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A DOE Retrospective for cVOC Bioremediation

Thirty (+) years of challenges, opportunities, and lessons learned

Brian Looney, Deborah Newby, Holly VerMeulen, Emily Fabricatore

Prepared in collaboration with

DOE Office of Environmental Management Office of Subsurface Closure

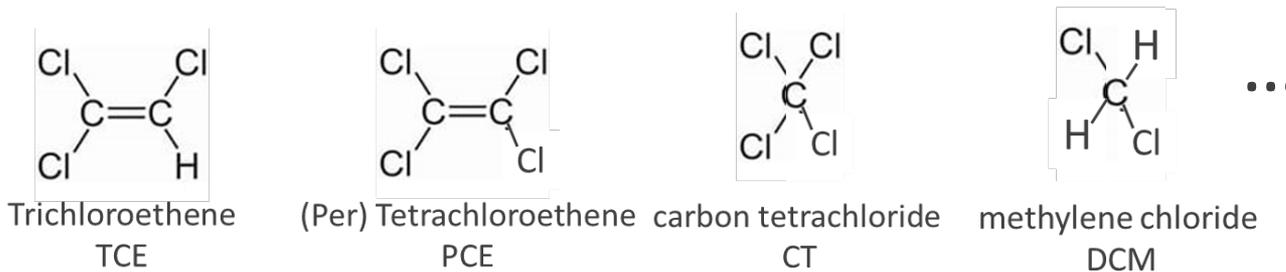
November 17, 2025

DOE EM Bioremediation Retrospective Background

- DOE has performed numerous bioremediation projects over three(+) decades
- Success of these projects has varied depending on contaminant, hydrogeologic conditions, available technologies, and evolving state of the science
- DOE's Environmental Management (EM) Office of Subsurface Closure (EM 4.12) was tasked with conducting a historical retrospective on cVOC bioremediation across the complex
- Technical support provided by Savannah River National Laboratory



cVOCs at DOE Sites



- **Industrial Sources –**

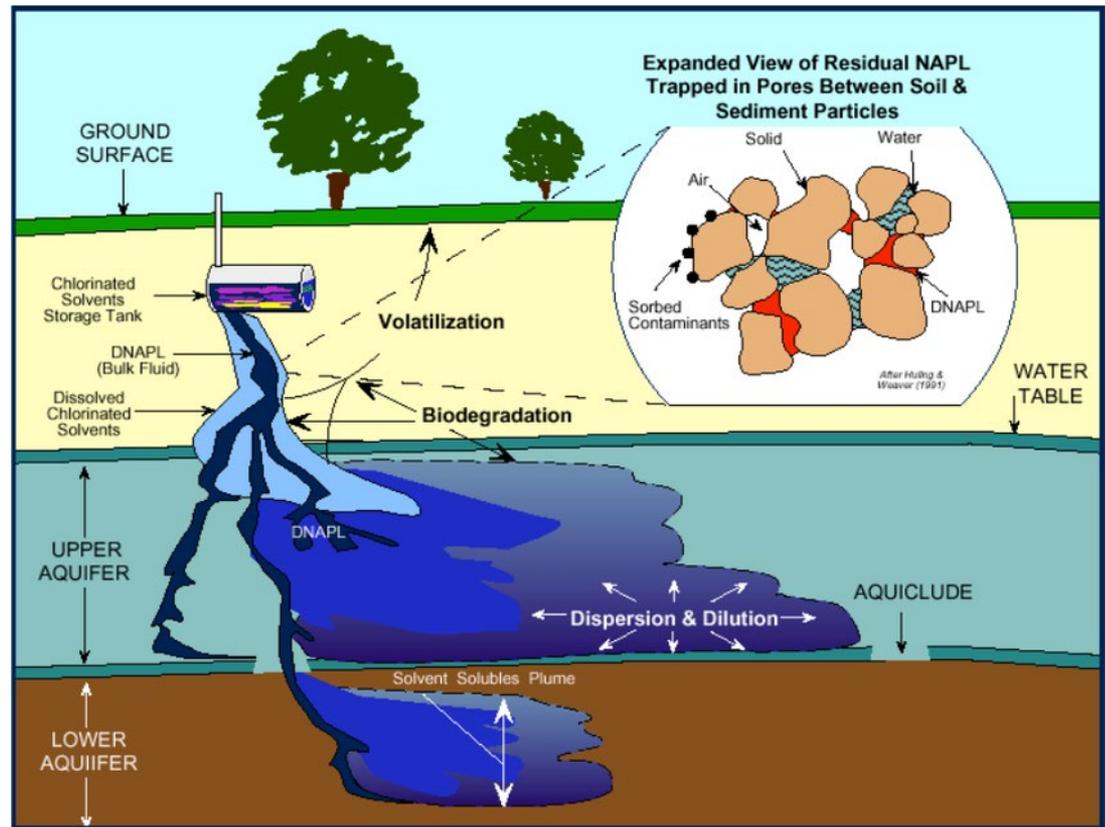
- **Degreasing agents** – trichloroethylene (TCE), tetrachloroethylene (PCE), carbon tetrachloride (CT) and methylene chloride (DCM) were some of the commonly used organic solvents used in DOE
- Solvents used in fabrication/manufacturing, maintenance, and service facilities
- Other common sources – laboratory chemicals, paint thinners, herbicides, pesticides, resins, glues and other mixing and thinning liquid

- **Releases to the Environment –**

- Leaks and spills – releases from tanks, pipes and facilities and nearfield disposal/releases
- Seepage Basins, Outfalls, Landfills and burial pits
- Disposal with polychlorinated biphenyls (PCBs) at some sites
- Radioactive/mixed waste disposal at some sites

Plume Dynamics

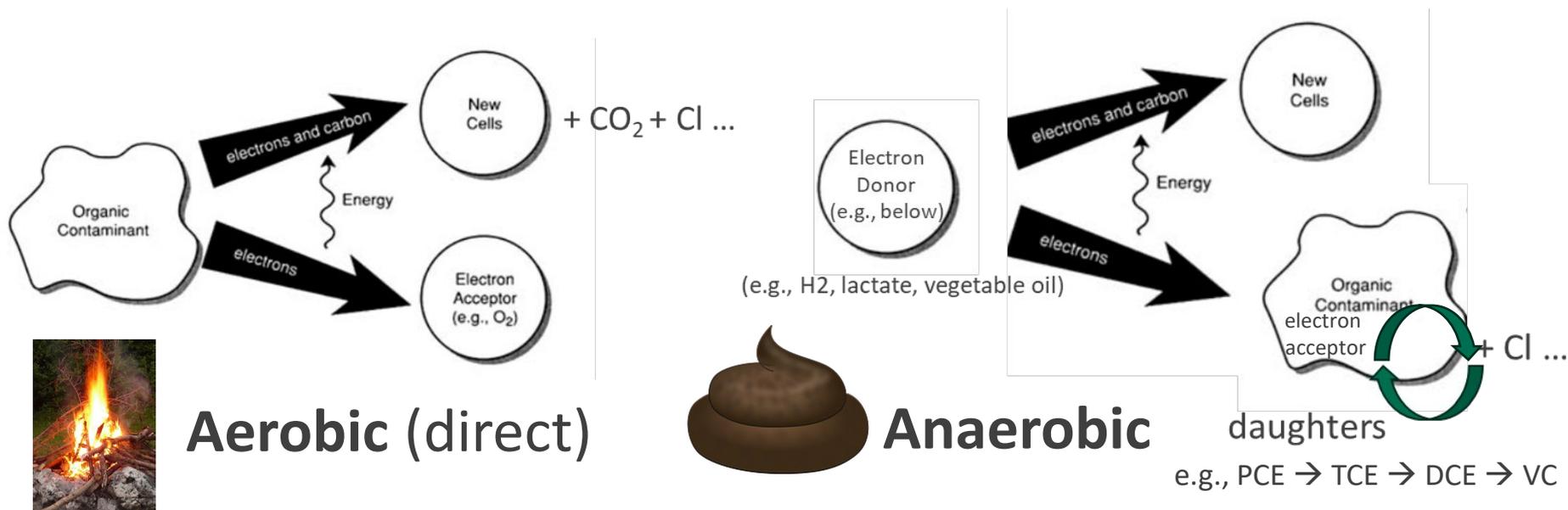
- Chlorinated solvents are heavier than water and sparingly soluble
- Initially present as Dense Non-Aqueous Phase Liquids (DNAPLs)
- Hydrogeology is a key factor in determining fate and transport
- Baseline treatment typically requires pumping water and treating at surface (pump & treat)



Schematic of chlorinated solvent pollution as dense non-aqueous phase liquids migrating downward in an aquifer and serving as a source for a solvent soluble plume. Also shown are natural attenuation processes (U.S. EPA 1999).

Bioremediation Basics

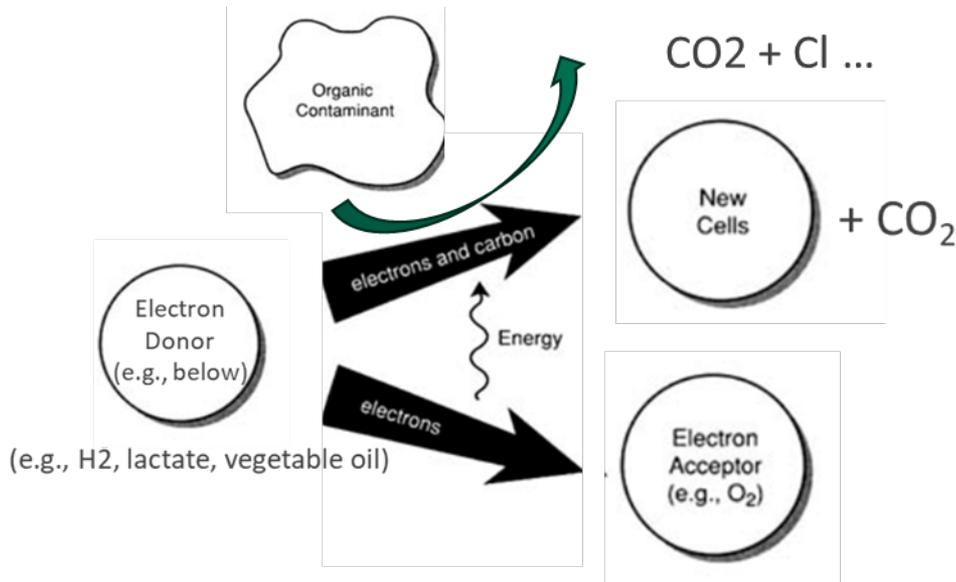
- Aerobic microbes – require oxygen (O_2) for metabolism
- Facultative microbes – grow in presence or absence of O_2
- Anaerobic microbe – grow in absence of oxygen
- Bioremediation relies on microorganisms to break down contaminants as they transfer electrons to provide energy for growth and survival – two major categories (Aerobic and Anaerobic) plus Aerobic Cometabolism



Bioremediation Basics (cont.)

- **Aerobic Cometabolism**

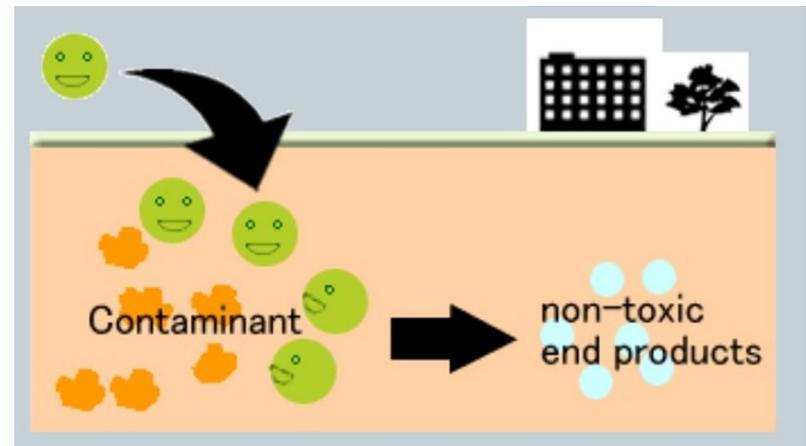
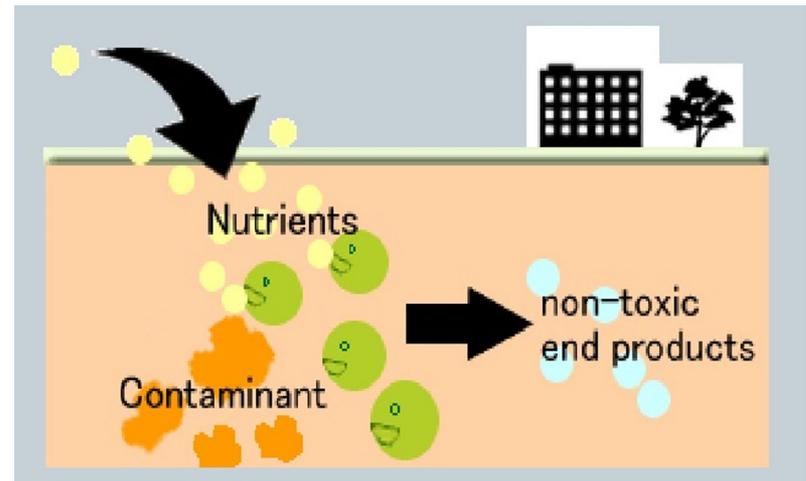
- Enzyme produced for breakdown of growth substrate also degrades another compound that the organism does not use for energy or growth
- Relies on the non-specificity of certain types of enzymes (enzymes that break down methane, toluene, phenol and other recalcitrant compounds)



hamburger and wrapper

Bioremediation Basics (cont.)

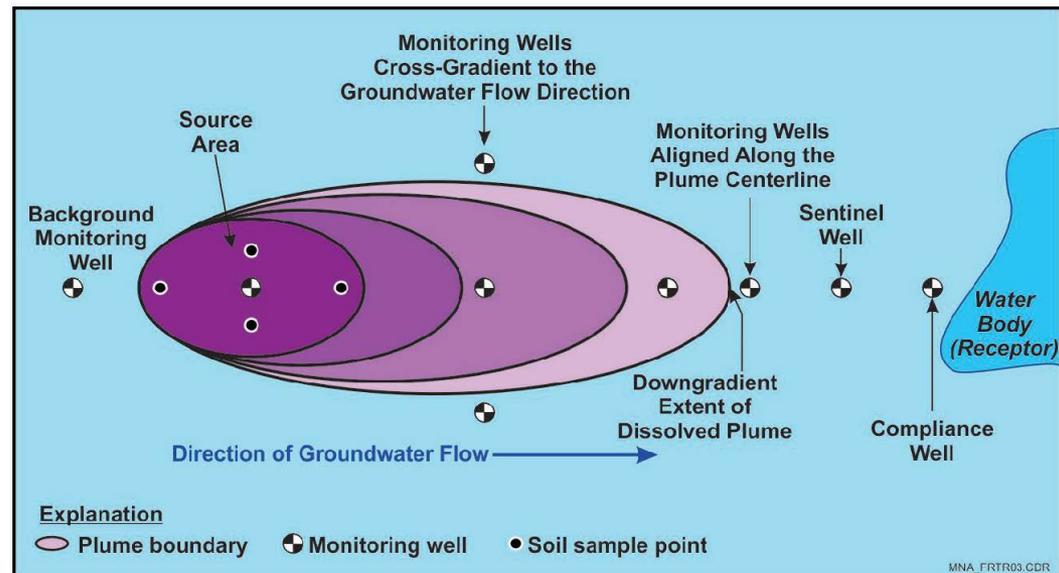
- Biostimulation – addition of nutrients to accelerate degradation
- Bioaugmentation – addition of microbes to accelerate degradation



[PPT - Bioremediation PowerPoint Presentation, free download - ID:1130330](#)

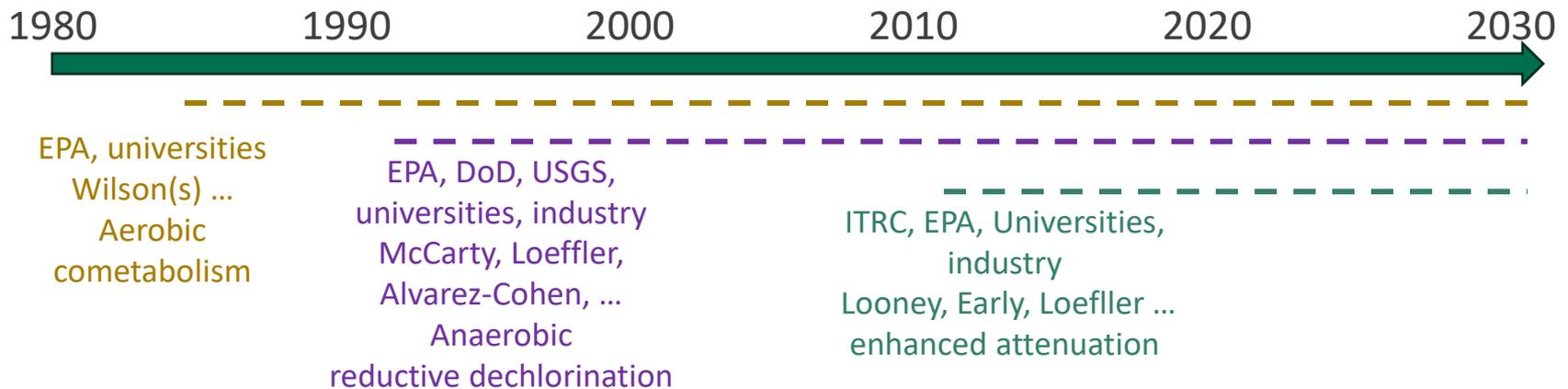
Bioremediation Basics (cont.)

- Active Bioremediation for cVOCs –
 - Uses biostimulation or/and bioaugmentation to support microbial processes to destroy cVOCs
 - Reduce risk and accelerate progress toward remedial goals
- Enhanced Attenuation –
 - Uses biostimulation or/and bioaugmentation to microbial process to destroy cVOCs sustainably alter and beneficially alter the plume mass balance
 - Goal: remedial objectives are met in a reasonable timeframe without further intervention
- Monitored Natural Attenuation –
 - Range of naturally-occurring physical, chemical, and/or biological processes that reduce groundwater contaminant concentrations over time without human intervention!



A Bit of History and Acknowledgement

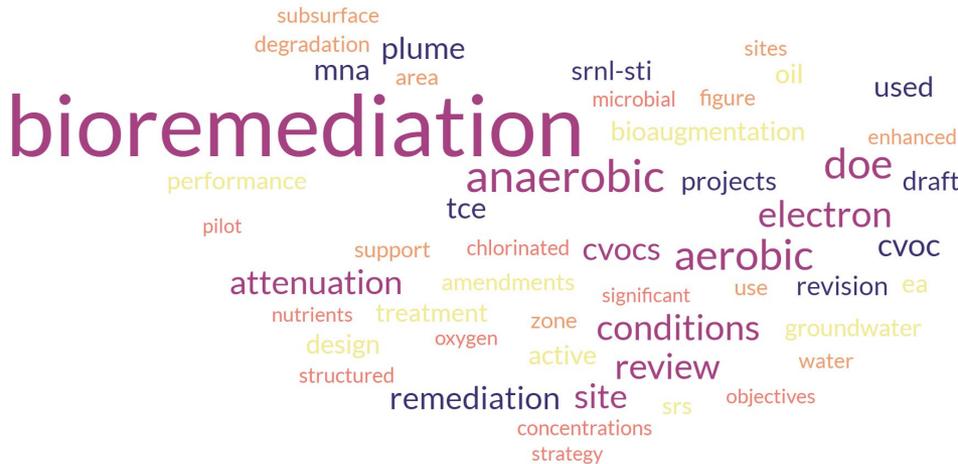
- DOE cVOC bioremediation experience extends from mid 1980s until present
- US Environmental Protection Agency (EPA) and US Department of Defense (DoD - e.g., SERDP and ESTCP) provided leadership in understanding and developing bioremediation science for cVOCs
- Several milestones and eras in the development of bioremediation:



- This DOE retrospective acknowledges and builds on efforts of EPA, DoD and others. Conclusions were focused on topics where DOE experiences provided supplemental, useful, or interesting/actionable information.

DOE EM Bioremediation Retrospective - Approach

- Performed as a “Systematic Review”
 - “A structured formal process examining the performance and outcomes of multiple separate projects/sites and then consolidating the information to provide actionable insights”
- Steps in review process:
 - Developed categories and standardized bins
 - Interviewed subject matter experts from Savannah River Site, Hanford, Idaho, Mound, and Pinellas
 - Consolidated information
 - Identified trends, relationships, and lessons learned



Bioremediation of Chlorinated Volatile Organic Compounds: DOE Experiences and Lessons Learned

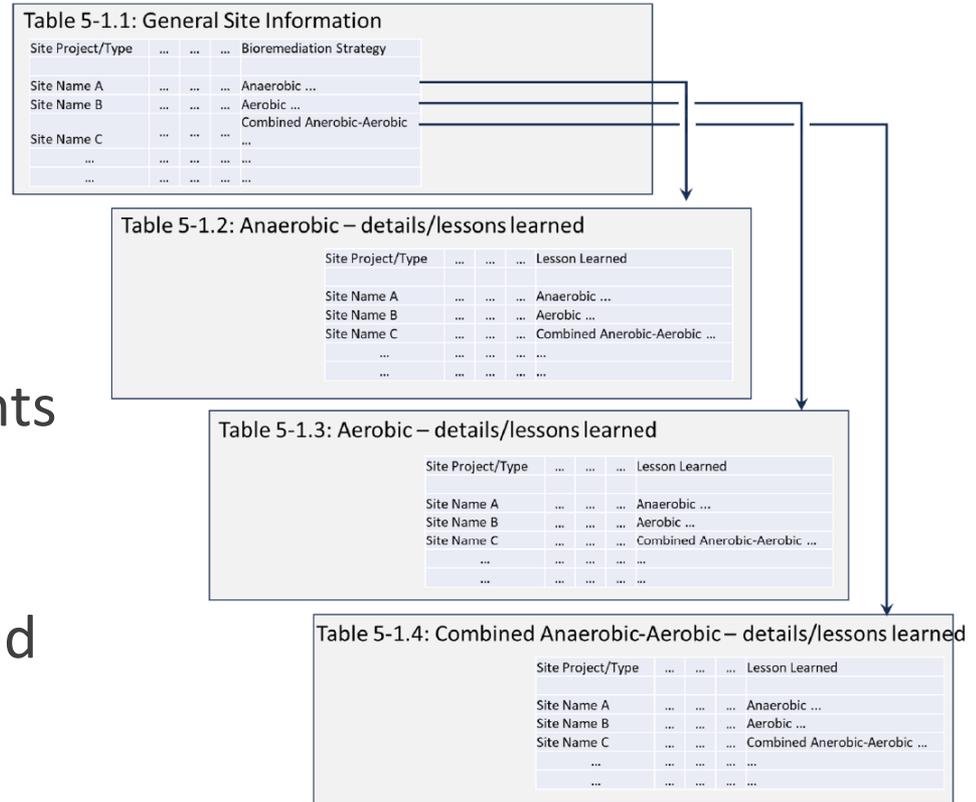
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Emily D. Fabricatore

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Some of the Factors Evaluated

- Type of contaminant: cVOCs
- Examine various parameters:
 - Design principles
 - Bioaugmentation
 - Electron donors and nutrients
 - Surface access and deployment
 - Monitoring performance and metrics



Schematic Depiction of Summary Table Structure

EM Bioremediation Retrospective – Key Findings

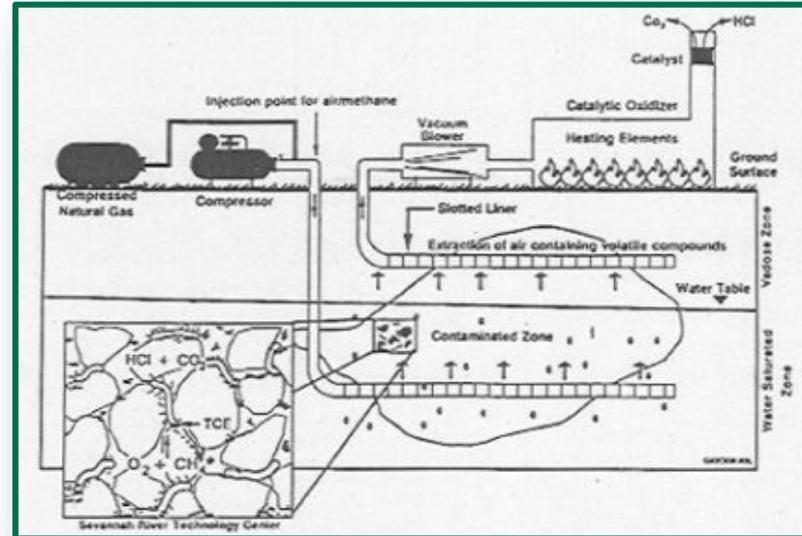
- Underperformance of bioremediation often resulted from poor access and delivery of amendments or from unfavorable biogeochemical conditions
- Implementation of the Enhanced Attenuation (EA) paradigm has been a game changer for cVOC bioremediation success
- Over time, there has been a trend toward longer lasting amendments
- The most successful projects have used a structured geochemical zone design that beneficially combines anaerobic and aerobic processes
- Use of “quantitative” microbial measurements (such as qPCR and arrays) to understand subsurface microbial ecology has proven to be cost effective and valuable for documenting the capabilities and evolution of the microbial community in response to bioremediation and changing conditions
- Most projects across the complex have performed well and provided value toward remedial objectives

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EM Bioremediation Retrospective – Improving Access and Delivery

- Underperformance of bioremediation often resulted from poor access and delivery of amendments
 - Bioremediation performance increased by ensuring adequate access and delivery of amendments to overcome physical limitations such as low permeability or inadequate well spacing
 - Close spacing and/or innovative access such as directional drilling (e.g., horizontal wells) proved beneficial at some sites

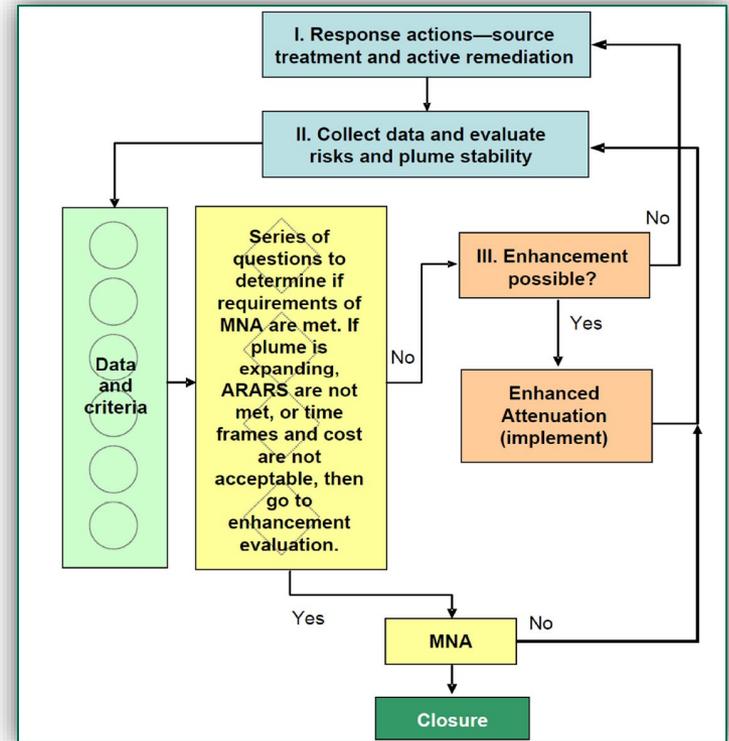
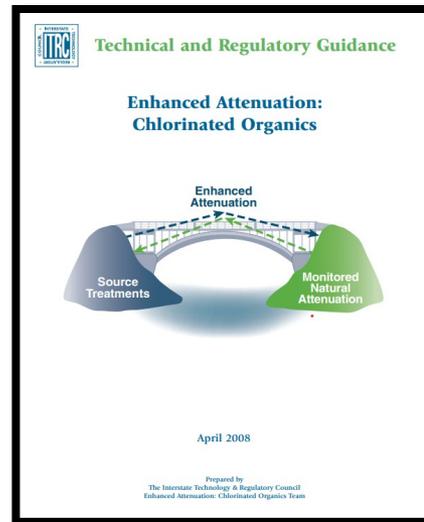


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EM Bioremediation Retrospective – Enhanced Attenuation (EA)

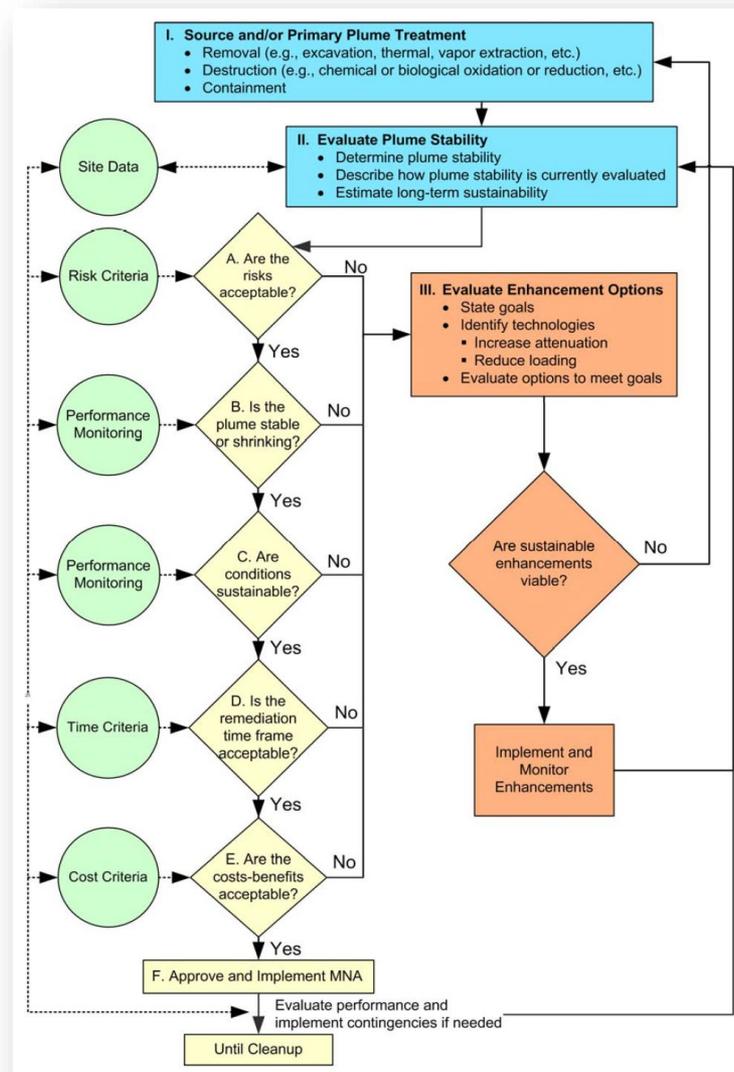
- EA is an engineered treatment that uses biostimulation or/and bioaugmentation to sustainably alter microbial process to destroy cVOCs and beneficially alter the plume mass balance such that remedial objectives are met in a reasonable timeframe without further intervention.
- DOE led national collaborative effort to implement EA for cVOCs – ITRC, state and federal regulators, other federal agencies, industry, and universities



Blue boxes – contaminated site initial characterization
Yellow boxes – viability of MNA
Orange boxes – EA

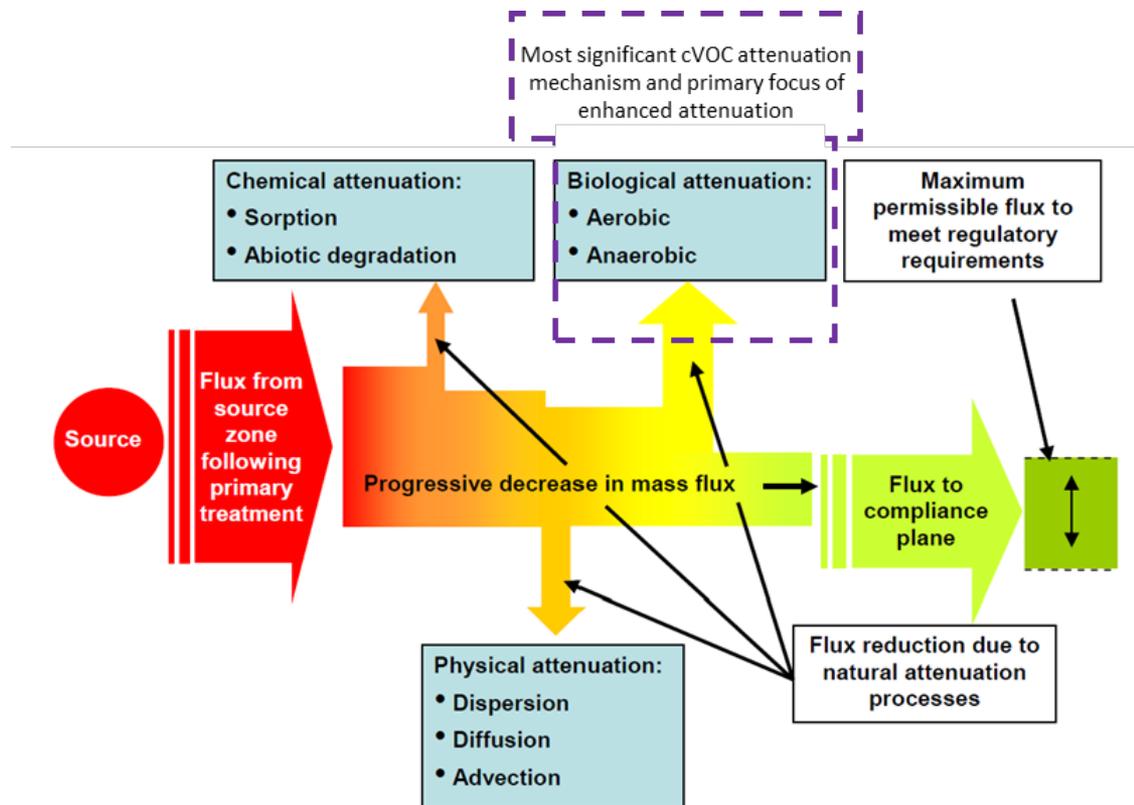
MNA/EA Decision Flowchart

- The ITRC Technical and Regulatory Guidance (and associated archived online training) provide a detailed description and discussion of the actions and basis for decision making throughout the flowchart



Plume Stability & Mass Balance – Basis of EA

- EA path – specific requirements to evaluate mass balance to optimize long-term plume stability/reduction and in selecting and designing EA treatment



Natural attenuation mass balance paradigm (ITRC, 2008)

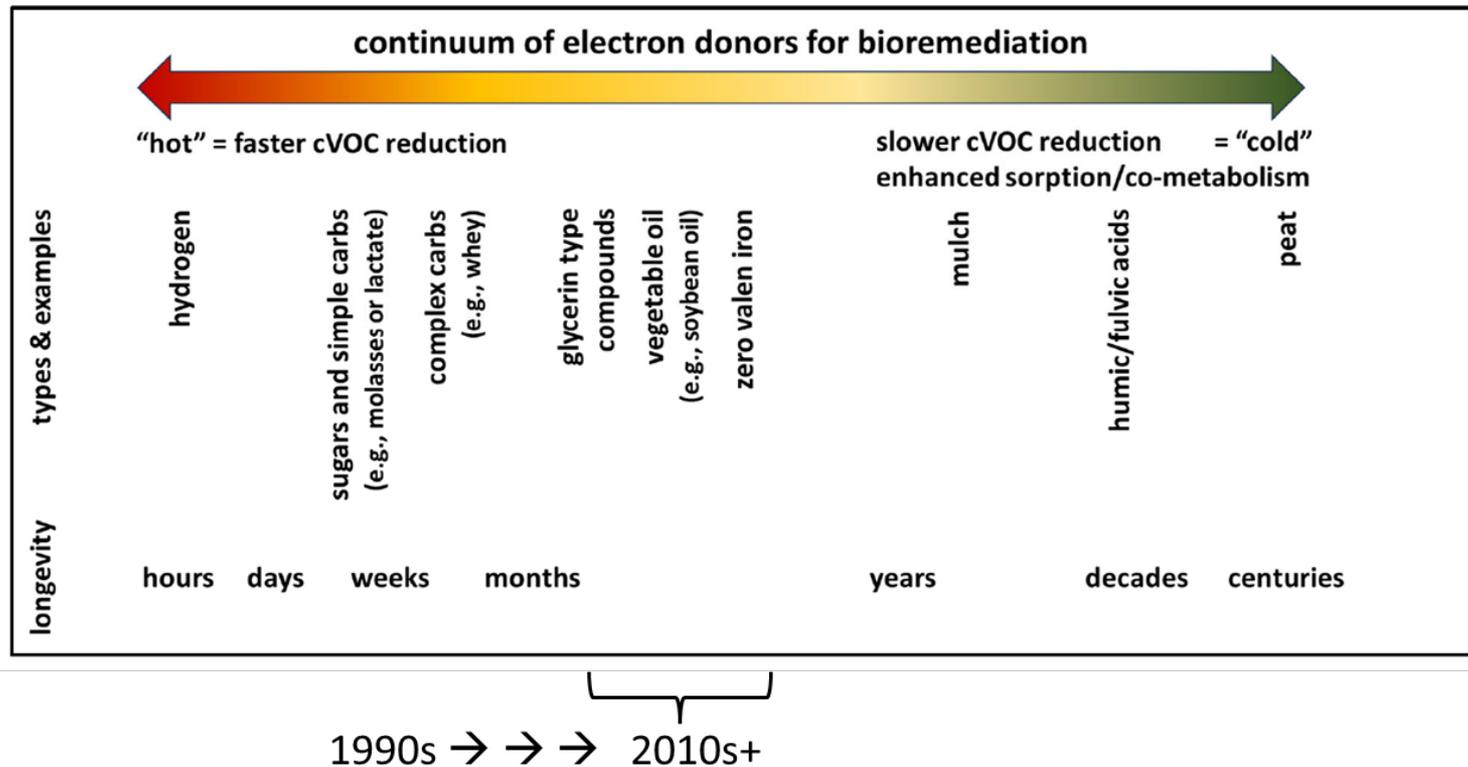
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EM Bioremediation Retrospective – Amendments

- Over time, there has been a trend toward longer lasting amendments
 - Bioremediation amendments have different properties
 - Improved outcomes with longer lasting amendments

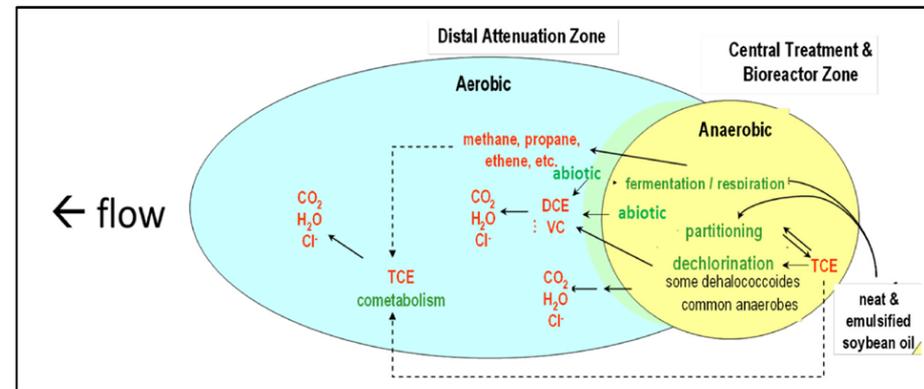
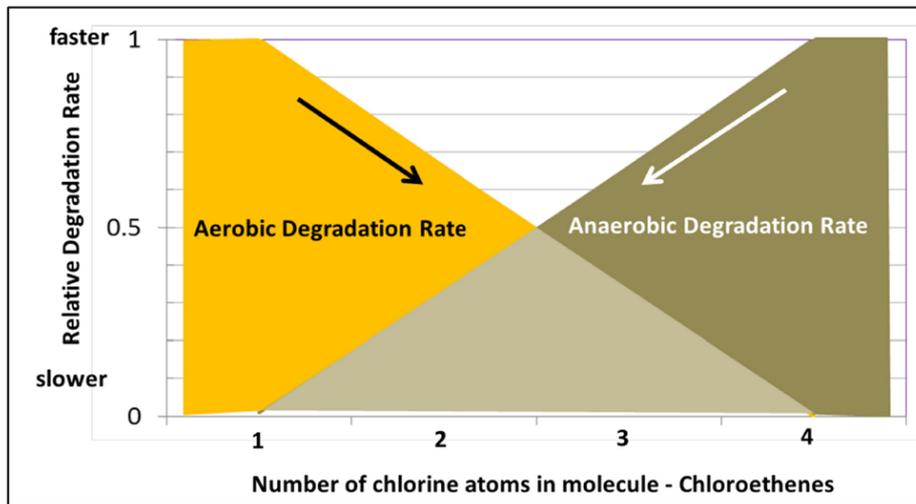


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EM Bioremediation Retrospective – Structured Geochemical Zones

- The most successful DOE cVOC bioremediation projects have used a structured geochemical zone design that beneficially combines anaerobic and aerobic processes
 - Adding specialized microorganisms is not needed at most sites



Left) Simplified depiction of the relative rates of chloroethene degradation under anaerobic and aerobic conditions. Right) Depiction of the relationship between anaerobic and aerobic treatment areas.



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Key Microorganisms and Enzymes

0*+ = confirmed contributor to attenuation of the listed contaminant; "-" = does not contribute to attenuation of listed contaminant (or no data available in the literature). The organisms and enzyme markers in the chart were detected in one or more samples during the monitoring period.

1 Dehalococcoides (DHC) is confirmed to reductively degrade most classes of cVOCs, including chlorinated ethenes, ethanes, methanes, phenols, benzenes and propanes.

2 Dehalobacter spp (DHBt) are confirmed to reductively degrade several classes of cVOCs, including chlorinated ethenes, ethanes, methanes, and benzenes. Dehalobacter DCM (DCM) is confirmed to reductively degrade chloroform.

3 Dehalogenimonas (DHG) is confirmed to reductively degrade chlorinated ethanes and propanes. Attenuation of chlorinated ethenes (e.g., PCE and TCE) has not been documented in the literature.

4 Desulfitobacterium spp. (DSB) are confirmed to reductively degrade several classes of cVOCs, including chlorinated ethenes, ethanes, phenols, and propanes.

5 Dehalobium chloroercoia (DECO) is confirmed to reductively degrade chlorinated benzenes.

6 Desulfuromonas spp. (DSM) are confirmed to reductively degrade PCE and TCE.

7 These enzymes that are associated with DHC spp and the reductive degradation of chlorinated ethanes (tceA Reductase (TCE)) or chlorinated ethene daughter products such as DCE and VC (BAVI Vinyl Chloride Reductase (BVC) and Vinyl Chloride Reductase (VCR))

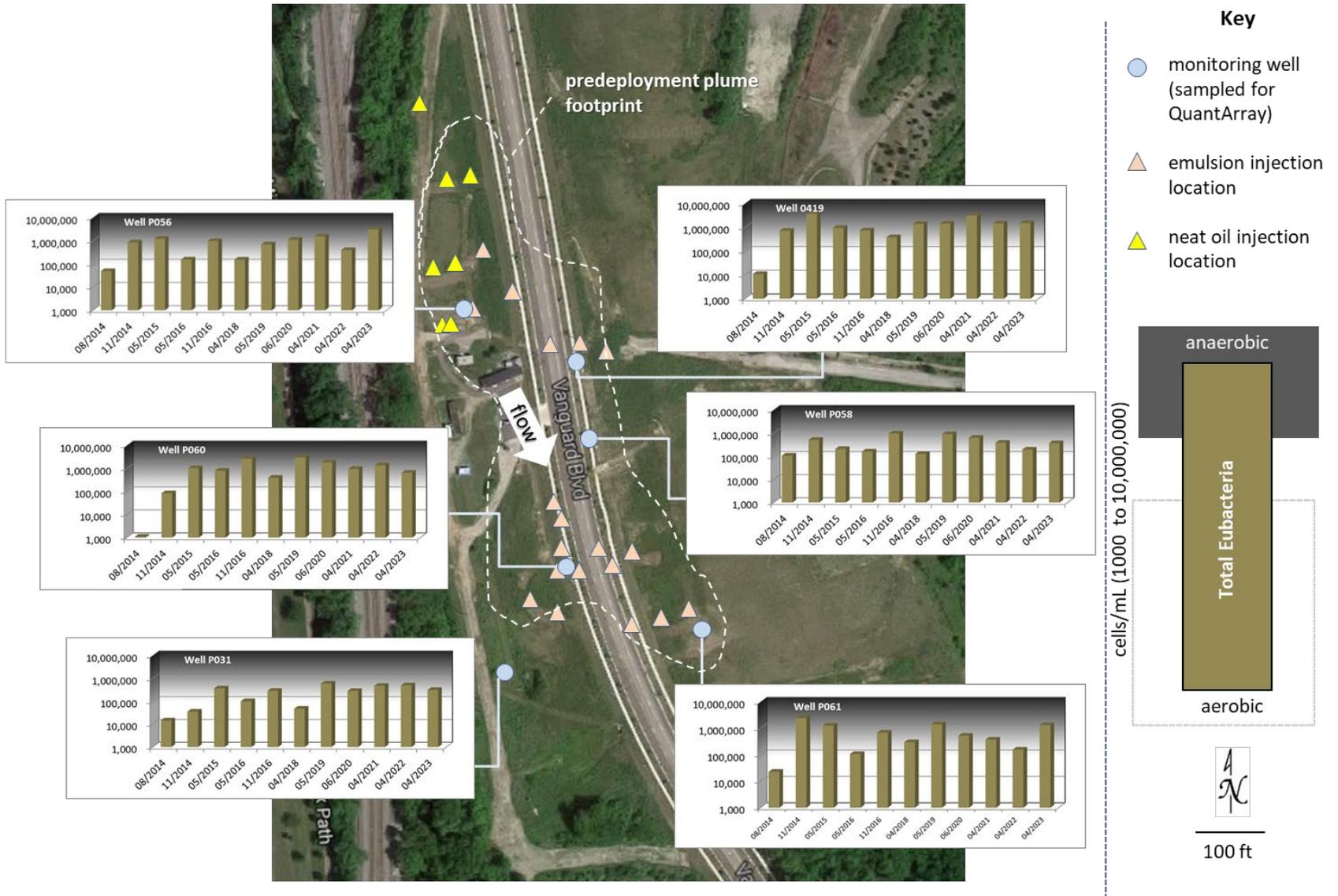
8 Chloroform reductase (CFR) is an enzyme associated with reductive degradation of chloroform. Two enzyme sequences (1,1 DCA Reductase (DCA) and 1,2 DCE reductase (DCAR)) are not listed in the table because they were not detected in the groundwater at Mound OU1.

9 These enzymes are associated with the cometabolism or metabolism of a wide range of cVOCs.

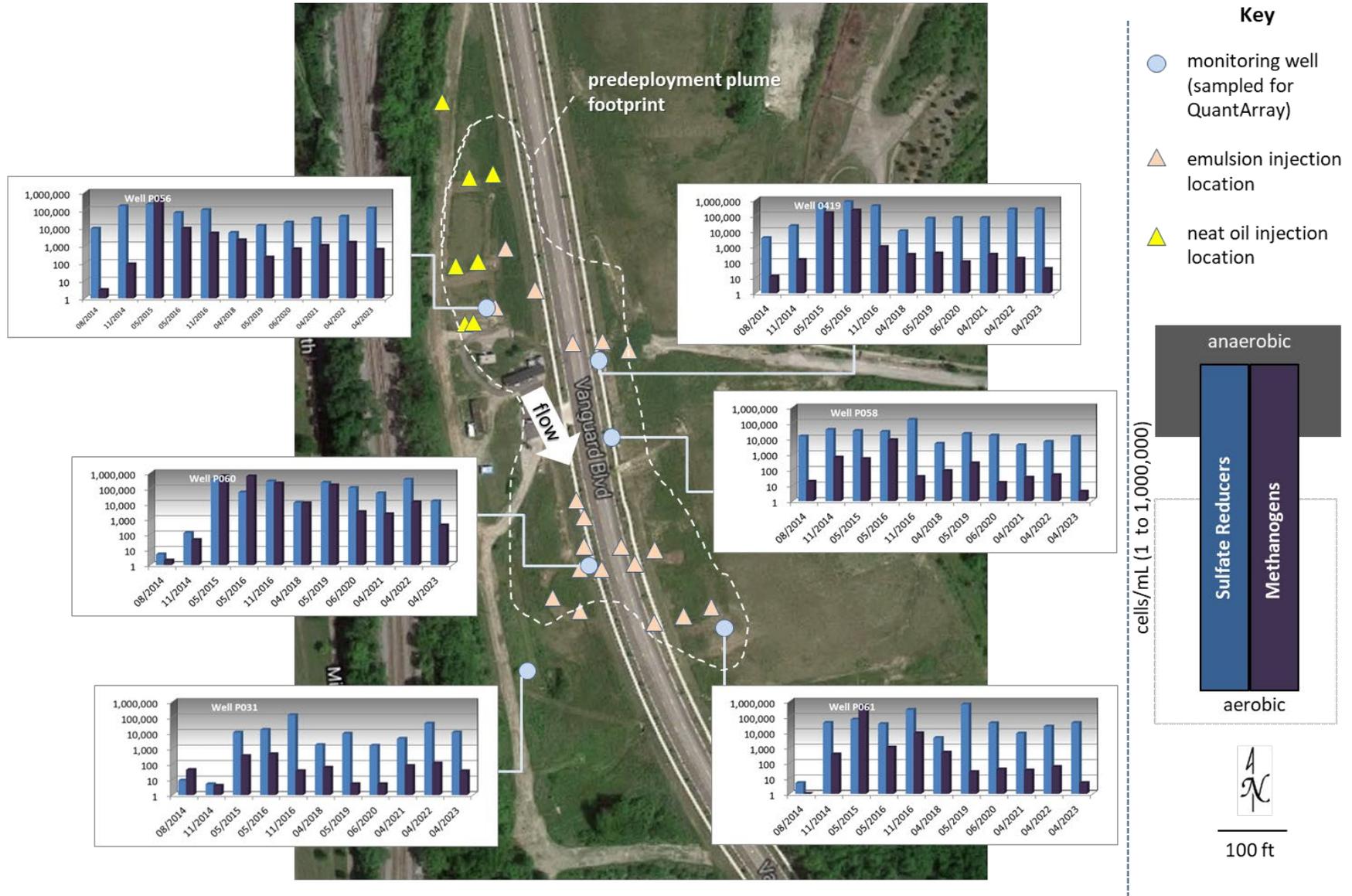
	Reductive Dechlorination and related Attenuation Processes											Aerobic (co)Metabolism								
	organisms						enzymes					Enzymes								
	Dehalococcoides spp. (DHC)	Dehalobacter spp. (DHBt)	Dehalobacter DCM (DCM)	Dehalogenimonas spp. (DHG)	Desulfitobacterium spp. (DSB)	Dehalobium chloroercoia (DECO)	Desulfuromonas spp. (DSM)	tceA Reductase (TCE)	BAVI Vinyl Chloride Reductase (BVC)	Vinyl Chloride Reductase (VCR)	Chloroform reductase (CFR)	Soluble Methane Monooxygenase (SMMO)	Particulate Methane Monooxygenase (PMMO)	Toluene Dioxygenase (TOD)	Phenol Hydroxylase (PHE)	Trichlorobenzene Dioxygenase (TCBO)	Toluene Monooxygenase 2 (RDEG)	Toluene Monooxygenase (RMC)	Ethene Monooxygenase (EtnC)	Epoxyalkane transferase (EtnE)
Chlorinated Ethenes																				
PCE	+	+	-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	+	-	-
TCE	+	+	-	-	+	-	+	-	-	-	+	+	+	+	+	+	+	+	-	-
cDCE	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	-	-
VC	+	-	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+
other cVOCs / Notes	1	2	2	3	4	5	6	7	7	7	8	9	9	9	9	9	9	9	9	9

- Microorganisms adapt to site specific and changing conditions
 - may not need bioaugmentation
 - can document development of organisms to support attenuation
 - can demonstrate that site adapts to changing needs/conditions

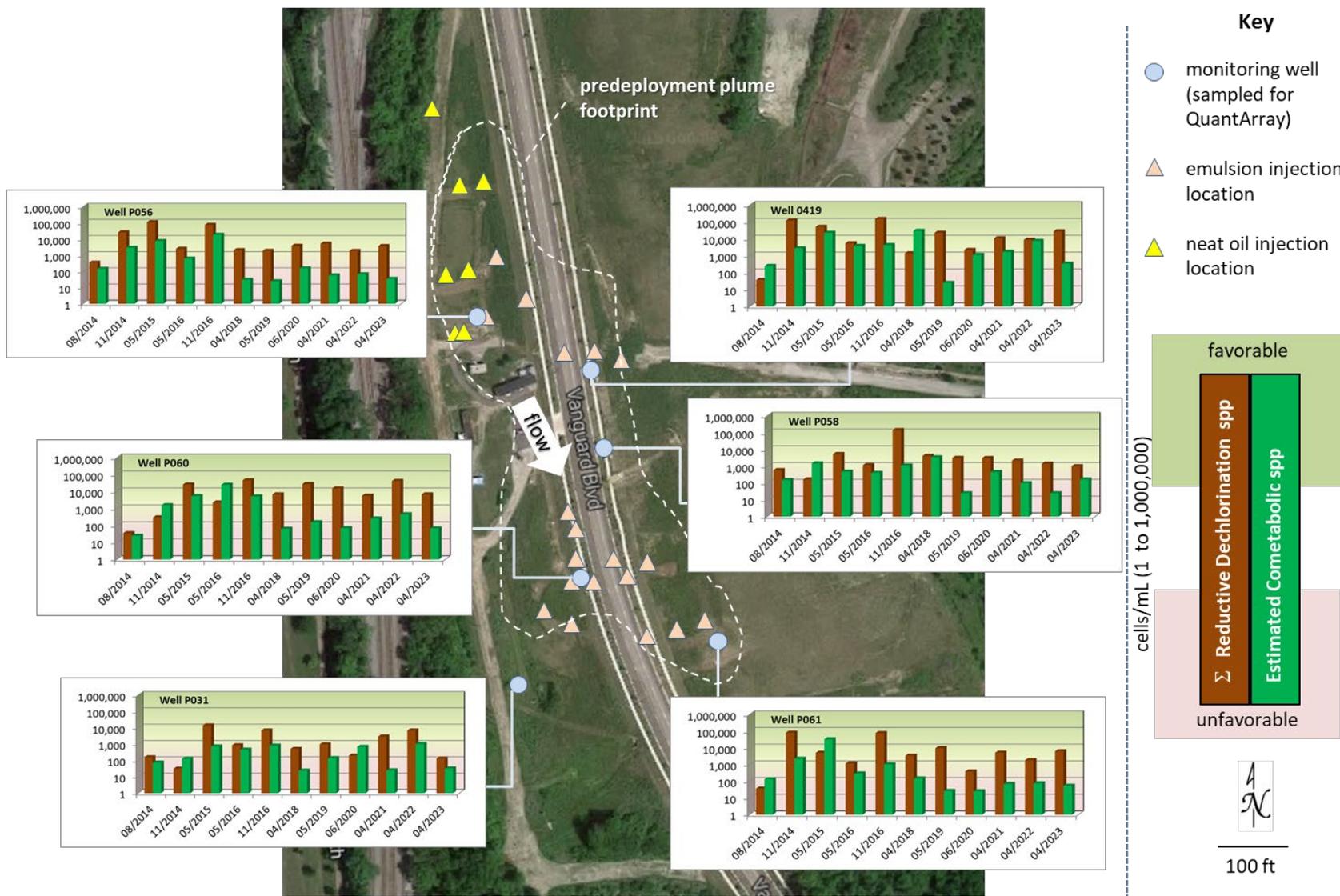
Example – Total Eubacteria Over Time at Mound OU1



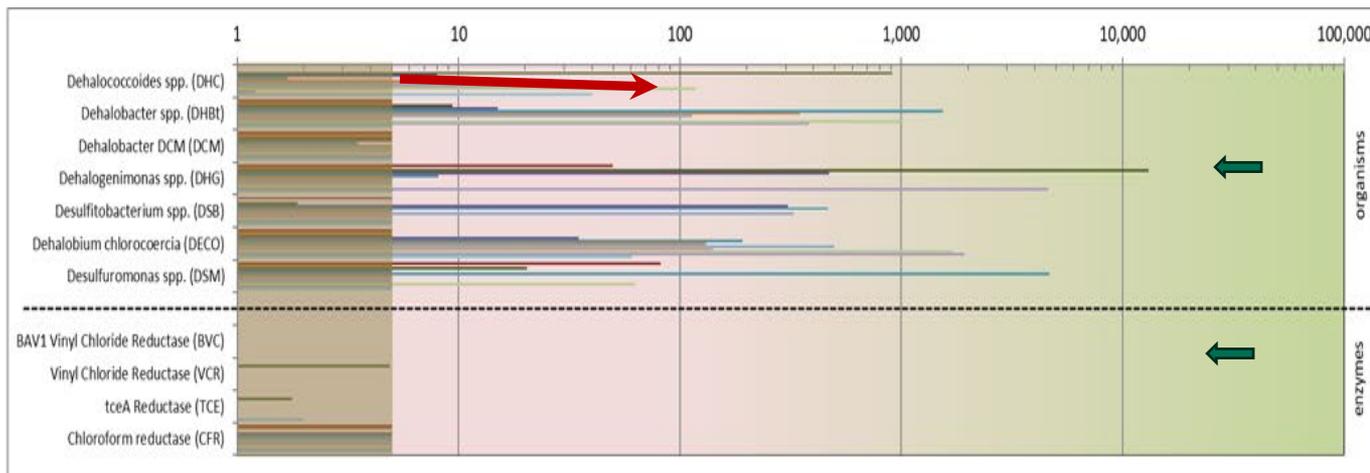
Example – Anaerobic Indicators Over Time at Mound OU1



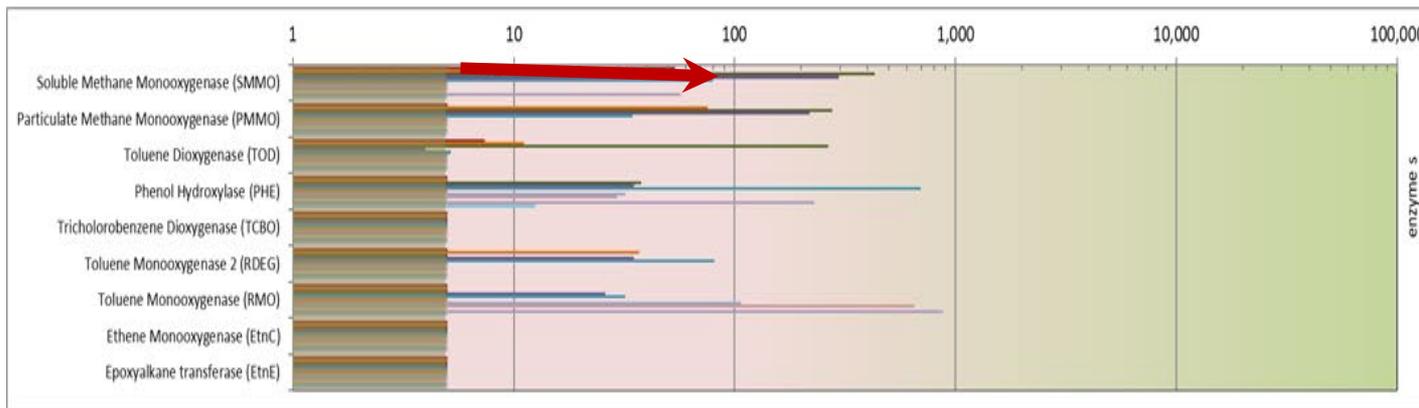
Example – Reductive and Cometabolic Bacteria Over Time at Mound OU1



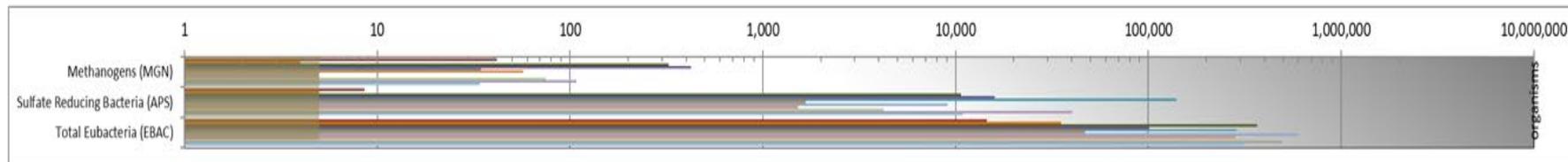
Example – Detailed Profile Over Time at Mound OU1



Anaerobic



Aerobic



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- ➔ • **Most projects have performed well and provided value toward remedial objectives**



Select Case Studies

- SRS cVOC Integrated Demonstration
- Mound OU1
- Pinellas
- Test Area North – INL
- SRS C-Area



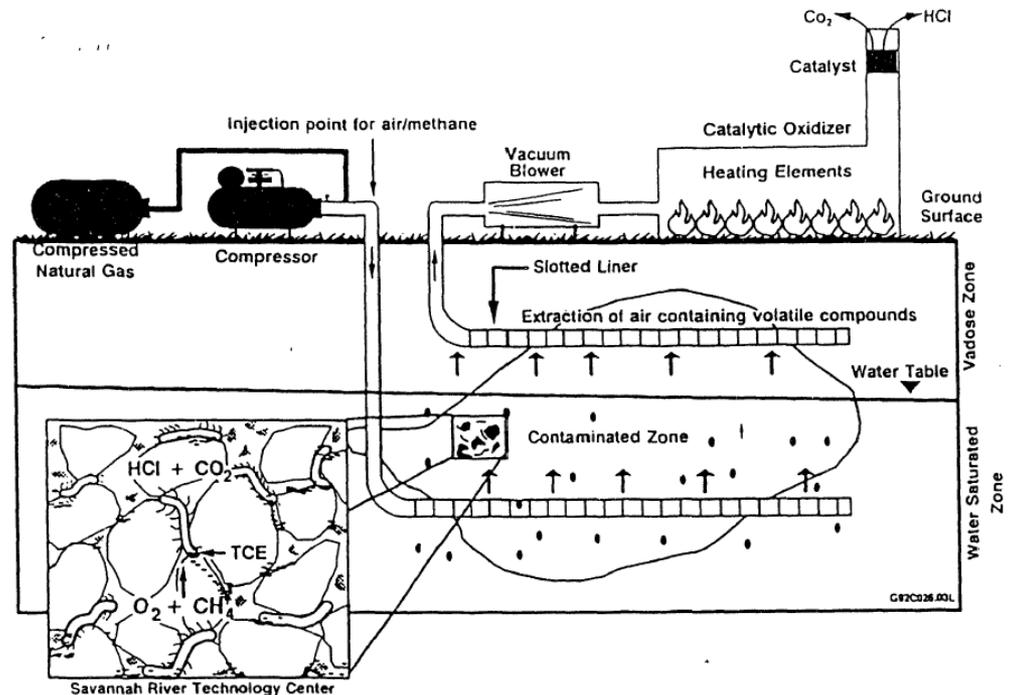
Case Studies – SRS cVOC Integrated Demonstration



Contaminant(s)	PCE and TCE
Plume Size	~ > 6 sq km (full scale pilot targeting residual sources in vadose zone and shallow groundwater)
Regulatory Drivers	<ul style="list-style-type: none">• Demonstrate technology to reduce residual sources and reduce future source mass flux• Support RCRA groundwater corrective action
Approach	Biosparging (cometabolism) below water table combined with SVE above water table using horizontal wells

Biosparging - Active Cometabolic Aerobic Bioremediation

- SRS integrated demonstration focused on synergistic technologies for improving characterization, access, monitoring and remediation of cVOCs (DNAPL sources and groundwater plumes)
- Primary bioremediation technology
 - Large-scale field pilot study in 1988-2002
 - Aerobic Cometabolism deployed using horizontal wells and air sparging
 - Cometabolite was methane and sparge process included macronutrients (P and N)



Results and Lessons Learned

- Bacteria capable of degrading TCE/PCE were stimulated
- Accelerated biodegradation occurred in situ without production of toxic daughter products
- Simple low-cost process -- gaseous nutrient injection was a viable delivery technique for in situ bioremediation in sandy aquifer sediments
- Combining cometabolism with physical removal (“in situ air stripping”) results in reduced costs and improved efficiency versus conventional baseline technologies

Based on the demonstration:

- Cometabolic biosparging was deployed at the SRS sanitary landfill (success) and piloted at Pinellas (underperformance)
- The gaseous nutrient injection method for phosphorus was patented, licensed, and used by industry
- The results supported development of ITRC EA technical guidance for cVOCs
- The results supported future development of combined anaerobic/aerobic remediation (“structured geochemical zone”) remediation strategies with successful deployment of this type of enhanced attenuation at DOE Sites (SRS TNX and Mound)

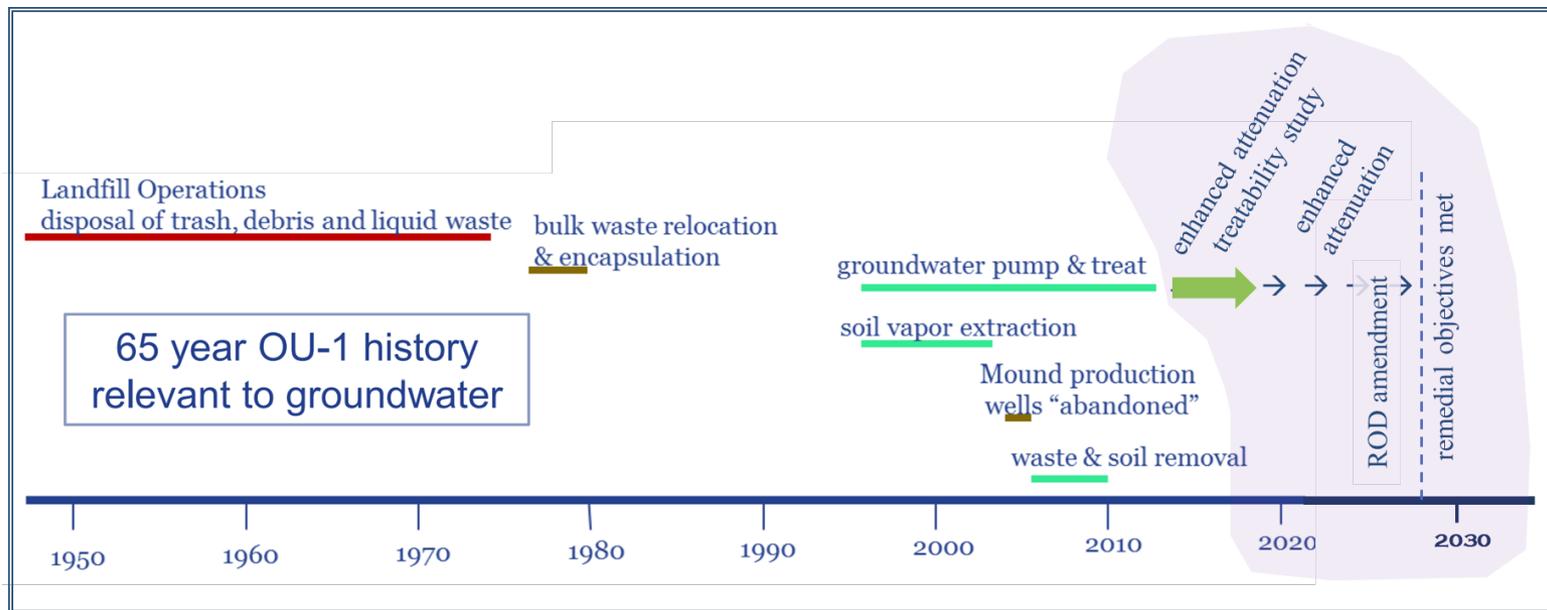
Case Studies – Mound OU1



Contaminant(s)	PCE and TCE
Plume Size	~ 2 acres
Regulatory Drivers	<ul style="list-style-type: none">• Accelerate progress towards remedial objectives compared to P&T• Assure plume is stable-shrinking• Demonstrate EA remedy is sustainable and durable
Approach	Enhanced Attenuation using combined anaerobic-aerobic structured geochemical zones

Mound OU1 - DOE Bioremediation Example

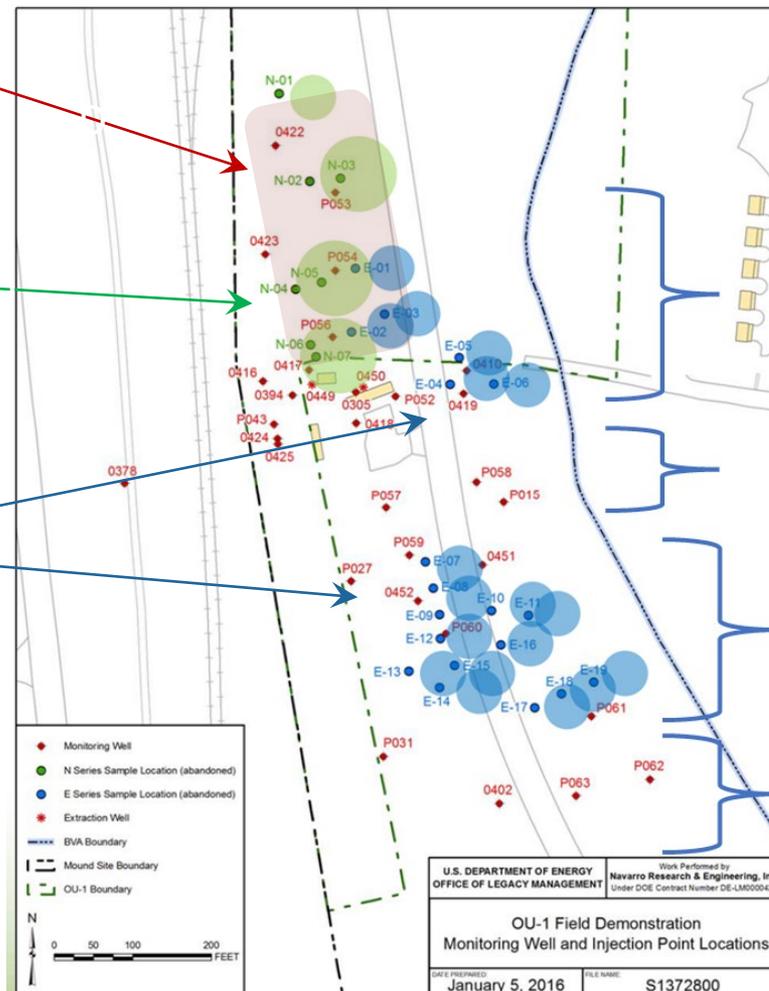
- Mound OU1 is a former landfill that has been remediated by removal/relocation of wastes and groundwater pump and treat
- Pump and treat was projected to operate until 2040 to reach remedial objectives
- A bioremediation (EA using aerobic and anaerobic structured geochemical zones) was proposed that is projected to meet objectives in 2028.



Mound OU1 Timeline

Mound OU1 - DOE Bioremediation Example

- Former Mound OU1 landfill
- Amendment locations
- N locations (green) – neat oil into vadose zone
- E locations (blue) – emulsified vegetable oil injected below the water table
- Structured Geochemical Zones



Anaerobic

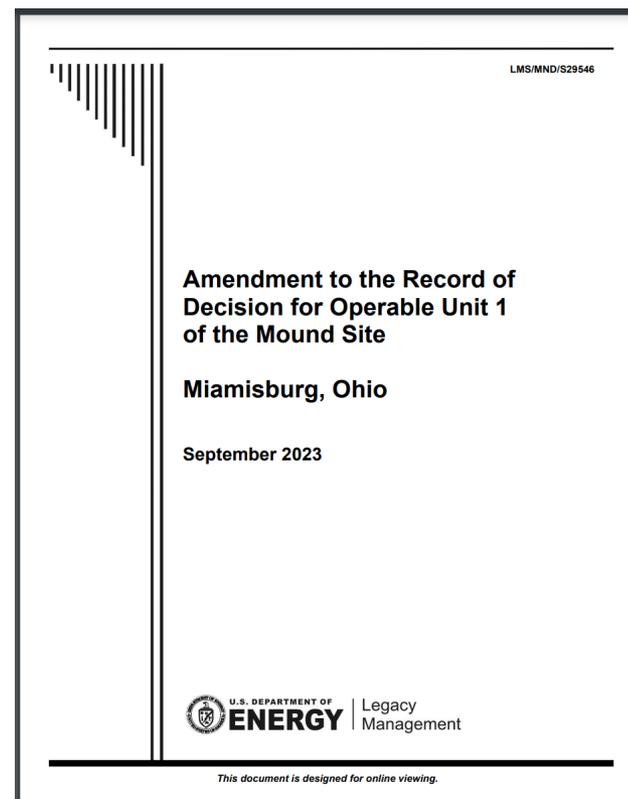
Aerobic

Anaerobic

Aerobic

Mound OU1 - DOE Bioremediation Success

- Completed CERCLA Treatability Study of Bioremediation
 - Implemented using ITRC & DOE developed EA Technical Guidance
 - Monitored using multiple lines of evidence (contaminant and plume trends, geochemistry, microbial community markers, etc.) consistent with EPA guidance
- Bioremediation performed exactly as projected
- Formal ROD Amendment to discontinue pump and treat and move to a passive attenuation-based remedy
 - Just monitoring and stewardship moving forward
- Significant cost savings (>\$5 million) for this one site
 - Savings from elimination of pump and treat with more than a decade reduction in remediation timeframe
- Second successful implementation of this strategy (SRD T Area had similar success and also resulted in formal ROD modification to eliminate pump and treat with similar cost savings....



Case Studies – Pinellas



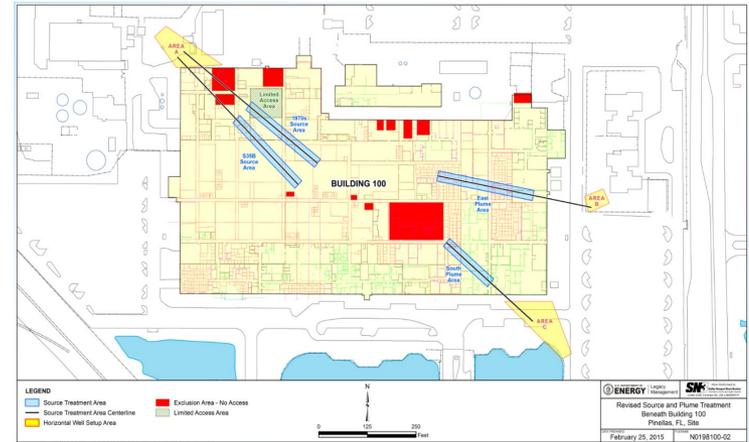
Contaminant(s)	TCE, PCE and related cVOCs
Plume Size	Approximately 30 acres Portion of plume under large building
Regulatory Drivers	Accelerate progress toward regulatory objectives CERCLA Remediate beneath large building
Approach	3 projects 1) Anaerobic Active Remediation (large scale pilot) 2) Aerobic Cometabolism Biosparging (large scale pilot) 3) Anaerobic (full scale permitted)

Pinellas Pilot Studies

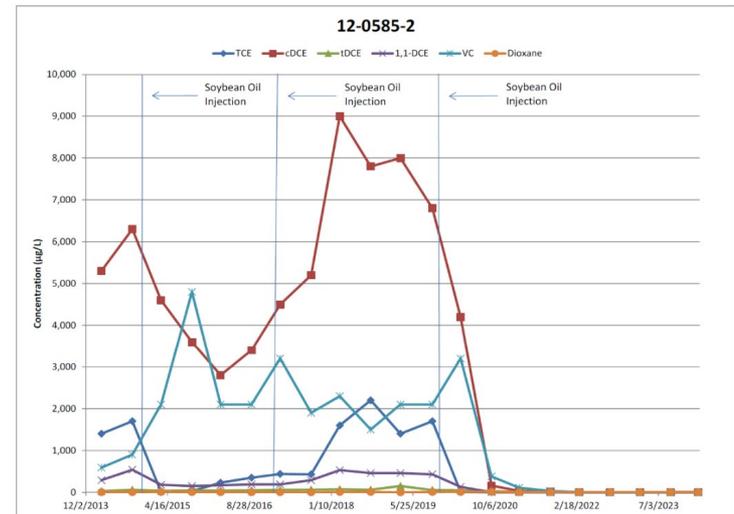
- Anaerobic reductive dichlorination bioremediation
 - Successful
 - Lactate/methanol/benzoate electron donor blend with no bioaugmentation
 - Increased degradation rates significantly
- Aerobic cometabolism and biosparging
 - “Underperformance” due to access, lithology, geochemistry
 - Minimal biodegradation, spread plume
 - Poor access and delivery, gas channeling, challenging geochemistry

Pinellas – Permitted Full Scale Anaerobic Bioremediation

- Emulsified oil
- Nutrients
- Bioaugmentation
- Nearing remedial goals in most wells
- Currently assessing opportunity to transition site to EA!



Horizontal well locations beneath Building 100.



Example monitoring well (12-0585-2) concentration trends in response to oil injections in horizontal wells.

Case Studies – Test Area North (INL)



Contaminant(s)	TCE
Co-contaminants	PCE, cis-1,2-DCE, trans-1,2-DCE, VC, tritium, Cs-137, Sr-90
Plume Details	2 miles long, 200-400 ft bgs
Regulatory Drivers	<ul style="list-style-type: none">• CERCLA• All cVOCs and radionuclides below MCLs by 2095
Approach	Multi-component remedy 1) Anaerobic reductive dechlorination – source zone 2) P&T medial zone 3) MNA distal zone

Test Area North – Idaho National Laboratory

MCLs

Contaminant	MCL (µg/L)
Perchloroethylene (PCE)	5
Trichloroethylene (TCE)	5
Cis-1,2-Dichloroethane (DCE)	70
Trans-1,2-Dichloroethane (DCE)	100
Vinyl chloride (VC)	2

Restoration by In Situ Bioremediation at the Hot Spot

- Amendments injected into the aquifer support dechlorination of VOCs
- Biological activity also degrades (removes) the secondary source of contamination
- Radionuclides not treated by biodegradation
- Radionuclides freed from secondary source not expected to migrate more than several hundred feet
- No waste except from groundwater monitoring

Restoration in Progress by Pump-and-Treat in the Medial Zone

- Contaminated groundwater remediation through pump-and-treat, with reinjection into the aquifer
- Treatment facility (NPTF) operations began in Fall 2001
- Radionuclides in this zone are below MCLs already and do not require treatment

Restoration by Monitored Natural Attenuation in the Distal Zone

- Natural degradation processes degrade VOCs
- Radionuclides in this zone are below MCLs already and do not require treatment
- No waste except from groundwater monitoring, and no construction or facility operation expenses

Institutional Controls

- Administrative controls
 - publish USGS map of area of contamination
 - prohibit industrial or residential wells in area of contamination
 - implement deed restrictions and land-use planning
 - use Agency five-year reviews to review performance and compliance monitoring efforts against forecasted levels
- Engineering controls
 - control access to facilities, area of contamination, and well heads
 - signs and postings
 - existing drinking water treated to be safe for human consumption
- Boundary of the institutional controls area has a buffer zone for conservative management of the plume

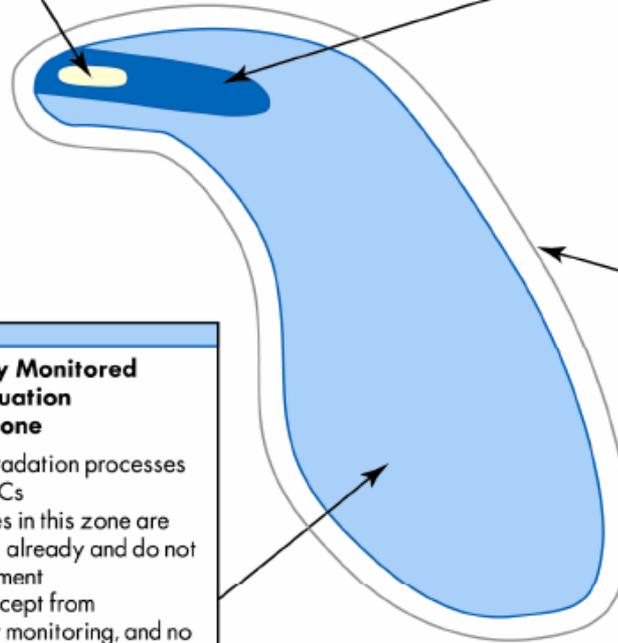
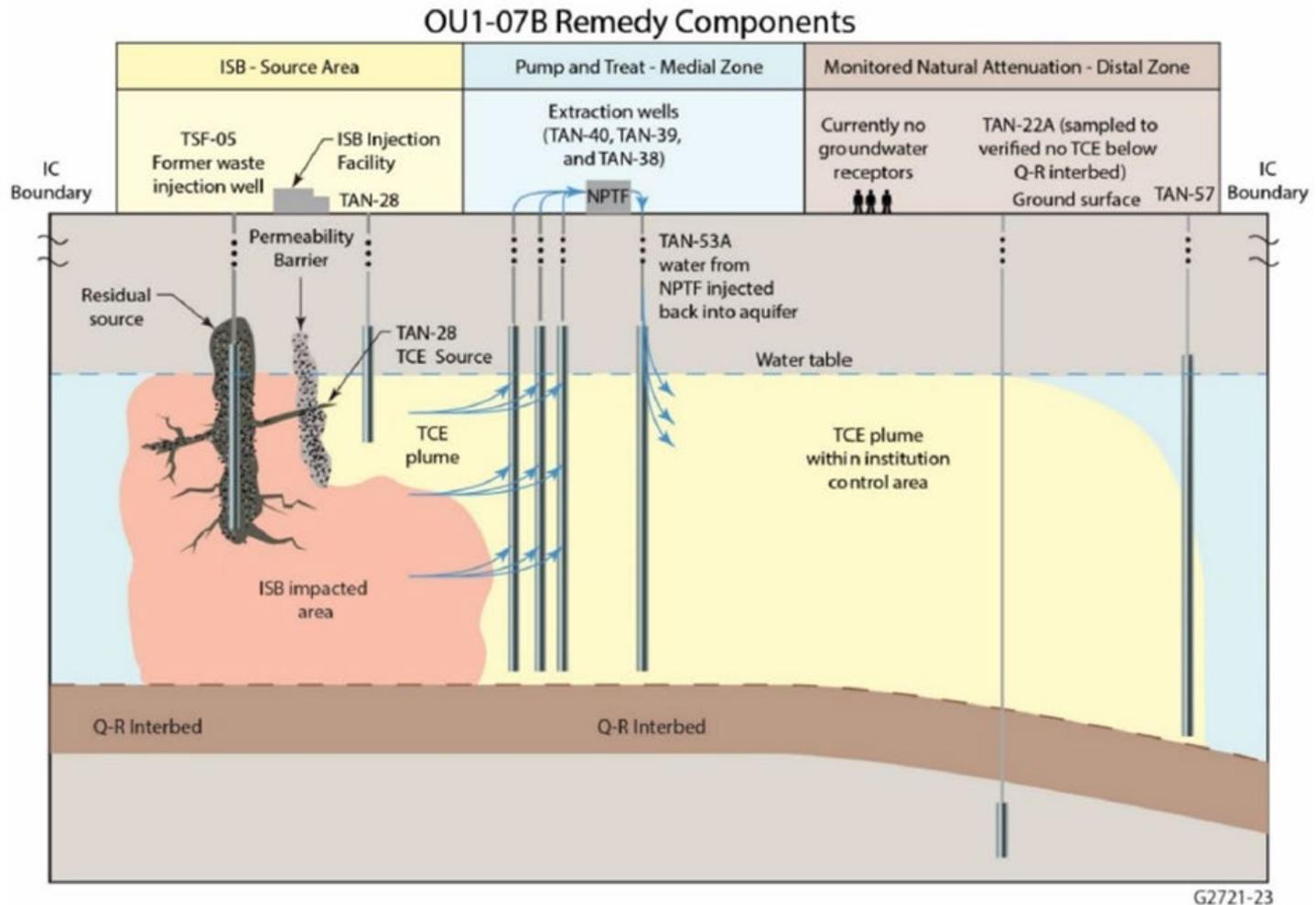


Illustration of the three zones of the TE plume at TAN (INL ICP, 2007)

Test Area North – Idaho National Laboratory



Conceptual site model of the trichloroethylene source and plume (DOE-ID, 2020)

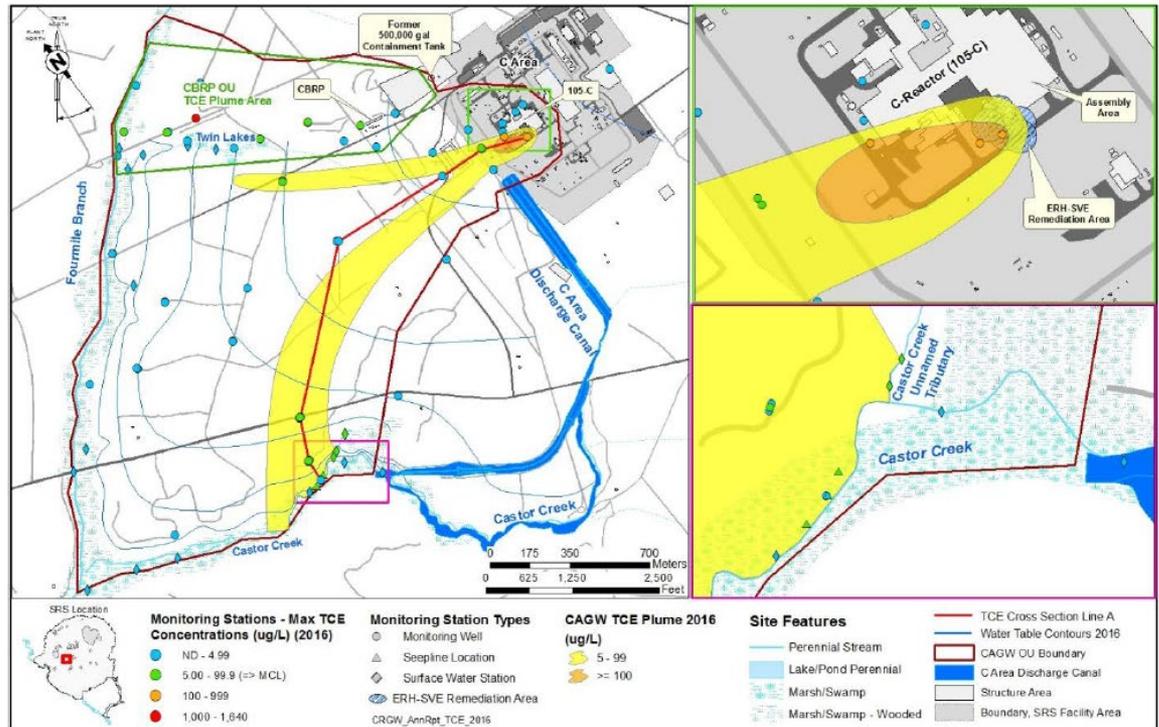
Case Studies – C-Area (SRS)



Contaminant(s)	TCE
Co-contaminants	Tritium
Plume Details	TCE conc. 15 ug/L in unnamed tributary to Castor Creek
Regulatory Drivers	DOE, EPA, SCDES, agreement for non-critical removal action Reduce TCE and Tritium below MCLs
Approach	Injection of emulsified oil, buffer, vitamin C, vitamin B

C-Area – Savannah River Site

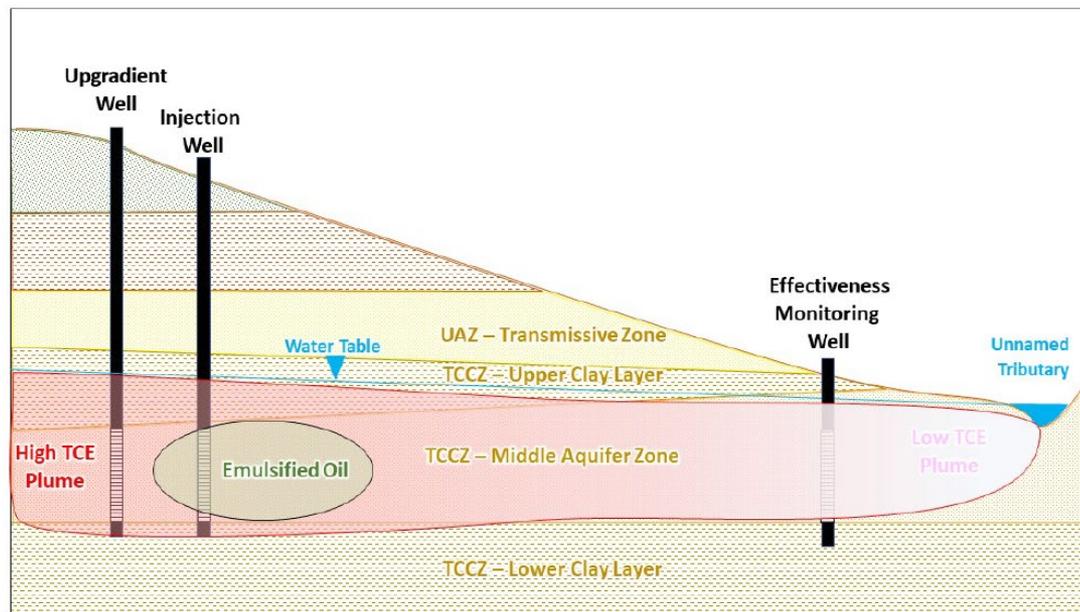
- CAGW Operable Unit Bio-Barrier, Non-Time Critical Removal Action
- Remediation expected to last 3-5 years
- TCE breaking down after 3 years



C-Area TCE Plume (2016) (SRNS, 2019)

C-Area – Savannah River Site

- Between 2020 and 2022, TCE concentrations dropped below 5 µg/L
- MNA will be used going forward as long as TCE and tritium drop/remain below MCLs
- Microbial community monitored using BioTraps (Microbial Insights)
- Lesson learned – oil should be injected prior to (and separate from) second injection of buffer/nutrients



Schematic of C-Area Operational Unit Removal Action Process (SRNS, 2019)

Cost Savings

- Bioremediation is often an appropriate and cost-effective solution for cVOC plume remediation
- Cost data is often limited, especially for CERCLA and RCRA sites
- Lower capital and operations/maintenance costs
- Variability and savings and approach – driving by site-specific characteristics
- Shifting from active to passive remediation saves significant costs

Summary of Cost-Savings Analyses

Site	Reported/Estimated Cost Savings *
SRS TNX	73% savings (EA vs P&T), estimated \$30 M
SRS Integrated Demonstration	10-20% savings, \$1.5 million for demonstration test site, time reduced by 5-7 years compared to SVE/P&T
Hanford	Targeted applicability only, cost prohibitive for entire CT plume due to scale and hydrogeology
Paducah	Demonstrated cost-effectiveness compared to P&T, not fully quantified
TAN (INL)	18% savings compared to P&T, in 2001 estimated \$23 M over 30 years
Mound Site	73% (EA vs P&T) savings, cleanup time reduced to approximately half, in 2016 savings estimated to be \$6 M
Pinellas	9-36% saving with upper end including EA in calculation vs P&T

*No cost data available for SRS A/M Area, Sanitary Landfill, or C-Area Operable Unit

Overarching Summary

The 30+ year period of DOE cVOC bioremediation experience as summarized in a systematic review will support DOE decision makers as well as contractors in their project management, project design, and field operations responsibilities for current and future cVOC bioremediation projects.

A few observations and path forward

- The portfolio of cVOC bioremediation projects in DOE has yielded significant success over the past three decades
 - Two sites have formally transitioned to EA from pump and treat and are nearing completion
 - Several sites nearing completion or ready to transition to EA
- DOE led the collaboration to develop technical guidance for implementing EA for cVOCs
- Systematic Review provided rapid, cost effective and actionable information
 - Review will support DOE decision makers and contractors in project management, project design, and field operations for current and future cVOC bioremediation projects- increasing the likelihood of success, reducing costs, and improving bioremediation performance

Future Work

- Recommended future work includes:
 - Webinar/workshop series development for LM and EM to discuss findings of the retrospective
 - Perform similar retrospective evaluations for other contaminant classes such as metals/radionuclides and emerging contaminants