fluorescens I-C

$\varepsilon^{15}N$	$\varepsilon^{13}C$
-12.7 ± 0.8 ‰	-2.7 ± 0.7 ‰

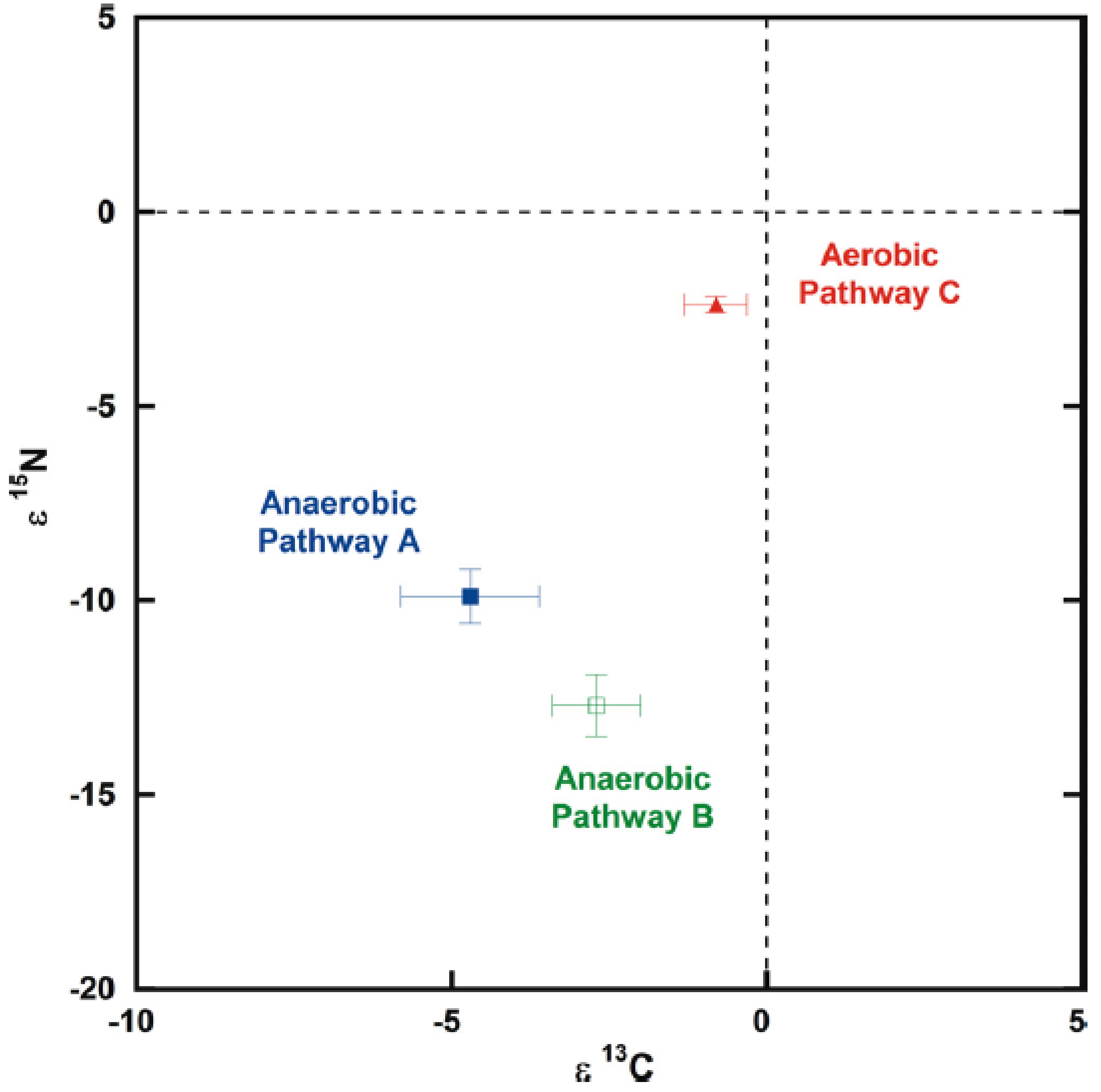
All anaerobic

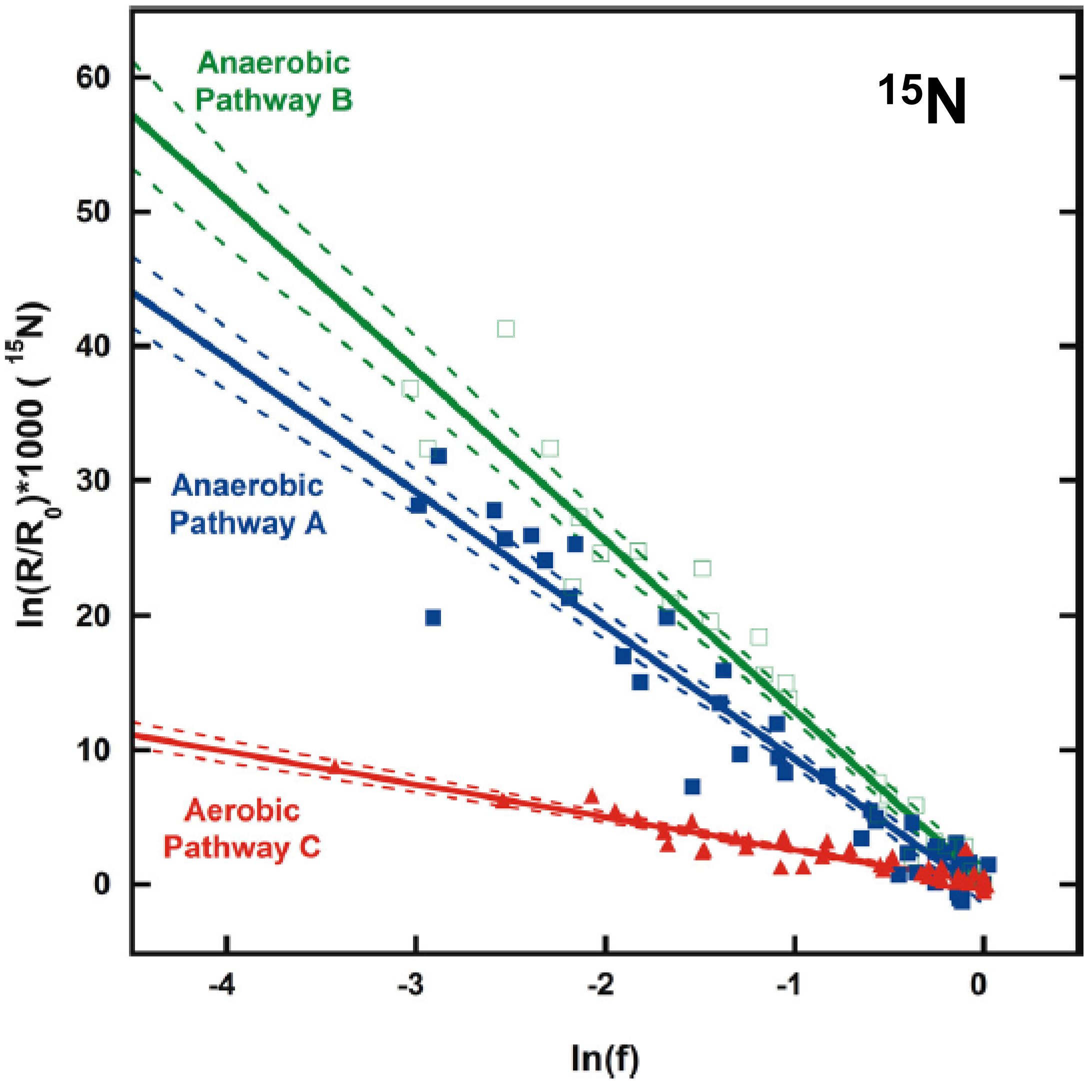
$\varepsilon^{15}N$	$\varepsilon^{13}C$
-11.9 ± 0.7 ‰	-4.0 ± 0.8 ‰

Shewanella sp. MR-1
Klebsiella sp. SCZ-1
Clostridium acetobutylicum ATCC824
Desulfovibrio spp.

$\varepsilon^{15}N$	$\varepsilon^{13}C$
-9.9 ± 0.7 ‰	-4.7 ± 1.1 ‰

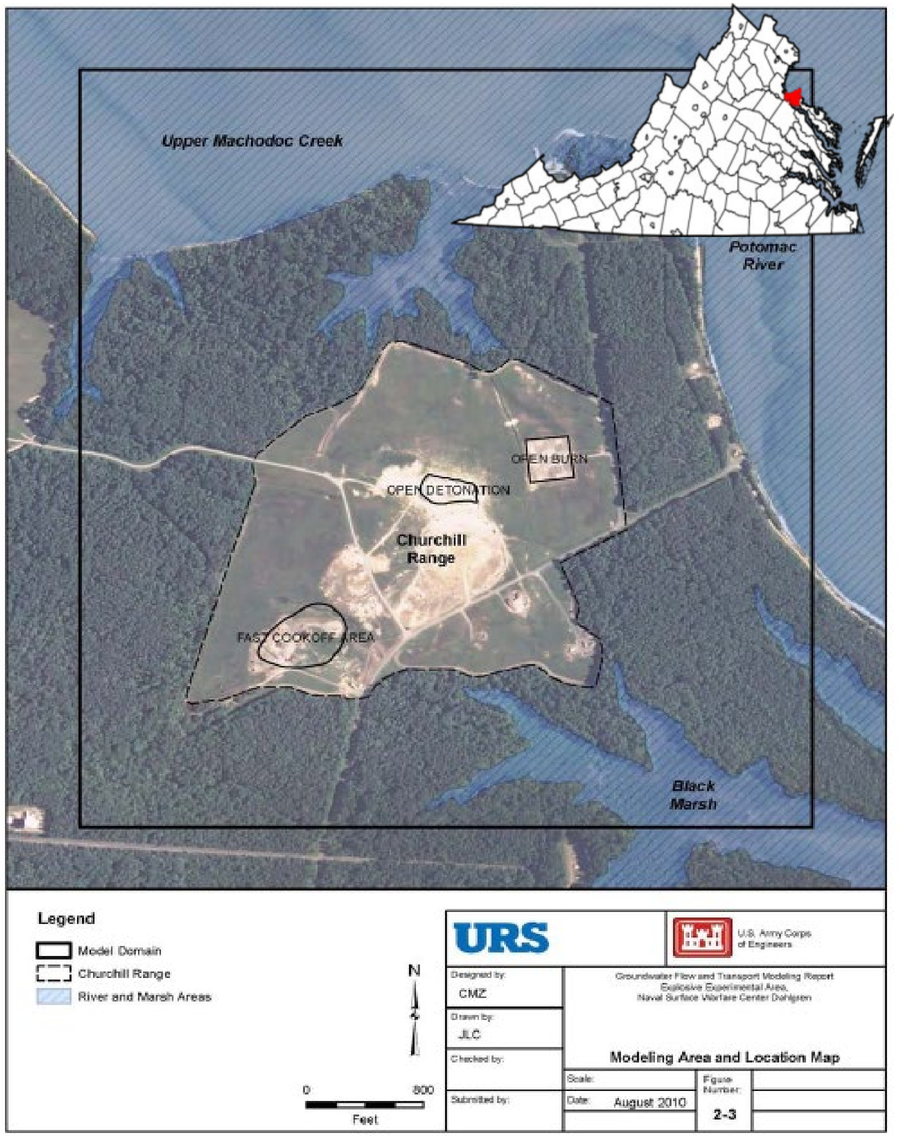
SUMMARY OF PURE CULTURE CSIA RESULTS





FIELD SITE FOR RDX CSIA – NSWC, DAHLGREN CHURCHILL RANGE

- Facility founded 1918
- Active US Navy testing range
- RDX and HMX in groundwater from OB/OD, fast cookoff, and other testing
- Shallow, acidic groundwater, generally aerobic, with some anoxic areas
- Site for ESTCP ER-201028 (In Situ Emulsified Oil Biobarrier)
- Conduct MNA evaluation using CSIA
  - RDX - N Isotopes
  - N and O isotopes in NO_3^-
- Measure C and N fractionation of RDX during enhanced anaerobic bioremediation.

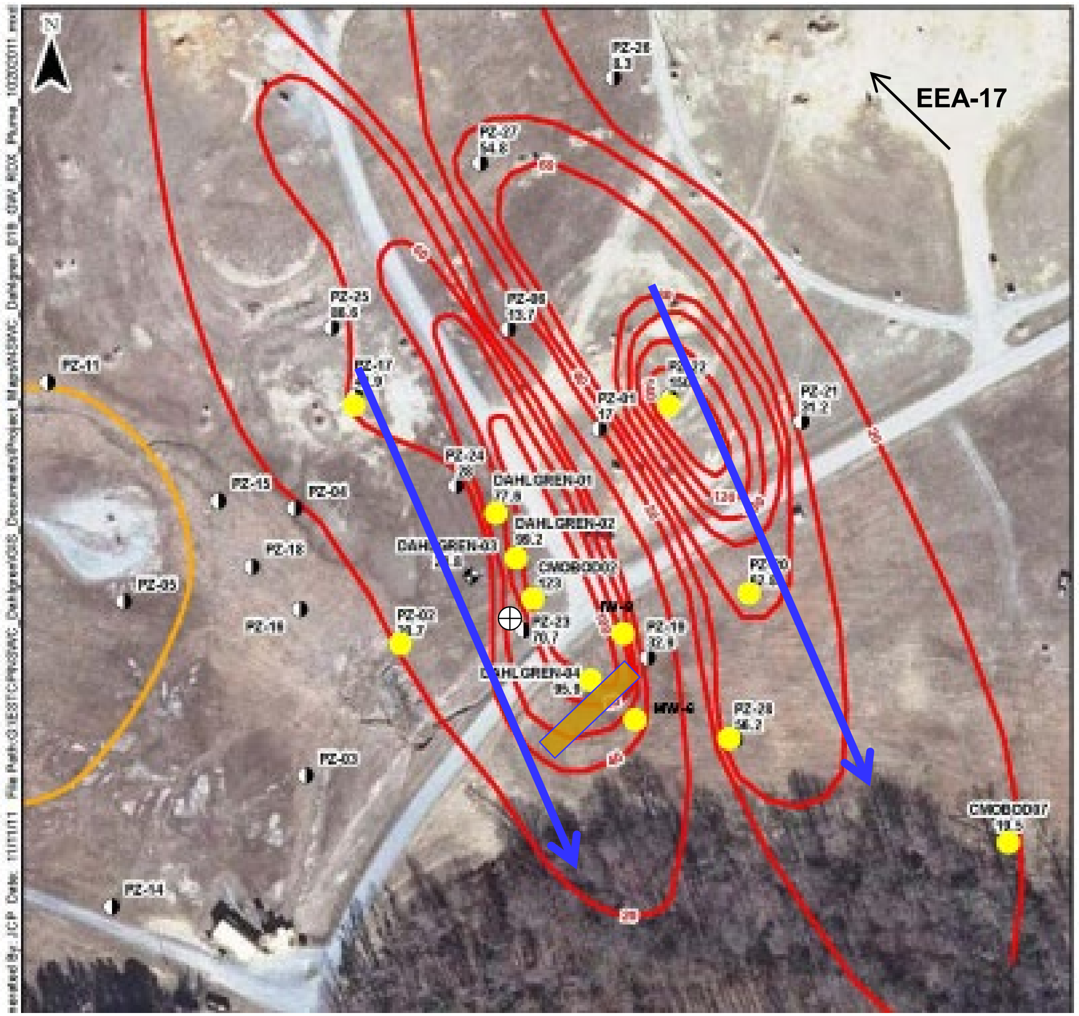


RDX MNA ANALYSIS

Sampling at Dahlgren NSWC

- Sampled 11 wells
  - ✓ RDX - $\delta^{15}N$ analysis
  - ✓ NO_3^- isotope analysis
  - ✓ Degradation products
  - ✓ Geochemistry
  - ✓ Field parameters

Parameter	Value
DO	1 – 8 mg/L
ORP	+230 – +340 mV
pH	4.3 – 4.9
NO_2^-	< 0.2
NO_3^-	1.7 – 4.2 mg/L
RDX	13 – 120 µg/L
MNX	0.2 – 0.9 µg/L
DNX, TNX	< 0.1 µg/L
NDAB	ND



RDX MONITORED MNA ANALYSIS - CSIA

$\delta^{15}N$ in RDX

<u>Western plume</u>

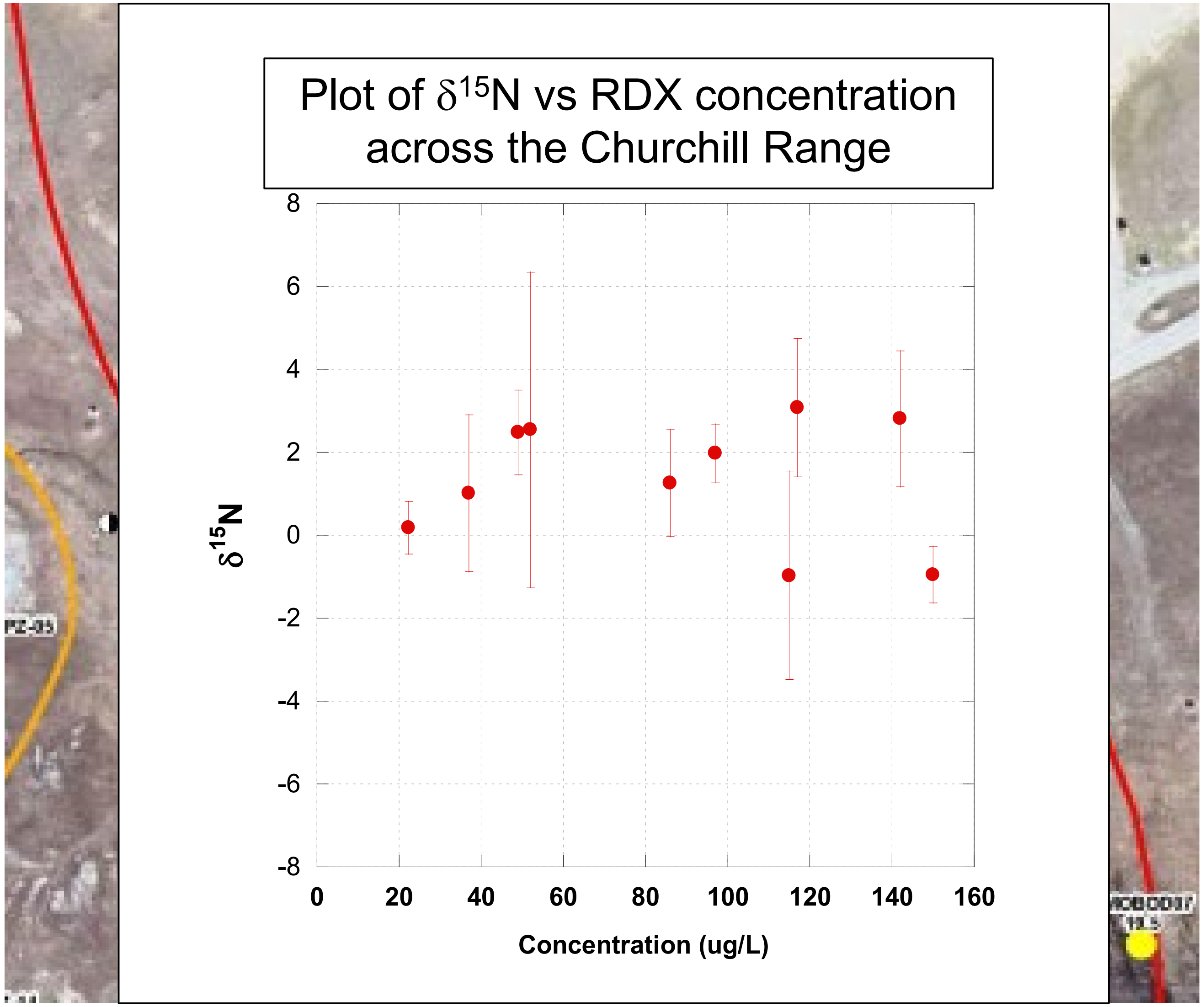
No enrichment with groundwater flow

No evidence of degradation

<u>Eastern Plume</u>

Lower $\delta^{15}N$ value in RDX

No evidence of degradation



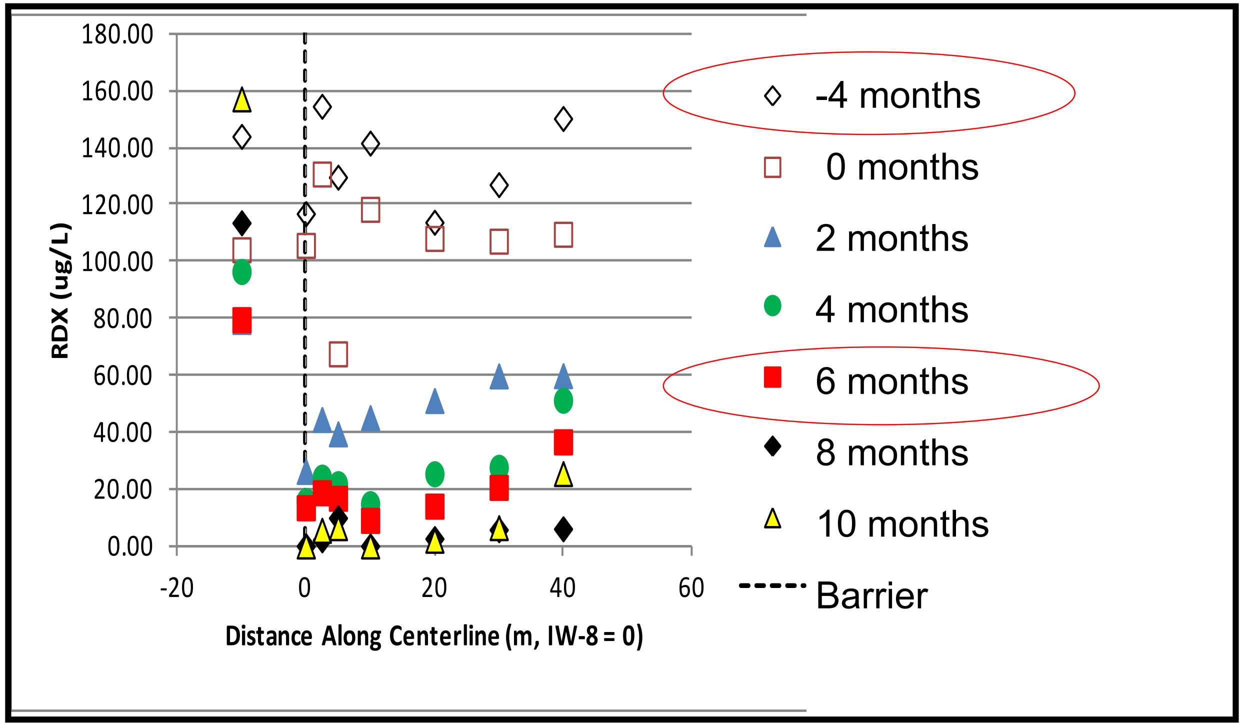
FIELD EVALUATION OF RDX ISOTOPES DURING BIOBARRIER STUDY

RDX Isotope Sampling at *In Situ* Biobarrier

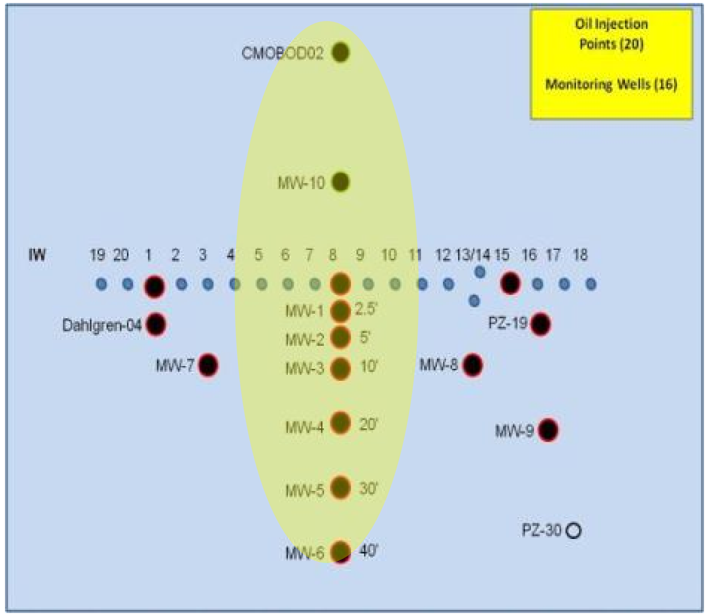
Clear evidence of anaerobic biodegradation

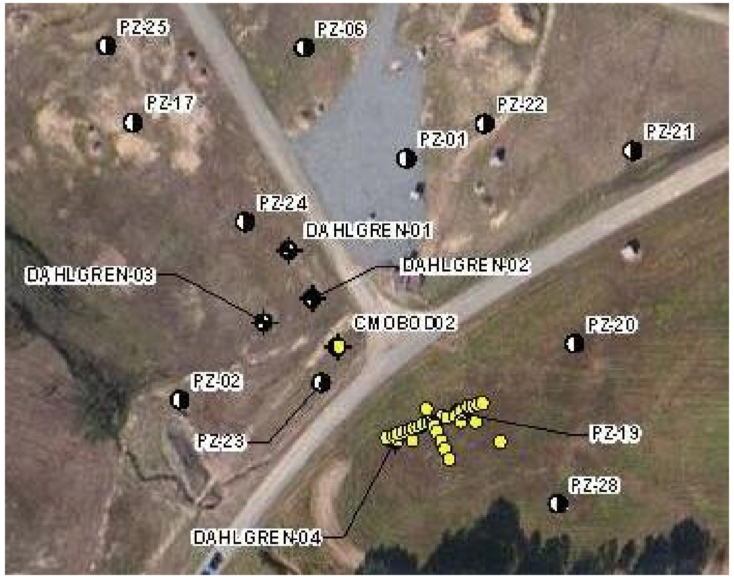
CSIA samples collected at select points

Pre-barrier 6 months post barrier



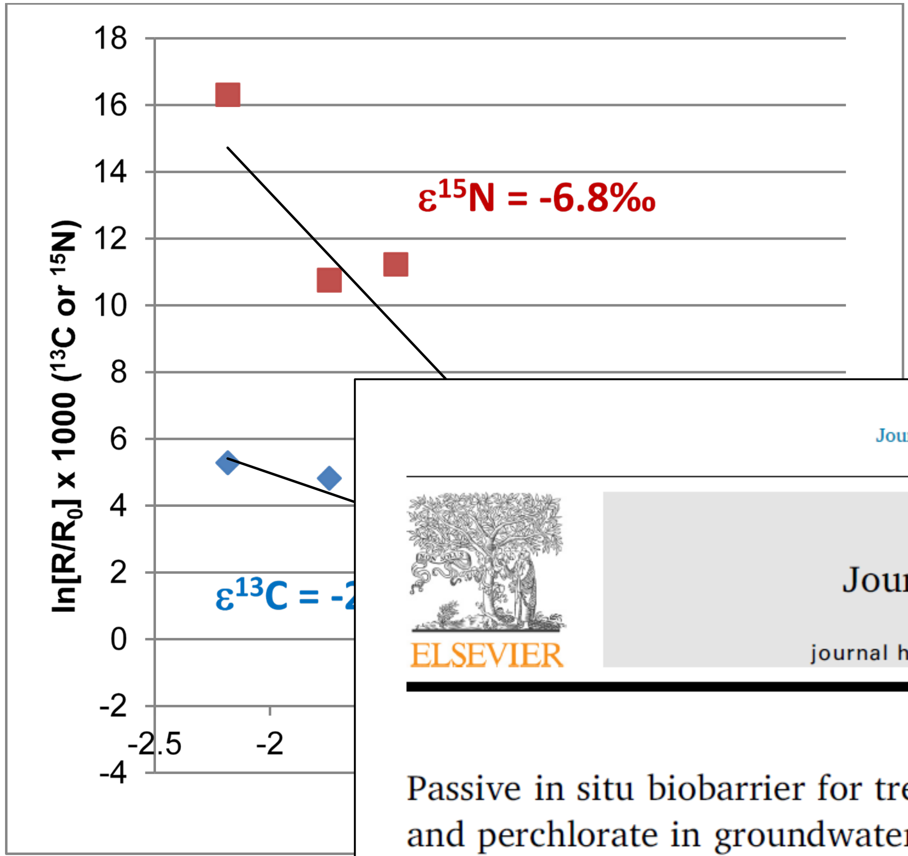
6 months: RDX: < 9 – 40 μg/L



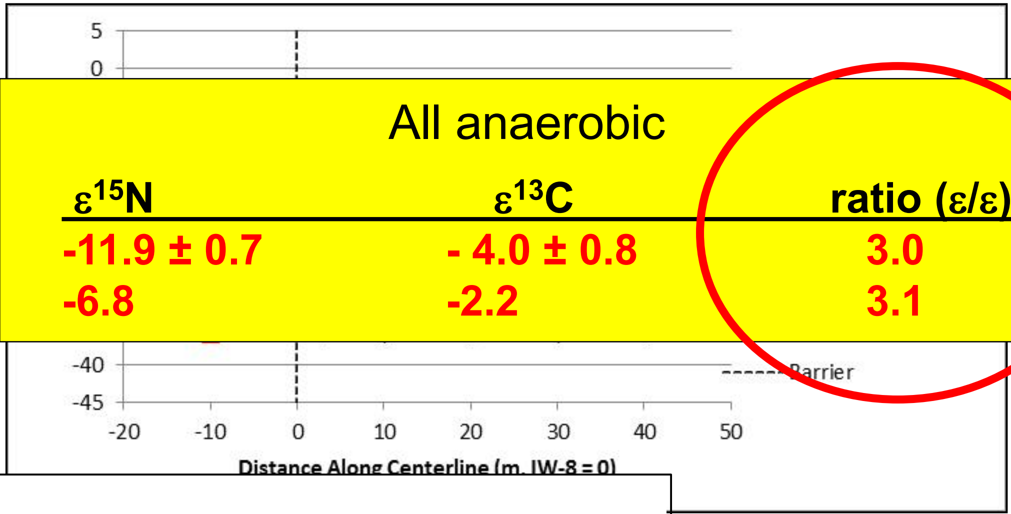


FIELD EVALUATION OF RDX ISOTOPES DURING BIOBARRIER STUDY

Plot of ln(R/R$_o$)*1000 vs ln(f) for N and C isotopes in RDX during pre-barrier and 6 months post-biobarrier

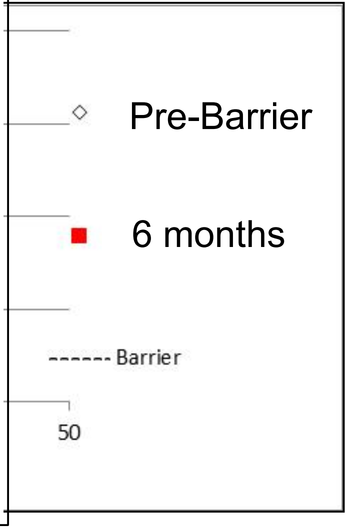


Plot of $\delta^{13}C$ (top) and $\delta^{15}N$ in RDX during pre-barrier and 6 months post-biobarrier



All anaerobic

$\varepsilon^{15}N$	$\varepsilon^{13}C$	ratio (ε/ε)
-11.9 ± 0.7	- 4.0 ± 0.8	3.0
-6.8	-2.2	3.1



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Passive in situ biobarrier for treatment of comingled nitramine explosives and perchlorate in groundwater on an active range

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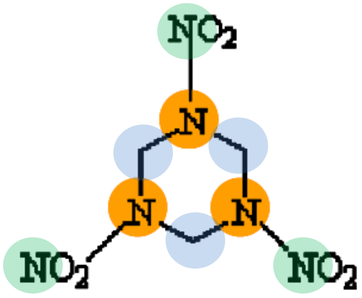
WHICH ORGANISMS ARE DEGRADING RDX? STABLE ISOTOPE PROBING (SIP)

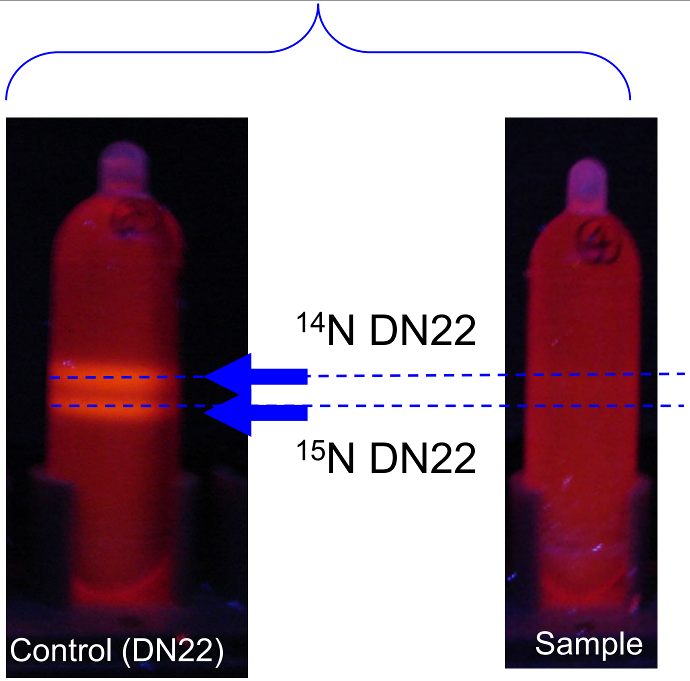


Isotopically enriched RDX

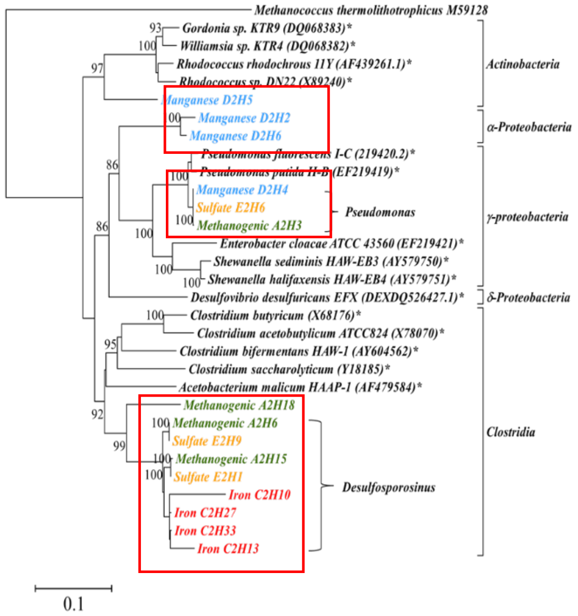
^{15}N - or ^{13}C-RDX added to microcosms or in the field

Incubate and extract community DNA

Separate ^{15}N- and ^{14}N-DNA via equilibrium centrifugation in CsCl-EtBr density gradient

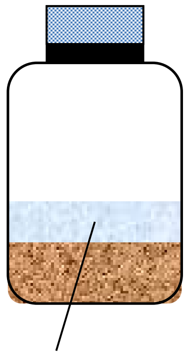


DNA sequencing and identification - ^{15}N or ^{13}C bands



STABLE ISOTOPE PROBING FOR RDX DEGRADERS UNDER DIFFERENT ELECTRON-ACCEPTING CONDITIONS – DAHLGREN NSWC

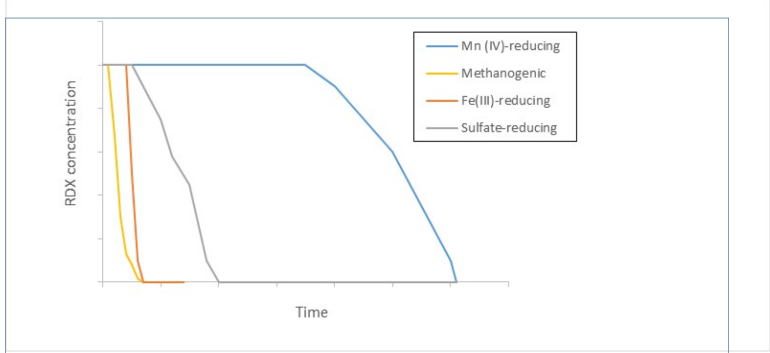
^{13}C-labeled RDX
or
^{15}N-labeled RDX
+



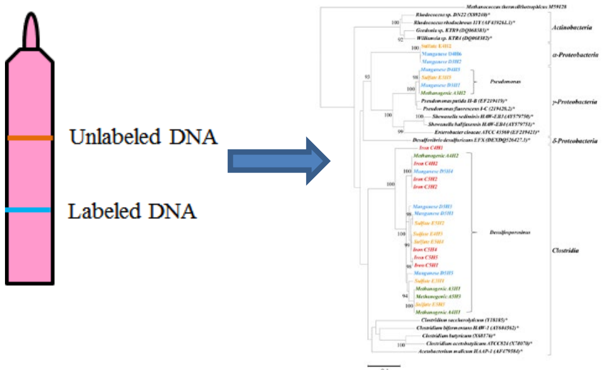
RDX-contaminated groundwater and soils

Different electron-accepting conditions:

- Aerobic and NO_3- reducing
- Mn(IV)- reducing condition
- Fe (III)- reducing condition
- SO_4^{2-}-reducing condition
- Methanogenic conditions

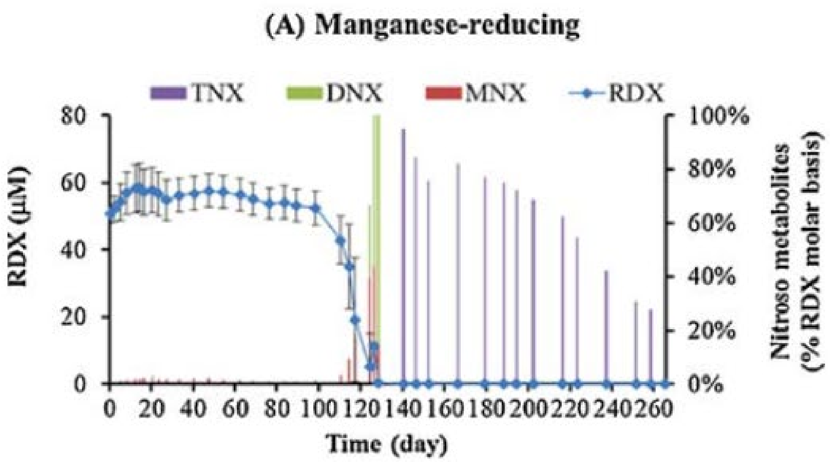


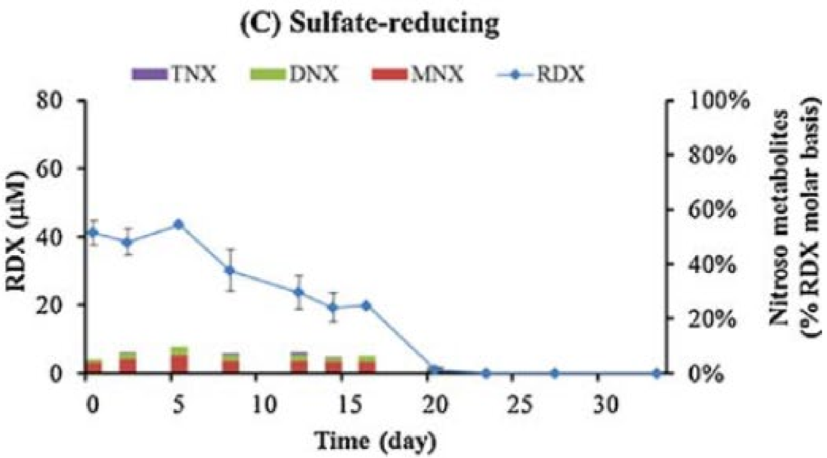
- RDX degradation rates and patterns were different under different electron-accepting conditions.

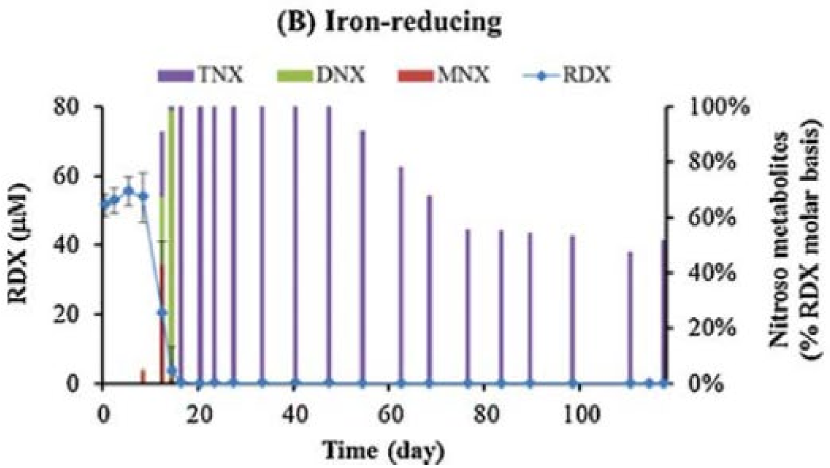


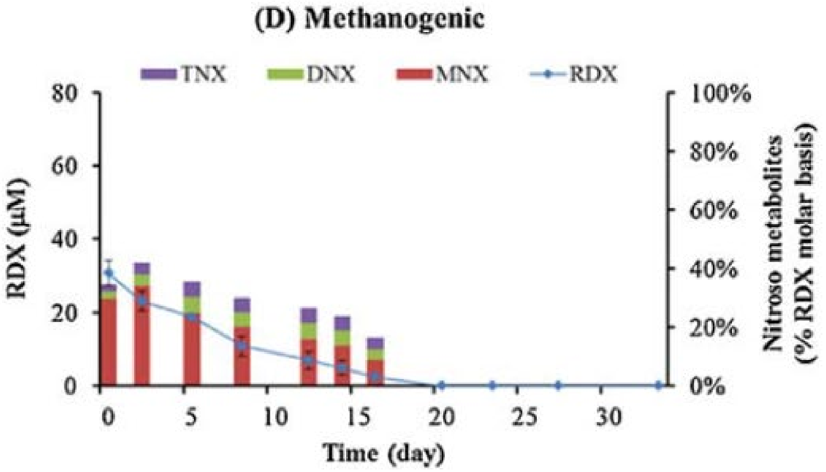
- Phylogenetic diverse RDX degraders were observed under different electron-accepting conditions

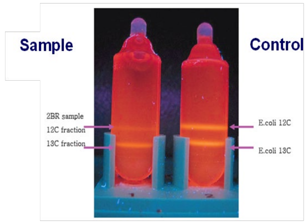
RDX BIODEGRADATION UNDER DIFFERENT ELECTRON-ACCEPTING CONDITIONS – DAHLGREN NSWC









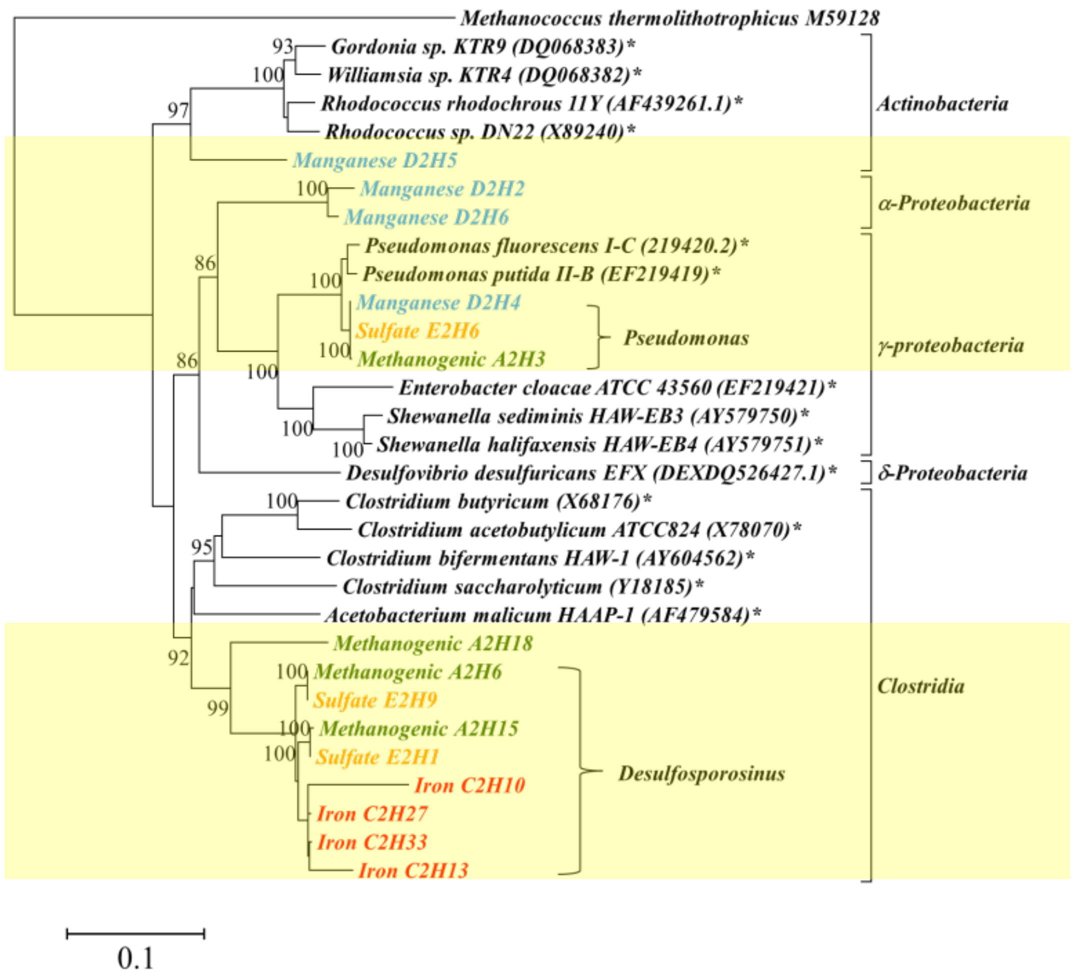


RDX BIODEGRADATION UNDER DIFFERENT ELECTRON-ACCEPTING CONDITIONS

Results (^{13}C- & ^{15}N-RDX)

1. Different dominant pathways
2. Predominance of *Clostridia* under Fe, CO_2, SO_4^- reducing conditions
3. *Desulfosporosinus* – Very diverse metabolism – different pathways?
4. α-*Proteobacteria* and others under Mn-reducing conditions

Phylogenetic Tree using SIP with ^{13}C-RDX



CONCLUSIONS

- A stable isotope analysis method has been developed and field-tested to quantify $\delta^{15}N$ and $\delta^{13}C$ in RDX.

- CSIA analysis of C and N can be used to document anaerobic degradation of RDX in the environment.

- CSIA analysis of N may be useful to document aerobic degradation of RDX in the environment – only at sites with extensive degradation.

- SIP can be applied to identify organisms incorporating C and N from RDX to further document biodegradation

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QUESTIONS

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