Chemical and Biological Degradation of Insensitive Munitions (IM) Mediated by Fe(III) Reducing Microorganisms

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Overview

- Graduate students that have worked on these data, and funding sources

- What are combined biological-chemical remediation strategies

- Why might these strategies be more appropriate in some situations

- Explosives and Insensitive Munitions (IM) Biodegradation
  - Iron mediated reactions
  - Electron shuttling reactions
  - RDX mineralization in aquifer material
  - Degradation pathway and intermediates
  - Activated carbon mediated reactions with RDX
  - Degradation of 2,4-dintroanisole
  - Photobiological degradation of RDX

- Future directions and conclusions

Up front acknowledgements, the real work!

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Dr. Jovan Popovic
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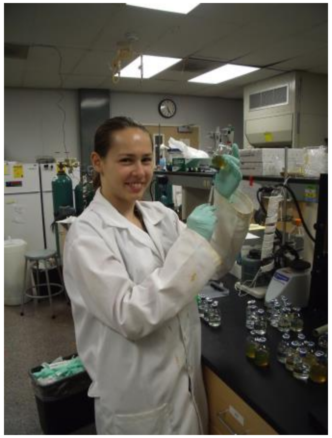
Post Doctoral Fellow



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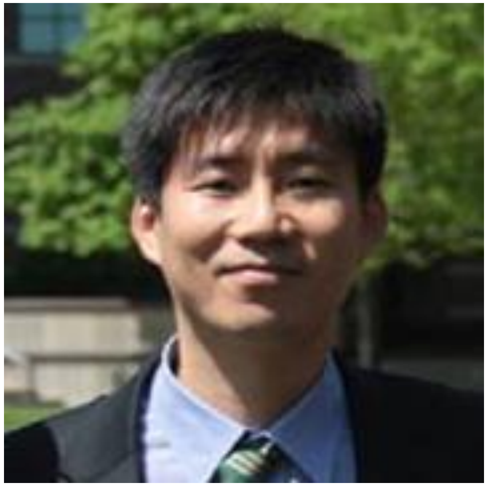

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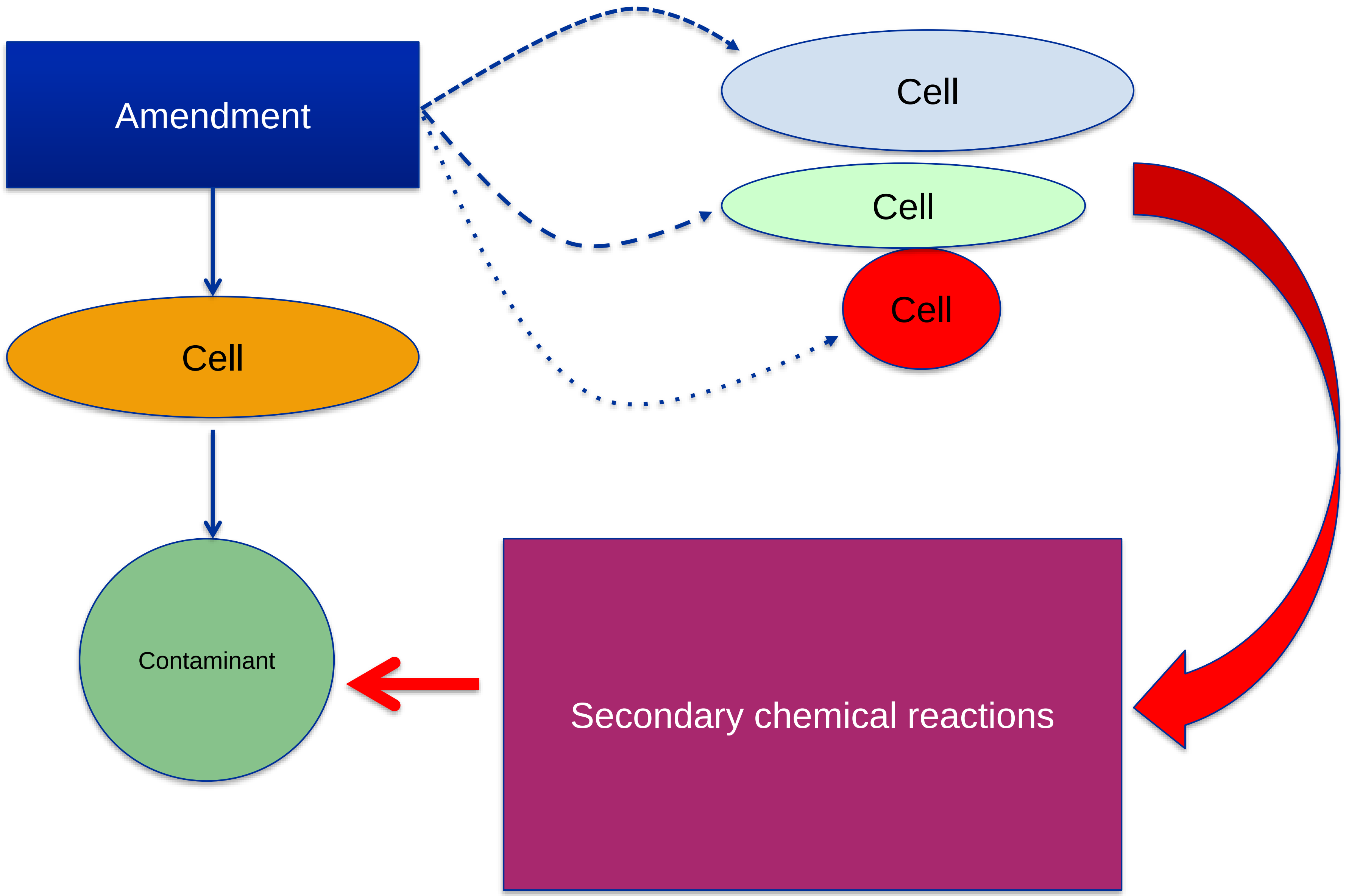
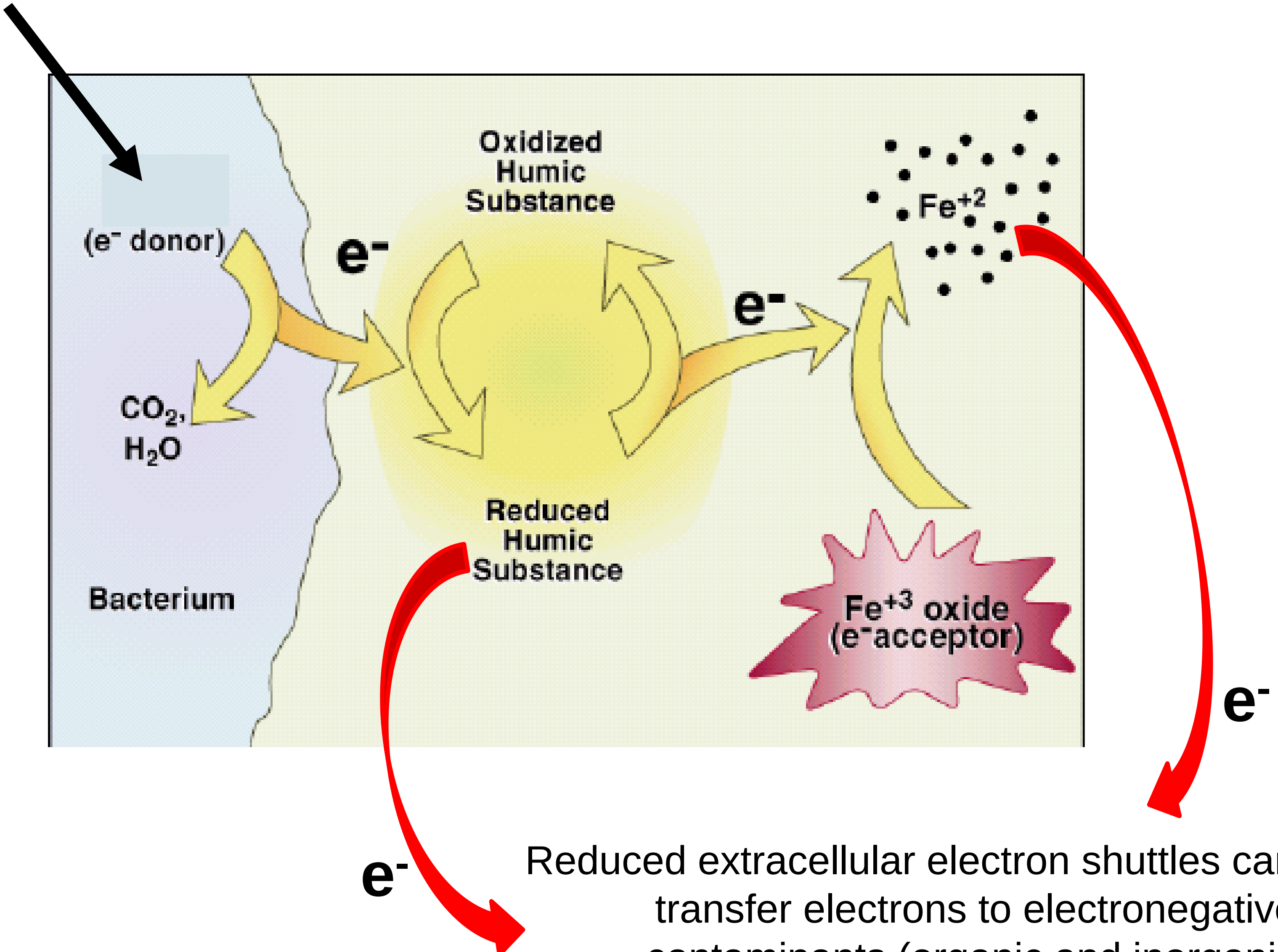
Masters Students: Yang Zhang (Consulting, China); Patricia Shin (Consulting, US); Neeraja Ramasubramanian (Consulting, India); Ayusha Khanna (Consulting, India); Sarah Galloway (Consulting, Ryder Truck Company Remediation, US), Kameryn McGee (in progress)

Undergraduates Trained on these projects: Ivy Choi, Anna Knussmann, Rachel Castillo, Erin Grubbs, Kathryn Fauerby, Jessica Bush, Gina Straga, Alexandra McIntyre, Kelsey Herring, and Olivia Felber

What do we mean by “combined reactions”?



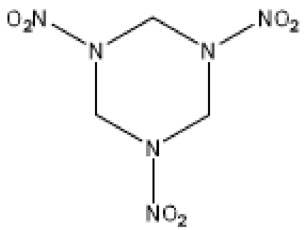
Electron donors can be
contaminants or labile substrates



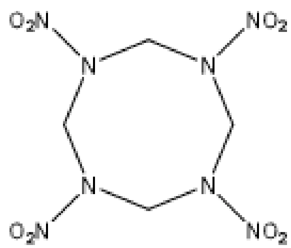
Reduced extracellular electron shuttles can transfer electrons to electronegative contaminants (organic and inorganic compounds)

Explosives and Insensitive Munitions

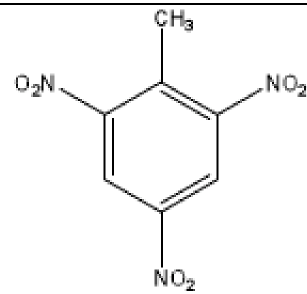
Over 1,200 sites in the U.S. and 2,000 sites in Europe have been contaminated by explosives



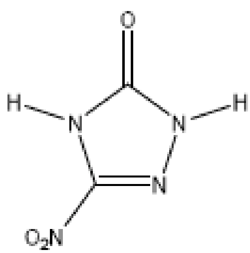
RDX
$C_3H_6N_6O_6$



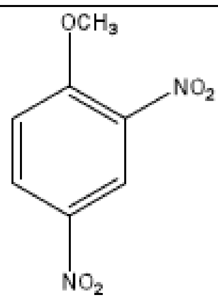
HMX
$C_4H_8N_8O_8$



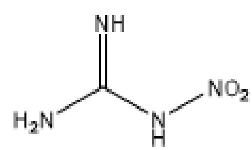
TNT
$C_7H_5N_3O_6$



NTO
$C_2H_2N_4O_3$



DNAN
$C_7H_6N_2O_5$



NQ
$CH_4N_4O_2$

Explosive	Molecular mass [g/mol]	Solubility in water [g/L]	Reduction potential [V]
RDX	222.12	38.9 mg/L (low to negligible)	-0.55
HMX	296.16	6.63 mg/L (low)	-0.66
TNT	227.13	insoluble	N/A
NTO	130.07	12.8	N/A
DNAN	198.13	sparingly soluble	-0.40
NQ	104.07	3	-0.70

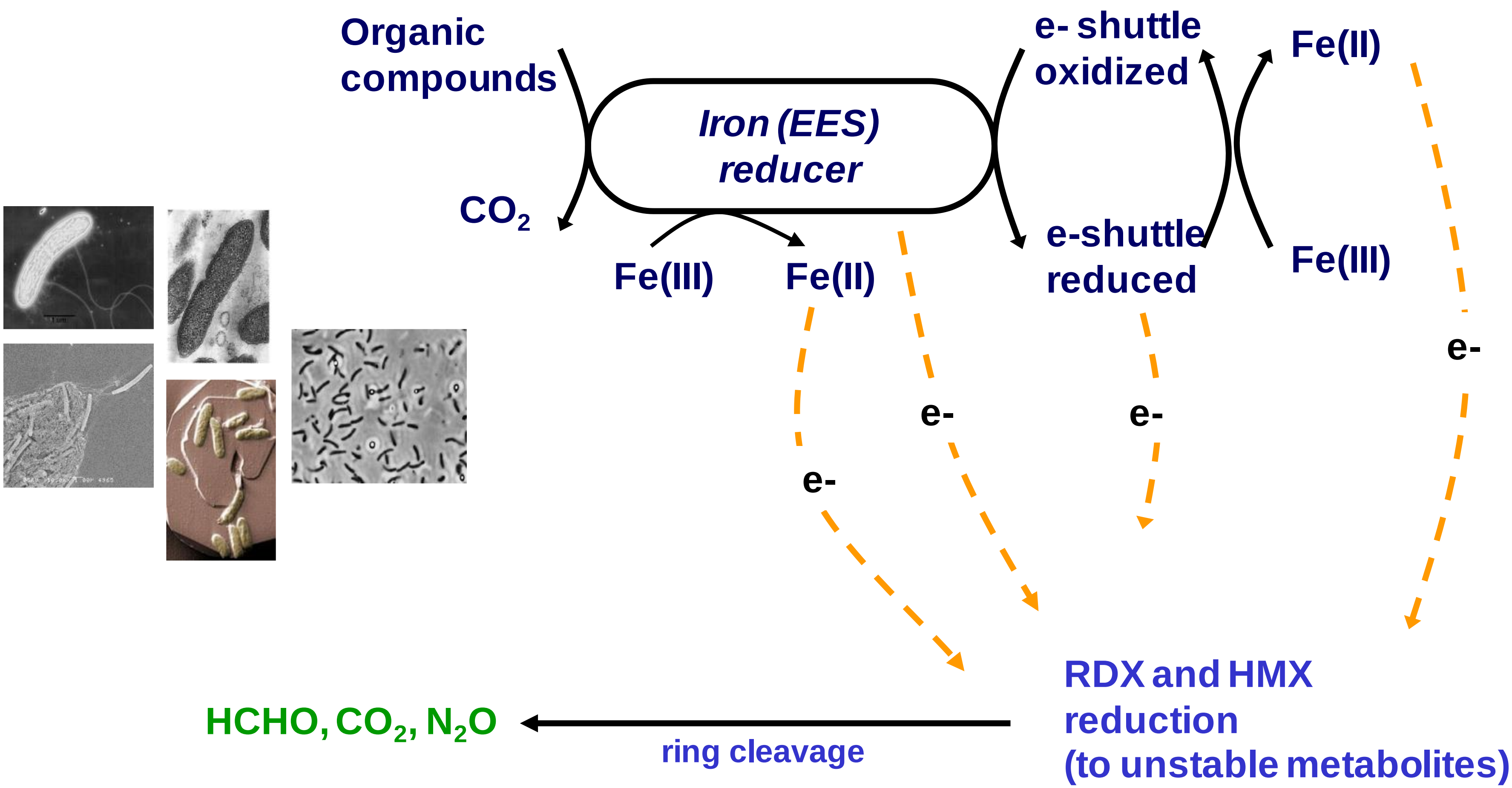
RDX is a possible human carcinogen (the lifetime health advisory in drinking water is 2μg/l)

HMX may damage the central nerve system (the lifetime health advisory in drinking water is 0.4mg/l)

IM such as 2,4-dinitroaniosole and 3-nitro-1,2,4-triazole-5-one (NTO) are currently being investigated in novel explosives composites for DoD use

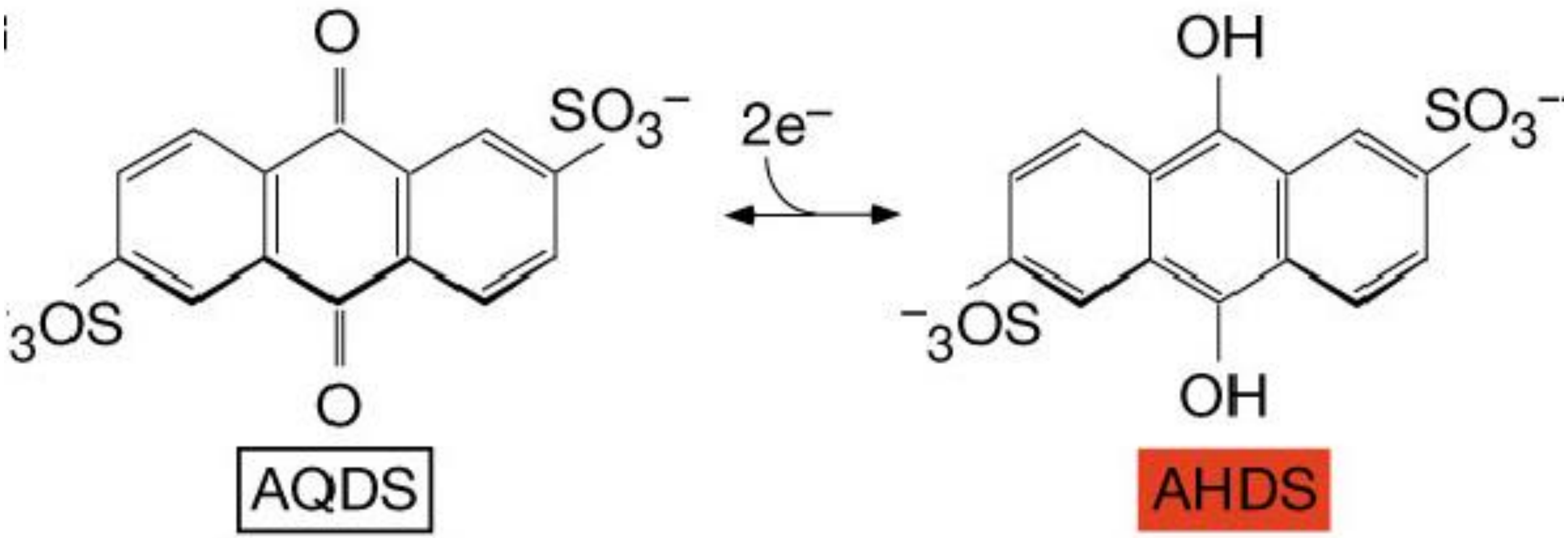
Niedzwiecka and Finneran , RSC ES:WR&T, 2015, V1(1), Page 34-39

Conceptual Model Summarized



Kwon and Finneran , Biodegradation, 2008 , V19(5), Page 705

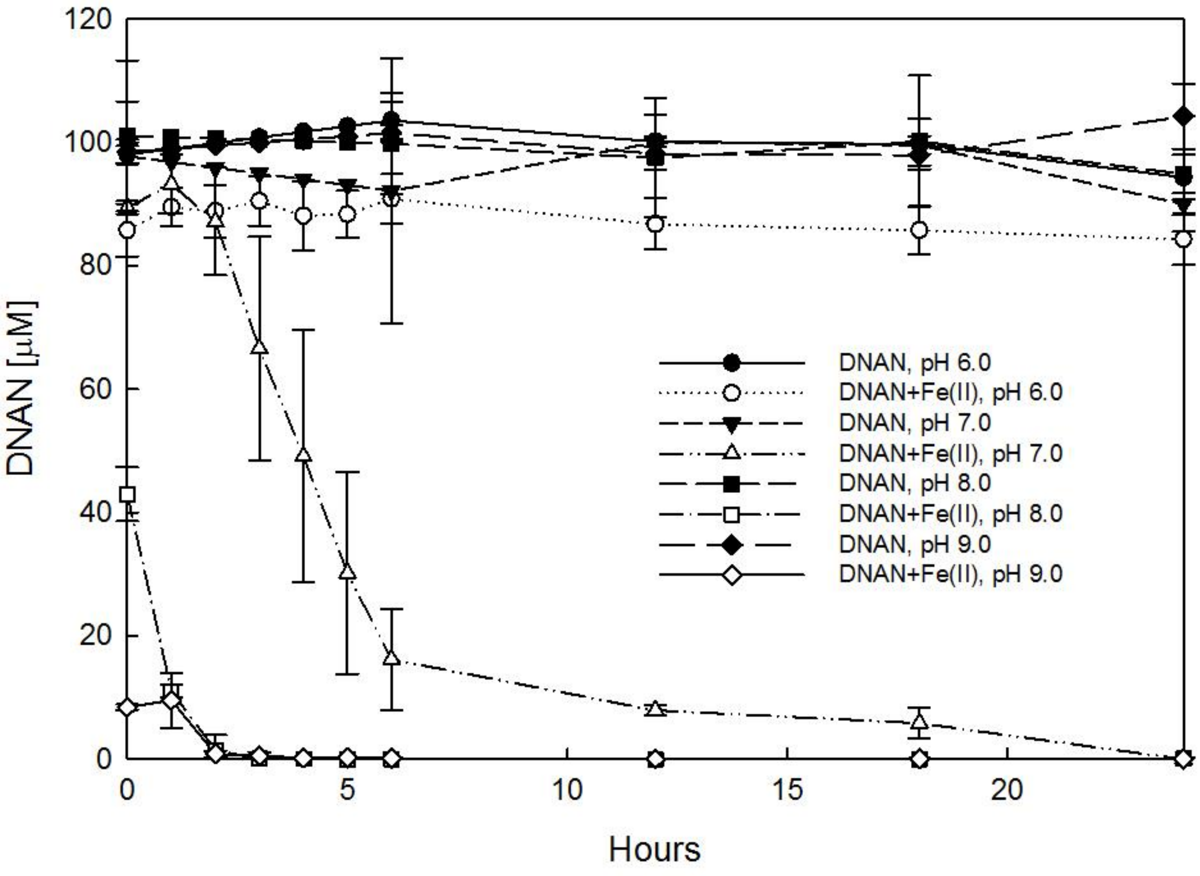
Anthraquinone-2,6-disulfonate (AQDS) Anthrahydroquinone-2,6-disulfonate (AH$_2$QDS)



The hydroquinone form has two dissociable protons

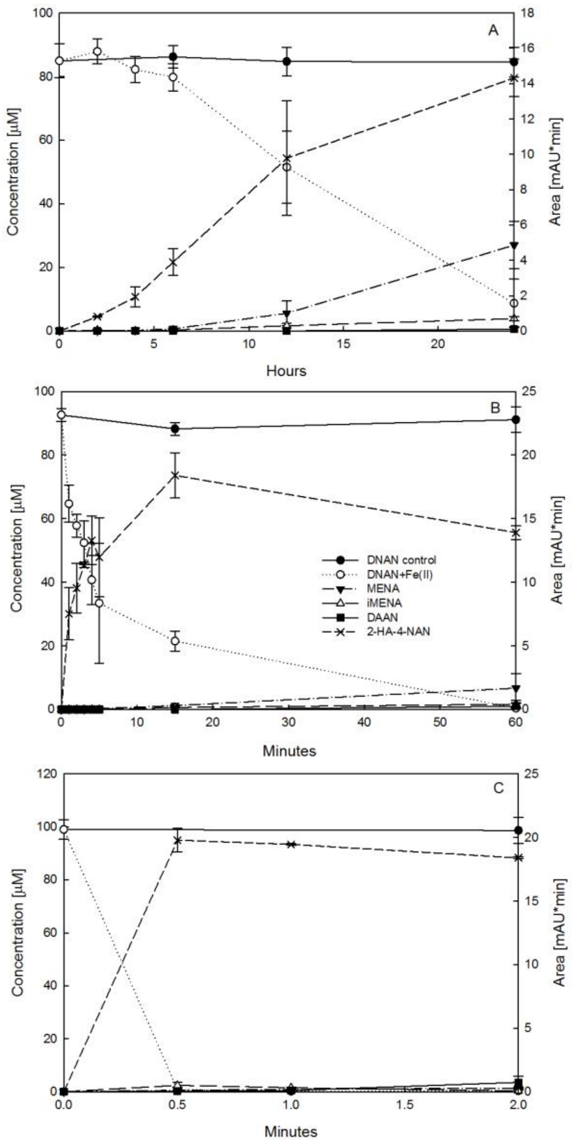
Combined reactions for next gen insensitive munitions

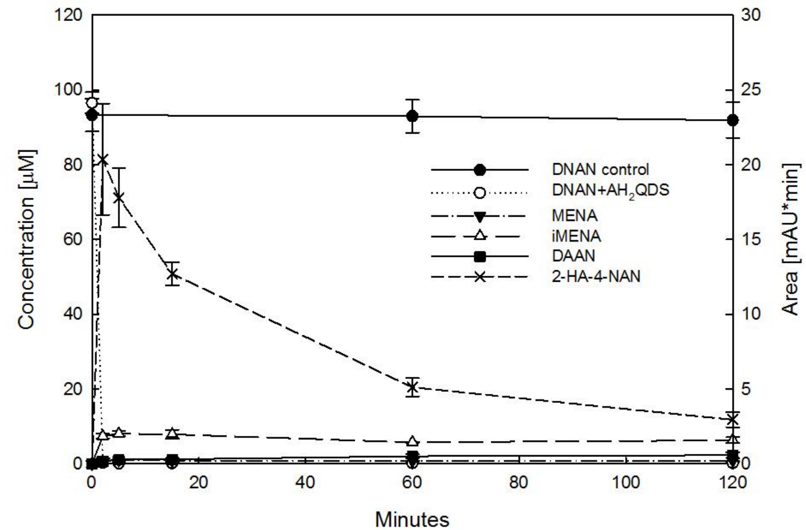
DNAN reduction by ferrous iron at pH 6, 7, 8, and 9

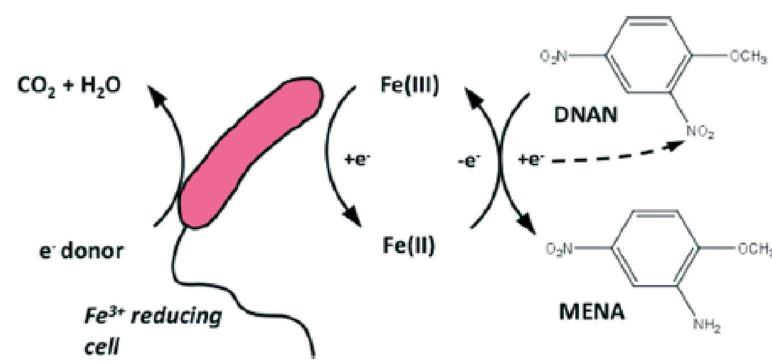


Adapted from Niedzwiecka and Finneran, 2015, ES-WR&T 1: 34-39

DNAN reduction by ferrous iron at pH 7, 8, and 9 (left), and AH_2QDS (right), with all reaction products shown

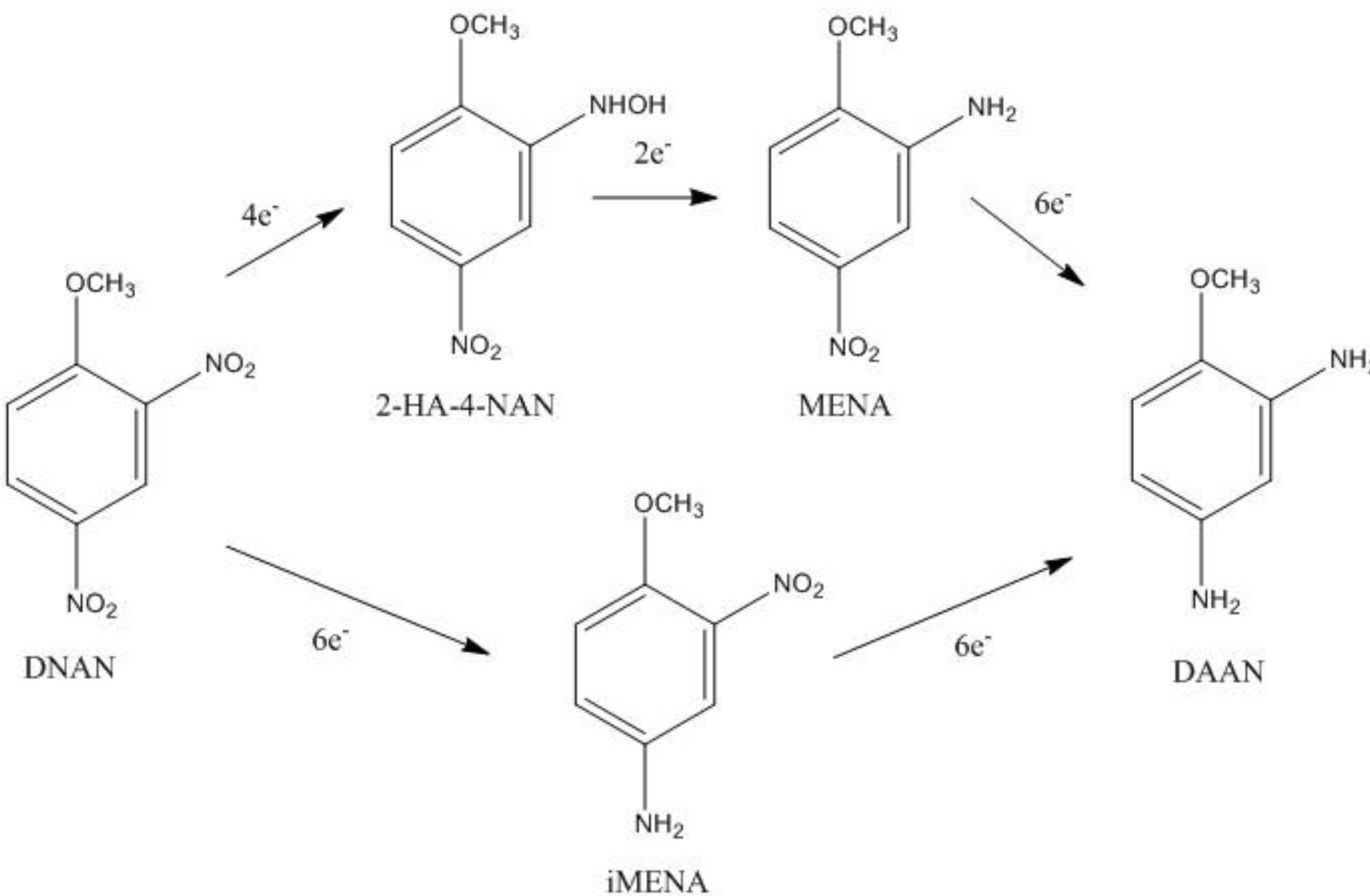




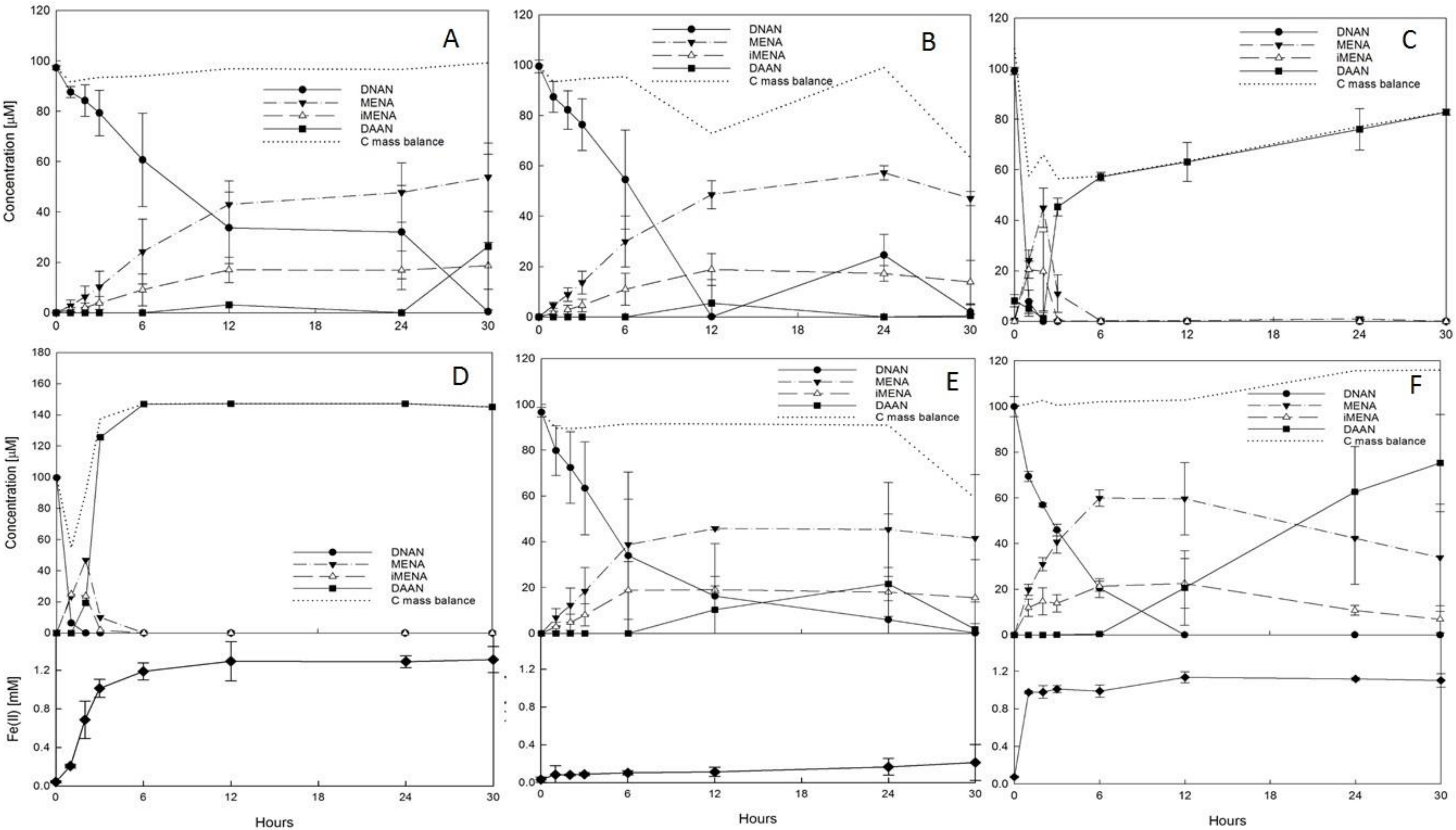


Adapted from Niedzwiecka and Finneran, 2015, ES-WR&T 1: 34-39

Proposed DNAN reduction pathway by ferrous iron, electron shuttling, and Fe(III)-reducing microorganisms



DNAN reduction GS-15 cells alone (A), cells plus acetate (B), with AQDS (C), with AQDS and Fe(III) (D), with Fe(III) (s) alone (E), and with Fe(III) citrate (aq) (F)



Short and long term mass balances for DNAN degradation by chemical or biological mechanisms

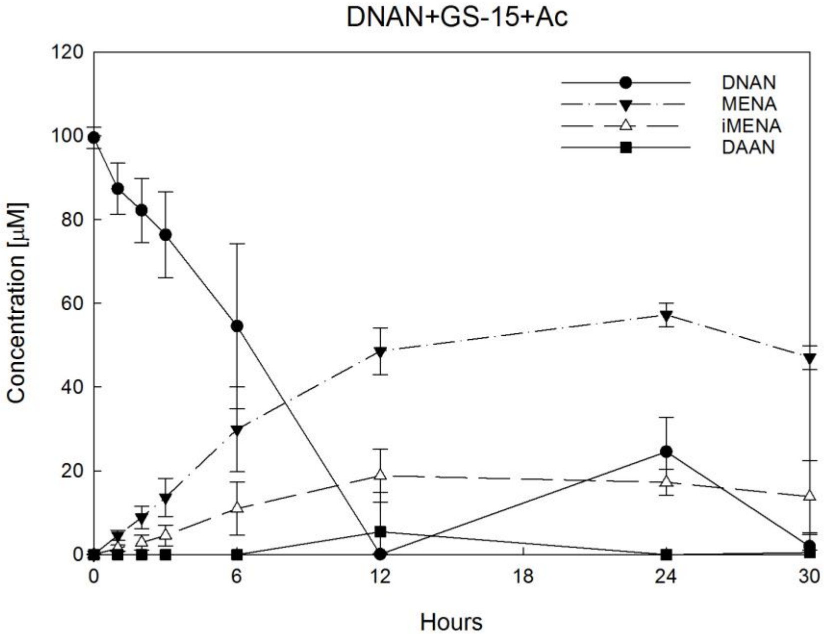
Table 1. Mass Balance (%) Following DNAN Reduction and Transformation Products Formation by Abiotic, Biological and Mixed Abiotic-Biological Pathways

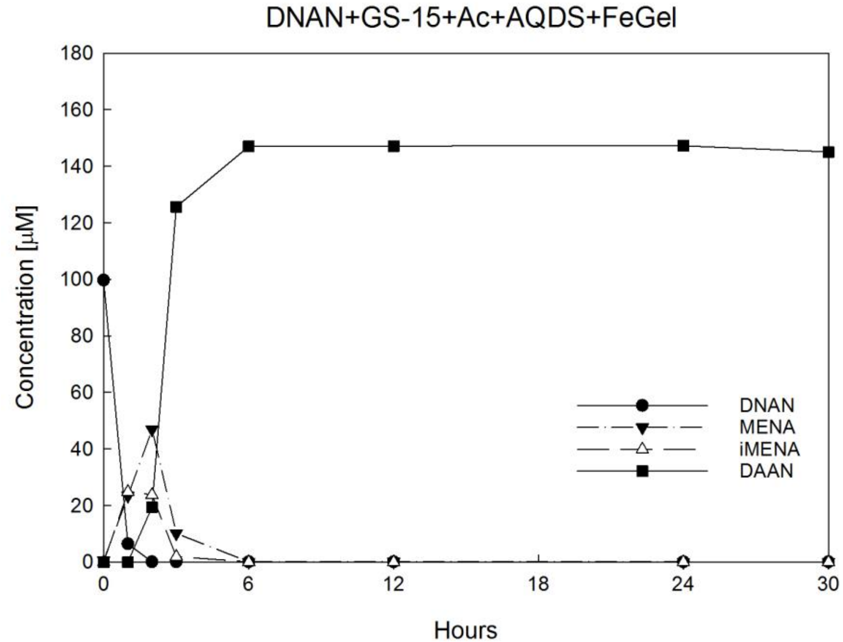
Compound	Short term mass balance (%)			Long term mass balance (%)		
	Fe(II) alone, pH 7 (abiotic)[a]	Fe(II) alone, pH 8 (abiotic)[b]	Fe(II) alone, pH 9 (abiotic)[c]	Fe(II) alone, pH 7 (abiotic)[d]	Fe(II) alone, pH 8 (abiotic)[e]	Fe(II) alone, pH 9 (abiotic)[f]
$C_7H_6N_2O_5$ (DNAN)	8.71	0.39	0.40	0.00	0.26	0.01
$C_7H_8N_2O_3$ (MENA)	27.02	6.68	1.42	53.58	12.69	1.04
$C_7H_8N_2O_3$ (iMENA)	3.91	1.62	0.34	-	-	-
$C_7H_{10}N_2O$ (DAAN)	0.59	0.97	3.56	-	63.74	91.58
total	40.23	9.66	5.72	53.58	76.69	92.63

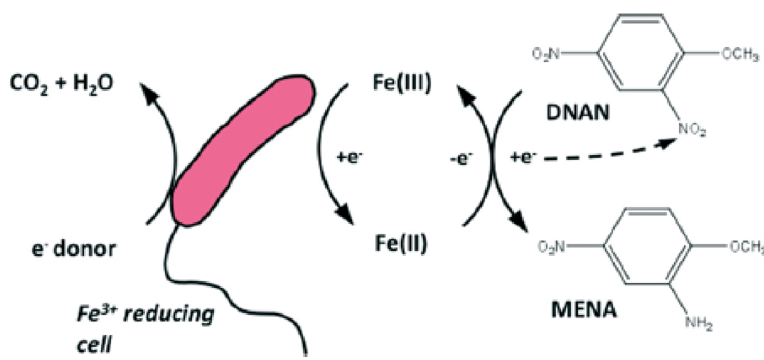
Compound	Mass balance (%)				
	cells alone (biological)[g]	cells + AQDS (mixed)[g]	cells + FeGel (mixed)[g]	cells + FeCit (mixed)[g]	cells + AQDS + FeGel (mixed)[g]
$C_7H_6N_2O_5$ (DNAN)	0.44	0.01	0.12	0.01	0.02
$C_7H_8N_2O_3$ (MENA)	53.78	0.08	41.53	33.83	0.00
$C_7H_8N_2O_3$ (iMENA)	18.67	0.00	15.53	6.89	0.00
$C_7H_{10}N_2O$ (DAAN)	26.32	82.79	1.81	75.23	145.07
total	99.21	82.88	58.99	115.95	145.09

Measured at [a]24 h, [b]1 h, [c]2 min, [d]96 h, [e]24 h, [f]52 min, [g]30 h.

DNAN reduction by *Geobacter metallireducens* in the presence and absence of Fe(III) and AQDS at pH 7

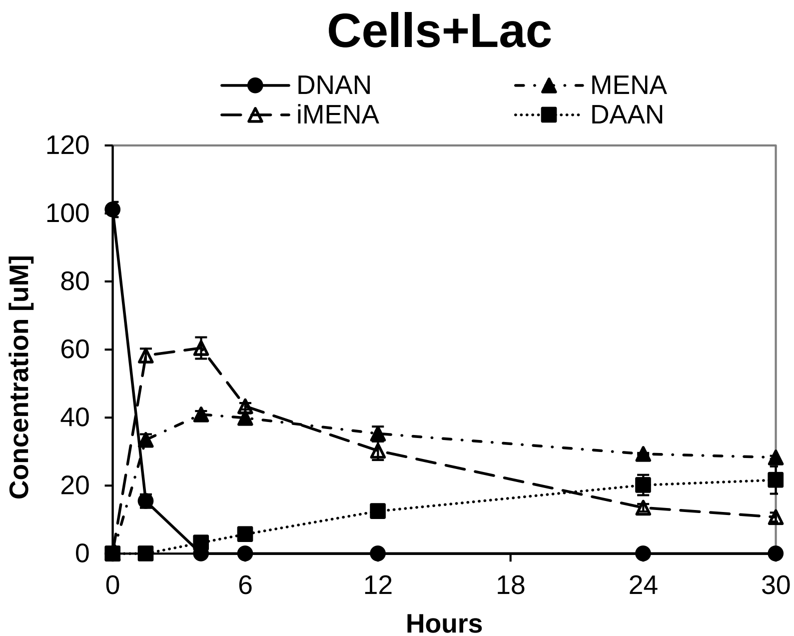


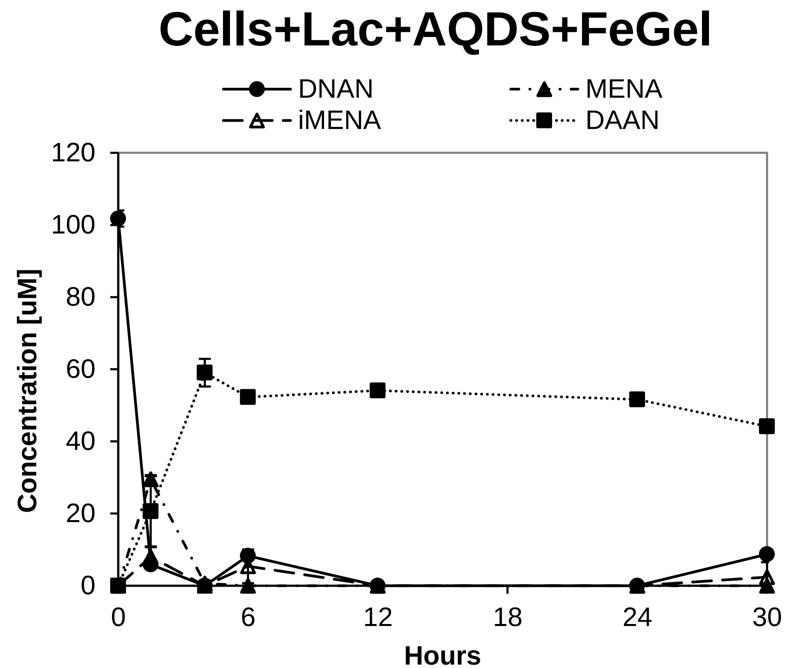


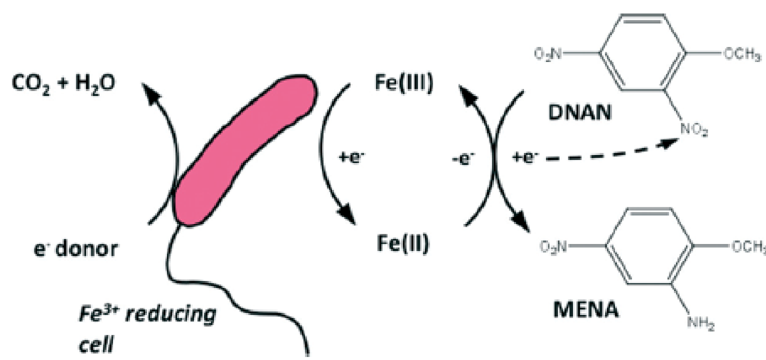


Adapted from Niedzwiecka and Finneran, 2015, ES-WR&T 1: 34-39

DNAN reduction by *Shewanella oneidensis* in the presence and absence of Fe(III) and AQDS at pH 7







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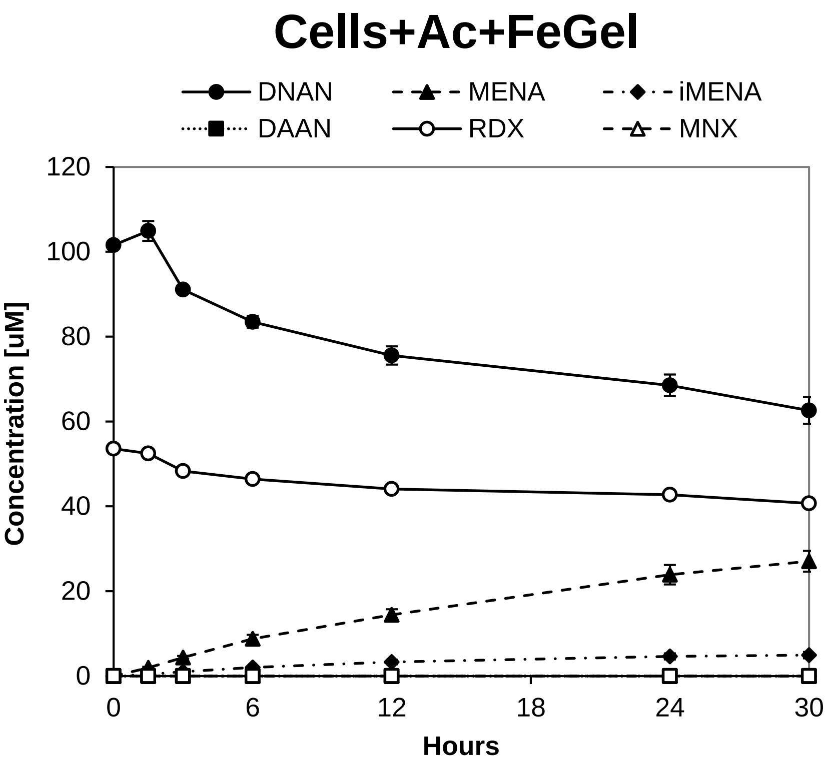
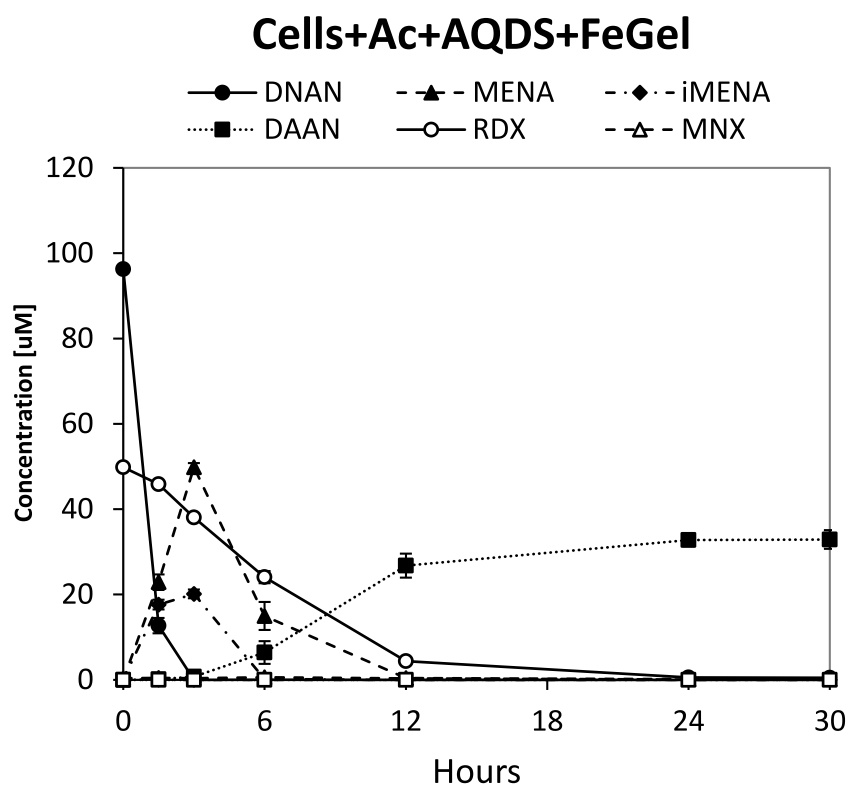
DNAN reduction by *Geobacter metallireducens* in the presence and absence of Fe(III) and AQDS at pH 7

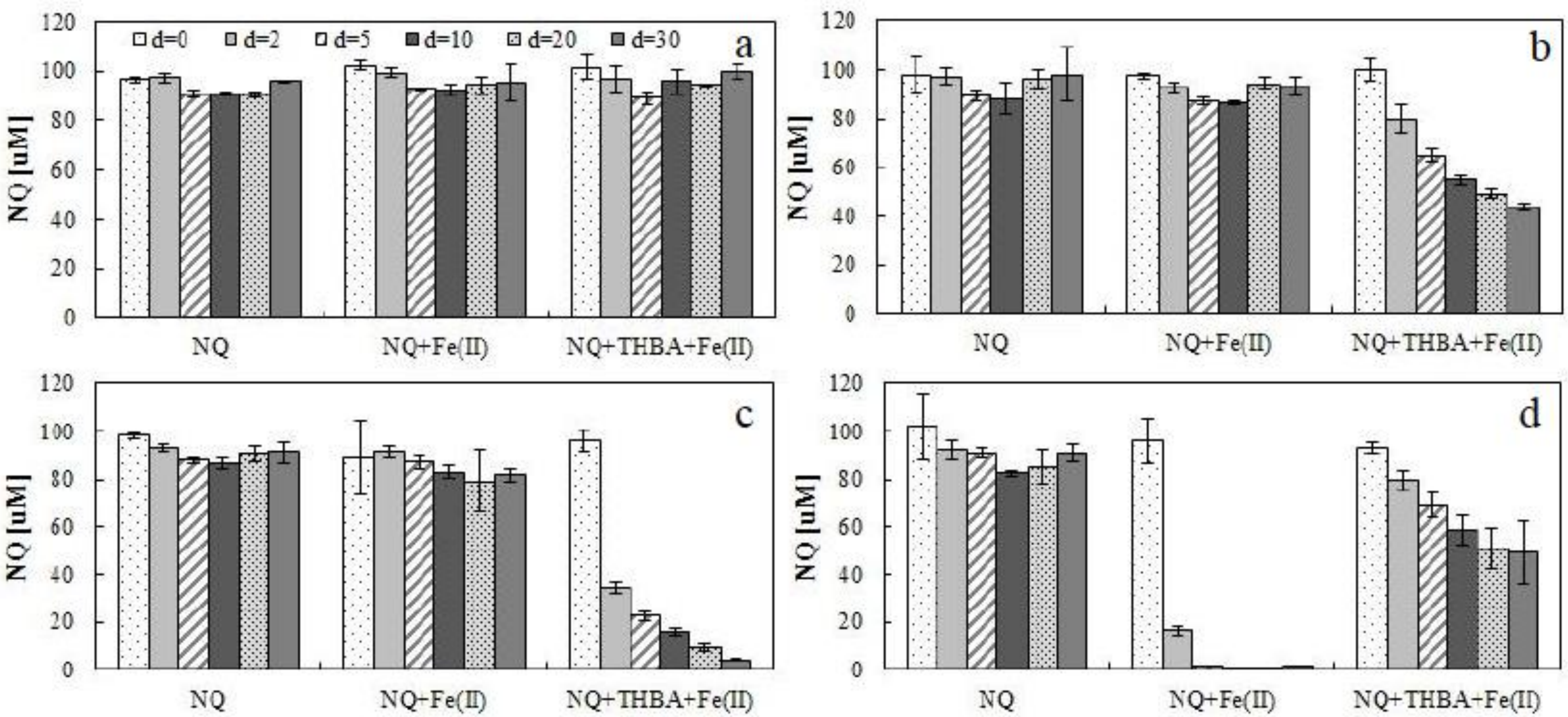




Adapted from Niedzwiecka and Finneran, 2015, ES-WR&T 1: 34-39

Nitroguanidine Degradation (NQ)

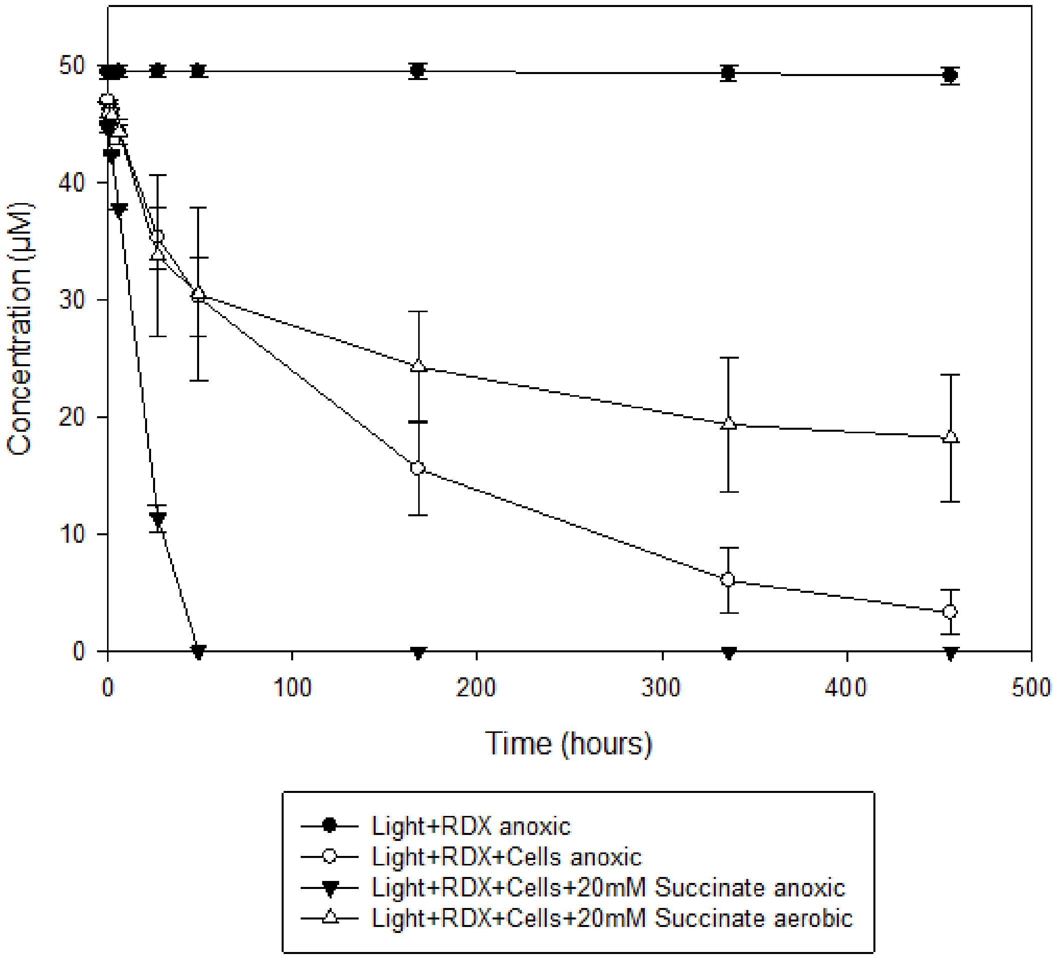
NQ reduction by ferrous iron alone, or in the presence of the ligand 2,3,4-trihydroxybenzoic acid (THBA) at pH 6, 7, 8, and 9 (A to D, respectively)



Photobiological Degradation of RDX

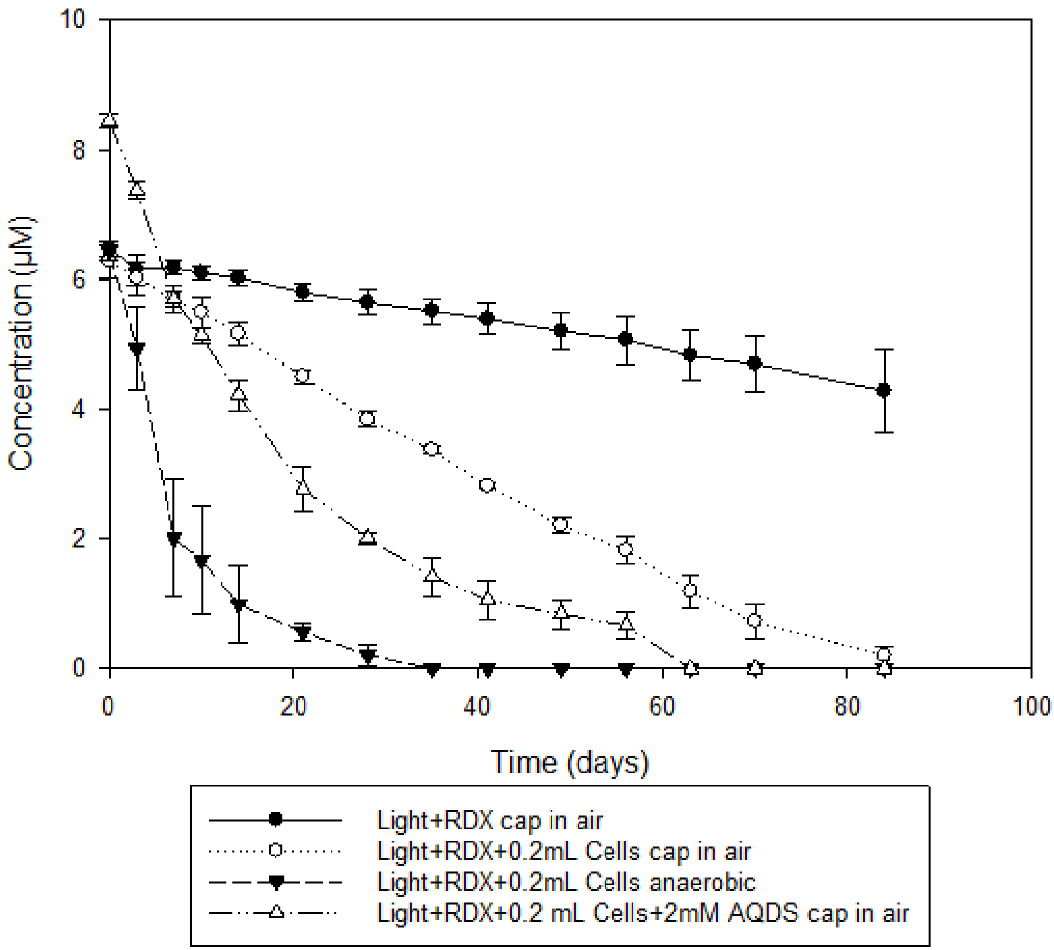
RDX reduction by *Rhodobacter sphaeroides* in the presence and absence of the electron shuttle AQDS

This is anoxygenic photosynthesis; it requires an organic or inorganic, but non-water, electron donor (in this case succinate)



RDX reduction by *Synechocystis strain PCC 6803* in the presence and absence of the electron shuttle AQDS

This is oxygenic photosynthesis; it requires water as the sole electron donor; so the system is essentially: *cells + contaminated water + light* = biodegradation!



Conclusions

1. Combined biological and chemical reactions can be more effective than either biological reaction or chemical reactions alone

2. Explosives and IM are readily reduced to innocuous or less toxic products by cells + iron, or cells + electron shuttles

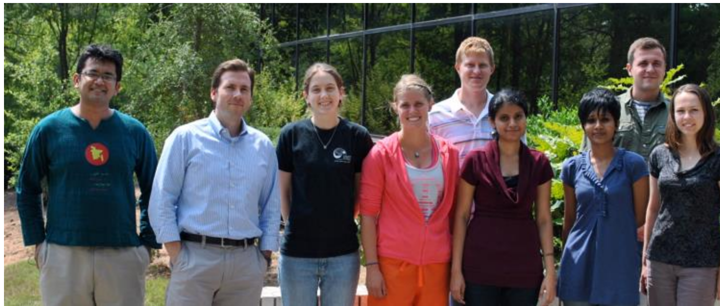
1. We developed the first photobiological RDX degradation system, in which water itself (with light energy) provides the electrons to reduce RDX to innocuous end products

2. These strategies are all predicated on a strong understanding of Fe(III)-reducing microorganisms

3. Although we did not discuss it, this also works in reverse, in which chemical remediation amendments stimulate microbial activity

4. Other contaminants including metals, metalloids, radionuclides, and chlorinated compounds can be degraded more quickly, and more completely, when biological and chemical reactions are combined

Acknowledgments



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