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Innovative Biological and Molecular Tools for Application to Mine Waste Issues

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U.S. DEPARTMENT OF ENERGY

Objective

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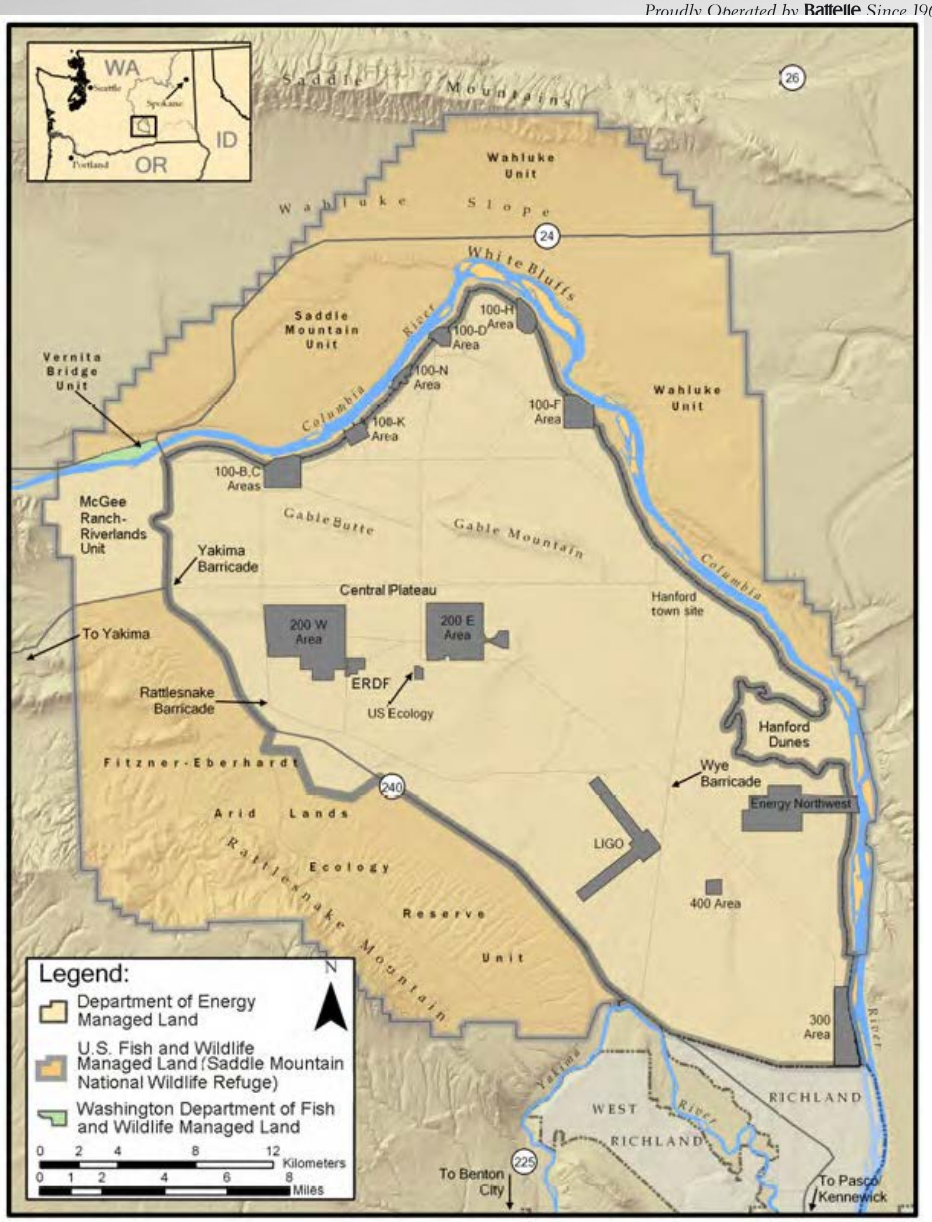
- To provide an overview of monitoring and remediation approaches developed by PNNL scientists that have the potential for application to surface and groundwater contaminated with metals and radionuclides
  - Look for potential opportunities to help mitigate hazards to the environment and restore watersheds and water resources
  - Provide technical information that will help in the decision making process for nearly 46,000 sites on abandoned mine lands and assist in minimizing impacts of ongoing mining operations

Outline

- Background
- Laboratory-scale
  - Microbial metal and radionuclide transformation
- Mesoscale (Intermediate scale) research tools
  - Interaction between soil and groundwater
- Field-scale
  - Application of constructed wetland for removal of inorganic compounds from water
- Monitoring
  - Molecular biological tools for monitoring microbial communities in the environment
- Decision making
  - Alternate end states analysis

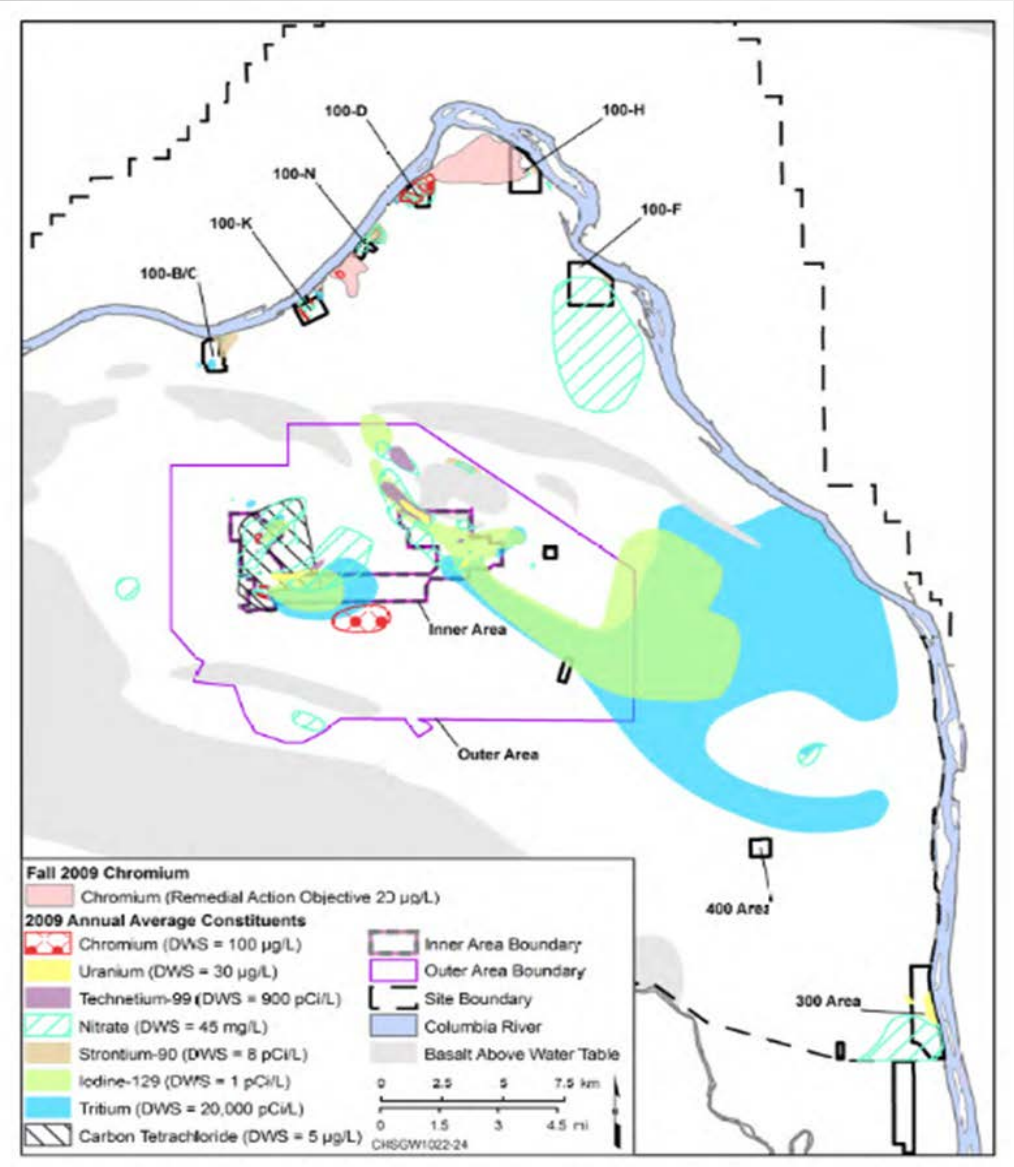
Hanford Site Background

- 586 sq. miles
  - Shrub steppe desert in southeast WA
- Began producing plutonium for nuclear weapons in 1947
- Last reactor ceased operation in 1987
- 110,000 tons of nuclear fuel was processed



Waste Issues

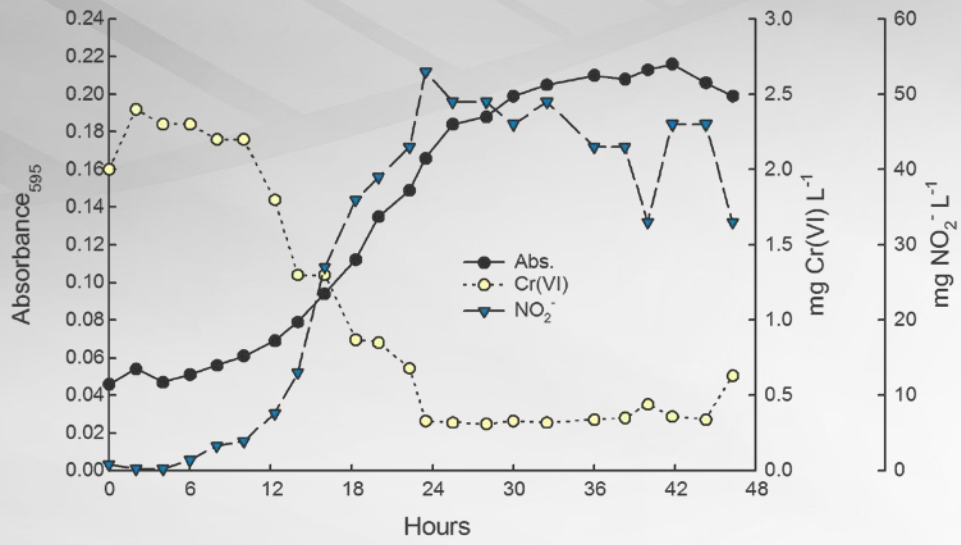
- An estimated 450 billion gallons of radioactive and hazardous waste released to subsurface
- Groundwater plume estimated to cover 150 square miles under site
- Mixed contaminant plumes:
  - Technetium-99
  - Uranium
  - Iodine-129
  - Carbon tetrachloride
  - Nitrate
  - Chromate
  - Tritium

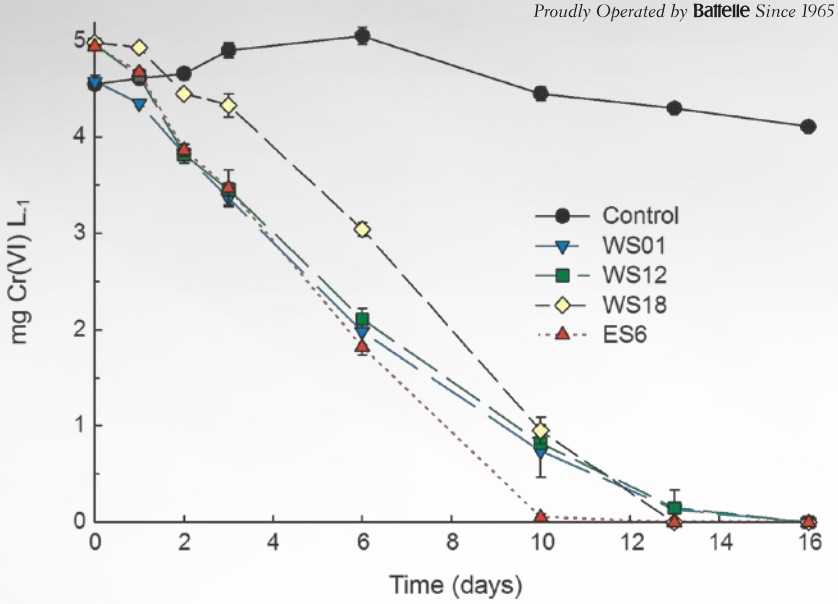


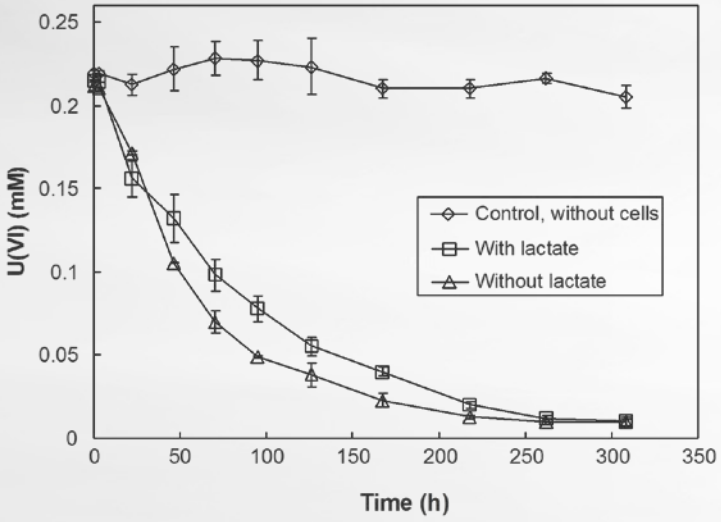
In Laboratory

- Microbes interact with inorganic compounds in their growth environment
  - Nitrate, sulfate, iron
  - Reductive conversion through metabolism
- Metabolism can be redirected for biotransformation of inorganic contaminants
  - Hexavalent uranium, hexavalent chromium, arsenic, selenium, etc.

A Few Examples



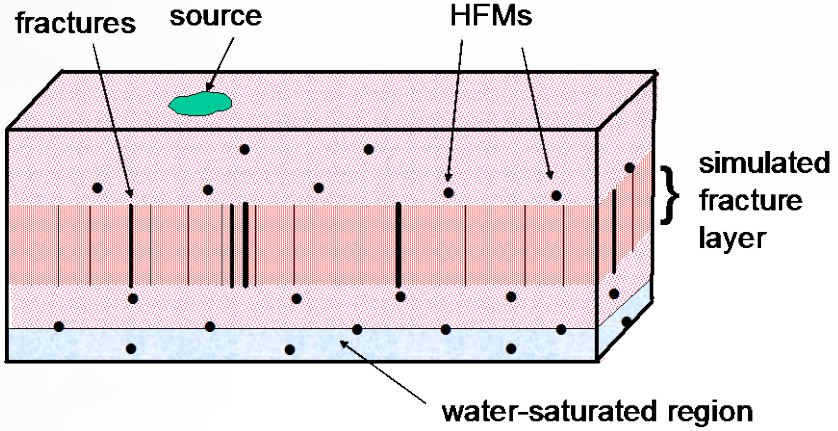




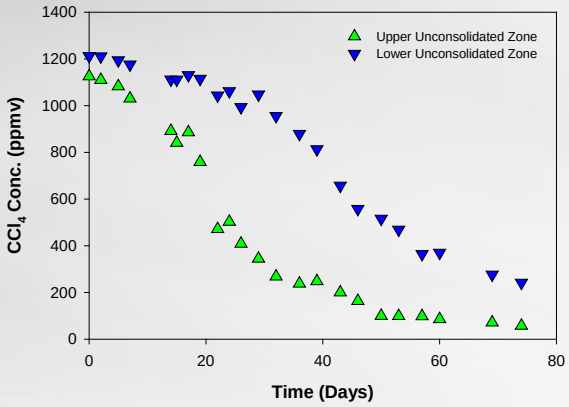
- Bacteria isolated from Hanford 100 Area

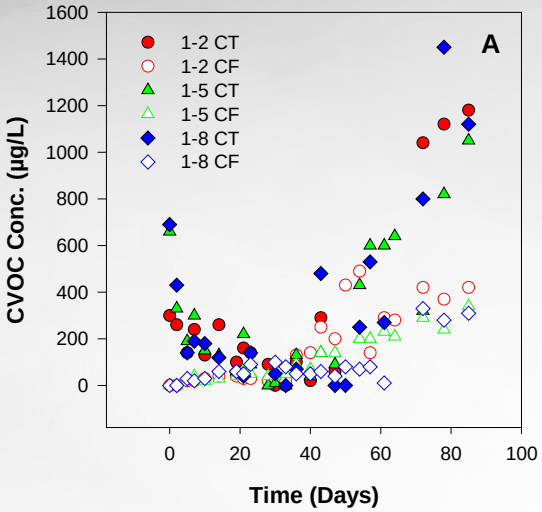
Mesoscale Tank Experiments

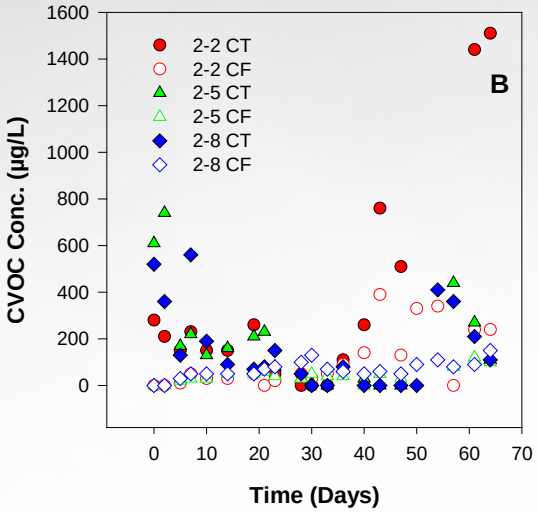
- Inoculum
  - Culture isolated from vapor extraction well in SDA located within RWMC at INL
    - Laboratory enrichment consisted of 8 *Pseudomonas* species
- Biostimulation with lactate
  - Batch initially
  - Continuous after 40 days

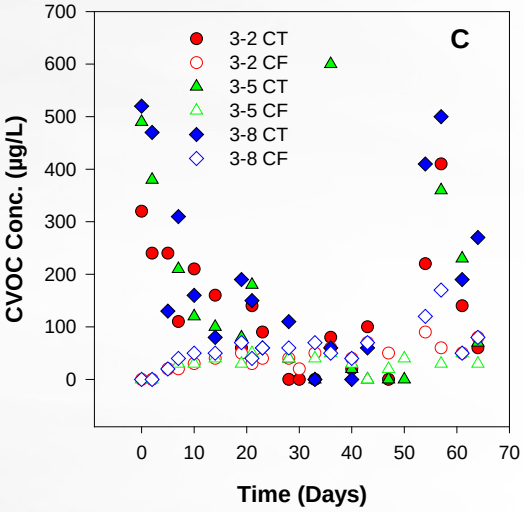


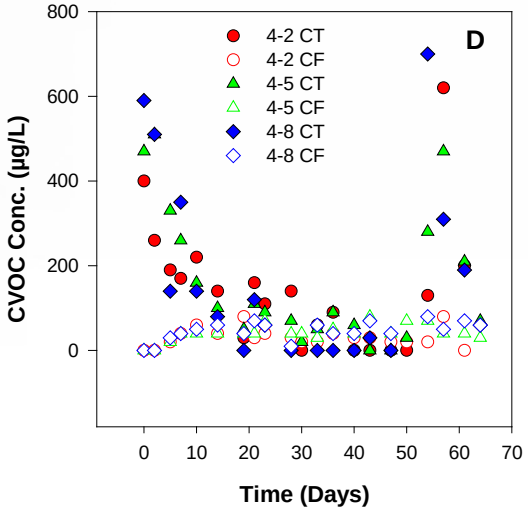
Experimental Results





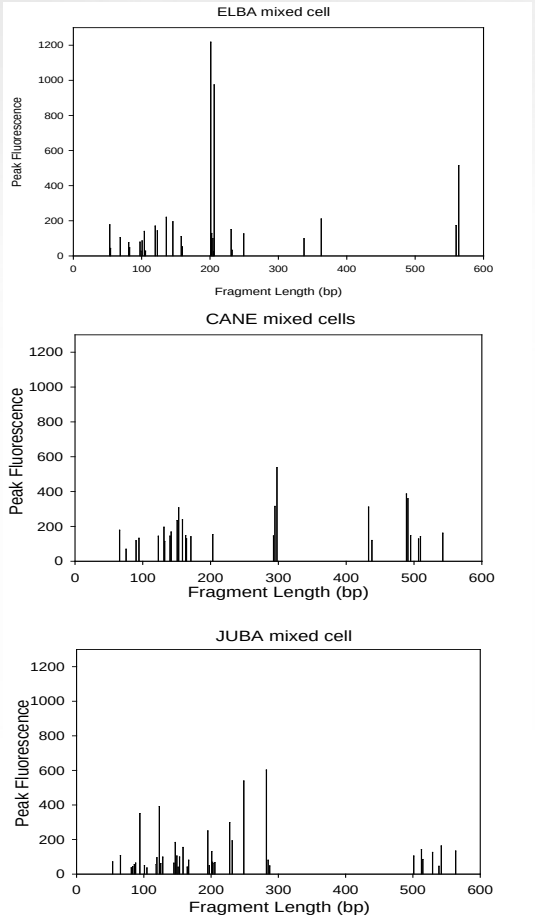






Constructed Wetlands

- Evaluation of the establishment and performance of wetland cells- primary and secondary filters, and settling, shallow or deep ponds.
- Evaluation of plant species, alone or in combination, for optimal treatment of surface run-off
- Molecular microbial characterization (i.e. evaluation of microbial diversity shifts as function of wetland function)
- Determination of the effectiveness of plant-microbe systems to capture sediment and nutrients (contaminants)



Molecular Biological Tools – Understanding the "black box"

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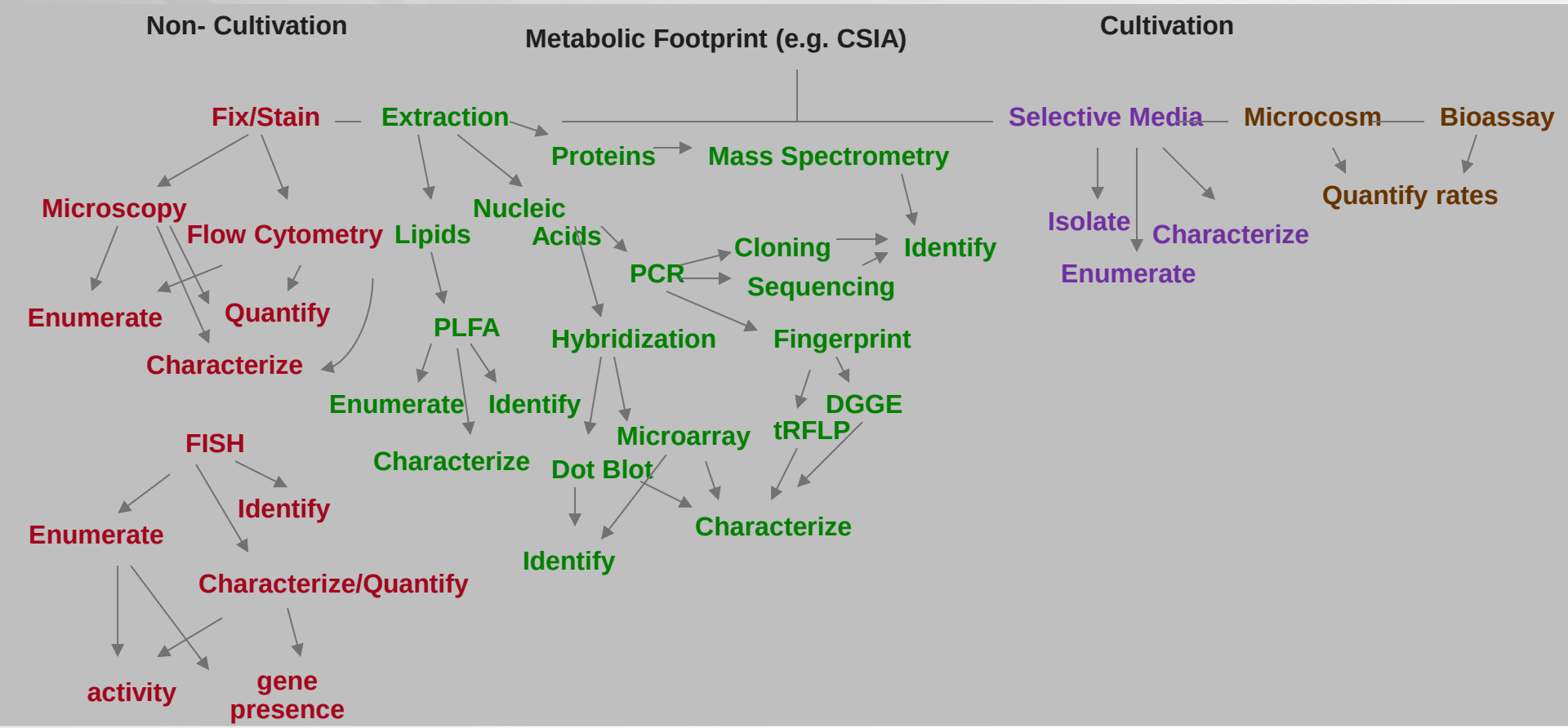

Molecular Tools Employed for Monitoring

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- Fluorescent in situ hybridization (FISH): Targets either 16S rDNA or RNA to determine the activity of the targeted organisms; wide array of target organisms

- qPCR: is a laboratory analytical method used to determine the number of copies of a specific, target gene present in a sample (functional genes, microbes, groups of related microorganisms)

- Enzyme Activity Probes (EAP): Detection of specific enzyme activities; probes validated target enzymes of interest (e.g., aromatic oxygenases, methane monooxyganase)

Sites Where MBTs Have Been Deployed

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- EAPs & qPCR:
  - 9 DOE, 15 DoD, 4 EPA, 8 Industrial sites (400 + MW locations)
  - 3 vapor impacted sites
  - Rates (microcosms) @ 12 sites
  - Coupled with CSIA @ 7 sites
  - Line of evidence for MNA @ >20 sites

- Co-contaminants
  - Carbon tetrachloride
  - 99Tc, Sr, I, U, Cr
  - PCE, DCE, VC
  - 1,1,1 TCA
  - Petroleum hydrocarbons
  - Nitrate
  - NDMA
  - 1,4 dioxane
  - MTBE

- Site heterogeneity
  - Varying depth to contamination
    - Surface to 500 ft bgs
  - Extensive plumes
    - > 3 miles in length
  - Varying hydrology
  - Varying geological strata

Application of MBTs to Contaminant Plume at Travis AFB

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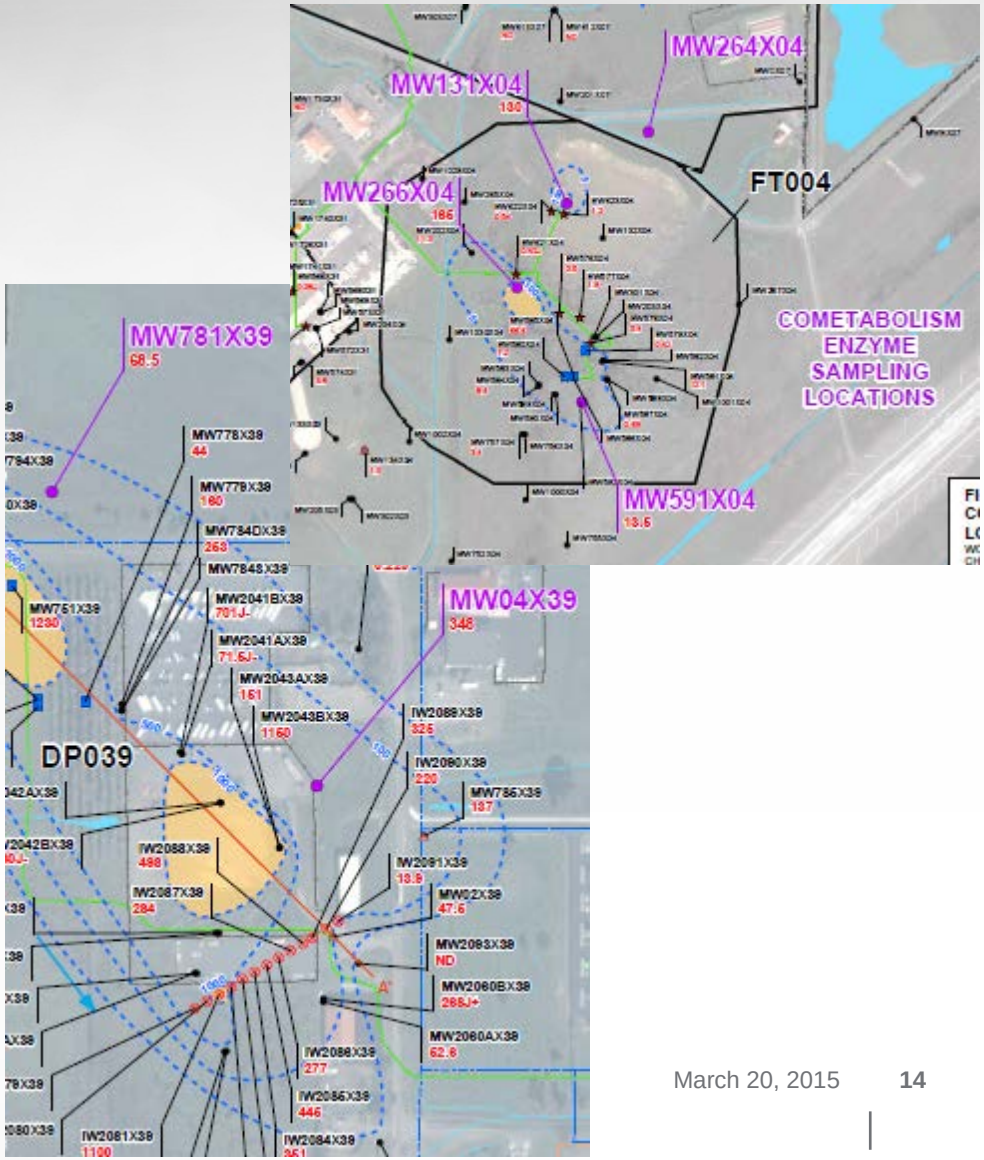
- Natural attenuation assessment
- Samples
  - Site 1 – FT004
    - Three in plume monitoring wells
    - One upgradient monitoring well
  - Site 2 – DP039
    - Two in plume monitoring wells
- Analyses
  - Enzyme activity probes
  - qPCR



Travis AFB

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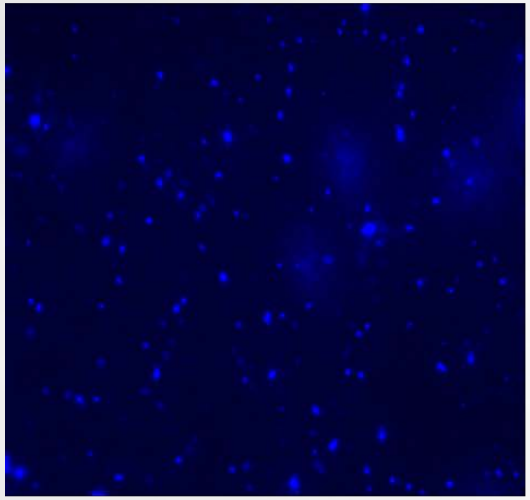
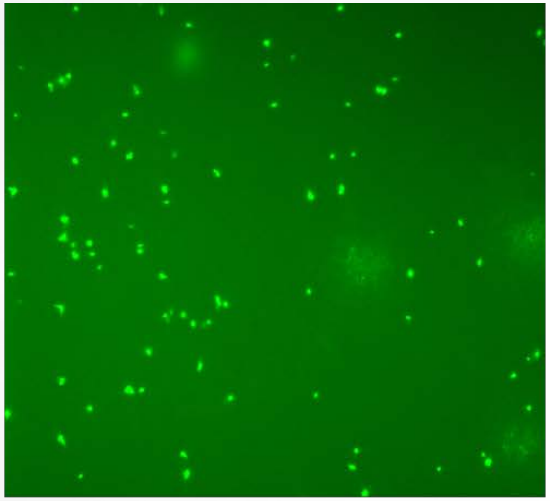
Enzyme Activity

	DAPI Total	*stdev*	3hpa T3-mono	*Stdev*	PA T2-mono	*stdev*	Cinn T23-di	stdev	Coumarin sMMO	Naphthalene MMOs
	Cells mL^{-1}		*Cells mL^{-1}*		*Cells mL^{-1}*		*Cells mL^{-1}*		*RFU*	*RFU*
MW131	4.52E+04	*1.02E+04*	1.17E+04	*4.87E+03*	3.55E+04	*7.56E+03*	4.17E+04	*7.99E+03*	Neg	Neg
MW591	2.23E+04	*6.19E+03*	1.62E+04	*6.11E+03*	1.54E+04	*4.25E+03*	1.68E+04	*5.72E+03*	Neg	Neg
MW266	3.67E+05	*1.22E+05*	1.01E+04	*3.70E+03*	5.48E+03	*2.30E+03*	0.00E+00	*0.00E+00*	Neg	Neg
MW264	7.64E+04	*1.59E+04*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	Neg	Neg
MW781B	5.74E+04	*1.37E+04*	1.48E+04	*6.04E+03*	9.89E+03	*4.76E+03*	5.48E+03	*1.97E+03*	Neg	Neg
MW04	1.11E+05	*2.04E+04*	1.87E+04	*2.50E+03*	1.78E+04	*5.65E+03*	0.00E+00	*0.00E+00*	Neg	Neg
MW781	8.65E+04	*2.54E+04*	1.59E+04	*6.40E+03*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	Neg	Neg
MWTB	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	0.00E+00	*0.00E+00*	Neg	Neg



Total Cells



(+) EAP

(-) EAP

Travis AFB (Cont)

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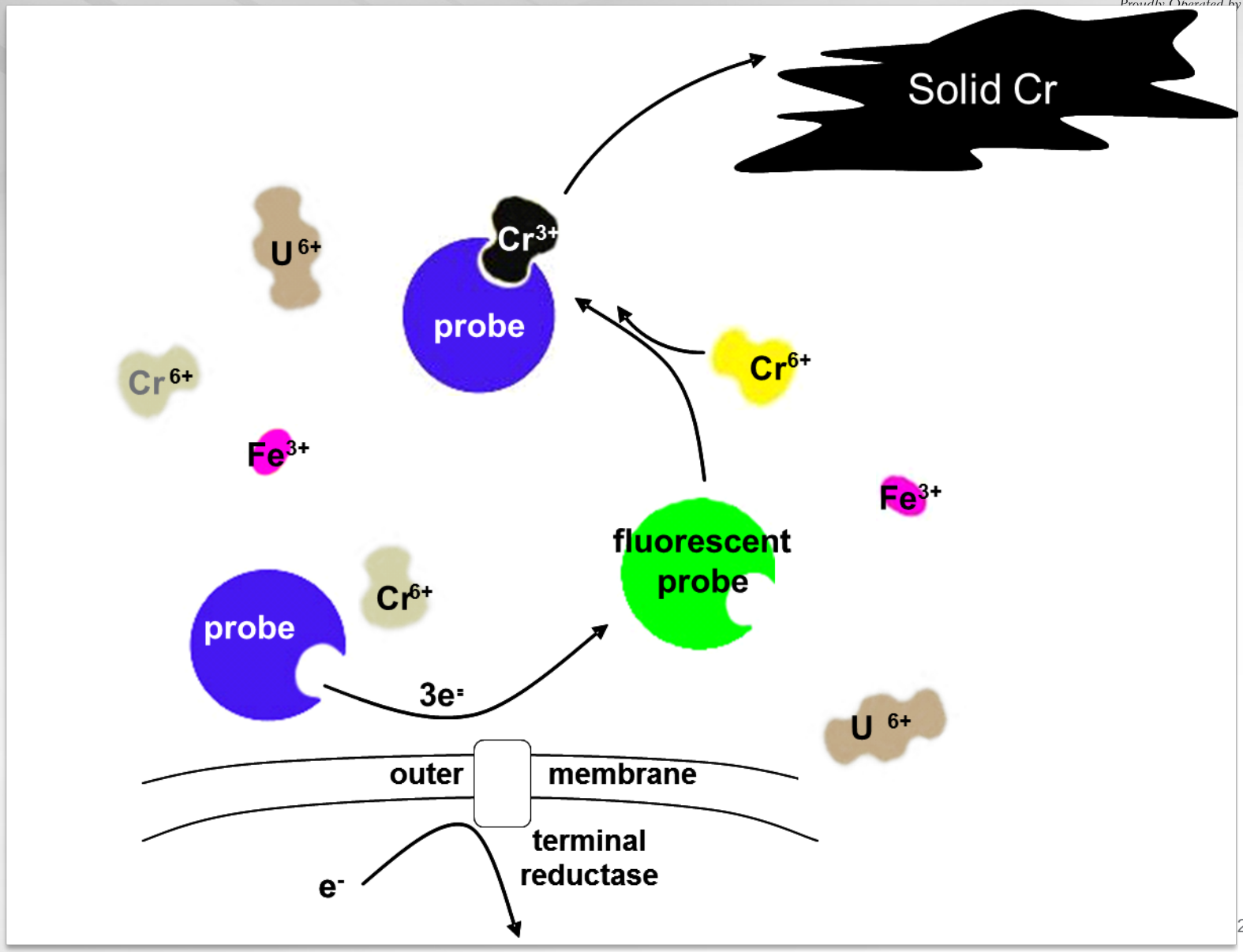
qPCR

	16S	PHE	RMO	TOD	23CAT	mmoX	pmoA	alkB
MW131	+	+	7.40E+2	1.70E+3	6.10E+3	ND	ND	ND
MW591	+	+++	4.02E+3	ND	3.75E+4	ND	ND	ND
MW266	+	+++	3.34E+3	ND	4.05E+5	ND	ND	ND
MW264	+	ND	ND	ND	ND	ND	ND	ND
MW781	+	++	8.44E+3	ND	6.35E+3	ND	ND	ND
MW04	+	+	5.84E+3	ND	4.36E+4	ND	ND	ND
MW781B	+	+++	8.44E+3	ND	1.94E+4	ND	ND	ND
MW-TB	-	ND	ND	ND	ND	ND	ND	ND
EC	-	ND	ND	ND	ND	ND	ND	ND

- **Application of molecular biological tools along with other site information was use by regulators in selection of monitored natural attenuation as the site remedy**

Second Generation Enzyme Activity Probes



Characterization of Fluidized Bed Reactor Microbial Community

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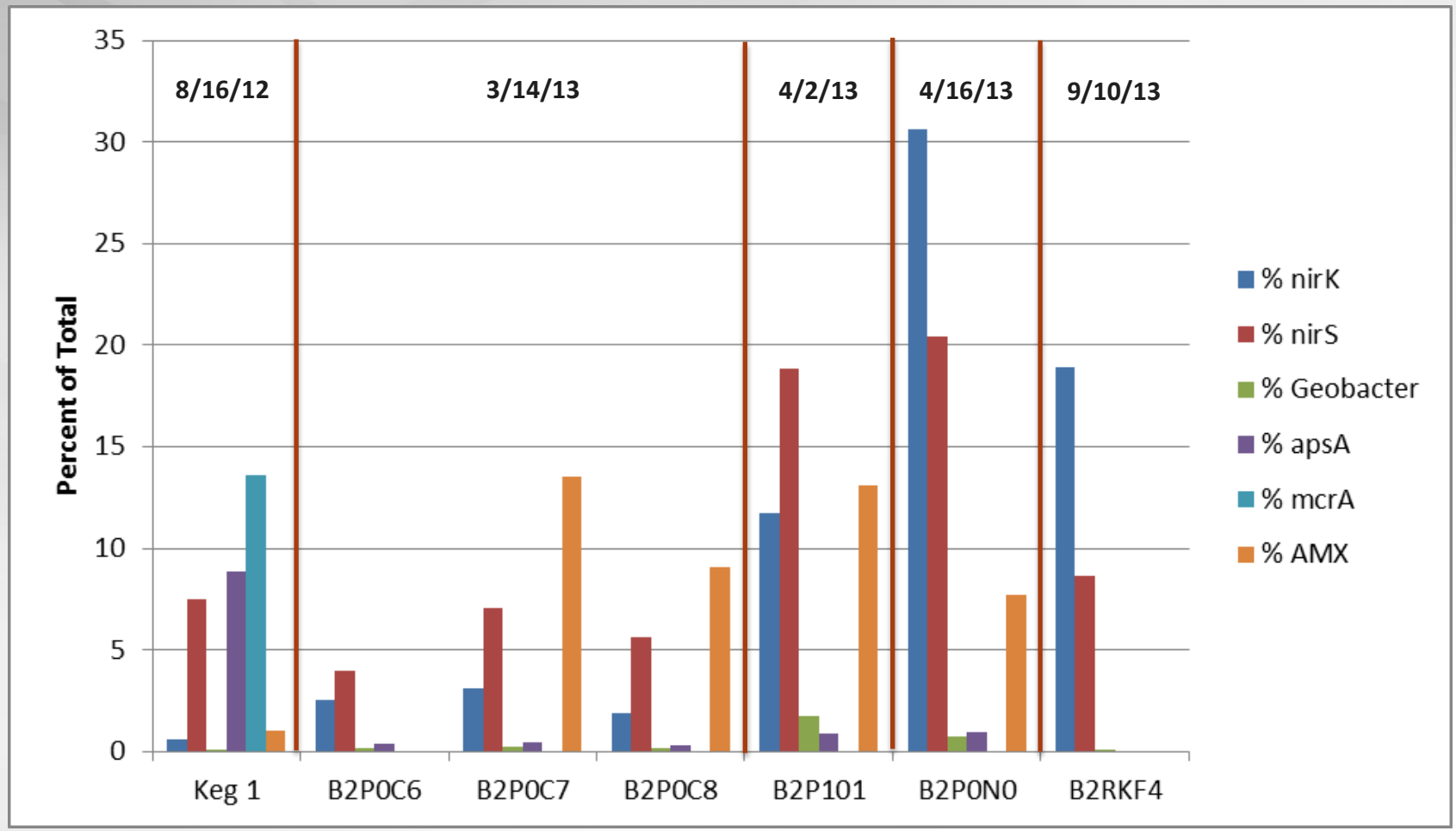
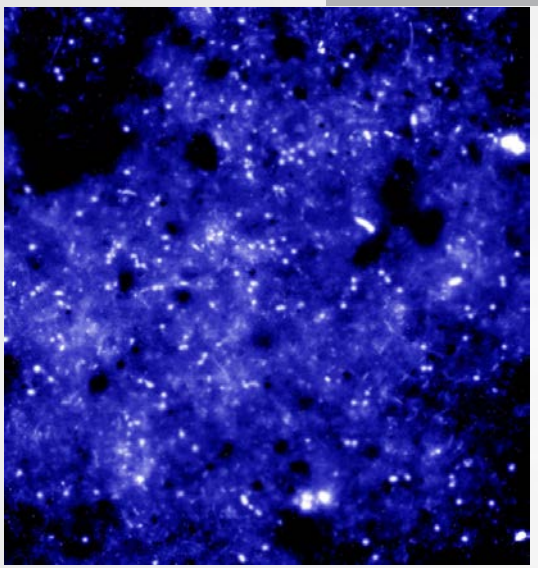
- Hanford 200 West Area Pump and Treat System
- Fluidized bed reactor using granular activated carbon support
  - Nitrate
  - Hexavalent chromate
  - Carbon tetrachloride
- Performance Issues
  - Build up of extracellular polymeric substances leading to support bulking
  - Less than optimal nitrate removal
- Application of molecular biological tools
  - Clone libraries
  - FISH for functional groups of bacteria
  - qPCR for functional genes

qPCR Results



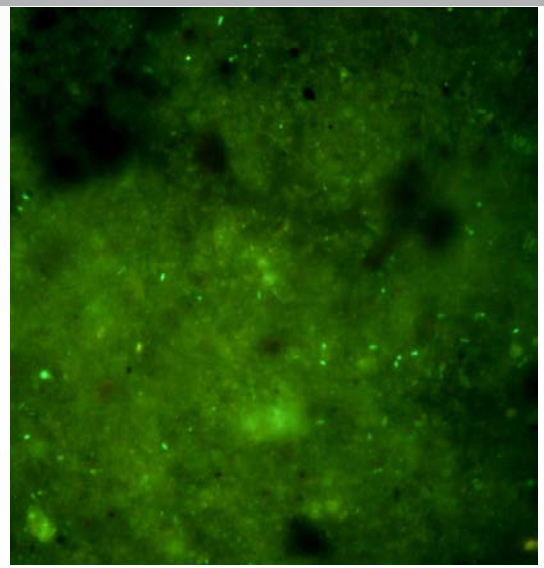
FISH Data

	B2P0C6	B2P0C7	BRP0C8	B2P1O1	B2P0N0	B2RKF4
Eubacteria	4.12E+09	3.41E+09	3.36E+09	3.38E+09	4.07E+09	6.01E+09
DAPI	7.16E+09	4.68E+09	4.41E+09	6.57E+09	5.84E+09	7.23E+09
Archaea	2.75E+07	2.06E+07	2.06E+07	2.75E+07	3.78E+07	2.75E+07
DAPI	9.08E+09	8.06E+09	6.24E+09	6.60E+09	6.06E+09	7.50E+09
Anammox	1.72E+07	6.87E+06	0.00E+00	6.87E+06	1.37E+07	6.87E+06
DAPI	8.13E+09	7.17E+09	6.56E+09	7.47E+09	6.52E+09	7.47E+09
SO4	2.75E+07	2.75E+07	3.43E+07	1.37E+08	7.21E+07	7.56E+07
DAPI	7.59E+09	5.70E+09	5.58E+09	5.36E+09	5.51E+09	6.36E+09
Fe	0.00E+00	0.00E+00	3.43E+06	0.00E+00	3.43E+06	0.00E+00
DAPI	6.70E+09	6.49E+09	4.77E+09	5.56E+09	4.87E+09	5.47E+09
CH4	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00	0.00E+00
DAPI	7.16E+09	4.68E+09	4.41E+09	6.57E+09	5.84E+09	7.23E+09



DAPI total cells

Methanogen



Dehalococcoides

Increasing Levels of Complexity

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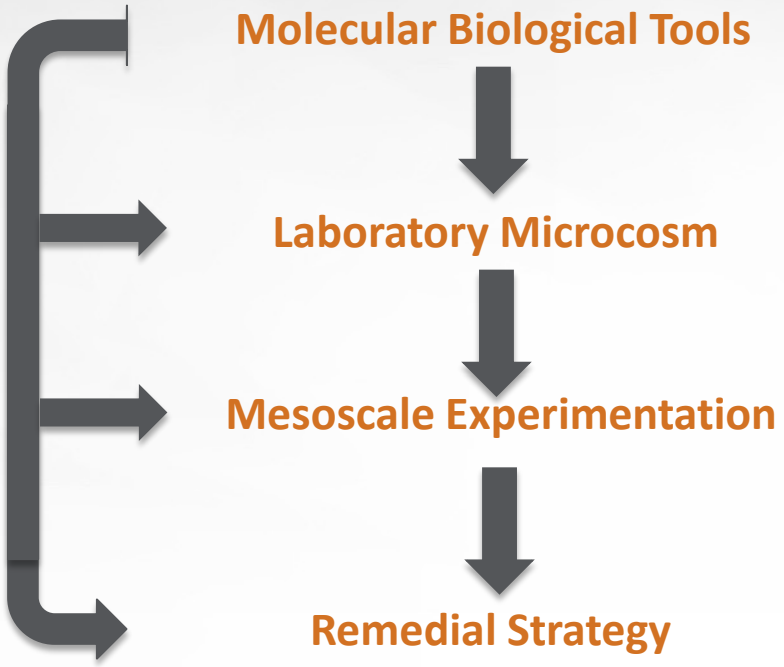
Complexity of Approach

Monitoring

Characterization
&
Predictive Understanding

Remedial Decision



System-Based Framework for Remediation Endpoints

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- An endpoint is:
  - risk-informed remediation goal or scenario permitted by regulations
  - protective of human health and the environment
  - scientifically and technically defensible
  - based on systematic, objective understanding of the contamination issue and a holistic remediation approach.

An endpoint framework enables establishing a path for cleanup that may include intermediate remedial milestones and transition points and/or regulatory alternatives to standards-based remediation.

All approaches for reaching an endpoint REMAIN protective of human health and the environment and meet regulatory requirements

Endpoints

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Are Not…	***Are…***
Walk-away approaches	**Active long-term management approach** that includes regular review of site conceptual model (SCM) to address residual contamination and employ new technologies and approaches as they become available
Quick or easy fixes	Based on robust **technically defensible** SCMs that support future predictions and risk-informed decisions
Un-Protective of human health and environment	Evaluated within the context of resource use, considering all aspects of present and future risk
Rigid and inflexible	Iterative, adaptive approach that provides transition of sites from 1) active to passive remediation or 2) intensive characterization and monitoring into efficient and cost effective LTM

Applicability of Endpoint Framework

- At all sites but especially complex ones with technical limitations to groundwater, surface water and soil/sediment restoration
- Extensive, recalcitrant, or long-lived contamination
  - Presence of non-aqueous phase liquid (NAPL), relatively immobile contaminants, metals and radionuclides
- Complex hydrogeological setting
  - Highly heterogeneous, low permeability geology, any environment difficult to characterize
- Other site specific circumstances

Endpoints Summary

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- Endpoint determination based on quantifying the “system” and estimating potential future risk from contaminants
  - Risk-informed understanding and defensibility of remedy decisions and means to verify performance
  - Incorporates mass flux/discharge concepts
  - Protective of human health and the environment
- Working to define technical gaps for implementation of the framework
- Regulatory and stakeholder engagement is critical
- Leveraging EPA and DoD efforts

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  - Pacific Northwest National Laboratory
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Questions

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